

- 1 -

OLIGONUCLEOTIDE SYNTHESIS USING CYCLIC-PHOSPHOROUS NUCLEOSIDES

TECHNICAL FIELD

[0001] The present disclosure provides methods for preparing oligonucleotides. In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using cyclic-phosphorous nucleosides. In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using substituted benzyl alcohol nucleophiles. In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using cyclic-phosphorous nucleosides and substituted benzyl alcohol nucleophiles.

DISCLOSURE

[0002] The demand for synthetic oligonucleotides is steadily increasing as advances in DNA and RNA technology continue to expand through the scientific and medical communities. Innovations in molecular biology, RNA-based therapeutics, DNA-based diagnostics, genetic engineering and treatments, and other similar methods continue to present a high demand for synthesized oligonucleotides that can be used to amplify, detect, analyze, quantify, modify, or target natural nucleic acids. However, current methods for synthesizing oligonucleotides (such as automated solid phase synthesis using phosphoramidite chemistry) can be expensive, limited in scalability, limited in portability (e.g., due to reliance on specialized packed bed reactors), and often require complex workflow systems.

[0003] A need therefore exists for oligonucleotide synthesis methods which are efficient, scalable, easily performed in batch or flow reactors, and reliably provide short, medium, or long-chain oligonucleotides of interest, including oligonucleotides for direct use or for use as synthetic building blocks.

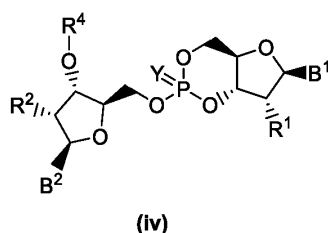
SUMMARY

[0004] The details of various embodiments of the present disclosure are set forth in the description below. In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide. In certain embodiments, the present disclosure provides a method for preparing a dinucleotide. In certain embodiments, the present disclosure provides a method for preparing a trinucleotide. In certain embodiments, the present

- 2 -

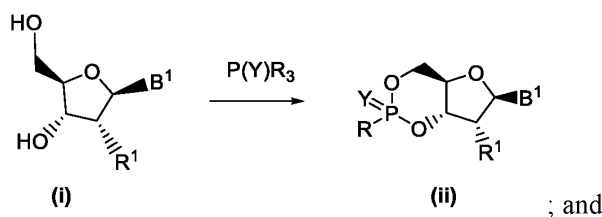
disclosure provides a method for preparing an oligonucleotide which is from 2-20 nucleotides in length.

[0005] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (iv) or a salt or solvate thereof,



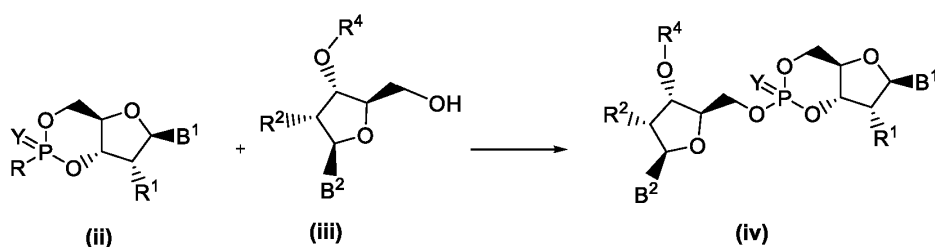
wherein: wherein Y is O or S, R^1 and R^2 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C_1-C_{20}) alkyl and (C_1-C_{20}) alkoxy group (including methoxy and methoxy ethyl); B^1 and B^2 are nucleobases, which may optionally be protected with one or more protecting groups; and R^4 is hydrogen or a protecting group.

[0006] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (iv) or a salt or solvate thereof, wherein the method comprises: (a) reacting a nucleoside of Formula (i) with $P(Y)R_3$, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C_1-C_6) alkoxy, (C_6-C_{12}) aryloxy, S- (C_1-C_6) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):



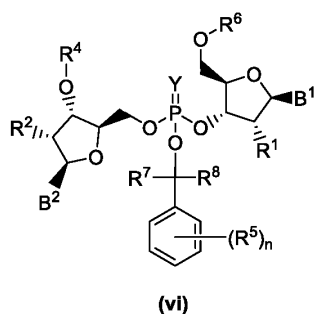
(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:

- 3 -



In certain embodiments, the method further comprises removing one or more of the protecting groups.

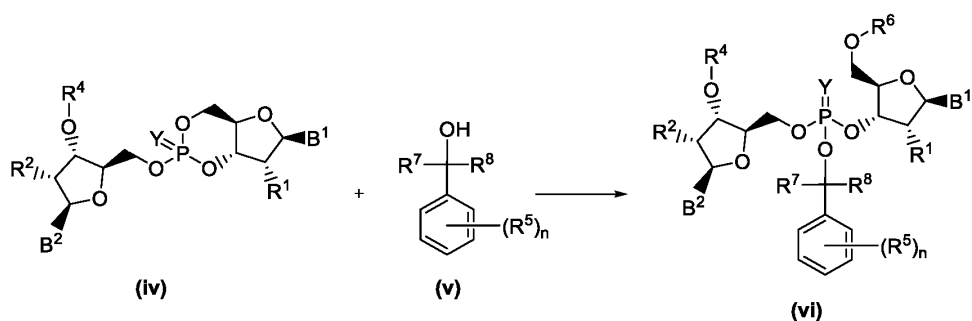
[0007] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof:



wherein: Y is O or S; R^1 and R^2 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (including methoxy and -O-methoxyethyl); B^1 and B^2 are nucleobases, which may optionally be protected; R^4 is hydrogen or a protecting group; n is chosen from 1, 2, 3, 4, and 5; each R^5 is independently a substituent on the phenyl ring of a benzyl protecting group; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl.

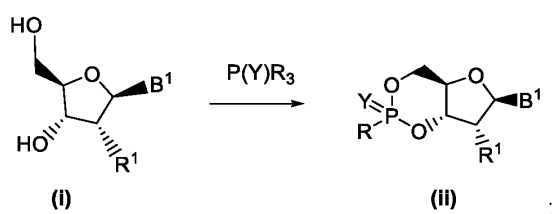
[0008] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof, wherein the method comprises: providing the cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group; and reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):

- 4 -

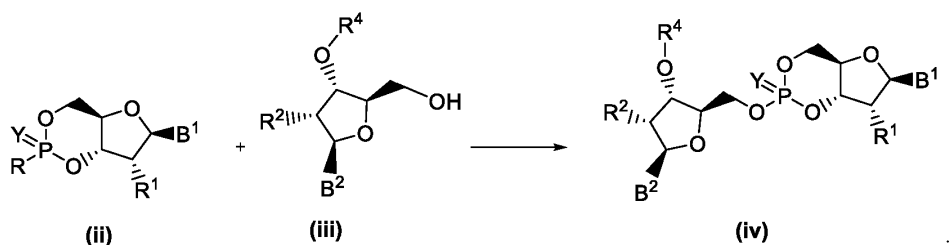


wherein: each R⁵ is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R⁶ is hydrogen or a protecting group; and R⁷ and R⁸ are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl. In certain embodiments, the method further comprises removing one or more of the protecting groups from the dinucleotide of Formula (vi).

[0009] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof, wherein the method comprises: (a) reacting a nucleoside of Formula (i) with P(Y)R₃, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):



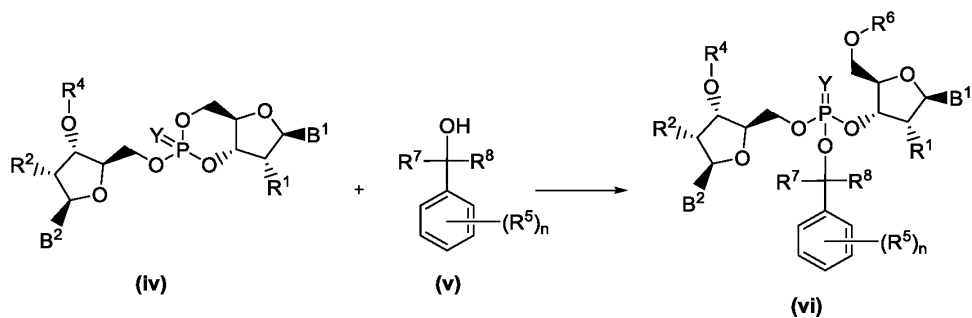
(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R⁴ is a protecting group:



and

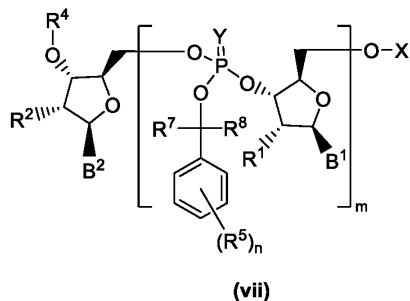
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(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



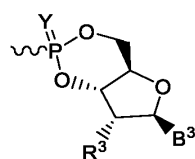
wherein: each R⁵ is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R⁶ is hydrogen or a protecting group; and R⁷ and R⁸ are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl. In certain embodiments, the method further comprises removing one or more of the protecting groups from the dinucleotide of Formula (vi).

[0010] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (vii):



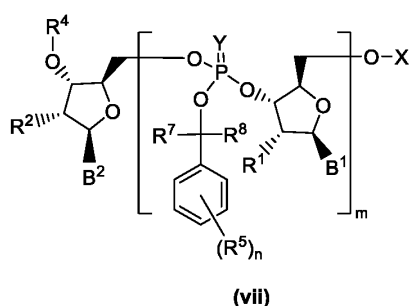
wherein: each Y is independently O or S; R¹, R², and R³ are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy (including methoxy and -O-methoxyethyl); R⁴ is H or a protecting group; n is chosen from 1, 2, 3, 4, and 5; each R⁵ is independently a substituent on the phenyl ring of the benzyl protecting group; each R⁷ and R⁸ is independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl; B¹, B², and B³ are nucleobases, which may optionally be protected; m is chosen from integers ranging from 1 to 18; and X is hydrogen, a

- 6 -



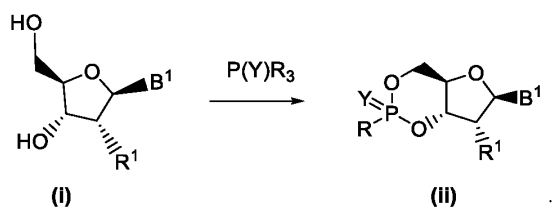
protecting group, or

[0011] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (vii), wherein the method comprises: providing the dinucleotide of Formula (vi); and repeating the reactions of steps (a), (b), and (c) from a method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof, as disclosed herein, one or more times to obtain an oligonucleotide of Formula (vii):



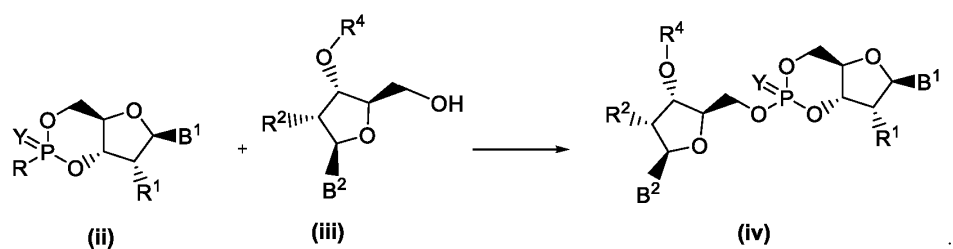
wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the dinucleotide or oligonucleotide. In certain embodiments, the method further comprises removing one or more of the protecting groups.

[0012] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (vii), wherein the method comprises: (a) reacting a nucleoside of Formula (i) with $P(Y)R_3$, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C_1-C_6) alkoxy, (C_6-C_{12}) aryloxy, S- (C_1-C_6) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):

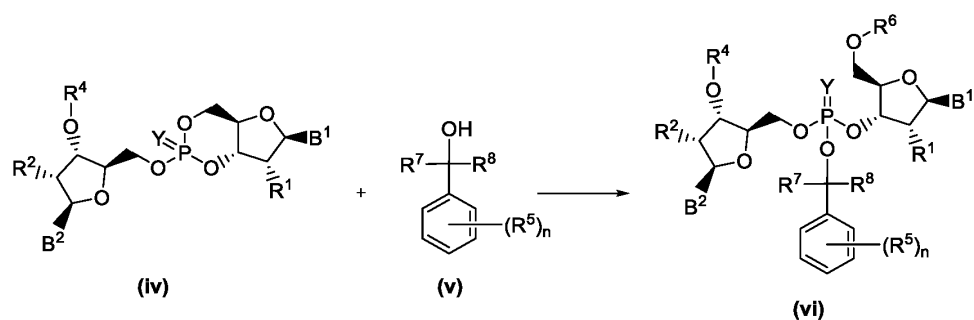


(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:

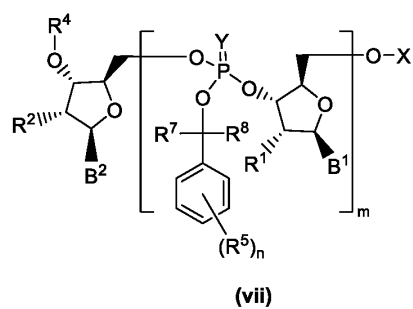
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(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



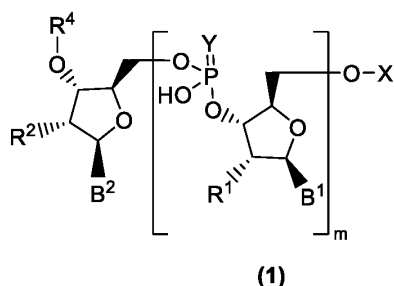
wherein: each R⁵ is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R⁶ is hydrogen or a protecting group; and R⁷ and R⁸ are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl; and (d) repeating the reactions of steps (a), (b), and (c) one or more times to obtain an oligonucleotide of Formula (vii):



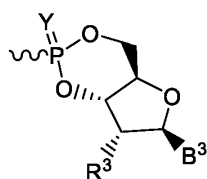
wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the dinucleotide or oligonucleotide. In certain embodiments, the method further comprises removing one or more of the protecting groups.

- 8 -

[0013] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof,



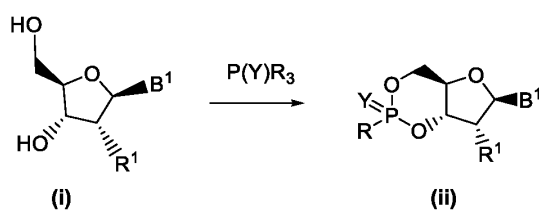
wherein: each Y is independently O or S; R¹, R², and R³ are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy (including methoxy and -O-methoxyethyl); B¹, B², and B³ are nucleobases, which may optionally be protected; R⁴ is hydrogen or a protecting group; m is chosen from integers ranging from 0 to 18; and X is hydrogen, a protecting group, or



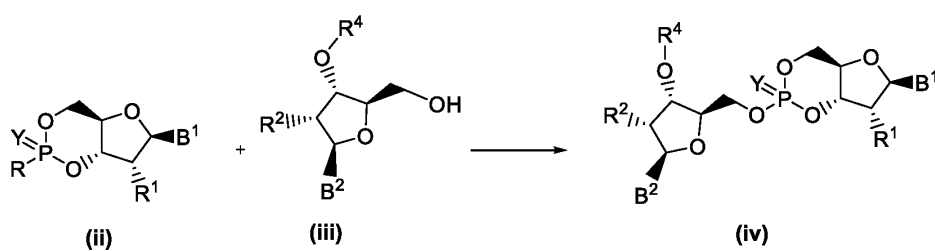
[0014] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof, wherein the method comprises: providing a dinucleotide of Formula (vi) or an oligonucleotide of Formula (vii); and hydrogenating the dinucleotide of Formula (vi) or the oligonucleotide of Formula (vii); wherein the method produces the oligonucleotide of Formula (1) or a salt or solvate thereof. In certain embodiments, the method further comprises removing one or more of the protecting groups.

[0015] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof, wherein the method comprises: (a) reacting a nucleoside of Formula (i) with P(Y)R₃, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):

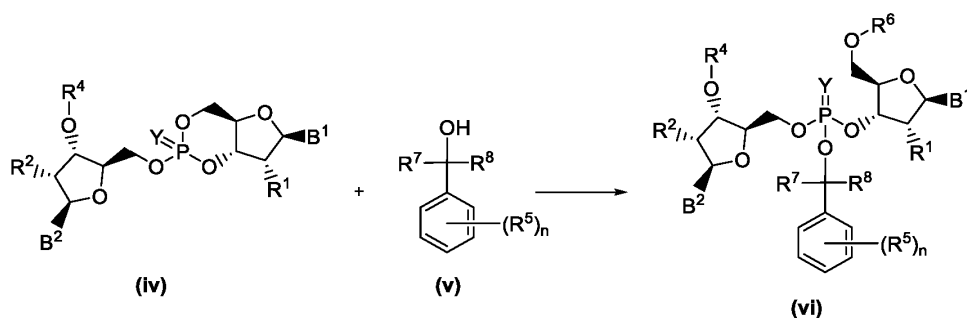
- 9 -



(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:

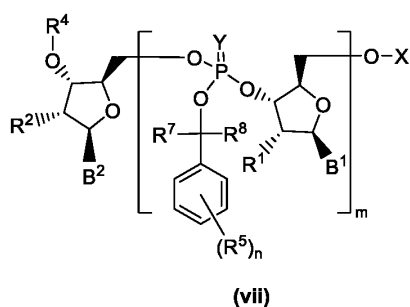


(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl; (d) optionally, repeating the reactions of steps (a), (b), and (c) one or more times to obtain an oligonucleotide of Formula (vii):

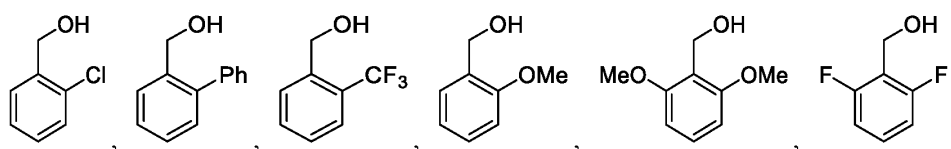
- 10 -



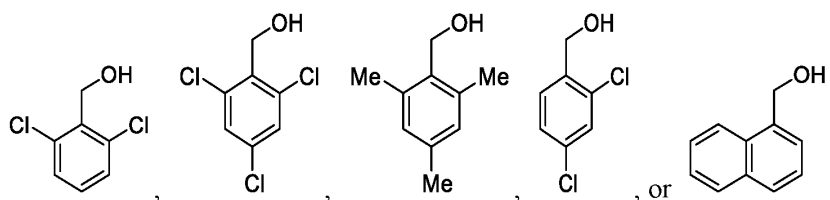
wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the oligonucleotide; and (e) hydrogenating the product of step (c) or step (d); wherein the method produces the oligonucleotide of Formula (1) or a salt or solvate thereof. In certain embodiments, the method further comprises removing one or more of the protecting groups.

[0016] In certain embodiments of the present disclosure, the cyclic phosphorus dinucleotide of Formula (iv) is obtained as a mixture of stereoisomers. In certain embodiments, the stereoisomers are separated prior to the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v).

[0017] In certain embodiments of the present disclosure, each instance of R^5 is an ortho- or para- substitution on the benzyl group. In certain embodiments, each instance of R^5 is an ortho- substitution on the benzyl group. In certain embodiments, each instance of R^5 is a para- substitution on the benzyl group. In certain embodiments, each instance of R^5 is independently chosen from halogen, (C_1-C_{20}) alkyl, (C_1-C_{20}) alkoxy, trifluoromethyl, and phenyl. In certain embodiments, each instance of R^5 is independently selected from phenyl, methyl, methoxy, fluoro, chloro, and CF_3 . In certain embodiments, each instance of R^5 is independently selected from chloro and CF_3 . In certain embodiments, R^5 is a fused phenyl ring forming a naphthyl ring system. In certain embodiments, the substituted benzyl alcohol nucleophile of Formula (v) is:



- 11 -



[0018] In certain embodiments of the present disclosure, the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) is carried out in a solvent system. In certain embodiments, the solvent comprises: tetrahydrofuran (THF); 2-methyltetrahydrofuran (2Me-THF); diethyl ether; 1,4-dioxane; dimethylsulfoxide (DMSO), N-methylpyrrolidone (NMP), dimethylacetamide (DMA), acetonitrile (MeCN), methyl tert-butyl ether (MTBE), tertAmyl alcohol, toluene, or a mixture thereof.

[0019] In certain embodiments of the present disclosure, the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) is carried out in the presence of a base. In certain embodiments, the base comprises: n-butyllithium (n-BuLi); lithium tertbutoxide (LiOtBu); lithium bis(trimethylsilyl)amide (LiHMDS); lithium tetramethylpiperidide (LiTMP); or Li-R_Z, where R_Z is alkyl or aryl; sodium t-butoxide (NaOtBu) + lithium chloride (LiCl); potassium t-butoxide (KOtBu) + lithium chloride (LiCl); triethylamine, Diazabicycloundecene (DBU), triazabicyclodecene (TBD), or a combination thereof. In certain embodiments, the base is pre-mixed with the substituted benzyl alcohol nucleophile of Formula (v) prior to the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v). In certain embodiments, the base is lithium tertbutoxide (LiOtBu).

[0020] In certain embodiments of the present disclosure, the dinucleotide of Formula (vi) is isolated by reacting with a silyl chloride.

[0021] In certain embodiments of the present disclosure, the cyclic phosphorus chloride nucleoside of Formula (ii) is obtained as a mixture of stereoisomers. In certain embodiments, the stereoisomers are separated prior to the step of reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii).

- 12 -

[0022] In certain embodiments of the present disclosure, R^2 is an alkoxy group linked to the adjacent O to form a ketal protecting group with R^4 .

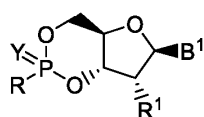
[0023] In certain embodiments of the present disclosure, R^4 is chosen from H, a silyl group, trimethylsilyl, triethylsilyl, t-butyl dimethylsilyl (TBS, TBDMS), t-butyl diphenylsilyl, triisopropylsilyl, or a ketal protecting group formed with R^2 .

[0024] In certain embodiments of the present disclosure, each nucleobase is chosen from pyrimidines and purines. In certain embodiments, each nucleobase is protected with one or more protecting groups. In certain embodiments, each nucleobase is protected with one or more protecting groups independently chosen from dimethyl formamide (DMF), formamidine, DMF-formamidine, phthalimido, chloro, bromo, acyl, allyl, benzyl, tert-butyloxycarbonyl (Boc), and benzyloxymethyl (BOM).

[0025] In certain embodiments of the present disclosure, the oligonucleotide synthesis method is a liquid phase process (i.e., conducted in liquid phase). In certain embodiments, the method is conducted using continuous flow. In certain embodiments, the method is carried out in a flow reactor.

[0026] In certain embodiments, the present disclosure provides an oligonucleotide (e.g., dinucleotide, trinucleotide) produced by a method of the present disclosure.

[0027] In certain embodiments, the present disclosure provides a cyclic phosphorous nucleoside of Formula (ii):

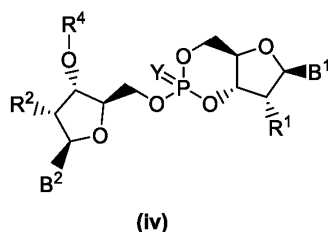


(ii)

or a salt or solvate thereof, wherein: Y is S or O; each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl; R^1 is chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy (including methoxy and -O-methoxyethyl); and B^1 is nucleobase, which may be optionally protected. In certain embodiments, the present disclosure provides a cyclic phosphorous nucleoside of Formula (ii) produced by a method of the present disclosure.

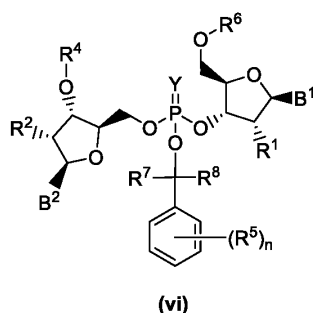
[0028] In certain embodiments, the present disclosure provides a cyclic phosphorus dinucleotide of Formula (iv):

- 13 -



or a salt or solvate thereof, wherein: Y is O or S; R^1 and R^2 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (including methoxy and -O-methoxyethyl); R^4 is H or a protecting group; and B^1 and B^2 are nucleobases, which may optionally be protected. In certain embodiments, the present disclosure provides a cyclic phosphorus dinucleotide of Formula (iv) produced by a method of the present disclosure.

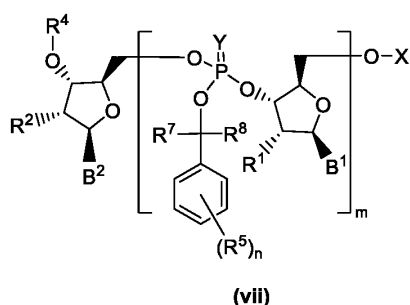
[0029] In certain embodiments, the present disclosure provides a dinucleotide of Formula (vi):



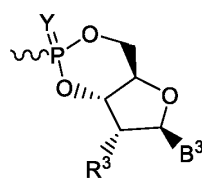
or a salt or solvate thereof, wherein: Y is O or S; R^1 and R^2 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (including methoxy and -O-methoxyethyl); R^4 is H or a protecting group; each R^5 is independently a substituent on the phenyl ring of the benzyl protecting group; R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl; B^1 and B^2 are nucleobases, which may optionally be protected; and n is chosen from 1, 2, 3, 4, and 5. In certain embodiments, the present disclosure provides a cyclic phosphorus dinucleotide of Formula (vi) produced by a method of the present disclosure.

[0030] In certain embodiments, the present disclosure provides an oligonucleotide of Formula (vii):

- 14 -

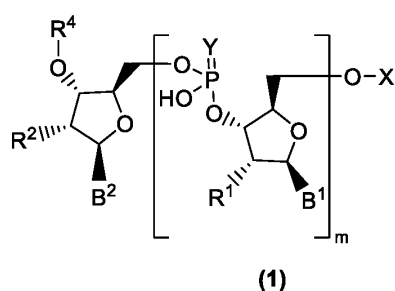


or a salt or solvate thereof, wherein: each Y is independently O or S; R^1 , R^2 , and R^3 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (including methoxy and -O-methoxyethyl); R^4 is H or a protecting group; n is chosen from 1, 2, 3, 4, and 5; each R^5 is independently a substituent on the phenyl ring of the benzyl protecting group; each R^7 and R^8 is independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl; B^1 , B^2 , and B^3 are nucleobases, which may optionally be protected; m is chosen from integers ranging from



1 to 18; and X is hydrogen, a protecting group, or
embodiments, the present disclosure provides an oligonucleotide of Formula (vii)
produced by a method of the present disclosure.

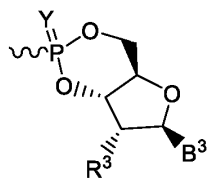
[0031] In certain embodiments, the present disclosure provides an oligonucleotide of Formula (1) or a salt or solvate thereof,



wherein: each Y is independently O or S; R^1 , R^2 , and R^3 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (including methoxy and -O-methoxyethyl); B^1 , B^2 , and B^3 are nucleobases, which may optionally be protected; R^4 is hydrogen or a protecting group; m is chosen

- 15 -

from integers ranging from 0 to 18; and X is hydrogen, a protecting group, or



. In certain embodiments, the present disclosure provides an oligonucleotide of Formula (1) produced by a method of the present disclosure.

[0032] In certain embodiments, the present disclosure provides a solution comprising a nucleoside (e.g., cyclic phosphorous nucleoside) of the present disclosure. In certain embodiments, the present disclosure provides a solution comprising a dinucleotide of the present disclosure. In certain embodiments, the present disclosure provides a solution comprising a trinucleotide of the present disclosure. In certain embodiments, the present disclosure provides a solution comprising an oligonucleotide of the present disclosure.

[0033] In certain embodiments, oligonucleotides of the present disclosure can be used in subsequent oligonucleotide or polynucleotide synthesis applications. In certain embodiments, oligonucleotides of the present disclosure can be used as a blockmer in solid phase synthesis of oligonucleotides or polynucleotides. In certain embodiments, oligonucleotides of the present disclosure can be used as blockmer amidites in solid phase synthesis of oligonucleotides or polynucleotides. In certain embodiments, oligonucleotides of the present disclosure can be used in enzymatic ligation reactions for the synthesis of oligonucleotides or polynucleotides.

BRIEF DESCRIPTION OF THE FIGURES

[0034] FIG. 1A and FIG. 1B present an example of an enzyme ligation reaction of shortmers to produce a ninemer. The demand for synthetic oligonucleotides is steadily increasing as advances in DNA and RNA technology

[0035] FIG. 2A, FIG. 2B, FIG. 2C, FIG. 2D, FIG. 2E, and FIG. 2F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 2HR; PhMe (Toluene).

[0036] FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D, FIG. 3E, and FIG. 3F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 2HR; ACN.

[0037] FIG. 4A, FIG. 4B, FIG. 4C, FIG. 4D, FIG. 4E, and FIG. 4F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 20HR; PhMe (Toluene).

- 16 -

[0038] FIG. 5A, FIG. 5B, FIG. 5C, FIG. 5D, FIG. 5E, and FIG. 5F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 20HR; ACN.

[0039] FIG. 6 presents a general overview for one embodiment of a solution-phase method for oligonucleotide synthesis of the present disclosure.

[0040] FIG. 7 presents a general overview for one embodiment of a continuous-flow oligonucleotide synthesis system of the present disclosure.

DETAILED DESCRIPTION

OLIGONUCLEOTIDE SYNTHESIS

Oligonucleotides

[0041] The present disclosure provides methods for preparing oligonucleotides. The present disclosure also provides oligonucleotides prepared by the methods of the present disclosure. Oligonucleotides are polymers of linked nucleotides, typically less than about 100 nucleotides in length. In certain embodiments, oligonucleotides of the present disclosure include at least 2 nucleotides. Thus, in certain embodiments, the oligonucleotides of the present invention include 2 to 100 nucleotides and all whole-number integer ranges from (e.g., 2 to 100, 2 to 50, 2 to 30, 2 to 20, 2 to 15, 2 to 10, 2 to 8, 2 to 6, 2 to 3 nucleotides, 3 to 100, 3 to 50, 3 to 30, 3 to 20, 3 to 15, 3 to 10, 3 to 8, or 3 to 6 nucleotides, etc.). In certain embodiments, the oligonucleotide includes 2 nucleotides (i.e., a dinucleotide). In certain embodiments, the oligonucleotide includes 3 nucleotides (i.e., a trinucleotide). In certain embodiments the oligonucleotide includes 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides.

[0042] In certain embodiments, oligonucleotides of the present disclosure include from 3 to 100 nucleotides. In certain embodiments, the oligonucleotide includes from 3 to 50 nucleotides. In certain embodiments, the oligonucleotide includes from 3 to 30 nucleotides. In certain embodiments, the oligonucleotide includes from 3 to 20 nucleotides. In certain embodiments, the oligonucleotide includes from 3 to 15 nucleotides. In certain embodiments, the oligonucleotide includes from 3 to 10 nucleotides. In certain embodiments, the oligonucleotide includes from 3 to 8 nucleotides. In certain embodiments, the oligonucleotide includes from 3 to 6 nucleotides. In certain embodiments, the oligonucleotide includes 3 nucleotides (i.e., a trinucleotide).

- 17 -

[0043] In certain embodiments, oligonucleotides of the present disclosure include from 5 to 20 nucleotides. In certain embodiments, oligonucleotides of the present disclosure include from 10 to 20 nucleotides. In certain embodiments, oligonucleotides of the present disclosure include from 15 to 20 nucleotides. In certain embodiments, the oligonucleotide includes 3 nucleotides. In certain embodiments, the oligonucleotide includes 5 nucleotides. In certain embodiments, the oligonucleotide includes 6 nucleotides. In certain embodiments, the oligonucleotide includes 7 nucleotides. In certain embodiments, the oligonucleotide includes 8 nucleotides. In certain embodiments, the oligonucleotide includes 9 nucleotides. In certain embodiments, the oligonucleotide includes 10 nucleotides. In certain embodiments, the oligonucleotide includes 11 nucleotides. In certain embodiments, the oligonucleotide includes 12 nucleotides. In certain embodiments, the oligonucleotide includes 13 nucleotides. In certain embodiments, the oligonucleotide includes 14 nucleotides. In certain embodiments, the oligonucleotide includes 15 nucleotides. In certain embodiments, the oligonucleotide includes 16 nucleotides. In certain embodiments, the oligonucleotide includes 17 nucleotides. In certain embodiments, the oligonucleotide includes 18 nucleotides. In certain embodiments, the oligonucleotide includes 19 nucleotides. In certain embodiments, the oligonucleotide includes 20 nucleotides.

[0044] The term "nucleotide" is defined herein (see below) as an organic compound having a nucleoside and a phosphate group. The term "nucleoside" is defined herein (see below) as an organic compound having a nucleobase (e.g., adenine, cytosine, guanine, thymine, or uracil) covalently attached to a pentose sugar (e.g., ribose or 2'-deoxyribose). The term "nucleobase" is defined herein (see below) as a heterocyclic moiety of a nucleoside. In certain embodiments, a nucleobase of the present disclosure is a canonical nucleobase. In certain embodiments, the nucleobase is a purine base -- adenine (A) or guanine (G). In certain embodiments, the nucleobase is a pyrimidine base -- thymine (T), cytosine (C), or uracil (U).

[0045] In certain embodiments, a nucleobase of the present disclosure is a noncanonical nucleobase (i.e., not a canonical nucleobase). Examples of noncanonical nucleobases include, but are not limited to: 3-methylcytosine; 5-methylcytosine (5-me-C); 5-ethylcytosine; 5-ethyluracil; 5-hydroxymethyl cytosine; 7-methylguanine; 1-methylguanine; 2-methylguanine; 8-methylguanine; 2,2-dimethylguanine; 1-

- 18 -

methyladenine; 2-methyladenine; N⁶-methyladenine; 7-methyladenine; N,N-dimethyladenine; alkyl derivatives (e.g., 6-methyl, 2-propyl) of adenine and guanine; 5-propyluracil; xanthine; hypoxanthine; 2-aminoadenine; 2-aminopyridine; 2-aminopurine; 2,6-diaminopurine; 8-aminoguanine; 2-thiouracil; 5-methyl-2-thiouracil; 4-thiouracil; 2-thiothymine; 2-thiocytosine; 6-thiopurine; 8-thioguanine; 5-halouracil; cytosine; alkynyl derivatives (e.g., 5-propynyl) of uracil and cytosine; 6-azo uracil; 5-uracil (pseudouracil); 8-substituted (e.g., 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-halo) adenines and guanines; 5-substituted (e.g., 5-chloro, 5-bromo, 5-trifluoromethyl) uracils and cytosines; 2-F-adenine; 8-azaguanine; 8-azaadenine; 7-deazaguanine; 7-deazaadenine; 3-deazaguanine; 3-deazaadenine; tricyclic pyrimidines; phenoxazine cytidine (1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one); phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one); substituted phenoxazine cytidines (e.g., 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one); carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one); pyridoindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one); 2-pyridone; N⁶-isopentyladenine; 2-methylthio-N⁶-isopentyladenine; 8-bromoadenine; 8-bromoguanine; 8-chloroguanine; 5-bromouracil; 5-fluorouracil; 5-chlorouracil; 5-iodouracil; 4-acetylcytosine; 5-methoxyuracil; 5-hydroxymethyluracil; 5-(carboxyhydroxymethyl)uracil, 5-(methylaminomethyl)uracil, 5-(carboxymethylaminomethyl)-uracil; 5-(2-bromovinyl)uracil; uracil-5-oxyacetic acid; uracil-5-oxyacetic acid methyl ester; pseudouracil, 1-methylpseudouracil; queosine; inosine; 1-methylinosine; and 6-hydroxyaminopurine. Examples of noncanonical nucleobases also include, but are not limited to: methylated purines or pyrimidines, alkylated purines or pyrimidines, acylated purines or pyrimidines, halogenated purines or pyrimidines, deazapurines, alkylated riboses, diaminopurine, inosine, and thiolated purines or pyrimidines.

[0046] In certain embodiments, the nucleobase is unprotected. In certain embodiments, the nucleobase is protected by a protecting group. The term “protecting group” is defined herein (see below) as a chemical moiety that protects reactive or labile groups on a molecule from undergoing undesired chemical reactions during synthetic procedures or other chemical reactions. A nucleobase protecting group refers to a protecting group applied to one or more functional groups of a nucleobase to prevent side reactions at the nucleobase during the synthesis of oligonucleotides. In certain

embodiments, nucleobase protecting groups are removed at the end of the oligonucleotide synthesis. Nucleobase protecting groups can include exocyclic amino groups or lactam groups. In certain embodiments, a nucleobase protecting group is applied to the N6-amino group of an adenine moiety. In certain embodiments, a nucleobase protecting group is applied to N4-amino group of a cytosine moiety. In certain embodiments, a nucleobase protecting group is applied to the N2-amino group, the N1-lactam (i.e., cyclic amide) group, and/or the O6-lactam groups of a guanine moiety. In certain embodiments, a nucleobase protecting group is applied to the N3-lactam group and/or the O4-lactam group of a uracil or thymine moiety. In certain embodiments, the nucleobase protecting group is base-labile. Examples of base-labile protecting group include Fmoc (fluorenylmethyloxycarbonyl). In certain embodiments, the nucleobase protecting group is acid labile. Examples of acid-labile protecting group include triarylmethyl protecting groups, 4, 4'-dimethoxytriphenyl methyl (DMT), and BOC carbamate (tert-butoxycarbonyl). In certain embodiments, the nucleobase protecting group is labile with a reagent other than bases or acids.

[0047] In certain embodiments, the nucleobase protecting group is an amino protecting group. As used herein, an amino protecting group refers to a protecting group which can be used to protect an amino group on a molecule. Examples of amino protecting groups include, but are not limited to, N-methylpyrrolidin-2-ylidenyl (PyA), pivaloyloxymethyl (POM), 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenyl)ethoxycarbonyl (Bpoc), t-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), benzyloxycarbonyl (Cbz), formyl, acetyl, trihaloacetyl, benzoyl, nitrophenylacetyl, 2-nitrobenzenesulfonyl, phthalimido, dithiasuccinoyl, methyl carbamate, ethyl carbamate, 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 2,7-di-t-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-phenylethyl carbamate (hZ), 1-(1-adamantyl)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-t-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-(3,5-di-t-butylphenyl)-1-methylethyl carbamate (t-Bumeoc), 2-(2'- and 4'-pyridyl)ethyl carbamate (Pyoc), 2-(N,N-dicyclohexylcarboxamido)ethyl carbamate, 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), 1-isopropylallyl

- 20 -

carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, N-hydroxypiperidinyl carbamate, alkylthio carbamate, p-methoxybenzyl carbamate (Moz), p-nitobenzyl carbamate, p-bromobenzyl carbamate, p-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate (Msz), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonyl ethyl carbamate, 2-(p-toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, m-chloro-p-acyloxybenzyl carbamate, p-(dihydroxyboryl)benzyl carbamate, 5-benzisoxazolylmethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcroc), m-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, o-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(o-nitrophenyl)methyl carbamate, phenothiazinyl-(10)-carbonyl derivative, N'-p-toluenesulfonylaminocarbonyl derivative, N'-phenylaminothiocarbonyl derivative, t-amyl carbamate, S-benzyl thiocarbamate, p-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, p-decyloxybenzyl carbamate, 2,2-dimethoxycarbonylvinyl carbamate, o-(N,N-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(N,N-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, p-(p'-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(p-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, p-(phenylazo)benzyl carbamate, 2,4,6-tri-t-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, 2,4,6-trimethylbenzyl carbamate, formamide, dimethyl formamide (DMF), formamidine, DMF-formamidine, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, N-benzoylphenylalanyl derivative, benzamide, p-phenylbenzamide, o-nitophenylacetamide, o-nitrophenoxyacetamide, acetoacetamide, (N'-dithiobenzyloxycarbonylamino)acetamide,

- 21 -

3-(p-hydroxyphenyl)propanamide, 3-(o-nitrophenyl)propanamide, 2-methyl-2-(o-nitrophenoxy)propanamide, 2-methyl-2-(o-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, o-nitrocinnamide, N-acetylmethionine derivative, o-nitrobenzamide, o-(benzoyloxymethyl)benzamide, 4,5-diphenyl-3-oxazolin-2-one, N-phthalimide, N-dithiasuccinimide (Dts), N-2,3-diphenylmaleimide, N-2,5-dimethylpyrrole, N-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, N-methylamine, N-allylamine, N-[2-(trimethylsilyl)ethoxy]methylamine (SEM), N-3-acetoxypyrrolamine, N-(1-isopropyl-4-nitro-2-oxo-3-pyrroline-3-yl)amine, quaternary ammonium salts, N-benzylamine, N-di(4-methoxyphenyl)methylamine, N-5-dibenzosuberylamine, N-triphenylmethylamine (Tr), N-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), N-9-phenylfluorenylamine (PhF), N-2,7-dichloro-9-fluorenylmethyleneamine, N-ferrocenylmethylamino (Fcm), N-2-picolylamino N'-oxide, N-1,1-dimethylthiomethyleneamine, N-benzylideneamine, N-p-methoxybenzylideneamine, N-diphenylmethyleneamine, N-[(2-pyridyl)mesityl]methyleneamine, N-(N',N'-dimethylaminomethylene)amine, N,N'-isopropylidenediamine, N-p-nitrobenzylideneamine, N-salicylideneamine, N-5-chlorosalicylideneamine, N-(5-chloro-2-hydroxyphenyl)phenylmethyleneamine, N-cyclohexylideneamine, N-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, N-borane derivative, N-diphenylborinic acid derivative, N-[phenyl(pentacarbonylchromium- or tungsten)carbonyl]amine, N-copper chelate, N-zinc chelate, N-nitroamine, N-nitrosoamine, amine N-oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, o-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, 3-nitropyridinesulfenamide (Npys), p-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6-trimethyl-4-methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-

sulfonamide (Pmc), methanesulfonamide (Ms), β -trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzyisulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamid.

[0048] In certain embodiments, the nucleobase protecting group is a keto/carboxylic acid protecting group. As used herein, a keto protecting group, a carboxylic acid protecting group, or a keto/carboxylic acid protecting group refers to a protecting group which can be used to protect a keto or carboxylic acid group on a molecule. Examples of keto/carboxylic acid protecting groups include, but are not limited to: silyl protecting groups such as trimethylsilyl, triethylsilyl, t-butyldimethylsilyl (TBS, TBDMS), t-butyldiphenylsilyl, or triisopropylsilyl; alkyl protecting groups such as methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, or tetrahydropyran-2-yl; alkenyl protecting groups such as allyl; aryl protecting groups such as optionally substituted phenyl, biphenyl, or naphthyl; and arylalkyl protecting groups such as substituted benzyl (e.g., p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl), or 2- and 4-picolyl.

[0049] In certain embodiments, the nucleobase protecting group is a hydroxyl protecting group. As used herein, a hydroxyl protecting group refers to a protecting group which can be used to protect a hydroxyl group on a molecule. Examples of protecting groups that can be used as a hydroxyl protecting group include, but are not limited to, acetyl, chloroacetyl, trichloroacetyl, trifluoroacetyl, t-butyl, t-butoxymethyl, methoxymethyl, bis(2-acetoxyethoxy)methyl (ACE), [(triisopropylsilyl)oxy]methyl (TOM), pivaloyl, benzoyl, p-phenylbenzoyl, monomethoxytrityl (MMTr), dimethoxytrityl (DMT), 4,4',4''-trimethoxytrityl (TMTr), 1(2-fluorophenyl)-4-methoxypiperidin-4-yl (FPMP), substituted pixyl, methyl, methoxymethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM), guaiacolmethyl (GUM), 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-

- 23 -

methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, a-naphthyl diphenylmethyl, p-methoxyphenyl diphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, t-butyl dimethylsilyl (TBS, TBDMS), t-butyl diphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butyl methoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate, 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl (TSE), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate, alkyl allyl carbonate, alkyl p-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl p-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl o-nitrobenzyl carbonate, alkyl p-nitrobenzyl carbonate, alkyl S-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-

- 24 -

(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (E)-2-methyl-2-butenolate, o-(methoxycarbonyl)benzoate, α -naphthoate, nitrate, alkyl N,N,N',N'-tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, 2-(2-nitrophenyl)ethyl, 2-(4-cyanophenyl)ethyl 2-(4-nitrophenyl)ethyl (NPE), 2-(4-nitrophenylsulfonyl)ethyl, 2-nitrophenyl, 4-nitrophenyl, 2,4,6-trimethylphenyl, 2-(2-nitrophenyl)ethyl, sulfate, methanesulfonate (mesylate), benzyisulfonate, tosylate (Ts), 2-cyanoethyl (CE or Cne), 3,5-dichlorophenyl, 2,4-dimethylphenyl, butylthiocarbonyl, 4,4',4''-tris(benzoyloxy)trityl, diphenylcarbamoyl, levulinyl, 2-(dibromomethyl)benzoyl (Dbmb), 2-(isopropylthiomethoxymethyl)benzoyl (Ptmt), 9-phenylxanthen-9-yl (pixyl) or 9-(p-methoxyphenyl)xanthine-9-yl (MOX).

[0050] In certain embodiments, the nucleobase protecting group is a thiol protecting group. As used herein, a thiol protecting group refers to a protecting group which can be used to protect a thiol group on a molecule. Examples of protecting groups that can be used as a thiol protecting group include, but are not limited to, acetyl, chloroacetyl, trichloroacetyl, trifluoroacetyl, t-butyl, t-butoxymethyl, methoxymethyl, bis(2-acetoxyethoxy)methyl (ACE), [(triisopropylsilyl)oxy]methyl (TOM), pivaloyl, benzoyl, p-phenylbenzoyl, monomethoxytrityl (MMTr), dimethoxytrityl (DMT), 4,4',4''-trimethoxytrityl (TMTr), 1(2-fluorophenyl)-4-methoxypiperidin-4-yl (FPMP), substituted pixyl, methyl, methoxymethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM), guaiacolmethyl (GUM), 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl,

tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, a-naphthyl diphenylmethyl, p-methoxyphenyl diphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, t-butyl dimethylsilyl (TBS, TBDMS), t-butyl diphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butyl methoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate, 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl (TSE), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl p-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl p-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl o-nitrobenzyl carbonate, alkyl p-nitrobenzyl carbonate, alkyl S-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-

- 26 -

(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (E)-2-methyl-2-butenolate, o-(methoxycarbonyl)benzoate, α -naphthoate, nitrate, alkyl N,N,N',N'-tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, 2-(2-nitrophenyl)ethyl, 2-(4-cyanophenyl)ethyl 2-(4-nitrophenyl)ethyl (NPE), 2-(4-nitrophenylsulfonyl)ethyl, 2-nitrophenyl, 4-nitrophenyl, 2,4,6-trimethylphenyl, 2-(2-nitrophenyl)ethyl, sulfate, methanesulfonate (mesylate), benzylsulfonate, tosylate (Ts), 2-cyanoethyl (CE or Cne), 3,5-dichlorophenyl, 2,4-dimethylphenyl, butylthiocarbonyl, 4,4',4''-tris(benzoyloxy)trityl, diphenylcarbamoyl, levulinyl, 2-(dibromomethyl)benzoyl (Dbmb), 2-(isopropylthiomethoxymethyl)benzoyl (Ptmt), 9-phenylxanthen-9-yl (pixyl) or 9-(p-methoxyphenyl)xanthine-9-yl (MOX).

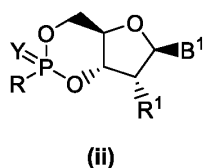
[0051] In certain embodiments, the nucleobase protecting group includes a protecting group for protecting 1,2- or 1,3-diols. Examples of protecting groups for protecting 1,2- or 1,3-diols include methylene acetal, ethylidene acetal, 1-t-butylethylidene ketal, 1-phenylethylidene ketal, (4-methoxyphenyl)ethylidene acetal, 2,2,2-trichloroethylidene acetal, acetonide, cyclopentylidene ketal, cyclohexylidene ketal, cycloheptylidene ketal, benzylidene acetal, p-methoxybenzylidene acetal, 2,4-dimethoxybenzylidene ketal, 3,4-dimethoxybenzylidene acetal, 2-nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1-methoxyethylidene ortho ester, 1-ethoxyethylidene ortho ester, 1,2-dimethoxyethylidene ortho ester, α -methoxybenzylidene ortho ester, 1-(N,N-dimethylamino)ethylidene derivative, α -(N,N'-dimethylamino)benzylidene derivative, 2-oxacyclopentylidene ortho ester, di-t-butylsilylene group (DTBS), 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene) derivative (TIPDS), tetra-t-butoxydisiloxane-1,3-diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

[0052] In certain embodiments, each nucleobase is protected with one or more protecting groups independently chosen from dimethyl formamide (DMF), N-methylpyrrolidin-2-ylidenyl (PyA), pivaloyloxymethyl (POM), formamidine, DMF-formamidine, phthalimido, chloro, bromo, acyl, allyl, benzyl, tert-butyloxycarbonyl (Boc), and benzyloxymethyl (BOM).

- 27 -

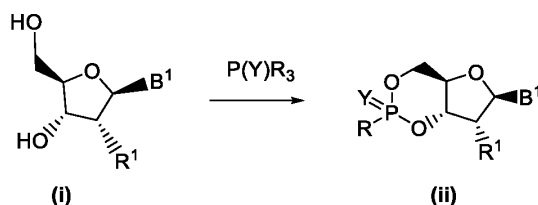
Cyclic-chlorophosphate nucleosides

[0053] In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using cyclic-phosphorous nucleosides, or a salt or solvate thereof. As used herein, a cyclic-phosphorous nucleoside refers to a nucleoside which includes a P(Y)R₃ group bound to the 3' and 5' oxygens of a nucleoside sugar to form a compound of Formula (ii):



or a salt or solvate thereof, wherein: Y is O or S; each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl; R¹ is chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy (including methoxy and -O-methoxyethyl); and B¹ is a nucleobase, which may be optionally protected. In certain embodiments, R¹ is (C₁-C₁₆) alkyl or (C₁-C₁₆) alkoxy. In certain embodiments, R¹ is (C₁-C₁₂) alkyl or (C₁-C₁₂) alkoxy. In certain embodiments, R¹ is (C₁-C₈) alkyl or (C₁-C₈) alkoxy. In certain embodiments, R¹ is (C₁-C₄) alkyl or (C₁-C₄) alkoxy. In certain embodiments, R¹ is fluorine. In certain embodiments, R¹ is methoxy. In certain embodiments, R¹ is O-methoxyethyl (MOE).

[0054] In certain embodiments, the present disclosure provides methods for preparing a cyclic-phosphorous nucleoside of Formula (ii), or a salt or solvate thereof. In certain embodiments, the method for preparing a cyclic-phosphorous nucleoside of Formula (ii) comprises: (a) reacting a nucleoside of Formula (i) with P(Y)R₃, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii)



- 28 -

[0055] In certain embodiments, the present disclosure provides a cyclic-phosphorous nucleoside of Formula (ii), or a salt or solvate thereof.

[0056] In certain embodiments, the nucleobase of the cyclic phosphorous nucleoside is a canonical nucleobase. In certain embodiments, the nucleobase is a purine base -- adenine (A) or guanine (G). In certain embodiments, the nucleobase is a pyrimidine base -- thymine (T), cytosine (C), or uracil (U). In certain embodiments, the nucleobase of the cyclic phosphorous nucleoside is a noncanonical nucleobase.

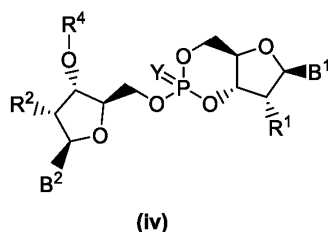
[0057] In certain embodiments, the nucleobase of the cyclic-phosphorous nucleoside is unprotected. In certain embodiments, the nucleobase of the cyclic-phosphorous nucleoside is protected with a nucleobase protecting group. In certain embodiments, the nucleobase of the cyclic-phosphorous nucleoside is protected with an amino nucleobase protecting group. In certain embodiments, the nucleobase of the cyclic-phosphorous nucleoside is protected with a keto/carboxylic acid nucleobase protecting group. In certain embodiments, the nucleobase of the cyclic-phosphorous nucleoside is protected with a hydroxyl nucleobase protecting group. In certain embodiments, the nucleobase of the cyclic-phosphorous nucleoside is protected with a thiol nucleobase protecting group. In certain embodiments, the nucleobase of the cyclic-phosphorous nucleoside is protected with one or more protecting groups independently chosen from dimethyl formamide (DMF), formamidine, DMF-formamidine, phthalimido, chloro, bromo, acyl, allyl, benzyl, tert-butyloxycarbonyl (Boc), and benzyloxymethyl (BOM).

[0058] In certain embodiments, the cyclic phosphorus chloride nucleoside of Formula (ii) is obtained as a mixture of stereoisomers. In certain embodiments, the stereoisomers are separated prior to subsequent reaction steps.

Dinucleotides

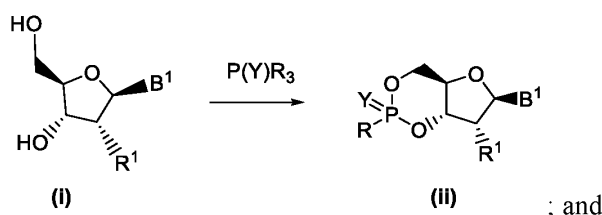
[0059] In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using a dinucleotide which comprises a cyclic-phosphorous nucleoside of the present disclosure, or a salt or solvate thereof. In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using a dinucleotide of Formula (iv) or a salt or solvate thereof:

- 29 -



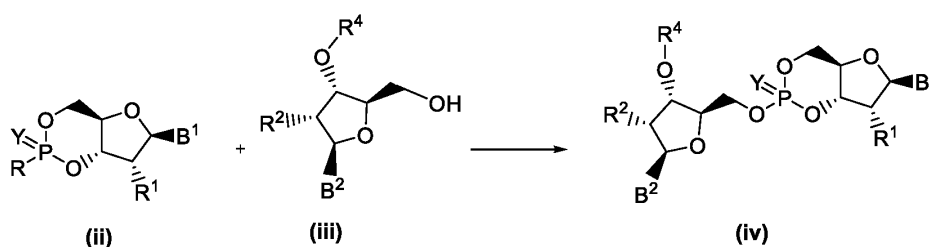
wherein: Y is O or S; R^1 and R^2 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy group (including methoxy and -O-methoxyethyl); B^1 and B^2 are nucleobases, which may optionally be protected with one or more protecting groups; and R^4 is hydrogen or a protecting group. In certain embodiments, R^1 and R^2 are independently chosen from (C₁-C₁₆) alkyl and (C₁-C₁₆) alkoxy. In certain embodiments, R^1 and R^2 are independently chosen from (C₁-C₁₂) alkyl and (C₁-C₁₂) alkoxy. In certain embodiments, R^1 and R^2 are independently chosen from (C₁-C₈) alkyl and (C₁-C₈) alkoxy. In certain embodiments, R^1 and R^2 are independently chosen from (C₁-C₄) alkyl and (C₁-C₄) alkoxy. In certain embodiments, R^1 and R^2 are fluorine. In certain embodiments, R^1 and R^2 are methoxy. In certain embodiments, R^1 and R^2 are O-methoxyethyl (MOE).

[0060] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (iv) or a salt or solvate thereof, wherein the method comprises: (a) reacting a nucleoside of Formula (i) with $P(Y)R_3$, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):



(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:

- 30 -



In certain embodiments, the method further comprises removing one or more of the protecting groups.

[0061] In certain embodiments, the cyclic phosphorus chloride nucleoside of Formula (ii) is obtained as a mixture of stereoisomers. In certain embodiments, the stereoisomers are separated prior to the step of reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii).

[0062] In certain embodiments, the present disclosure provides a dinucleotide of Formula (iv) or a salt or solvate thereof.

[0063] In certain embodiments, R² is an alkoxy group linked to the adjacent 3' oxygen to form a ketal protecting group with R⁴.

[0064] In certain embodiments, R⁴ is hydrogen. In certain embodiments, R⁴ is a protecting group. In certain embodiments, R⁴ is a hydroxyl protecting group. In certain embodiments, R⁴ is chosen from a silyl group, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl (TBS, TBDMS), t-butyldiphenylsilyl, or triisopropylsilyl. In certain embodiments, R⁴ forms a ketal protecting group with R².

[0065] In certain embodiments, the cyclic phosphorus dinucleotide of Formula (iv) is obtained in an (R) stereoisomer configuration. In certain embodiments, the cyclic phosphorus dinucleotide of Formula (iv) is obtained in an (S) stereoisomer configuration. In certain embodiments, the cyclic phosphorus dinucleotide of Formula (iv) is obtained as a mixture of stereoisomers. In certain embodiments, the cyclic phosphorus dinucleotide of Formula (iv) is obtained as a mixture of (R) and (S) stereoisomers. In certain embodiments, the stereoisomers are separated prior to subsequent synthesis steps. In certain embodiments, the stereoisomers are separated prior to the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v).

[0068] In certain embodiments, the mixture of stereoisomers comprises a ratio of (R) configuration product to (S) configuration product. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 1.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 2.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 3.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 4.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 5.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 6.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 7.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 8.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 9.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 10.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 11.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 12.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 13.0. In certain embodiments, the mixture of stereoisomers has an (R)/(S) ratio of at least about 14.0.

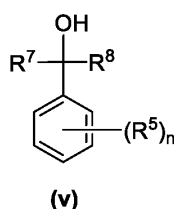
[0069] In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 1.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 2.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 3.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 4.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 5.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 6.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 7.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 8.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 9.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 10.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 11.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 12.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 13.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 14.0.

- 33 -

ratio of at least about 13.0. In certain embodiments, the mixture of stereoisomers has an (S)/(R) ratio of at least about 14.0.

Substituted benzyl alcohol nucleophiles

[0070] In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using a substituted benzyl alcohol nucleophile. As used herein, a substituted benzyl alcohol nucleophile or "BnOH" refers to a benzyl alcohol which includes at least one substitution on the benzene ring. A substituted benzyl alcohol nucleophile can include a primary benzyl alcohol ($\text{Bn-CH}_2\text{-OH}$), a secondary benzyl alcohol ($\text{Bn-CH(R}^7\text{)-OH}$), or a tertiary benzyl alcohol ($\text{Bn-C(R}^7\text{)(R}^8\text{)-OH}$). In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using a substituted benzyl alcohol nucleophile of Formula (v):

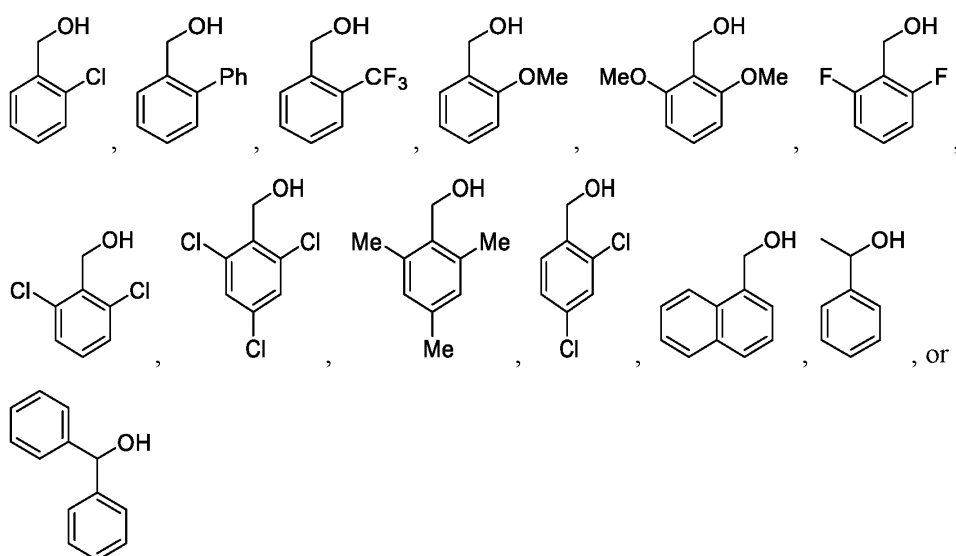


wherein: n is chosen from 1, 2, 3, 4, and 5; each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; and R^7 and R^8 are independently selected from hydrogen, $(\text{C}_1\text{-C}_{20})$ alkyl and aryl.

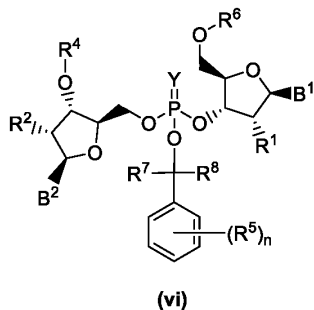
[0071] In certain embodiments, each instance of R^5 is an ortho- or para- substitution on the benzyl group. In certain embodiments, each instance of R^5 is an ortho- substitution on the benzyl group. In certain embodiments, each instance of R^5 is a para- substitution on the benzyl group. In certain embodiments, each instance of R^5 is independently chosen from: halogen, $(\text{C}_1\text{-C}_{20})$ alkyl, $(\text{C}_1\text{-C}_{20})$ alkoxy, trifluoromethyl, and phenyl (substituted or unsubstituted). In certain embodiments, R^5 is a fused phenyl ring forming a naphthyl ring system. In certain embodiments, R^5 is $(\text{C}_1\text{-C}_{16})$ alkyl or $(\text{C}_1\text{-C}_{16})$ alkoxy. In certain embodiments, R^5 is $(\text{C}_1\text{-C}_{12})$ alkyl or $(\text{C}_1\text{-C}_{12})$ alkoxy. In certain embodiments, R^5 is $(\text{C}_1\text{-C}_8)$ alkyl or $(\text{C}_1\text{-C}_8)$ alkoxy. In certain embodiments, R^5 is $(\text{C}_1\text{-C}_4)$ alkyl or $(\text{C}_1\text{-C}_4)$ alkoxy.

[0072] In certain embodiments, the substituted benzyl alcohol nucleophile of Formula (v) is:

- 34 -



[0073] In certain embodiments, the present disclosure provides methods for preparing oligonucleotides using cyclic-phosphorous nucleosides and substituted benzyl alcohol nucleophiles. In certain embodiments, the present disclosure provides methods for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof:

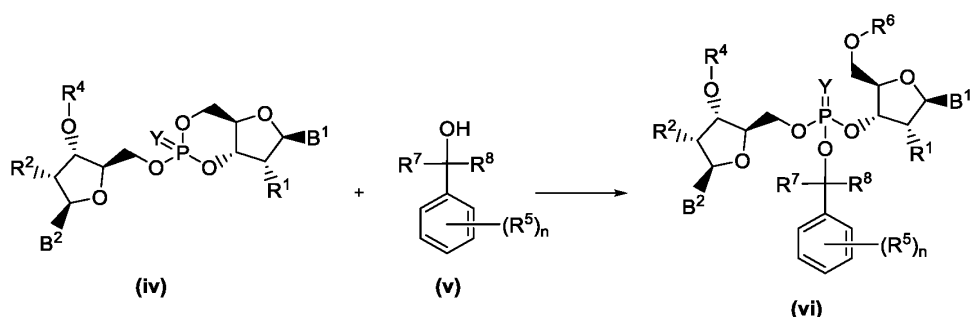


wherein: Y is O or S; R¹ and R² are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy (including methoxy and -O-methoxyethyl); B¹ and B² are nucleobases, which may optionally be protected; R⁴ is hydrogen or a protecting group; n is chosen from 1, 2, 3, 4, and 5; each R⁵ is independently a substituent on the phenyl ring of the benzyl protecting group; R⁶ is hydrogen or a protecting group; and R⁷ and R⁸ are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl. In certain embodiments, R¹ and R² are independently chosen from (C₁-C₁₆) alkyl and (C₁-C₁₆) alkoxy. In certain embodiments, R¹ and R² are independently chosen from (C₁-C₁₂) alkyl and (C₁-C₁₂) alkoxy. In certain

- 35 -

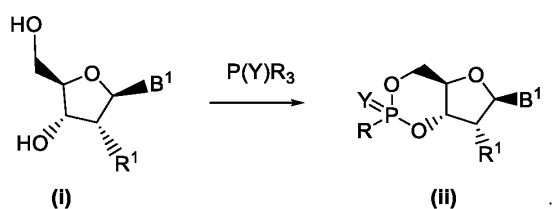
embodiments, R^1 and R^2 are independently chosen from (C_1-C_8) alkyl and (C_1-C_8) alkoxy. In certain embodiments, R^1 and R^2 are independently chosen from (C_1-C_4) alkyl and (C_1-C_4) alkoxy. In certain embodiments, R^1 and R^2 are fluorine. In certain embodiments, R^1 and R^2 are methoxy. In certain embodiments, R^1 and R^2 are -O-methoxyethyl (MOE).

[0074] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof, wherein the method comprises: providing the cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group; and reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



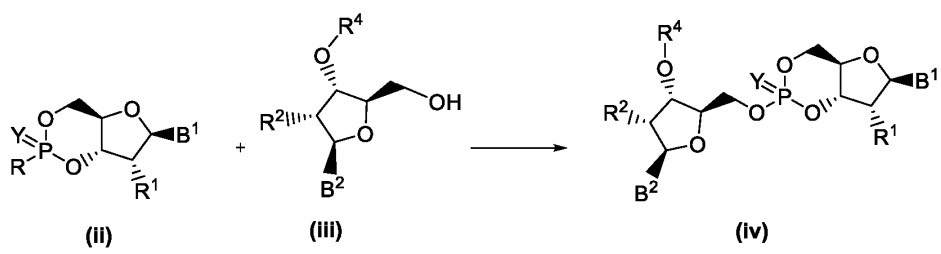
wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl. In certain embodiments, the method further comprises removing one or more of the protecting groups from the dinucleotide of Formula (vi).

[0075] In certain embodiments, the present disclosure provides a method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof, wherein the method comprises: (a) reacting a nucleoside of Formula (i) with $P(Y)R_3$, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C_1-C_6) alkoxy, (C_6-C_{12}) aryloxy, S- (C_1-C_6) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):



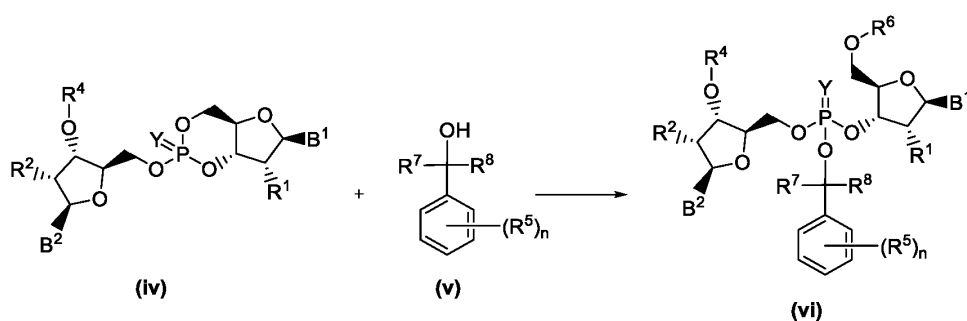
- 36 -

(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:



and

(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl. In certain embodiments, the method further comprises removing one or more of the protecting groups from the dinucleotide of Formula (vi).

[0076] In certain embodiments, the present disclosure provides a dinucleotide of Formula (vi) or a salt or solvate thereof.

[0077] In certain embodiments, R^6 is hydrogen. In certain embodiments, R^6 is a protecting group. In certain embodiments, R^6 is a hydroxyl protecting group. In certain embodiments, R^6 is chosen from a silyl group, trimethylsilyl, triethylsilyl, t-butyl dimethylsilyl (TBS, TBDMS), t-butyl diphenylsilyl, or triisopropylsilyl.

[0078] In certain embodiments, the process of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v)

- 37 -

forms a dinucleotide with [3'-5']-linkage (e.g., a dinucleotide of Formula (vi)). In certain embodiments, the process of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) forms a dinucleotide with [5'-5']-linkage. In certain embodiments, the process of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) forms a mixture of isomers (e.g., constitutional isomers). In certain embodiments the mixture of isomers includes a mixture of [3'-5']-linkage and [5'-5']-linkage stereoisomers. In certain embodiments, the isomers are separated prior to subsequent synthesis steps.

[0079] In certain embodiments, the mixture of isomers comprises at least about 10% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 20% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 30% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 40% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 50% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 60% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 70% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 75% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 80% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 85% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 90% of [3'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 95% of [3'-5']-linkage product.

[0080] In certain embodiments, the mixture of isomers comprises at least about 10% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 20% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 30% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 40% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 50% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 60% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 70% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers

- 38 -

comprises at least about 75% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 80% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 85% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 90% of [5'-5']-linkage product. In certain embodiments, the mixture of isomers comprises at least about 95% of [5'-5']-linkage product.

[0081] In certain embodiments, the mixture of isomers comprises an [S] ratio of [3'-5']-linkage over [5'-5']-linkage. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 1.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 2.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 3.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 4.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 5.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 6.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 7.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 8.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 9.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 10.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 11.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 12.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 13.0. In certain embodiments, the mixture of isomers has an [S] ratio of at least about 14.0.

[0082] In certain embodiments, the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) is carried out in a solvent system. In certain embodiments, the solvent comprises: tetrahydrofuran (THF); 2-methyltetrahydrofuran (2Me-THF); diethyl ether; 1,4-dioxane; dimethylsulfoxide (DMSO), N-methylpyrrolidone (NMP), dimethylacetamide (DMA), acetonitrile (MeCN), methyl tert-butyl ether (MTBE), tertAmyl alcohol, toluene, or a mixture thereof.

[0083] In certain embodiments, the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) is carried out in the presence of a base. In certain embodiments, the base comprises: n-butyllithium (n-

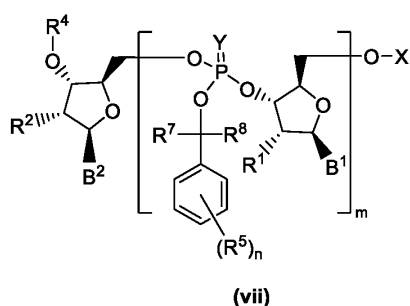
- 39 -

BuLi); lithium tertbutoxide (LiOtBu); lithium bis(trimethylsilyl)amide (LiHMDS); lithium tetramethylpiperidide (LiTMP); or Li-R_Z, where R_Z is alkyl or aryl; sodium t-butoxide (NaOtBu) + lithium chloride (LiCl); potassium t-butoxide (KOtBu) + lithium chloride (LiCl); triethylamine, Diazabicycloundecene (DBU), triazabicyclodecene (TBD), or a combination thereof. In certain embodiments, the base is pre-mixed with the substituted benzyl alcohol nucleophile of Formula (v) prior to the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v). In certain embodiments, the base is lithium tertbutoxide (LiOtBu).

[0084] In certain embodiments, the dinucleotide of Formula (vi) is isolated by reacting with a silyl chloride.

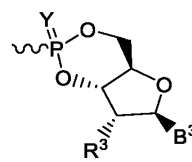
Protected oligonucleotides

[0085] In certain embodiments, the present disclosure provides a method for preparing a phospho-protected oligonucleotide. In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (vii):



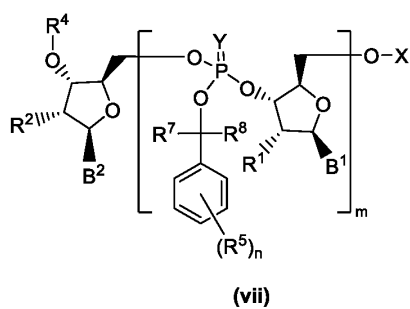
wherein: each Y is independently O or S; R¹, R², and R³ are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy (including methoxy and -O-methoxyethyl); R⁴ is H or a protecting group; n is chosen from 1, 2, 3, 4, and 5; each R⁵ is independently a substituent on the phenyl ring of the benzyl protecting group; each R⁷ and R⁸ is independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl; B¹, B², and B³ are nucleobases, which may optionally be protected; m is chosen from integers ranging from 1 to 18; and X is hydrogen, a

- 40 -



protecting group, or a cyclic-phosphorous nucleoside . In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C₁-C₁₆) alkyl and (C₁-C₁₆) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C₁-C₁₂) alkyl and (C₁-C₁₂) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C₁-C₈) alkyl and (C₁-C₈) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C₁-C₄) alkyl and (C₁-C₄) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are fluorine. In certain embodiments, R^1 , R^2 , and R^3 are methoxy. In certain embodiments, R^1 , R^2 , and R^3 are -O-methoxyethyl (MOE).

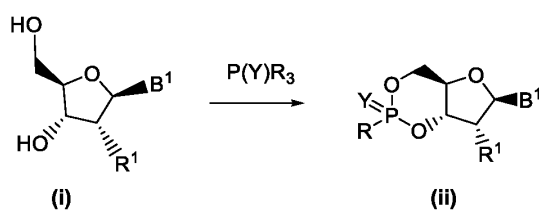
[0086] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (vii), wherein the method comprises: providing the dinucleotide of Formula (vi); and repeating the reactions of steps (a), (b), and (c) from a method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof, as disclosed herein, one or more times to obtain an oligonucleotide of Formula (vii):



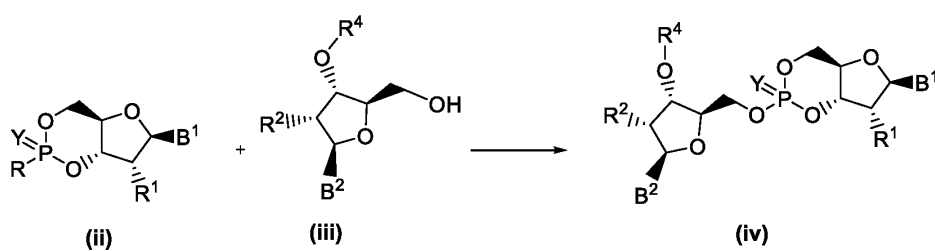
wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the dinucleotide or oligonucleotide. In certain embodiments, the method further comprises removing one or more of the protecting groups.

[0087] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (vii), wherein the method comprises: (a) reacting a nucleoside of Formula (i) with P(Y)R₃, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):

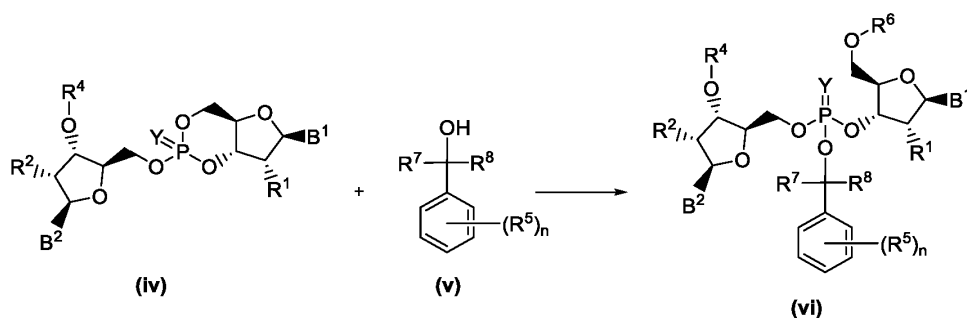
- 41 -



(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:

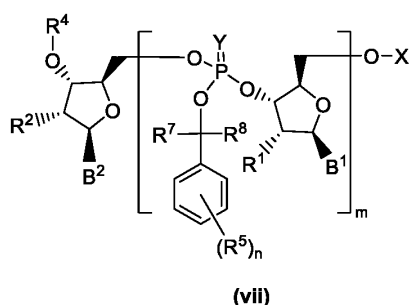


(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl; and (d) repeating the reactions of steps (a), (b), and (c) one or more times to obtain an oligonucleotide of Formula (vii):

- 42 -

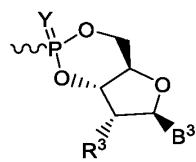


wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the dinucleotide or oligonucleotide. In certain embodiments, the method further comprises removing one or more of the protecting groups.

[0088] In certain embodiments, the present disclosure provides an oligonucleotide of Formula (vii) or a salt or solvate thereof.

[0089] In certain embodiments, X is hydrogen. In certain embodiments, R6 of the terminal nucleoside is deprotected and X is hydrogen. In certain embodiments, X is a protecting group. In certain embodiments, X is a hydroxyl protecting group. In certain embodiments, X is chosen from a silyl group, acyl group, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl (TBS, TBDMS), t-butyldiphenylsilyl, or triisopropylsilyl.

[0090] In certain embodiments, X is a cyclic-phosphorous nucleoside

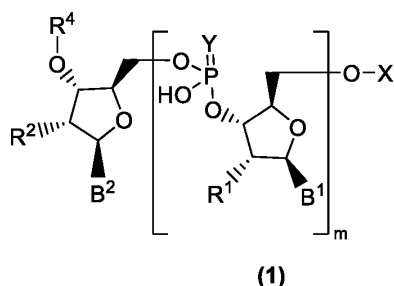


, wherein Y is O or S. In certain embodiments, X is a cyclic-phosphorous nucleoside which can be used to continue elongation of the oligonucleotide by repeating the reactions of steps (a), (b), and (c) from a method for preparing a dinucleotide of Formula (vi) as disclosed herein.

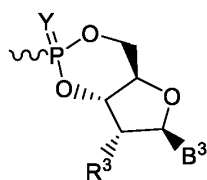
Synthesized oligonucleotides

[0091] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide. In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof,

- 43 -



wherein: each Y is independently O or S; R^1 , R^2 , and R^3 are independently chosen from hydrogen, hydroxy (protected), halogen (including fluorine), N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (including methoxy and -O-methoxyethyl); B^1 , B^2 , and B^3 are nucleobases, which may optionally be protected; R^4 is hydrogen or a protecting group; m is chosen from integers ranging from 0 to 18; and X is hydrogen, a protecting group, or



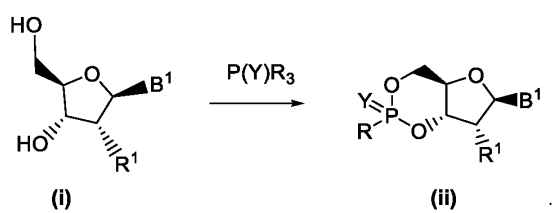
. In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C_1-C_{16}) alkyl and (C_1-C_{16}) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C_1-C_{12}) alkyl and (C_1-C_{12}) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C_1-C_8) alkyl and (C_1-C_8) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are independently chosen from (C_1-C_4) alkyl and (C_1-C_4) alkoxy. In certain embodiments, R^1 , R^2 , and R^3 are fluorine. In certain embodiments, R^1 , R^2 , and R^3 are methoxy. In certain embodiments, R^1 , R^2 , and R^3 are -O-methoxyethyl (MOE).

[0092] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof, wherein the method comprises: providing a dinucleotide of Formula (vi) or an oligonucleotide of Formula (vii); and hydrogenating the dinucleotide of Formula (vi) or the oligonucleotide of Formula (vii); wherein the method produces the oligonucleotide of Formula (1) or a salt or solvate thereof. In certain embodiments, the method further comprises removing one or more of the protecting groups.

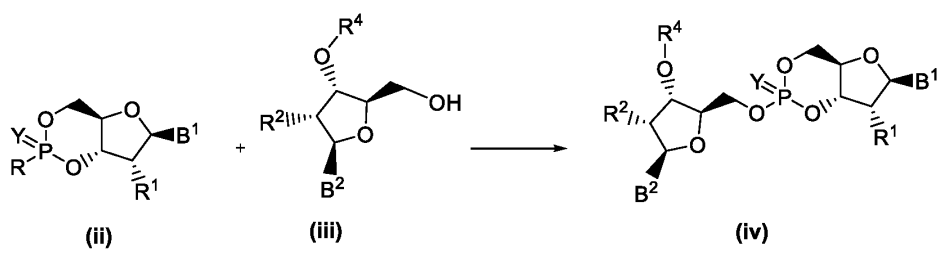
[0093] In certain embodiments, the present disclosure provides a method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof, wherein the method

- 44 -

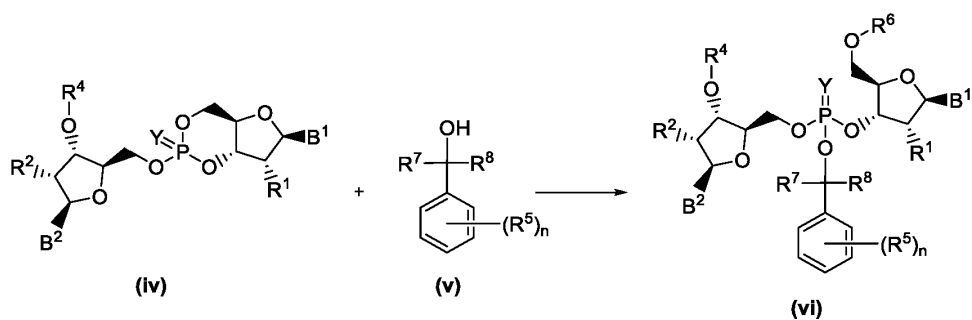
comprises: (a) reacting a nucleoside of Formula (i) with $P(Y)R_3$, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C_1-C_6) alkoxy, (C_6-C_{12}) aryloxy, S- (C_1-C_6) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):



(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:

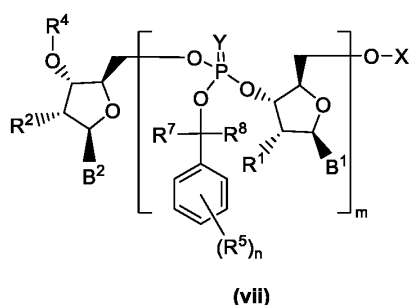


(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl; (d) optionally, repeating the reactions of steps (a), (b), and (c) one or more times to obtain an oligonucleotide of Formula (vii):

- 45 -



wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the oligonucleotide; and (e) hydrogenating the product of step (c) or step (d); wherein the method produces the oligonucleotide of Formula (1) or a salt or solvate thereof. In certain embodiments, the method further comprises removing one or more of the protecting groups.

[0094] In certain embodiments, the oligonucleotide is a blockmer. As used herein, the term blockmer refers to an oligonucleotide which includes at least two consecutive nucleotide units sharing a common structural feature (i.e., chemistry or stereochemistry) at the nucleobase, sugar and/or internucleotide linkage. The at least two consecutive nucleotide units sharing a common structural feature can be referred to as a "block".

[0095] In certain embodiments, oligonucleotides of the present disclosure can be used in subsequent oligonucleotide or polynucleotide synthesis applications. In certain embodiments, oligonucleotides of the present disclosure can be used as blockmer amidites in solid phase synthesis of oligonucleotides or polynucleotides. In certain embodiments, oligonucleotides of the present disclosure can be used in enzymatic ligation reactions for the synthesis of oligonucleotides or polynucleotides.

[0096] In certain embodiments, the present disclosure provides a polynucleotide of Formula (1) or a salt or solvate thereof, wherein m is chosen from integers ranging from 0 to 100. In certain embodiments, m is chosen from integers ranging from 1 to 18. In certain embodiments, m is chosen from integers ranging from 1 to 3.

[0097] In certain embodiments of the present disclosure, the oligonucleotide synthesis method is a liquid phase process (i.e., conducted in liquid phase). As used herein, a liquid phase process refers to a process in which all reaction components (e.g., starting materials, reagents, catalysts, bases) are dissolved fully in a solvent mixture, such that there are no solid particles present.

- 46 -

[0098] In certain embodiments, the method is conducted using continuous flow. As used herein, continuous flow refers to a process in which starting materials, reagents, catalysts, bases, and all other reaction components are continuously added to a reactor while the product, side-products, unreacted starting materials, and other reaction components are continuously removed from the reactor.

[0099] In certain embodiments, the method is carried out in a flow reactor. As used herein, flow reactor refers to a pipe, a tube, a continuously stirred tank reactor (CSTR), a cascade of multiple CSTRs, an intermittently filled and emptied batch reactor, glass microreactor, or other reactors which can be operated continuously. In certain embodiments, the method is carried out in a flow reactor at ambient temperature (e.g., 20-25 °C). In certain embodiments, the method is carried out in a flow reactor with a reaction time between 45-120 minutes. In certain embodiments, the method is carried out in a flow reactor with a reaction time between 45-60 minutes.

DEFINITIONS

[0100] Unless stated otherwise, the following terms and phrases have the meanings described below. The definitions are not meant to be limiting in nature and serve to provide a clearer understanding of certain aspects of the present disclosure.

[0101] *Approximately/About*: As used herein, the terms "approximately" and "about" are used interchangeably herein and refer to a value that is within +/- 10% of the recited value as applied to one or more values of interest. In certain embodiments, the term refers to a range of values that fall within +/- 10%, +/- 9%, +/- 8%, +/- 7%, +/- 6%, +/- 5%, +/- 4%, +/- 3%, +/- 2%, +/- 1%, or less of the stated reference value, unless otherwise expressly stated or otherwise clearly evident from the context.

[0102] *Complementary*: As used herein, the terms "complementary" or "complementarity" refer a structural relationship between two nucleotides, nucleosides, or nucleobases (e.g., on two opposing nucleic acids or on opposing regions of a single nucleic acid strand such as a hairpin), wherein the relationship permits the two nucleotides to form base pairs with one another. For example, a purine nucleotide of one nucleic acid that is complementary to a pyrimidine nucleotide of an opposing nucleic acid may base pair together by forming hydrogen bonds with one another. Complementary nucleotides can base pair in the canonical Watson-Crick manner (i.e., adenine pairing with thymine or uracil, and guanine pairing with cytosine), or in any other manner that

- 47 -

allows for the formation of stable duplexes. Likewise, two nucleic acids may have regions of multiple nucleotides that are complementary with each other to form regions of complementarity.

[0103] *Deoxyribonucleotide*: As used herein, the term "deoxyribonucleotide" refers a nucleotide having a hydrogen in place of a hydroxyl at the 2' position of its pentose sugar when compared with a ribonucleotide. A modified deoxyribonucleotide has one or more modifications or substitutions of atoms other than hydroxyl at the 2' position, including modifications or substitutions in or of the nucleobase, sugar, or phosphate group.

[0104] *Double-stranded oligonucleotide*: As used herein, the terms "double-stranded oligonucleotide" or "ds oligonucleotide" refer to an oligonucleotide that is in a duplex form. The complementary base-pairing of duplex region(s) of a ds oligonucleotide can be formed between antiparallel sequences of nucleotides of covalently separate nucleic acid strands. Likewise, complementary base-pairing of duplex region(s) of a ds oligonucleotide can be formed between antiparallel sequences of nucleotides of nucleic acid strands that are covalently linked. Moreover, complementary base-pairing of duplex region(s) of a ds oligonucleotide can be formed from single nucleic acid strand that is folded (*e.g.*, via a hairpin) to provide complementary antiparallel sequences of nucleotides that base pair together. A ds oligonucleotide can include two covalently separate nucleic acid strands that are fully duplexed with one another. A ds oligonucleotide can include two covalently separate nucleic acid strands that are partially duplexed (*e.g.*, having overhangs at one or both ends). A ds oligonucleotide can include an antiparallel sequence of nucleotides that are partially complementary, and thus, may have one or more mismatches, which may include internal mismatches or end mismatches.

[0105] *Duplex*: As used herein, the terms "duplex" and "duplex region" in reference to nucleic acids (*e.g.*, oligonucleotides), refer to a structure formed through complementary base pairing of two antiparallel sequences of nucleotides, whether formed by two covalently separate nucleic acid strands or by a single, folded strand (*e.g.*, via a hairpin). A duplex may form despite not having full complementarity between the two strands, or when an abasic moiety is present.

[0106] *Modified internucleotide linkage*: As used herein, the term "modified internucleotide linkage" refers to an internucleotide linkage having one or more chemical

- 48 -

modifications when compared with a reference internucleotide linkage having a phosphodiester bond. A modified internucleotide linkage is a non-naturally occurring linkage.

[0107] *Modified nucleotide*: As used herein, the term "modified nucleotide" refers to a nucleotide having one or more chemical modifications when compared with a corresponding reference nucleotide selected from: adenine ribonucleotide, guanine ribonucleotide, cytosine ribonucleotide, uracil ribonucleotide, adenine deoxyribonucleotide, guanine deoxyribonucleotide, cytosine deoxyribonucleotide, and thymidine deoxyribonucleotide. A modified nucleotide is a non-naturally occurring nucleotide. A modified nucleotide can have, for example, one or more chemical modification in its sugar, nucleobase, and/or phosphate group. Additionally, or alternatively, a modified nucleotide can have one or more chemical moieties conjugated to a corresponding reference nucleotide.

[0108] *Nucleobase*: As used herein, the term "nucleobase" refers to a heterocyclic moiety of a nucleoside (e.g., adenine in adenosine, cytosine in cytidine, guanine in guanosine, thymine in thymidine, and uracil in uridine). A nucleobase can be a "canonical nucleobase" or "primary nucleobase," which are used interchangeably herein and include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). A nucleobase can be a "noncanonical nucleobase," which include synthetic or natural nucleobases which are not a canonical nucleobase.

[0109] *Nucleoside*: As used herein, the term "nucleoside" refers to an organic compound having a nucleobase -- for example, adenine, cytosine, guanine, thymine, or uracil -- covalently attached to a pentose sugar -- for example, ribose or 2'-deoxyribose.

[0110] *Nucleotide*: As used herein, the term "nucleotide" refers to a nucleoside in a phosphorylated form (e.g., a phosphate ester or thiophosphate ester of a nucleoside). A nucleotide can serve as a monomeric unit of nucleic acid polymers (e.g., oligonucleotides) such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

[0111] *Oligonucleotide*: As used herein, the term "oligonucleotide" refers to a polymer of linked nucleotides, each of which can include a canonical or noncanonical nucleobase. An oligonucleotide is typically less than about 100 nucleotides in length. An oligonucleotide may be single-stranded (ss) or double stranded (ds). An oligonucleotide

- 49 -

may or may not have duplex regions. Oligonucleotides can include dinucleotide or trinucleotide polymers. As used herein, the term "polynucleotide" refers to a polymer of linked nucleotides which can be more than about 100 nucleotides in length. As used herein, the term dinucleotide includes two linked nucleosides with a one or more phosphate-based linkages (e.g., a dinucleoside with two nucleosides and a cyclic phosphorus, phosphate ester, or thiophosphate ester linkage). As used herein, the term trinucleotide includes three linked nucleosides with two or more phosphate-based linkages (e.g., a trinucleoside with three nucleosides and cyclic phosphorus, phosphate ester, or thiophosphate ester linkages).

[0112] *Phosphate analog*: As used herein, "phosphate analog" refers to a chemical moiety that mimics the electrostatic and/or steric properties of a phosphate group. Examples of phosphate analogs include, but are not limited to, 5' phosphonates, such as 5' methylene phosphonate (5'-MP) and 5'-(E)-vinylphosphonate (5'-VP). An oligonucleotide can have a phosphate analog at a 4'-carbon position of the sugar (referred to as a "4'-phosphate analog") or at a 5'-terminal nucleotide in place of a 5'-phosphate. An example of a 4'-phosphate analog is oxymethylphosphonate, in which the oxygen atom of the oxymethyl group is bound to the sugar moiety (e.g., at its 4'-carbon) or analog thereof. See, e.g., Intl. Patent Application Publication No. WO 2018/045317. Other modifications have been developed for the 5' end of oligonucleotides (see, e.g., Intl. Patent Application No. WO 2011/133871; US Patent No. 8,927,513; and Prakash et al. (2015) Nuc. Acids Res. 43:2993-3011).

[0113] *Protecting group*: As used herein, the terms "protecting group" or "protective group" refer to a chemical moiety which is known in the art to protect reactive or labile groups on a molecule (e.g., hydroxyl, amino, and thiol groups) against undesired reactions during synthetic procedures and other chemical reactions. Protecting groups are typically used selectively and/or orthogonally to protect labile sites in a molecule from undergoing undesired modifications during chemical reactions at other reactive sites within the molecule, and can then be removed to leave the labile group unprotected and available for further reactions.

[0114] *Ribonucleotide*: As used herein, the term "ribonucleotide" refers to a nucleotide having a ribose as its pentose sugar, which contains a hydroxyl group at its 2' position. A modified ribonucleotide is a ribonucleotide having one or more modifications or

- 50 -

substitutions of atoms other than hydrogen at the 2' position, including modifications or substitutions in or of the nucleobase, sugar, or phosphate group.

[0115] *Substituted benzyl alcohol nucleophile*: As used herein, the terms "substituted benzyl alcohol nucleophile" or "BnOH" refer to a benzyl alcohol which includes at least one substitution on the benzene ring. A substituted benzyl alcohol nucleophile can include a primary benzyl alcohol (Bn-CH₂-OH), a secondary benzyl alcohol (Bn-CH(R⁷)-OH), or a tertiary benzyl alcohol (Bn-C(R⁷)(R⁸)-OH).

[0116] *Strand*: As used herein, the term "strand" refers to a single, contiguous sequence of nucleotides linked together through internucleotide linkages (*e.g.*, phosphodiester linkages or phosphorothioate linkages). A strand can have two free ends (*e.g.*, a 5' end and a 3' end).

[0117] *Synthetic*: As used in the context of a product herein (*e.g.*, synthetic oligonucleotide, synthetic nucleobase), the term "synthetic" refers to a nucleic acid or other compound that is artificially synthesized (*e.g.*, using a machine such as, for example, a solid phase nucleic acid synthesizer) or that is otherwise not derived from a natural source (*e.g.*, a cell or organism) that normally produces the nucleic acid or other compound.

General Considerations

[0118] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, one or more equivalents to the specific embodiments in accordance with the present disclosure described herein. The scope of the present disclosure is not intended to be limited to the above Description or the following Examples.

[0119] At various places in the present disclosure, substituents, or properties of compounds of the present disclosure are disclosed in groups or in ranges. It is intended that the present disclosure comprise each and every individual or sub-combination of the members of such groups and ranges, and that such groups or ranges include the endpoints. By way of nonlimiting example, if a group or range is from about 1 to about 10, then the group or range includes both the value of about 1 and the value of about 10.

[0120] Articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that comprise "or" between one or more members of a group are considered satisfied if

- 51 -

one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The present disclosure can include embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The present disclosure can include embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[0121] The term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" and "consisting essentially of" is also encompassed and disclosed.

[0122] The abbreviation, "*e.g.*," is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation "*e.g.*," is synonymous with the term "for example." The abbreviation, "*i.e.*," is derived from the Latin *id est*, and is used herein to indicate a non-limiting rewording or clarification. Thus, the abbreviation "*i.e.*," is synonymous with the term "that is."

[0123] Any particular embodiment of the present disclosure that falls within the prior art may be explicitly excluded from any one or more of the claims. Any particular embodiment of the agents, methods, and/or compositions of the present disclosure can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[0124] The present specification will control in instances where publications, patent applications, patents, and other references mentioned herein are incorporated by reference and are in conflict with the present specification.

[0125] Section headings, materials, methods, and examples are illustrative only and not intended to be limiting.

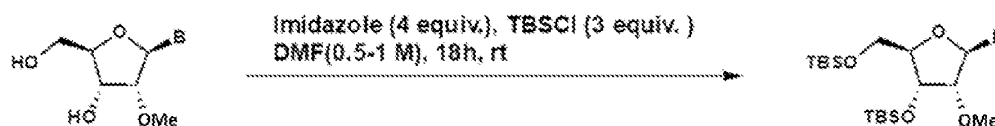
EXAMPLES

[0126] Unless noted otherwise, all reactions in the following Examples were set up inside of a chemical fume hood and run under a nitrogen or argon atmosphere. Anhydrous acetonitrile (MeCN) was purchased from Sigma-Aldrich. HPLC-grade toluene was purchased from Sigma-Aldrich and was further purified by successive filtration through neutral alumina and CuO columns under argon pressure. Reactions were run in oven-

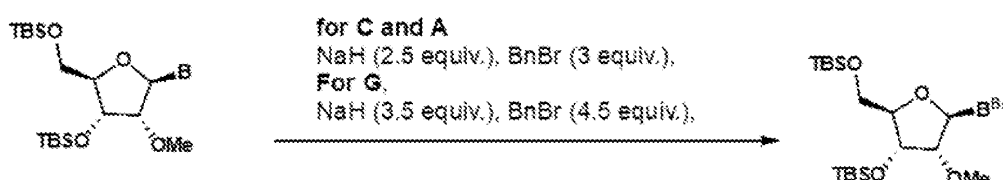
- 52 -

dried reaction tubes (Fisherbrand, 16 x 125 mm, product no. 1495935A) sealed with a screw cap (Thermo Fisher Scientific, catalog no. B7995-18), and fitted with a Teflon-lined septum (Thermo Fisher Scientific, catalog no. C47995-15). Solvents used for extractions, crystallizations, and column chromatography were purchased from Sigma-Aldrich as ACS grade, except for hexanes, which were HPLC grade. Lithium tert-butoxide (LiOt-Bu) was purchased from Alfa Aesar and stored in a nitrogen-filled glovebox. All preparative reactions were set up in the nitrogen filled glovebox and closed reaction vials were taken outside and stirred for noted time. Screening of reaction conditions and mechanistic experiments were carried out with the aid of a nitrogen-filled glovebox. Organic compounds were purified by flash chromatography using Silicycle SiliaFlash P60 (230–400 mesh) silica gel either manually or using a CombiFlash NextGen 300 automated chromatography system.

[0127] For general analytics, CDCl_3 and d_6 -DMSO were purchased from Cambridge Isotope Labs. NMR spectra were collected on Bruker Avance III HD 400 or 500 MHz spectrometer. ^1H (CDCl_3 : δ 7.26; d_6 -DMSO: δ 2.50) and ^{13}C NMR shifts (CDCl_3 : δ 77.16; d_6 -DMSO: δ 39.52) were referenced to residual solvent peaks. The following abbreviations were used to characterize multiplicities: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, p = pentet, sept = septet, m = multiplet. ^{13}C and ^{19}F spectra were obtained with ^1H decoupling. Gas chromatography (GC) analyses were performed on an Agilent 7890A gas chromatograph with an FID detector using a J&W DB-1 column (10 m, 0.1 mm I.D.). LC/MS was recorded on an Agilent 6120 Quadrupole LC/MS. Elemental analyses were performed by Atlantic Microlabs Inc., Norcross, GA, USA. High-resolution mass spectra were recorded on a JEOL AccuTOF LC-Plus 46 DART system and on an Agilent Technologies 6545 Q-TOF LC/MS system. IR spectra were recorded on a Nicolet iS5 spectrometer equipped with an iD5 diamond laminate ATR accessory from Thermo Fisher Scientific. IR spectra were acquired from neat samples. Melting points were obtained using a Stanford Research Systems EZ-Melt melting point apparatus.

Example 1. Preparation of substrates -- C, A, G, U**a. Synthesis of 3',5'-Bis-TBS-protected nucleosides (GP1)**

[0128] A 50 mL round bottom flask equipped with a magnetic stir bar was charged with nucleosides 'B' (U, C, A or G, 1 equiv.) and imidazole (4 equiv.) and dried in vacuo by using standard Schlenk line technique. This mixture was dissolved in anhydrous DMF (0.5-1 M, depending on solubility) and *t*-butyldimethylsilyl chloride (3 equiv.) was added. The reaction mixture was stirred for 18 hours at room temperature under an inert atmosphere. Upon completion of reaction (monitored by TLC or LC-MS analysis), reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×100 mL). The combined organic layer was washed with brine (100 mL) and dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by silica gel column chromatography and eluted with ethyl acetate/hexane, 6:4. Fractions containing the product were pooled and evaporated.

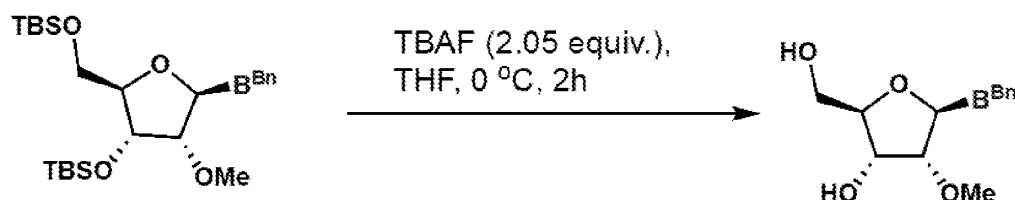
b. Synthesis of Benzyl-protected nucleosides (GP2)

[0129] A 100-mL round bottom flask equipped with a magnetic stir bar was charged with Bis-TBS-protected cytidine (1 equiv.) and dry DMF (0.3 M). The resulting solution was cooled down to 0 °C and NaH was added. The resulting solution was stirred for 5 minutes and benzyl bromide was added dropwise at 0 °C. The progress of reaction was monitored by LC-MS analysis. After 45 minutes, second portion of NaH was added and stirred for additional 1 hour at 0 °C. The reaction mixture was warmed to RT and stirred for 30 minutes before quenched with NH₄Cl (50 mL). The resulting mixture was extracted with EtOAc (100 mL×3). The combined organic layer was washed with brine (100 mL), dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography with hexanes/ethyl acetate as eluants (0-

- 54 -

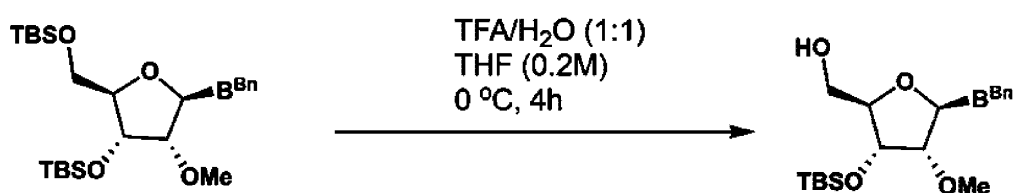
100% gradient) by using a Combi-Flash NextGen 300 automated chromatography system.

c. Synthesis of 3',5'-dihydroxy nucleoside via tbs-deprotection (GP3)



[0130] A 100 mL round bottom flask equipped with a magnetic stir bar was charged with Bis-TBS-multibenzyprotected nucleoside (4 mmol) and dry THF (20 ml). The reaction mixture was cooled to 0 °C and tetrabutylammonium fluoride (TBAF, 1M solution in THF, 8.2 mmol, 8.2 mL) was added dropwise. The reaction was stirred for 1 hour at 0 °C. The reaction was monitored via LC-MS. Upon completion of the reaction, it was quenched with NH₄Cl (10 mL) and diluted with water (10 mL). The resulting mixture was extracted with ethyl acetate (100 mL x3). The mixture was then concentrated under reduced pressure. The crude product was purified by flash chromatography on a silica gel column using methylene chloride-methanol (0 to 5% gradient) (for C) or hexane-ethyl acetate (0 to 100% gradient, for A, G) to give the final product compound (87-97% yield) as a white solid.

d. Synthesis of 3-OTBS-5-OH- nucleosides via selective deprotection of 5'-Siloxy of bis-TBS-nucleoside (GP4)

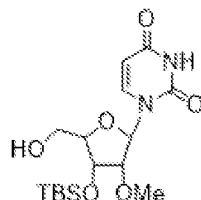


[0131] Isolated or crude bis-TBS-protected nucleosides (U, dibenzyl-C, -A, and tri-benzyl G; 1 equiv.) were dissolved in THF (0.2 M) and treated with TFA (5 equiv.) in H₂O (TFA:H₂O, 1:1 mixture) at 0 °C. The reaction was monitored via LC-MS. Upon completion after 4-6 hours, the reaction was diluted with equal volume water and pH adjusted to 7 to 8 with solid sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (3x150 mL) and washed with brine (100 mL). The combined organic layer was dried over sodium sulfate and concentrated with the aid of rotavapor. The residue

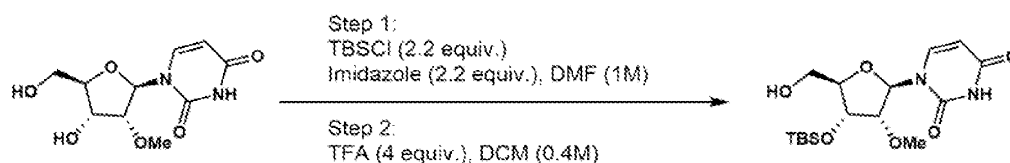
- 55 -

purified by silica gel column chromatography and eluted with hexane-ethyl acetate [0 to 100% gradient] to give 5'-OH, 3-TBS-nucleoside (70-80% yields).

e. One pot-procedure to synthesize 3-OTBS-5-OH-nucleosides from nucleoside (GP5)



1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione, S1



[0132] To a solution of 2'-O-methyl uridine (10 g, 39 mmol) in DMF (30 mL) was added imidazole (5.80 g, 85.0 mmol) and the solution was cooled to 5 °C. To the solution was added TBSCl (12.8 g, 85.0 mmol) slowly over 15 minutes. The reaction was then warmed to 23 °C and found to reach completion within 20 hours. The reaction was added to a separatory funnel containing 100 mL ethyl acetate and washed 5 x 250 mL with water to remove the DMF. The organic layer was dried over sodium sulfate and concentrated to dryness. The white solids were redissolved in dichloromethane (100 mL) and treated with TFA (18.2 g, 160 mmol). After 20 hours, the reaction was poured into a flask containing 300 mL water and pH adjusted to pH = 8 with solid sodium bicarbonate. The aqueous layer was extracted with dichloromethane (3x150 mL). The dichloromethane layer was dried over sodium sulfate and concentrated to 30 mL or until solids begin to precipitate. The concentrated solution was then transferred to a flask equipped with overhead stirring and treated with 70 mL hexanes. The solution was aged for 3 hours and the solids were collected via filtration funnel and the cake was rinsed with 100 mL hexanes. The solids were dried in a vacuum oven resulting white solids (9.9 g, 68%). The crude solids were found to be 95% pure by HPLC and used without further

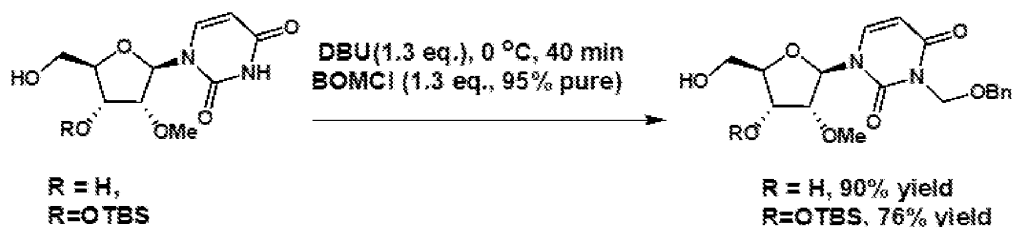
- 56 -

purification. The identity was confirmed with proton NMR and MS analysis and found to be consistent with data in literature (*Chem. Commun.* 2021, 57 (55), 6808-6811).

[0133] ^1H NMR (400 MHz, CDCl_3) δ 8.34 (s, 1H), 7.68 (d, $J = 8.1$ Hz, 1H), 5.75 (dd, $J = 8.1, 2.3$ Hz, 1H), 5.68 (d, $J = 4.1$ Hz, 1H), 4.38 (t, $J = 5.1$ Hz, 1H), 4.09 (dt, $J = 5.0, 2.2$ Hz, 1H), 4.06 – 3.95 (m, 2H), 3.85 – 3.72 (m, 1H), 3.51 (s, 3H), 2.61 – 2.52 (m, 1H), 0.94 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H).

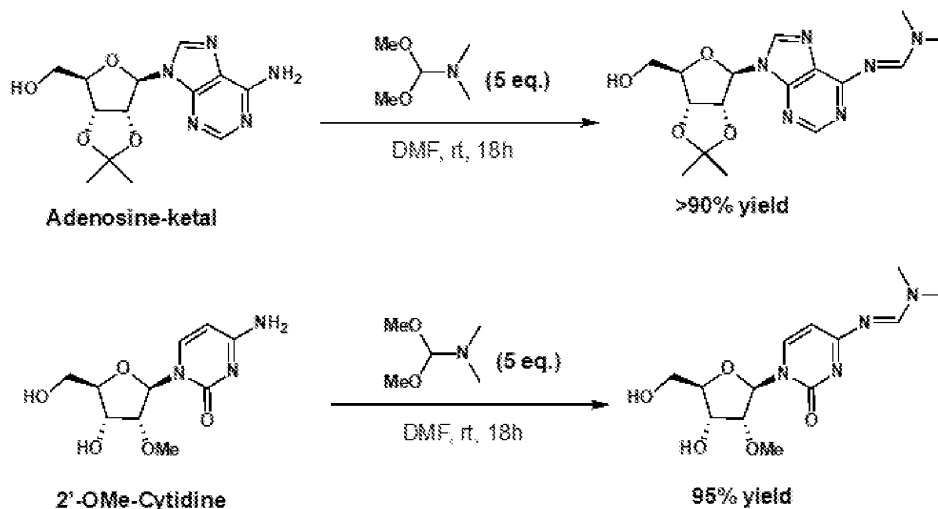
[0134] ^{13}C NMR (101 MHz, CDCl_3) δ 163.44, 150.24, 142.10, 102.31, 91.11, 85.33, 82.55, 69.57, 61.06, 58.47, 25.71, 25.65, 18.15, -4.72, -4.82.

f. Benzyloxymethyl acetal (BOM)-protection of nucleoside (GP6)

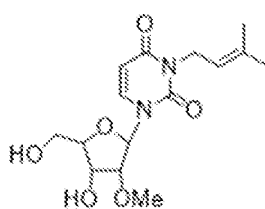


[0135] To 100-mL round bottom flask equipped with a magnetic stir bar containing 50.0 mL of DMF, was added 1-(4-hydroxy-5-(hydroxymethyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (7.11 g, 25.0 mmol). The resulting solution was treated with DBU (4.85 mL, 32.5 mmol, 1.3 equiv.) and BOMCl (4.50 mL, 32.5 mmol, 1.3 equiv.) at 0 °C for 40 minutes. The progress of reaction was monitored by TLC analysis. Upon completion, the reaction was quenched with H_2O (50 mL), and the resulting mixture was extracted with EtOAc (150 mL $\times$ 4). The combined organic layer was washed with brine (100 mL), dried over Na_2SO_4 , filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography with DCM/MeOH as eluants (0-5% MeOH-DCM gradient) by using a Combi-Flash NextGen 300 automated chromatography system.

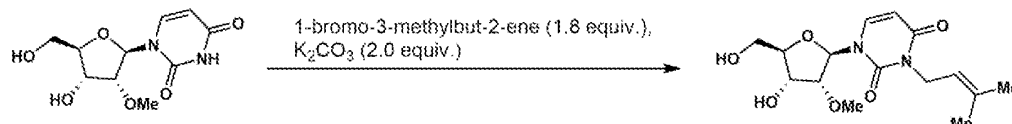
- 57 -

g. DMF-formamidine protection of NH₂ of nucleoside (GP7)

[0136] An oven-dried 100-mL RB-flask equipped with a magnetic stir bar was charged with adenosine-ketal (1 equiv) or 2'-OMe-cytidine (1 equiv), and then 1, 1-dimethoxy-N,N- dimethylmethanamine (5 equiv, 32.5 mmol) in DMF (10 mL, 0.65 M). After stirring for 18 hours at room temperature, the reaction mixture was concentrated to dryness (20 x 2 mL toluene was added to aid the evaporation of DMF under reduced pressure) and the crude white solid triturated with ether (3 x 100 mL). The solid was collected by filtration and dried under high vacuum for 12 hours to yield >90% pure product (90-95%) as off-white solid.

h. N-Alkylations of uridine

1-((2*R*,3*R*,4*R*,5*R*)-4-hydroxy-5-(hydroxymethyl)-3-methoxytetrahydrofuran-2-yl)-3-(3-methylbut-2-en-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione



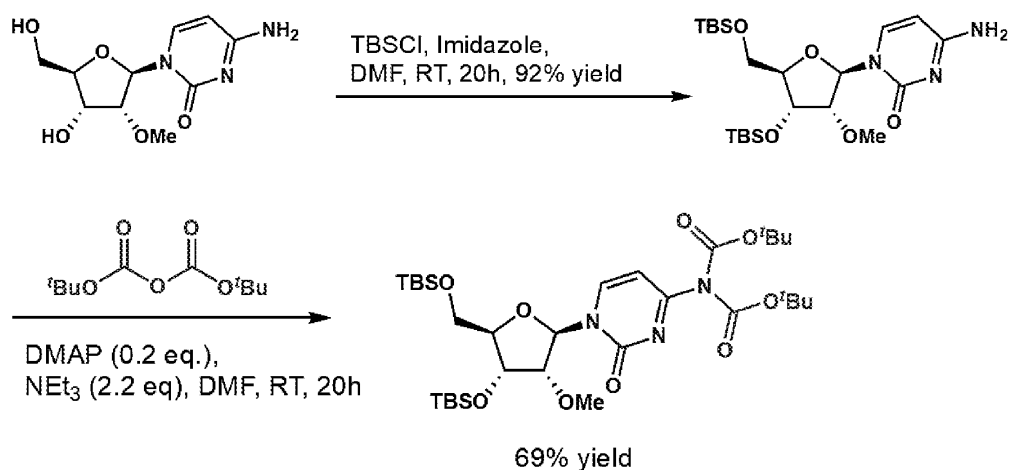
- 58 -

[0137] To a 50-mL round bottom flask equipped with a magnetic stir bar containing 30.0 mL of DMF was added 2'-O-methyl uridine (5.96 g, 23.069 mmol, 1.0 equiv.), 1-bromo-3-methylbut-2-ene (4.77 mL, 6.15 mmol, 1.79 equiv.) and potassium carbonate (6.35 g, 45.96 mmol, 2.0 equiv.). The reaction mixture was allowed to stir at 60 °C for 4h before it was diluted with 30 mL of water and extracted with dichloromethane (3×20 mL). The organic layer was collected, dried over magnesium sulfate, filtered, concentrated, and purified by silica gel column chromatography with acetone/hexane = 1:1 as eluent followed by drying under vacuum overnight to give titled compound as a white solid with a yield of 54%. The identity was confirmed with proton NMR and MS analysis and found to be consistent with data in literature (*J. Am. Chem. Soc.* **2017**, *139*, 5467–5473).

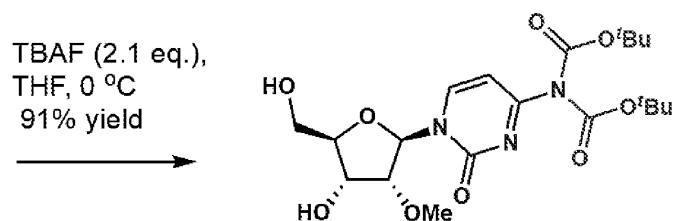
[0138] ^1H NMR (500 MHz, CDCl_3) δ 7.73 (d, J = 8.1 Hz, 1H), 5.81 (d, J = 3.1 Hz, 1H), 5.78 (d, J = 8.1 Hz, 1H), 5.24 (td, J = 7.7, 7.0, 1.6 Hz, 1H), 4.61 – 4.48 (m, 2H), 4.35 (dt, J = 7.2, 5.6 Hz, 1H), 4.08 – 3.98 (m, 3H), 3.88 (ddd, J = 12.7, 6.1, 2.8 Hz, 1H), 3.61 (s, 3H), 2.74 (d, J = 6.2 Hz, 1H), 2.71 (d, J = 7.3 Hz, 1H), 1.82 (d, J = 1.4 Hz, 3H), 1.72 (d, J = 1.6 Hz, 3H).

[0139] ^{13}C NMR (126 MHz, CDCl_3) δ 162.65, 150.90, 139.01, 137.41, 118.28, 102.23, 90.19, 84.85, 82.98, 68.61, 61.32, 58.87, 39.41, 25.85, 18.25.

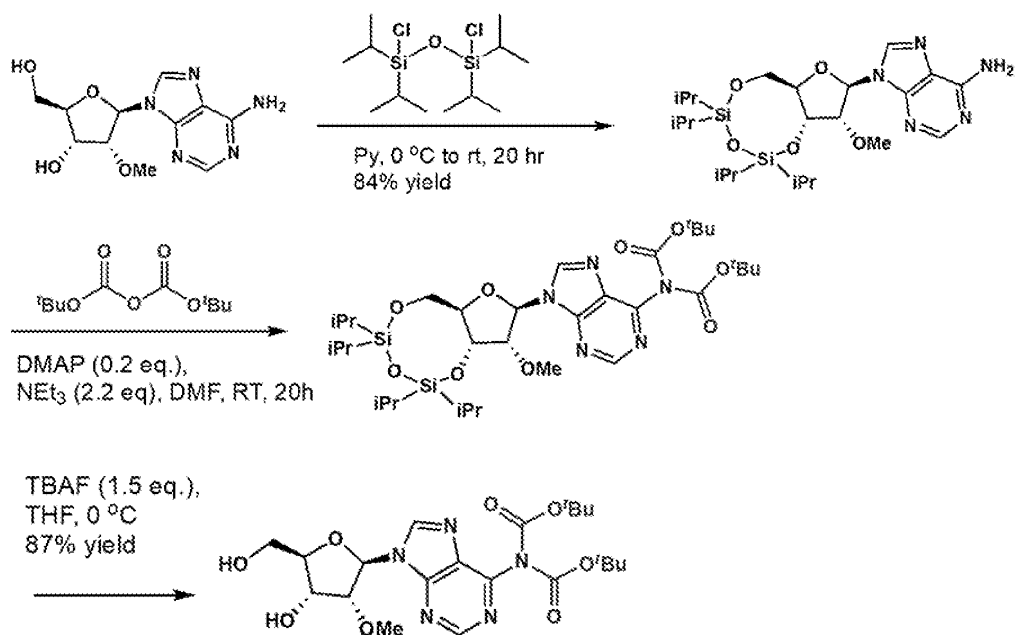
i. Bis-Boc protection of Cytidine



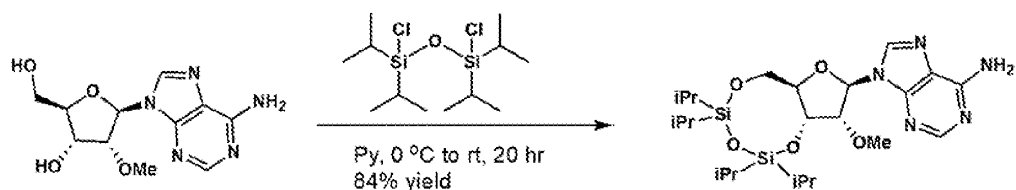
- 59 -



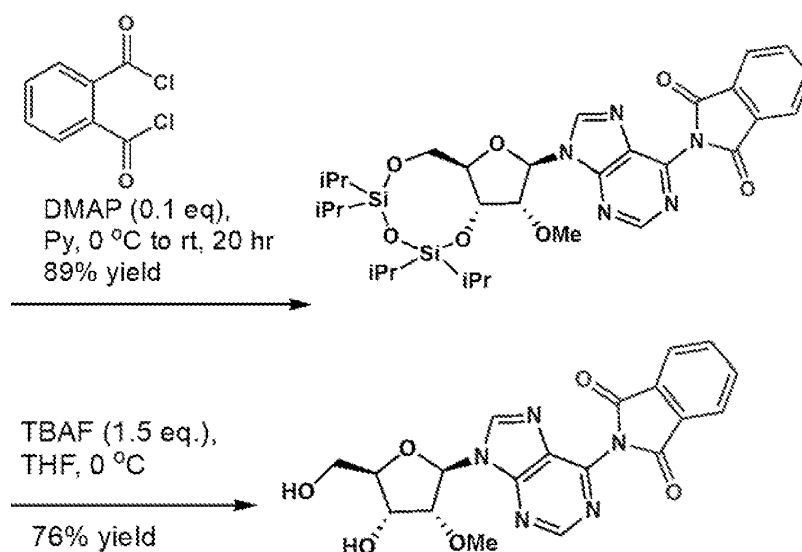
j. Bis-Boc protection of Adenosine



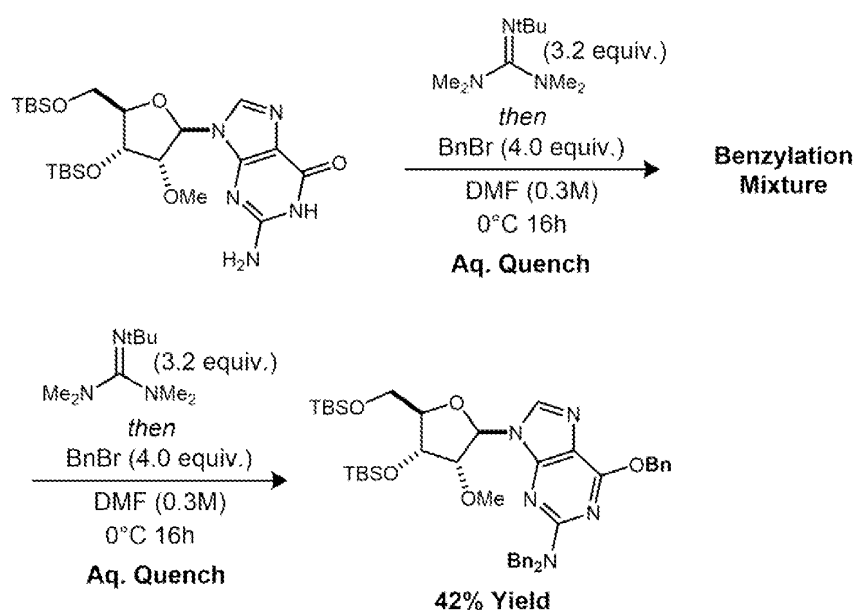
k. Phthalimido protection of Adenosine



- 60 -



1. Guanosine protection



Example 2. Spectra of specific monomers

a. DMF-A-diol

[0140] ^1H NMR (500 MHz, DMSO) δ 8.92 (t, $J = 0.6$ Hz, 1H), 8.51 (s, 1H), 8.42 (s, 1H), 6.06 (d, $J = 5.9$ Hz, 1H), 5.33 – 5.26 (m, 2H), 4.41 – 4.31 (m, 2H), 3.98 (q, $J = 3.6$

- 61 -

Hz, 1H), 3.68 (ddd, $J = 12.0, 4.9, 3.8$ Hz, 1H), 3.57 (ddd, $J = 12.0, 6.5, 3.8$ Hz, 1H), 3.31 (s, 3H), 3.20 (d, $J = 0.5$ Hz, 3H), 3.13 (d, $J = 0.6$ Hz, 3H).

[0141] ^{13}C NMR (126 MHz, DMSO) δ 159.37, 158.09, 151.90, 151.11, 141.33, 125.77, 86.28, 85.63, 82.47, 68.74, 61.36, 57.48, 54.92, 40.69, 34.58.

b. 3-TBS-G-DMF-AMIDE

[0142] ^1H NMR (500 MHz, CDCl_3) δ 8.46 (s, 1H), 7.71 (s, 1H), 7.16 – 7.13 (m, 1H), 5.80 (d, $J = 7.4$ Hz, 1H), 4.50 (dd, $J = 4.9, 1.4$ Hz, 1H), 4.42 (dd, $J = 7.5, 4.9$ Hz, 1H), 4.15 (p, $J = 1.4$ Hz, 1H), 3.90 (dd, $J = 12.6, 2.1$ Hz, 1H), 3.68 (dd, $J = 12.6, 1.5$ Hz, 1H), 3.27 (s, 3H), 3.18 (t, $J = 2.2$ Hz, 3H), 3.12 – 3.05 (m, 3H), 0.93 (t, $J = 1.1$ Hz, 9H), 0.12 (dt, $J = 8.5, 1.0$ Hz, 6H).

c. 3-TBS-ABn₂

[0143] ^1H NMR (500 MHz, CDCl_3) δ 8.36 (s, 1H), 7.79 (s, 1H), 7.36 – 7.24 (m, 10H), 5.86 (d, $J = 8.0$ Hz, 1H), 5.80 – 4.75 (m, 4H), 4.72 (dd, $J = 8.0, 4.6$ Hz, 1H), 4.61 (d, $J = 4.6$ Hz, 1H), 4.22 (d, $J = 1.6$ Hz, 1H), 3.97 (dd, $J = 13.1, 1.6$ Hz, 1H), 3.74 (d, $J = 13.0$ Hz, 1H), 3.29 (s, 3H), 0.96 (s, 9H), 0.16 (d, $J = 8.6$ Hz, 6H).

d. 3-TBS-T-BOM

[0144] ^1H NMR (400 MHz, CDCl_3) δ 7.38 – 7.16 (m, 7H), 6.10 (t, $J = 6.7$ Hz, 1H), 5.44 (s, 2H), 4.65 (s, 2H), 4.43 (dt, $J = 6.8, 3.7$ Hz, 1H), 3.87 (dd, $J = 9.8, 3.4$ Hz, 2H), 3.71 (ddd, $J = 12.1, 6.4, 3.5$ Hz, 1H), 2.36 – 2.10 (m, 3H), 0.85 (s, 9H).

[0145] ^{13}C NMR (101 MHz, CDCl_3) δ 163.54, 151.13, 138.11, 135.70, 128.41, 127.80, 127.75, 110.46, 87.68, 87.61, 72.37, 71.71, 70.66, 62.24, 40.69, 25.84, 18.08, 13.40, -4.55, -4.72.

e. 3-TBS-N-Allyl-U-diol

[0146] ^1H NMR (400 MHz, CDCl_3) δ 7.24 (s, 1H), 5.38 – 5.30 (m, 2H), 4.80 (tp, $J = 6.9, 1.4$ Hz, 1H), 4.16 – 4.02 (m, 2H), 3.90 (dt, $J = 7.2, 5.6$ Hz, 1H), 3.66 – 3.55 (m, 3H), 3.43 (ddd, $J = 12.6, 6.2, 2.8$ Hz, 1H), 3.17 (s, 3H), 2.23 (d, $J = 7.2$ Hz, 1H), 2.16 (s, 1H), 1.37 (s, 3H), 1.28 (q, $J = 1.1$ Hz, 3H).

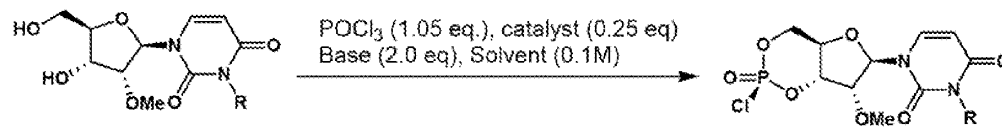
[0147] ^{13}C NMR (101 MHz, CDCl_3) δ 162.73, 162.62, 150.89, 139.25, 137.06, 118.38, 101.85, 90.10, 85.06, 83.28, 69.56, 60.66, 58.46, 39.30, 36.56, 31.49, 25.76, 18.19, 18.15, -4.68, -4.79.

- 62 -

f. Adenosine-N-Formamidinium

[0148] ^1H NMR (500 MHz, CDCl_3) δ 8.93 (s, 1H), 8.47 (s, 1H), 7.90 (s, 1H), 6.57 – 6.52 (m, 1H), 5.86 (d, $J = 4.9$ Hz, 1H), 5.20 (t, $J = 5.4$ Hz, 1H), 5.10 (dd, $J = 5.9, 1.3$ Hz, 1H), 4.51 (q, $J = 1.6$ Hz, 1H), 3.96 (dd, $J = 12.7, 1.5$ Hz, 1H), 3.80 – 3.73 (m, 1H), 3.19 (s, 3H), 1.62 (s, 3H), 1.35 (s, 3H).

[0149] ^{13}C NMR (126 MHz, CDCl_3) δ 160.43, 158.43, 152.16, 150.14, 141.38, 127.69, 114.04, 94.41, 86.12, 83.05, 81.81, 63.53, 41.48, 35.36, 27.75, 25.34.

Example 3. Synthesis of cyclic-chlorophosphate nucleosides (GP8)a. Study of conditions for synthesis of cyclic chlorophosphate

Yields and dr are determined based on ^{31}P NMR. R = 1,1-dimethylallyl

[0150] An oven-dried 100 mL Schlenk flask equipped with a magnetic stir bar, was charged with protected diol-nucleosides (1 equiv., 2 mmol), DMAP (0.1-0.2 equiv., 60-120 mg, 0.25-0.5 mmol) in DCM (18 mL, 0.1 M). To the resulting suspension triethylamine or S-Collidine (2.2 equiv, 4.4 mmol in 1 mL DCM) was added via syringe. The resulting solution was cooled to $-20\text{ }^{\circ}\text{C}$, and freshly distilled POCl_3 (1.05 equiv., 2.1 mmol in 1 mL DCM) was added in one portion. After letting the solution stir for one hour, it was warmed to $0\text{ }^{\circ}\text{C}$ and stirred overnight. The diastereoselectivity of the crude product was monitored by taking aliquots of reaction mixture and by analyzing with ^{31}P NMR. The reaction mixture was further stirred at room temperature for 2-4 hours until the diastereomeric ratio (dr) of crude product reached to $>20:1$. Once the crude dr achieved $>20:1$, reaction mixture was diluted with 10 mL of freshly distilled diethyl ether to precipitate the amine salt. The resulting suspension was filtered via frit filter funnel and the residue was rinsed with diethyl ether (10x2 mL). The filtrate was concentrated with the aid of rotary evaporator. The crude product was purified via silica gel (crude product was loaded on column by dissolving in minimum amount of DCM, height, ~ 2 -inch, diameter, ~ 0.9 -inch) column chromatography with hexanes: ethyl acetate (0 to 80% gradient) by using a Combi-Flash NextGen 300 automated chromatography system.

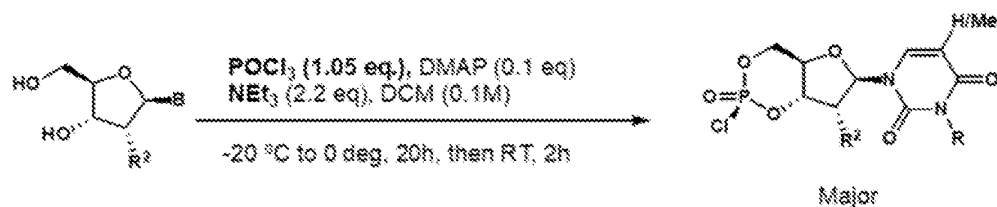
- 63 -

[0151] Variations in synthesis conditions are summarized in Table 1. Resulting cyclic-chlorophosphate nucleoside yields and diastereomeric ratios (dr) are also summarized in Table 1.

Table 1 - Cyclic-chlorophosphate nucleoside synthesis conditions

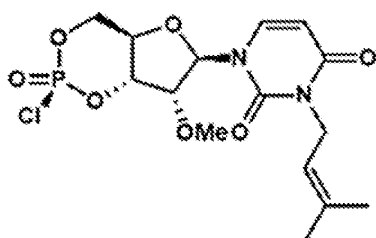
Entry	Catalyst	Base	Solvent	Temp (C), Time	Yield (dr)
1	None	n-BuLi	MTBE	-20, 1h	< 5%
2	None	LiHMDS	THF	-20, 1h	20% (10:1)
3	DAMP	KHMDS	THF	-20, 1h	trace
4	DAMP	DMAP	MeCN	-20, 1h	20% (10:1)
5	DMAP	S-Collidine	MeCN	-20, 1h	20% (3:1)
6	DMAP	DMAP	DCM	-20, 2h	40% (15:1)
7	DMAP	S-Collidine	DCM	-20, 1h	40% (1: 2.5)
8	DMAP	S-Collidine	DCM	-20, 20h	75% (2.5:1) (50% isolated yield, dr 5:1)
9	DMAP	S-Collidine	DCM	-20, 1h then 0, 20h	78% (15:1) (49% isolated yield, dr 20:1)
10	DMAP	Triethylamine	DCM	-20, 1h then 0, 20h	78% (20:1) (49% isolated yield, dr 20:1)
11	DMAP	Triethylamine	DCM	-20, 1h then 0, 20h, rt 3h	79% (30:1) (56% isolated yield, dr 35:1)

b. Study of nucleosides for synthesis of cyclic chlorophosphite

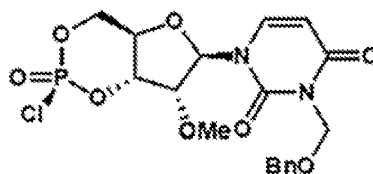


[0152] Cyclic-chlorophosphate nucleosides were synthesized according to the general procedures in Examples 3a, but using various nucleosides. Resulting cyclic-chlorophosphate nucleosides with their yield and diastereomeric ratio (dr) were as follows:

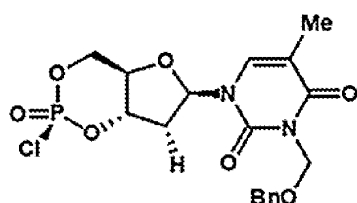
- 64 -



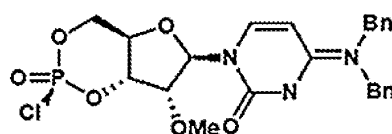
2'-OMe-U-Allyl-Cl-CP,
50% yield, 20:1 dr



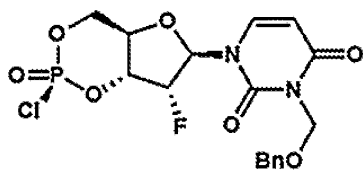
2'-OMe-U-BOM-Cl-CP,
62% yield, 36:1 dr
with collidine, 46% yield, >20:1 dr



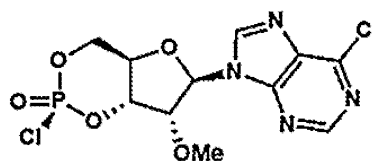
2'-deoxy-TBOM-Cl-CP,
55% yield, 16:1 dr
with collidine, 51% yield, 4:1 dr



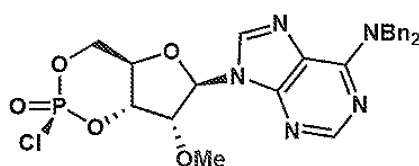
2'-OMe-C(N-Bn₂)-Cl-CP,
30% yield, 40:1 dr



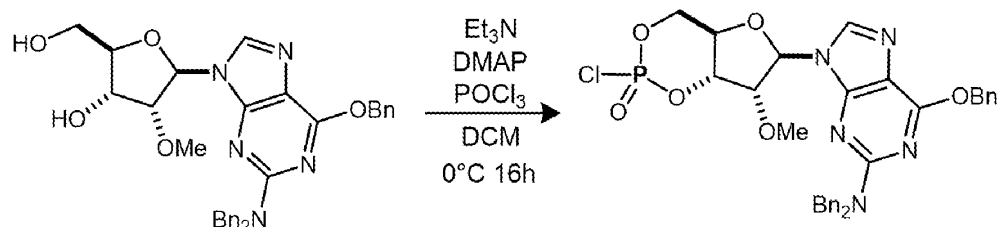
2'-F-U-BOM-Cl-CP,
with collidine, 48% yield, 18:1 dr



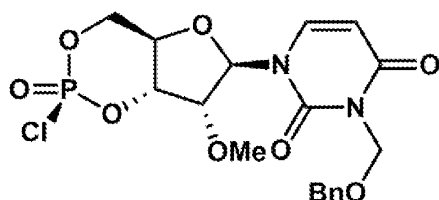
2'-OMe-6Cl-purine-Cl-CP,
47% yield, 10:1 dr



2'-OMe-A(N-Bn₂)-Cl-CP,
47% yield, 10:1 dr

c. Guanosine cyclic phosphate synthesis**Example 4. Synthesis of specific cyclic-chlorophosphate nucleosides**

a. 3-((benzyloxy)methyl)-1-((2*S*,4*aR*,6*R*,7*R*,7*aR*)-2-chloro-7-methoxy-2-oxidotetrahydro-4*H*-furo[3,2-*d*][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1*H*,3*H*)-dione



[0153] The compound was synthesized by following general procedure **GP8** using U-BOM (1.85 g, 4.9 mmol, 1 equiv.), triethylamine (1.09 g, 10.76 mmol, 2.2 equiv.), DMAP (120 mg, 0.098 mmol, 0.2 equiv.) and POCl₃ (824 mg, 5.37 mmol, 1.1 equiv.), in DCM (35 mL, 0.14M). Column conditions. SiO₂-25g column, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 80%), 1380 mg of desired product isolated as white solid. Yield = 62%, dr = 38:1.

[0154] ³¹P NMR (162 MHz, CDCl₃) δ -1.12, -1.72.

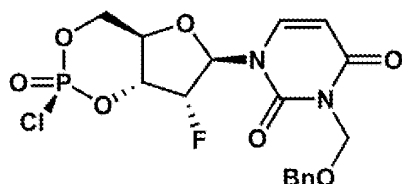
[0155] ¹H NMR (400 MHz, CDCl₃) δ 7.41 – 7.27 (m, 5H), 7.13 (d, *J* = 8.1 Hz, 1H), 5.79 (d, *J* = 8.1 Hz, 1H), 5.50 – 5.41 (m, 2H), 5.30 (q, *J* = 0.9 Hz, 1H), 4.92 (ddd, *J* = 9.9, 5.1, 3.5 Hz, 1H), 4.78 – 4.64 (m, 3H), 4.46 (ddd, *J* = 11.2, 9.7, 1.7 Hz, 1H), 4.32 (d, *J* = 5.1 Hz, 1H), 4.25 (td, *J* = 10.3, 4.7 Hz, 1H), 3.57 (s, 3H).

[0156] ¹³C NMR (101 MHz, CDCl₃) δ 162.22, 150.38, 141.12, 137.83, 128.52, 128.01, 127.82, 103.03, 97.69, 80.02, 79.95, 79.55, 79.46, 72.67, 70.93, 70.83, 70.51, 70.30, 70.24, 59.87.

[0157] HR-MS is not determined as the compound hydrolyzed in the process of analysis.

- 66 -

b. 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-chloro-7-fluoro-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



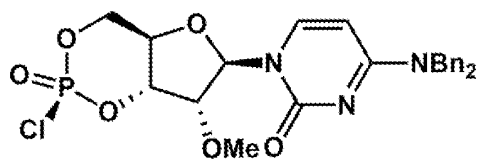
[0158] The compound was synthesized by following general procedure **GP8** using U-BOM (900 mg, 2.46 mmol, 1 equiv.), triethylamine (522 mg, 5.17 mmol, 2.1 equiv.), DMAP (60 mg, 0.049 mmol, 0.2 equiv.) and POCl₃ (414 mg, 2.70 mmol, 1.1 equiv.), in DCM (25 mL, 0.1M). Column conditions. SiO₂-10g column, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 80%), 530 mg of desired product isolated as white solid. Yield = 48%, dr = 18:1.

[0159] ³¹P NMR (162 MHz, CDCl₃) δ -1.62, -2.04.

[0160] ¹H NMR (400 MHz, CDCl₃) δ 7.38 – 7.27 (m, 5H), 7.12 (d, *J* = 8.0 Hz, 1H), 5.82 (d, *J* = 8.0 Hz, 1H), 5.65 – 5.36 (m, 3H), 5.31 (s, 1H), 5.30 (s, 2H), 5.15 (ddt, *J* = 22.9, 8.6, 4.0 Hz, 1H), 4.76 (dd, *J* = 9.7, 4.6 Hz, 1H), 4.69 (s, 2H), 4.51 (t, *J* = 10.0 Hz, 1H), 4.24 (td, *J* = 10.3, 4.6 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 1H).

[0161] HR-MS is not determined as the compound hydrolyzed in the process of analysis.

c. 1-((2S,4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-4-(dibenzylamino)pyrimidin-2(1H)-one



[0162] The compound was synthesized by following general procedure **GP8** using C-Bn₂ (870 mg, 2.46 mmol, 1 equiv.), triethylamine (443 mg, 4.37 mmol, 2.2 equiv.), DMAP (24 mg, 0.02 mmol, 0.1 equiv.) and POCl₃ (335 mg, 2.19 mmol, 1.1 equiv.), in DCM (21 mL, 0.1M). Column conditions. SiO₂-10g column, wet loading by dissolving in

- 67 -

minimum amount of DCM, Hex-EA gradient (0% to 100%), 304 mg of desired product isolated as white solid. Yield = 30%, dr = 22:1.

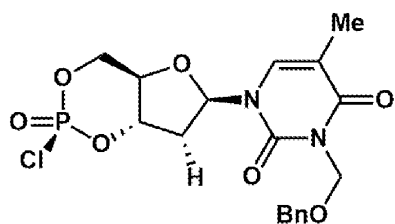
[0163] ^{31}P NMR (162 MHz, CDCl_3) δ -0.96, -1.11.

[0164] ^1H NMR (400 MHz, CDCl_3) δ 7.34 – 7.17 (m, 12H), 7.07 (d, J = 7.3 Hz, 2H), 5.78 (d, J = 7.7 Hz, 1H), 5.26 (d, J = 14.4 Hz, 1H), 5.18 (ddd, J = 9.6, 5.0, 3.6 Hz, 1H), 5.02 – 4.90 (m, 2H), 4.63 (ddd, J = 27.1, 9.6, 4.7 Hz, 1H), 4.51 – 4.39 (m, 5H), 4.21 (td, J = 10.3, 4.7 Hz, 1H), 3.53 (s, 3H).

[0165] ^{13}C NMR (101 MHz, CDCl_3) δ 164.53, 154.59, 144.22, 136.64, 135.55, 129.25, 128.82, 128.65, 128.03, 127.91, 126.26, 98.34, 92.95, 80.30, 80.23, 79.84, 79.75, 77.36, 71.24, 71.14, 70.40, 70.35, 59.70, 50.85, 50.57.

[0166] HR-MS is not determined as the compound hydrolyzed in the process of analysis.

d. 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7aS)-2-chloro-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-5-methylpyrimidine-2,4(1H,3H)-dione



[0167] The compound was synthesized by following general procedure **GP8** using T-BOM (2.5 g, 7.18 mmol, 1 equiv.), triethylamine (1.598 g, 15.79 mmol, 2.2 equiv.), DMAP (175 mg, 1.43 mmol, 0.1 equiv.) and POCl_3 (1.21 g, 7.89 mmol, 1.1 equiv.), in DCM (60 mL, 0.12M). Column conditions. SiO_2 -25g column, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 85%), 1700 mg of desired product isolated as white solid. Yield = 55%, dr = 16:1.

[0168] ^{31}P NMR (162 MHz, CDCl_3) δ -1.48, -1.98.

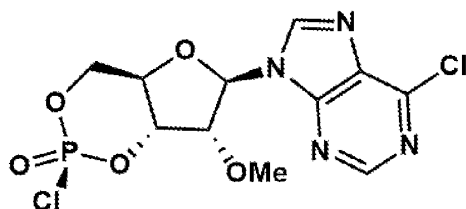
[0169] ^1H NMR (400 MHz, CDCl_3) δ 7.40 – 7.28 (m, 5H), 7.28 – 7.22 (m, 1H), 6.92 (q, J = 1.2 Hz, 1H), 5.97 (dd, J = 8.8, 3.0 Hz, 1H), 5.48 (d, J = 1.7 Hz, 2H), 5.04 – 4.91 (m, 1H), 4.79 – 4.61 (m, 3H), 4.53 (ddd, J = 10.6, 9.7, 1.7 Hz, 1H), 3.95 (dddd, J = 10.7, 9.1, 4.6, 0.7 Hz, 1H), 2.74 – 2.53 (m, 2H), 1.95 (d, J = 1.2 Hz, 3H).

- 68 -

[0170] ^{13}C NMR (126 MHz, CDCl_3) δ 163.13, 150.39, 137.95, 135.67, 128.47, 127.92, 127.82, 111.49, 88.37, 79.12, 79.07, 77.36, 73.71, 73.66, 72.57, 71.07, 70.99, 70.75, 34.70, 34.62.

[0171] HR-MS is not determined as the compound hydrolyzed in the process of analysis.

e. (2*S*,4*aR*,6*R*,7*R*,7*aR*)-2-chloro-6-(6-chloro-9*H*-purin-9-yl)-7-methoxytetrahydro-4*H*-furo[3,2-*d*][1,3,2]dioxaphosphinine 2-oxide



[0172] The compound was synthesized by following general procedure GP8 using 2-OMe-A(6Cl)-diol (1050 mg, 3.5 mmol, 1 equiv.), triethylamine (777 mg, 7.68 mmol, 2.2 equiv.), DMAP (43 mg, 1.43 mmol, 0.1 equiv.) and POCl_3 (589 mg, 3.84 mmol, 1.1 equiv.), in DCM (30 mL, 0.12M). Column conditions. SiO_2 -10 g column, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 85%), 620 mg of desired product isolated as white solid. Yield = 47%, dr = 10:1.

[0173] ^{31}P NMR (162 MHz, CDCl_3) δ -1.16, -1.76.

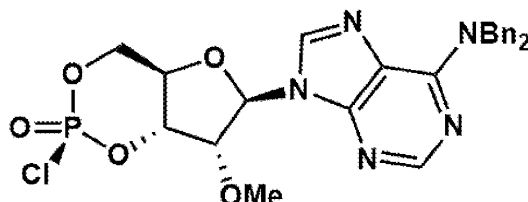
[0174] ^1H NMR (400 MHz, CDCl_3) δ 8.80 (s, 1H), 8.18 (s, 1H), 6.01 (s, 1H), 5.60 (dt, J = 8.9, 4.2 Hz, 1H), 4.90 – 4.37 (m, 4H), 3.63 (s, 3H).

[0175] ^{13}C NMR (101 MHz, CDCl_3) δ 152.55, 144.61, 132.93, 91.84, 80.24, 80.15, 80.03, 79.97, 71.01, 70.91, 70.64, 70.58, 60.20.

[0176] HR-MS is not determined as the compound hydrolyzed in the process of analysis.

- 69 -

f. (2S,4aR,6R,7R,7aR)-2-chloro-6-(6-(dibenzylamino)-9H-purin-9-yl)-7-methoxytetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinine 2-oxide



[0177] The compound was synthesized by following general procedure GP8 using A-Bn₂ (2.2 g, 4.77 mmol, 1 equiv.), triethylamine (1.061 g, 10.49 mmol, 2.2 equiv.), DMAP (58 mg, 0.47 mmol, 0.1 equiv.) and POCl₃ (804 mg, 5.24 mmol, 1.1 equiv.), in DCM (45 mL, 0.11M). Column conditions. SiO₂-25g column, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 100%), 1220 mg of desired product isolated as white solid. Yield = 47%, dr = 18:1.

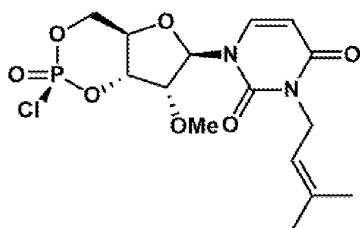
[0178] ³¹P NMR (203 MHz, CDCl₃) δ -1.01, -1.26.

[0179] ¹H NMR (500 MHz, CDCl₃) δ 8.37 (s, 1H), 7.73 (s, 1H), 7.33 – 7.24 (m, 11H), 5.90 (s, 1H), 5.81 (ddd, *J* = 9.7, 4.9, 3.6 Hz, 1H), 5.00 (s, 2H), 4.70 (ddd, *J* = 27.2, 9.6, 4.7 Hz, 1H), 4.63 (d, *J* = 4.9 Hz, 1H), 4.51 (ddd, *J* = 11.0, 9.6, 1.6 Hz, 1H), 4.38 (td, *J* = 10.3, 4.6 Hz, 1H), 3.60 (s, 3H).

[0180] ¹³C NMR (101 MHz, CDCl₃) δ 152.41, 150.19, 138.39, 128.84, 127.95, 127.69, 120.56, 91.79, 80.31, 80.22, 80.12, 80.05, 71.22, 71.12, 70.53, 70.47, 60.00.

[0181] HR-MS is not determined as the compound hydrolyzed in the process of analysis.

g. 1-((2S,4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-3-(3-methylbut-2-en-1-yl)pyrimidine-2,4(1H,3H)-dione



[0182] The title compound was synthesized by following general procedure GP8 using U-Allyl-diol (650 mg, 2 mmol, 1 equiv.), S-collidine (507 mg, 4.18 mmol, 2.1 equiv.),

- 70 -

DMAP (49 mg, 0.4 mmol, 0.2 equiv.) and POCl₃ (804 mg, 5.24 mmol, 1.1 equiv.), in DCM (20 mL, 0.1M). Column conditions. SiO₂-10 g column, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 100%), 400 mg of desired product isolated as white solid. Yield = 49%, dr = 14:1.

[0183] ³¹P NMR (162 MHz, CDCl₃) δ -1.08, -1.71.

[0184] ¹H NMR (400 MHz, CDCl₃) δ 7.12 (d, *J* = 8.1 Hz, 1H), 5.81 (d, *J* = 8.0 Hz, 1H), 5.35 (d, *J* = 1.1 Hz, 1H), 5.20 (tdd, *J* = 6.8, 2.8, 1.4 Hz, 1H), 4.93 (ddd, *J* = 9.8, 5.1, 3.5 Hz, 1H), 4.72 (ddd, *J* = 27.1, 9.7, 4.7 Hz, 1H), 4.57 – 4.42 (m, 3H), 4.36 (d, *J* = 5.0 Hz, 1H), 4.26 (td, *J* = 10.3, 4.7 Hz, 1H), 3.58 (s, 3H), 1.81 (s, 3H), 1.72 (d, *J* = 1.2 Hz, 3H).

[0185] HR-MS is not determined as the titled compound hydrolyzed in the process of analysis.

Example 5. Synthesis of cyclic dinucleotides

a. Multi-pot synthesis – Method A (GP9)

[0186] Inside the N₂-filled glovebox, a 20 mL reaction tube A equipped with a magnetic stir-bar, was charged with LiOtBu (1 equiv., 1 mmol), and the reaction tube was then taken out of the glovebox, put under nitrogen atmosphere via the Schlenk-line technique and kept at -20 °C with aid of a cryocooler. A stock of solution with 5'-OH-nucleoside (1 equiv., 1 mmol) in Toluene (5 mL, 0.2 M) was added to the reaction tube A. The resulting reaction was stirred at -20 °C for 30 min.

[0187] Another 20 mL reaction tube B equipped with magnetic stir bar was charged with cyclic-chlorophosphate nucleosides (1.2 equiv., 1.2 mmol) and 5 mL of MeCN. The reaction mixture was cooled to -20 °C with aid of a cryocooler. The pre-stirred solution of alkoxide from reaction tube A was transferred to a round bottom flask via syringe dropwise over the period of 5 minutes while stirring under nitrogen atmosphere. The resulting reaction mixture was stirred for 18 hours at -20 °C. In cases where incomplete conversion was observed, the reaction was warmed to room temperature for 30 minutes to ensure 100% conversion. The reaction was tracked by ³¹P NMR. Upon completion of the reaction, the crude reaction mixture was concentrated under reduced pressure and the crude product was purified via Combi-Flash NextGen 300 automated chromatography system with hexane: ethyl acetate (0% to 100% gradient). Alternatively, DCM:

- 71 -

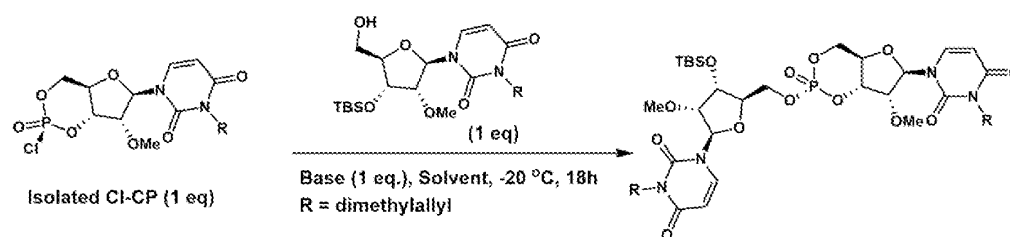
Acetonitrile (2% isopropanol) or DCM: Ethyl acetate (2% MeOH) was used for more polar compounds. For adenosine and cytidine containing cyclic dinucleotide, DCM: MeOH was used as an eluant (0% to 10%).

b. Multi-pot synthesis – Method B (GP10)

[0188] A solution of 5'-OH-nucleoside (1 equiv., 1 mmol) and triethylamine (1.1 equiv., 1.1 mmol) in dichloromethane (2.5 mL) was added to reaction tube A containing a solution of POCl₃ (1 equiv., 1 mmol) in DCM (2.5 mL), dropwise over a period of 25 minutes while stirring at -20 °C. The resulting solution was stirred for 30 minutes.

[0189] Another reaction tube B equipped with magnetic stir bar was charged with protected diol-nucleosides (1 equiv., 1 mmol) and DMAP (2.2 equiv., 2.2 mmol) in DCM (5 mL, 0.2M). The resulting suspension was cooled to -78 °C. The pre-stirred solution from reaction tube A was cannula transferred to reaction tube B. The resulting mixture was stirred for an additional 1 hour at -78 °C and slowly warmed to room temperature, while stirring for another 1 hour. The reaction was monitored by ³¹P NMR and upon completion, it was diluted with diethyl ether to precipitate the salts and filtered with frit filter funnel. The resulting filtrate was concentrated under reduced pressure. The crude product was purified via Combi-Flash NextGen 300 automated chromatography system with silica gel column with hexanes:acetone (0% to 100% gradient, Crude product was loaded on the column by dissolving in minimum amount of DCM (height, ~3-inch, diameter, ~0.9-inch). Alternatively, DCM:Acetone was used for more polar compounds. For adenosine containing cyclic dinucleotide, DCM:MeOH was used as an eluant (0% to 5%)

c. Study of reaction conditions for synthesizing cyclic dinucleotides

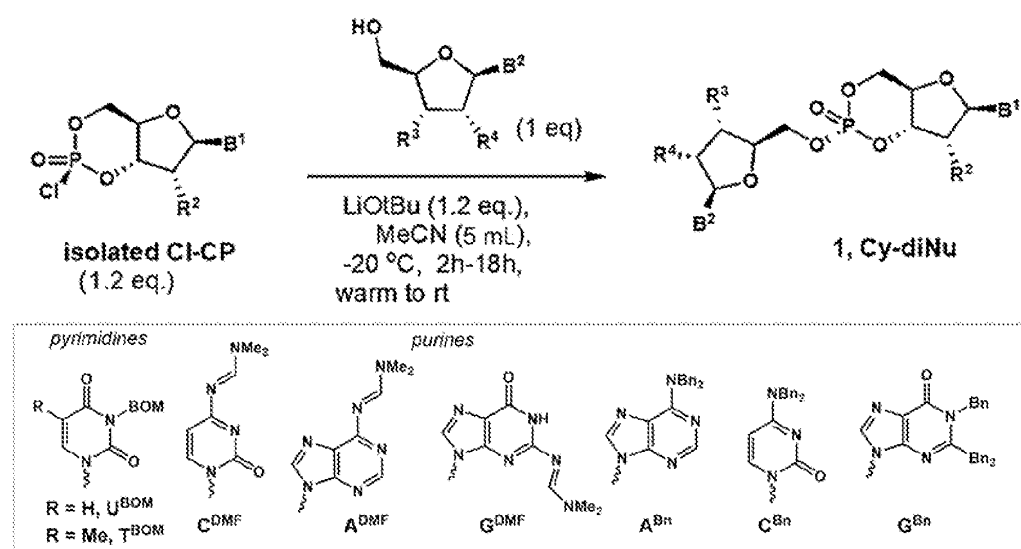


[0190] Cyclic dinucleotides were synthesized according to the general procedures of Example 5b, but using various synthesis conditions as summarized in Table 2. Resulting cyclic dinucleotides yields (based on ³¹P NMR) are also summarized in Table 2.

Table 2 – Cyclic dinucleotide synthesis conditions

dr of Cl-CP	Base	Solvent	dr of product (major is shown)	Yield ³¹ P NMR
15:1	LiOtBu	MeCN	4:1	92%
5:1	LiOtBu	MeCN	3:1	90%
5:1	LiOtBu	Toluene	4:1	91%
5:1	NaOtBu	Toluene	2:1	84%
5:1	KOtBu	Toluene	1:1	78%
15:1	LiOtBu	Toluene	5:1	90%
Scaled up 5:1	LiOtBu	Toluene	4:1	90% (70% isolated yield)
20:1	LiOtBu	Toluene	6:1	> 90% (88% isolated yield)
34:1 (1.1 equiv)	LiOtBu	ACN/Toluene	> 10:1	> 90% (86% isolated yield)

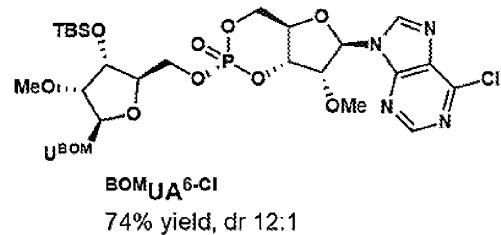
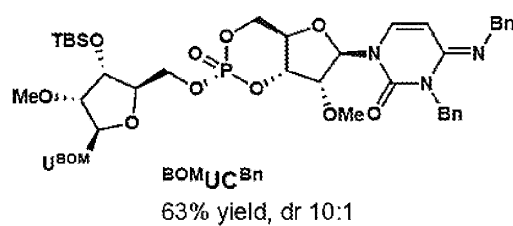
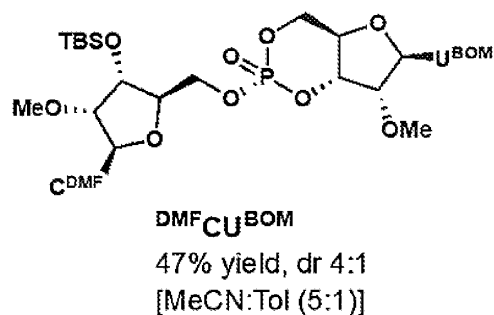
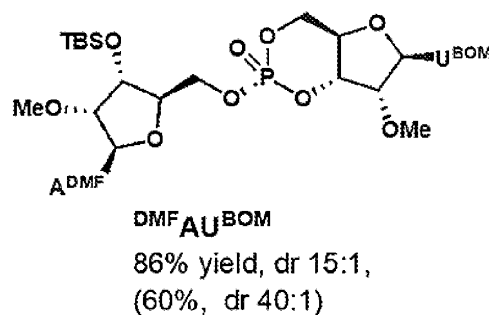
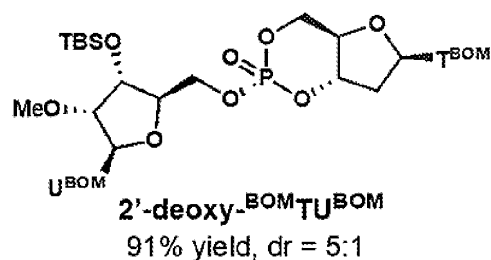
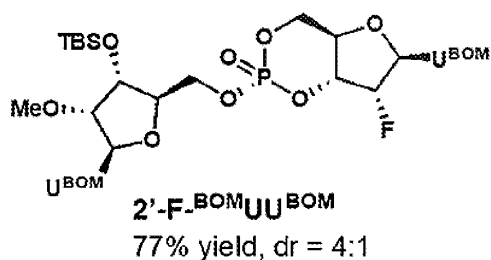
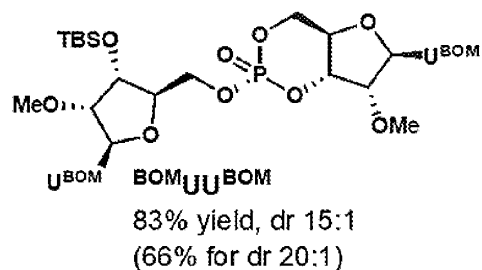
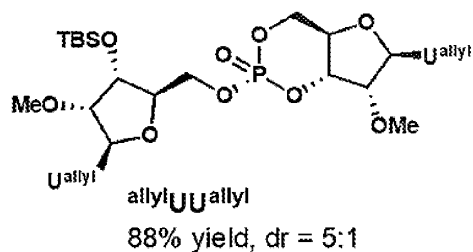
d. Study of nucleosides for synthesis of cyclic dinucleotides



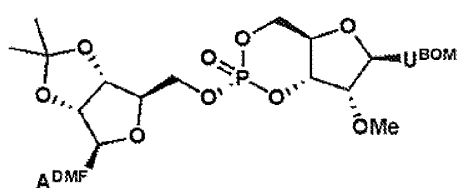
[0191] Cyclic-dinucleotides were synthesized according to the general procedures in Examples 5b, but using various nucleosides. Resulting cyclic dinucleotides with their

- 73 -

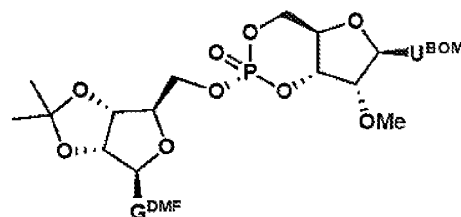
yield and/or diastereomeric ratio (dr) were as follows [yields and dr are reported for isolated materials; structure of major diastereomer shown]



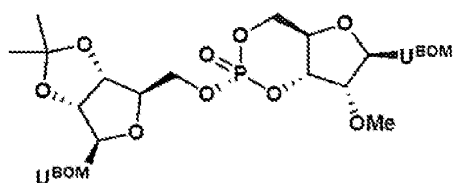
- 74 -



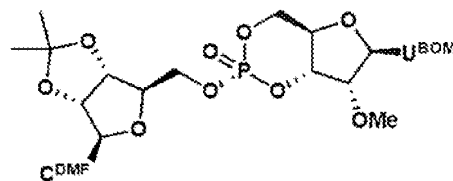
Ketal-DMFAUBOM
85% yield, dr 12:1
F1 dr 10:1, F2 dr 23:1



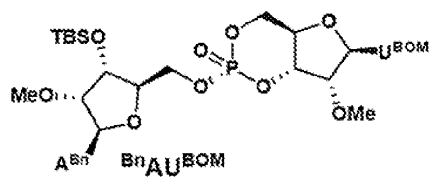
Ketal-DMFGUBOM
54% yield, dr 20:1
[MeCN:Tol (5:1)]



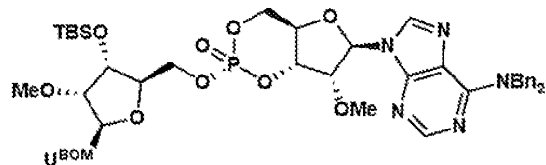
Ketal-BOMUUBOM
90% yield, dr 15:1



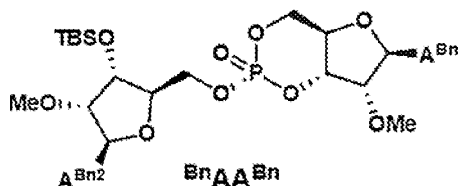
Ketal-DMFCUBOM
50% yield,
dr 14:1
[MeCN:Tol (5:1)]



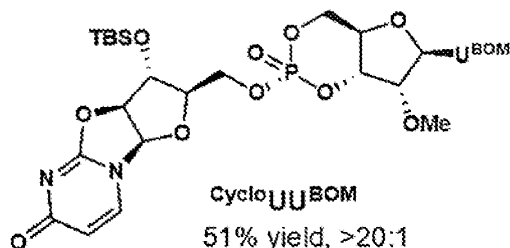
BOMUA^{Bn}BOM
87% yield, dr 22:1



BOMUA^{Bn}
84% yield, dr 30:1



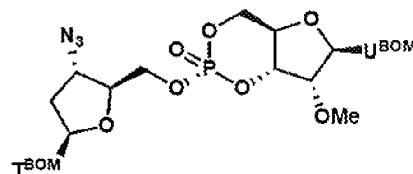
BnAA^{Bn}
86% yield, dr 14:1



CycloUUBOM
51% yield, >20:1

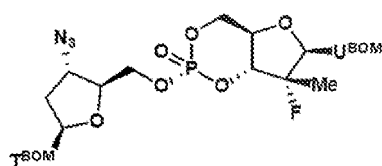


BOMTUUBOM
89% yield, dr >20:1,
65% yield, dr 34:1



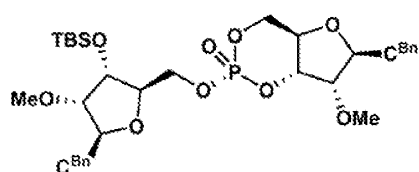
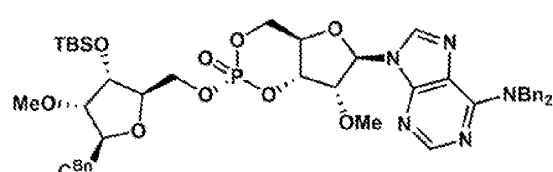
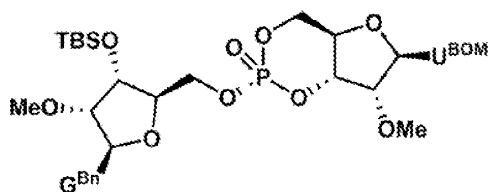
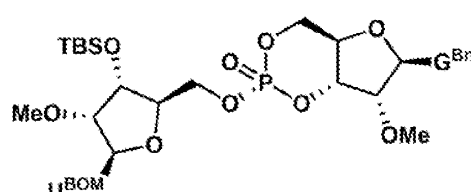
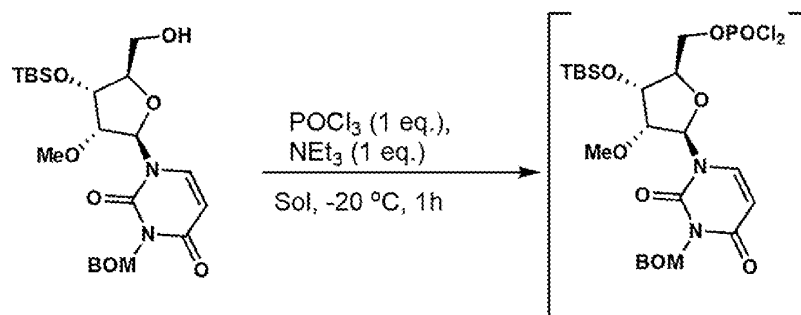
BOMTUUBOM
83% yield, dr >30:1,

- 75 -

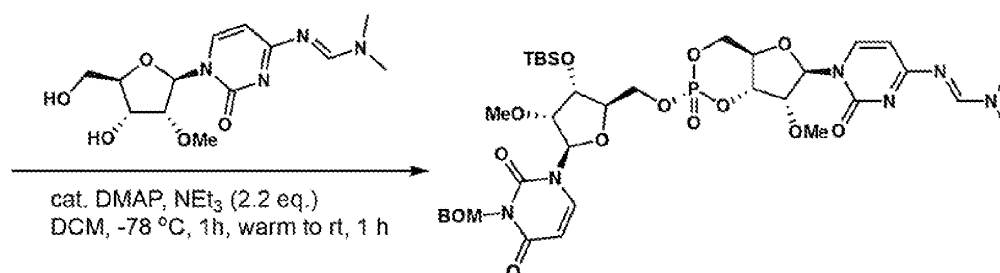
**BOMTU(F/Me)^{BOM}**

50% yield, dr 10:1,

32% yield, dr 1:10

**mCmC** , dr 3:1**mCmA** , dr 2:1**mGmU** , dr 3:1**mUmG** , dr 2.3:1e. One-pot synthesis – Method C (GP11)

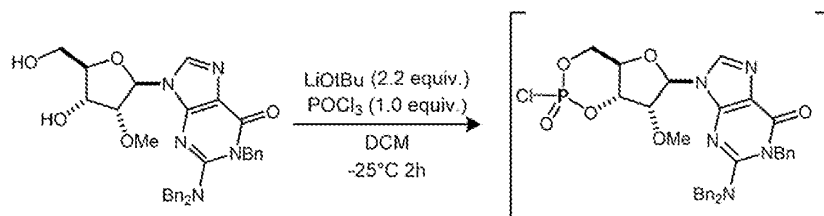
- 76 -



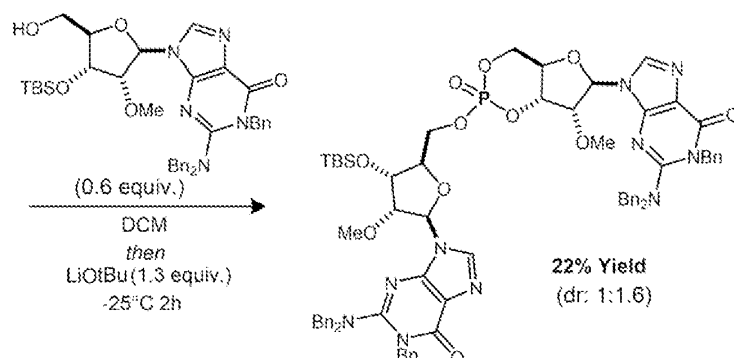
[0192] A solution of 5'-OH-nucleoside (1 equiv., 1 mmol) and triethylamine (1.1 equiv., 1.1 mmol) in dichloromethane (2.5 mL) was added to reaction tube A containing a solution of POCl₃ (1 equiv., 1 mmol) in DCM (2.5 mL), dropwise over a period of 25 minutes while stirring at -20 °C. The resulting solution was stirred for 30 minutes.

[0193] Another reaction tube B equipped with magnetic stir bar was charged with diol (1 equiv., 1 mmol) and DMAP (2.2 equiv., 2.2 mmol) in DCM (5 mL, 0.2M). The resulting suspension was cooled to -78 °C. The pre-stirred solution from reaction tube A was cannula transferred to reaction tube B. The resulting mixture was stirred for additional 1 hour at -78 °C and slowly warmed to room temperature while stirring for another 1 hour. The reaction was monitored by ³¹P NMR and upon completion, it was diluted with diethyl ether to precipitate the salts and filtered with frit filter funnel. The resulting filtrate was concentrated under reduced pressure. The crude product was purified via Combi-Flash NextGen 300 automated chromatography system with silica gel column with hexanes:acetone (0% to 100% gradient). Crude product was loaded on column by dissolving in minimum amount of DCM, height, ~3-inch, diameter, ~0.9-inch. Alternatively, DCM:Acetone can be used for more polar compounds. For adenosine containing cyclic dinucleotide, DCM:MeOH was used as an eluant (0% to 5%).

f. Guanosine dimer synthesis

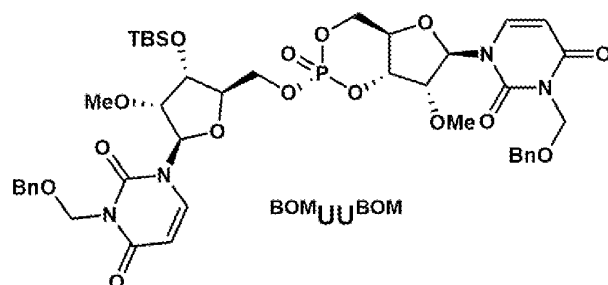


- 77 -



Example 6. Synthesis of specific cyclic dinucleotides

a. 3-((benzyloxy)methyl)-1-((2R,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyl dimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



[0194] The compound was synthesized by following general procedure GP10 using 3-((benzyloxy)methyl)-1-((2R,3R,4R,5R)-4-((tert-butyl dimethylsilyl)oxy)-5-(hydroxymethyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (3-OTBS-U-BOM, 400 mg, 0.81 mmol, 1 equiv.), LiOtBu (72 mg, 0.89 mmol, 1.1 equiv.), 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (U-BOM-Cl-CP, 410 mg, 0.89 mmol, 1.1 equiv.), and in Toluene:ACN (1:1). Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 100%), desired product was isolated as white solid in two fractions. Yield = F2, 66%, dr = 20:1, F1, 17%, 15:1. Overall yield = 83%.

- 78 -

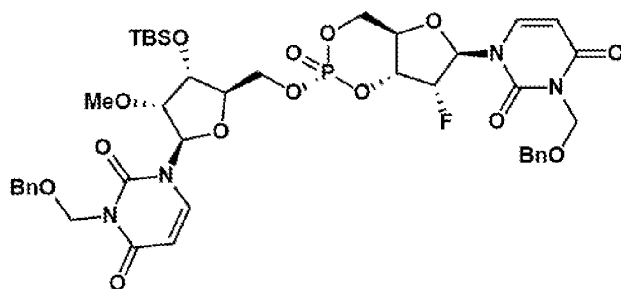
[0195] ^{31}P NMR (162 MHz, CDCl_3) δ -3.76.

[0196] ^1H NMR (400 MHz, CDCl_3) δ 7.61 (d, J = 8.2 Hz, 1H), 7.44 – 7.26 (m, 10H), 7.14 (d, J = 8.2 Hz, 1H), 5.91 (d, J = 2.0 Hz, 1H), 5.81 (t, J = 8.5 Hz, 2H), 5.60 – 5.43 (m, 5H), 4.80 (ddd, J = 10.0, 5.1, 1.3 Hz, 1H), 4.73 (s, 4H), 4.70 – 4.59 (m, 1H), 4.59 – 4.48 (m, 2H), 4.39 – 4.23 (m, 2H), 4.22 – 4.11 (m, 3H), 3.69 (dd, J = 4.2, 2.0 Hz, 1H), 3.59 (s, 3H), 3.59 (s, 3H), 0.94 (s, 9H), 0.14 (d, J = 2.3 Hz, 6H).

[0197] ^{13}C NMR (101 MHz, CDCl_3) δ 162.66, 162.15, 150.94, 150.38, 139.34, 138.23, 137.96, 137.93, 128.50, 128.47, 127.94, 127.88, 127.84, 127.73, 102.93, 102.10, 95.36, 89.13, 83.36, 81.44, 81.36, 80.31, 80.23, 78.07, 78.03, 77.36, 72.67, 72.44, 70.99, 70.93, 70.61, 70.41, 69.39, 69.31, 69.15, 66.51, 66.45, 59.45, 58.58, 25.77, 18.20, -4.53, -4.83.

[0198] HR-MS (Q-TOF, ESI) calculated for $[\text{C}_{42}\text{H}_{55}\text{N}_4\text{O}_{15}\text{PSi}] [\text{M}+\text{H}^+]$: 937.3063, found 937.3009.

b. 3-(((benzyloxy)methyl)-1-((2R,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-fluoro-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



[0199] The compound was synthesized by following general procedure GP10 using 3-OTBS-U-BOM (200 mg, 0.41 mmol, 1 equiv.), LiOtBu (34 mg, 0.43 mmol, 1.05 equiv.), F-U-BOM-Cl-CP (200 mg, 0.45 mmol, 1.1 equiv.), and in Toluene: ACN (1:1). Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 100%), desired product was isolated as white solid. Yield = 77%, dr = 5:1.

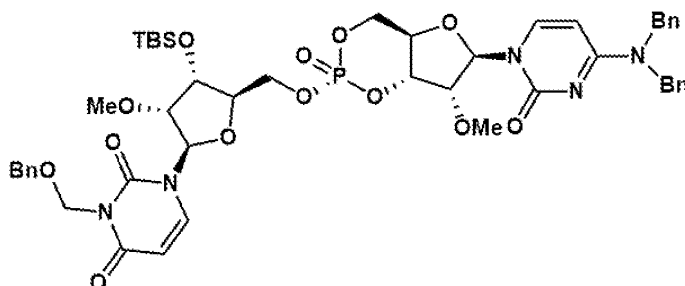
[0200] ^{31}P NMR (162 MHz, CDCl_3) δ -4.29, -6.86.

- 79 -

[0201] ^1H NMR (400 MHz, CDCl_3) δ 7.55 (d, $J = 8.2$ Hz, 1H), 7.40 – 7.27 (m, 12H), 7.08 (d, $J = 8.2$ Hz, 1H), 5.86 (d, $J = 2.1$ Hz, 1H), 5.82 – 5.72 (m, 3H), 5.53 – 5.33 (m, 8H), 4.70 (d, $J = 4.8$ Hz, 4H), 4.58 – 4.45 (m, 3H), 4.36 (ddd, $J = 11.6, 6.2, 2.3$ Hz, 2H), 4.28 – 4.10 (m, 4H), 3.70 (dd, $J = 4.5, 2.2$ Hz, 1H), 3.56 (s, 4H), 0.92 (d, $J = 1.7$ Hz, 10H), 0.12 (d, $J = 1.2$ Hz, 6H).

[0202] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{41}\text{H}_{52}\text{FN}_4\text{O}_{14}\text{PSi}$ [$\text{M} + \text{H}^+$]: 903.3044, found 903.3038.

c. 3-((benzyloxy)methyl)-1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((2R,4aR,6R,7R,7aR)-6-(4-(dibenzylamino)-2-oxopyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione



[0203] The compound was synthesized by following general procedure GP using 3-OTBS-U-BOM (200 mg, 0.41 mmol, 1 equiv.), LiOtBu (36 mg, 0.44 mmol, 1.1 equiv.), C-Bn2-Cl-CP (235 mg, 0.45 mmol, 1.12 equiv.), and in Toluene:ACN (1:1). Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM-EA (1% MeOH) gradient (0% to 100%), desired product was isolated as white solid. Yield = 250 mg, 63%, dr = 10:1.

[0204] ^{31}P NMR (162 MHz, CDCl_3) δ -3.55, -6.36.

[0205] ^1H NMR (400 MHz, CDCl_3) δ 7.60 (d, $J = 8.2$ Hz, 1H), 7.40 – 7.26 (m, 15H), 7.12 (d, $J = 7.3$ Hz, 2H), 5.90 (d, $J = 2.1$ Hz, 1H), 5.82 (dd, $J = 9.0, 8.0$ Hz, 2H), 5.75 (s, 1H), 5.54 – 5.40 (m, 2H), 5.03 (q, $J = 14.7$ Hz, 2H), 4.75 – 4.58 (m, 4H), 4.53 (s, 1H), 4.53 – 4.43 (m, 4H), 4.34 (ddd, $J = 11.3, 9.6, 5.5$ Hz, 2H), 4.25 – 4.11 (m, 3H), 3.69 – 3.61 (m, 4H), 3.61 – 3.51 (m, 4H), 0.91 (s, 10H), 0.11 (d, $J = 2.4$ Hz, 6H).

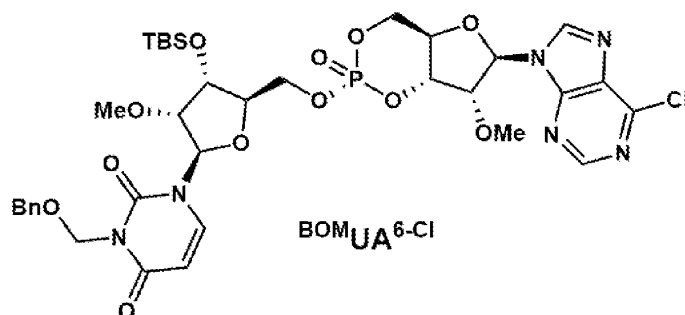
[0206] ^{13}C NMR (101 MHz, CDCl_3) δ 164.50, 164.36, 162.75, 162.67, 154.70, 150.98, 141.29, 138.20, 137.98, 136.76, 135.65, 129.25, 128.80, 128.61, 128.45, 128.00,

- 80 -

127.87, 127.84, 126.29, 102.14, 94.62, 92.69, 88.94, 83.37, 81.53, 81.45, 80.63, 80.56, 78.09, 78.05, 72.42, 70.95, 70.89, 70.40, 69.70, 69.62, 69.13, 66.43, 66.38, 59.29, 58.55, 58.53, 50.79, 50.49, 25.81, 25.77, 18.19, -4.45, -4.54, -4.73, -4.84.

[0207] HR-MS (Q-TOF, ESI) calculated for $C_{48}H_{60}N_5O_{13}PSi$, $[M+H]^+$: 974.3768, found 974.3761.

d. 3-(((benzyloxy)methyl)-1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((2R,4aR,6R,7R,7aR)-6-(6-chloro-9H-purin-9-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione



[0208] The compound was synthesized by following general procedure GP10 using 3-OTBS-U-BOM (400 mg, 0.81 mmol, 1 equiv.), LiOtBu (72 mg, 0.89 mmol, 1.1 equiv.), A(6Cl)-Cl-CP (371 mg, 0.97 mmol, 1.2 equiv.), and in Toluene:ACN (1:1). Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, Hex-EA gradient (0% to 100%), desired product was isolated as white solid in two fractions. Yield = 500 mg, 74%, dr = 15:1.

[0209] ^{31}P NMR (162 MHz, $CDCl_3$) δ -4.02, -6.82.

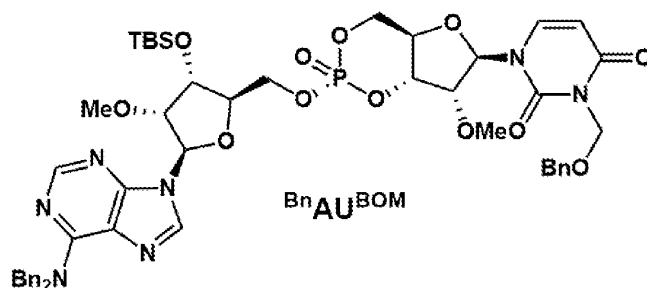
[0210] 1H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, J = 0.6 Hz, 1H), 8.17 (d, J = 1.4 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.39 – 7.27 (m, 5H), 6.02 (d, J = 0.9 Hz, 1H), 5.89 (d, J = 1.9 Hz, 1H), 5.82 (d, J = 8.2 Hz, 1H), 5.57 – 5.41 (m, 3H), 4.71 (s, 2H), 4.69 – 4.49 (m, 5H), 4.37 (ddd, J = 10.9, 9.5, 5.5 Hz, 2H), 4.21 – 4.13 (m, 2H), 3.72 – 3.66 (m, 1H), 3.59 (s, 4H), 3.57 (s, 3H), 0.92 (s, 9H), 0.13 (d, J = 1.3 Hz, 6H).

[0211] ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.65, 152.70, 152.22, 150.93, 150.79, 144.26, 138.22, 137.94, 132.72, 128.47, 127.88, 127.78, 102.11, 91.65, 89.25, 83.38, 81.41, 81.33, 80.57, 80.49, 78.39, 72.45, 71.37, 71.31, 70.41, 69.54, 69.46, 69.18, 66.54, 66.48, 59.87, 58.62, 25.78, 18.22, -4.51, -4.82.

- 81 -

[0212] HR-MS (Q-TOF, ESI) calculated for $C_{35}H_{46}ClN_6O_{12}PSi$, $[M+Na^+]$: 859.2261, found 859.2256.

e. 3-((benzyloxy)methyl)-1-((2R,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-3-((tert-butyl)dimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



[0213] The compound was synthesized by following general procedure GP10 using 3-OTBS-A-Bn (350 mg, 0.6 mmol, 1 equiv.), LiOtBu (59 mg, 0.73 mmol, 1.2 equiv.), U-BOM-Cl-CP (335 mg, 0.73 mmol, 1.2 equiv.), and Toluene:ACN (1:1, 7 mL). Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:MeOH gradient (0% to 5%), desired product was isolated as white solid. Yield = 530 mg, 87%, dr = 22:1.

[0214] ^{31}P NMR (162 MHz, $CDCl_3$) δ -3.99, -6.90.

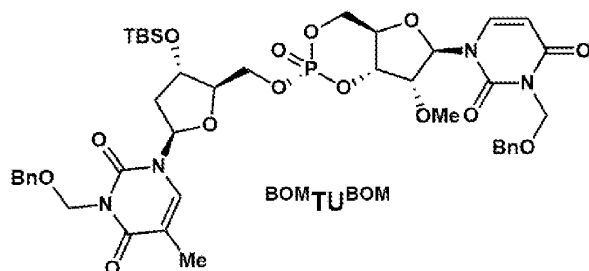
[0215] 1H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.95 (s, 1H), 7.36 – 7.21 (m, 17H), 6.92 (d, J = 8.1 Hz, 1H), 6.11 (d, J = 3.8 Hz, 1H), 5.64 (d, J = 8.1 Hz, 1H), 5.42 (d, J = 1.9 Hz, 3H), 4.96 (s, 2H), 4.74 – 4.63 (m, 4H), 4.55 – 4.37 (m, 3H), 4.36 – 4.16 (m, 4H), 4.04 (d, J = 5.0 Hz, 1H), 3.52 (s, 3H), 3.50 (s, 3H), 0.94 (s, 9H), 0.17 (d, J = 2.9 Hz, 6H).

[0216] ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.17, 155.13, 152.92, 150.85, 150.29, 139.56, 137.95, 137.64, 128.73, 128.50, 128.03, 127.93, 127.72, 127.49, 120.36, 102.68, 95.58, 87.27, 82.53, 82.23, 82.15, 80.19, 80.12, 78.22, 78.18, 72.63, 70.62, 70.56, 70.39, 69.16, 69.08, 66.89, 66.84, 59.41, 58.77, 25.89, 18.28, -4.55, -4.72.

[0217] HR-MS (Q-TOF, ESI) calculated for $C_{49}H_{60}N_7O_{12}PSi$, $[M+H^+]$: 998.388, found 998.3893.

- 82 -

f. 3-((benzyloxy)methyl)-1-((2R,4S,5R)-5-((((2R,4aR,6R,7R,7aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-4-((tert-butyltrimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione



[0218] The compound was synthesized by following general procedure GP10 using 3-OTBS-T-BOM (250 mg, 0.52 mmol, 1 equiv.), LiOtBu (51 mg, 0.63 mmol, 1.2 equiv.), U-BOM-Cl-CP (289 mg, 0.63 mmol, 1.2 equiv.), and Toluene:ACN (1:1, 6 mL). Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, Hex:EA gradient (0% to 100%), desired product was isolated as white solid. Yield = 420 mg, 89%, dr = 22:1.

[0219] ^{31}P NMR (162 MHz, CDCl_3) δ -3.60, -6.41.

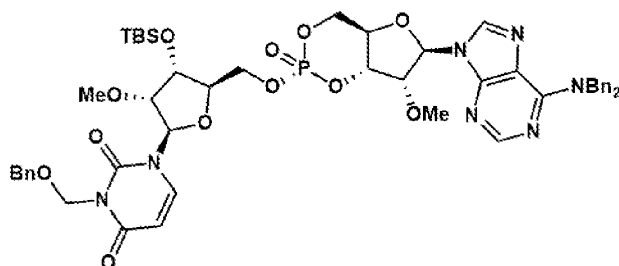
[0220] ^1H NMR (400 MHz, CDCl_3) δ 7.41 – 7.31 (m, 10H), 7.29 (d, J = 7.1 Hz, 3H), 7.13 (d, J = 8.2 Hz, 1H), 6.34 (t, J = 6.7 Hz, 1H), 5.80 (dd, J = 8.1, 0.7 Hz, 1H), 5.57 – 5.42 (m, 6H), 4.82 – 4.70 (m, 6H), 4.65 (ddd, J = 17.7, 9.7, 5.3 Hz, 2H), 4.58 – 4.38 (m, 4H), 4.38 – 4.23 (m, 3H), 4.16 – 4.03 (m, 2H), 3.58 (d, J = 0.7 Hz, 3H), 2.34 (ddd, J = 13.6, 6.1, 3.4 Hz, 1H), 2.16 – 2.04 (m, 2H), 2.01 – 1.96 (m, 3H), 0.92 (s, 9H), 0.12 (d, J = 1.7 Hz, 6H).

[0221] ^{13}C NMR (126 MHz, CDCl_3) δ 163.50, 163.43, 162.23, 151.17, 151.15, 151.09, 151.07, 150.44, 150.39, 139.53, 139.40, 138.04, 137.99, 137.89, 137.86, 135.54, 135.25, 133.72, 128.50, 128.44, 128.42, 127.95, 127.84, 127.81, 127.72, 110.88, 110.70, 110.46, 102.85, 95.31, 95.23, 87.18, 87.15, 86.99, 85.74, 85.69, 85.66, 85.64, 85.59, 85.54, 85.37, 80.64, 80.60, 80.22, 80.21, 80.16, 80.15, 79.75, 79.71, 78.10, 78.07, 78.04, 72.65, 72.41, 72.40, 72.38, 71.48, 70.71, 70.69, 70.66, 70.59, 70.53, 69.57, 69.51, 69.44, 69.38, 63.65, 63.38, 62.47, 62.17, 62.09, 59.67, 59.40, 59.38, 26.04, 26.03, 25.89, 25.82, 25.77, 13.40, 13.39, 13.34, -2.83, -3.46, -5.28, -5.37.

- 83 -

[0222] HR-MS (Q-TOF, ESI) calculated for $C_{42}H_{55}N_4O_{14}PSi$, $[M+H]^+$: 899.3295, found 899.3288.

g. 3-((benzyloxy)methyl)-1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((2R,4aR,6R,7R,7aR)-6-(6-(dibenzylamino)-9H-purin-9-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione



[0223] The compound was synthesized by following general procedure GP10 using 3-OTBS-U-BOM (400 mg, 0.81 mmol, 1 equiv.), LiOtBu (78 mg, 0.97 mmol, 1.2 equiv.), A-Bn2-Cl-CP (528 mg, 0.97 mmol, 1.2 equiv.), and Toluene:ACN (1:1, 8 mL). Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, Hex:EA gradient (0% to 100%), desired product was isolated as white solid. Yield = 680 mg, 87%, dr = 30:1.

[0224] 1H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.73 (s, 1H), 7.62 (d, J = 8.2 Hz, 1H), 7.37 (d, J = 7.1 Hz, 2H), 7.34 – 7.20 (m, 16H), 5.94 – 5.88 (m, 2H), 5.84 (d, J = 8.2 Hz, 1H), 5.67 (ddd, J = 10.0, 5.2, 1.1 Hz, 1H), 5.48 (q, J = 9.7 Hz, 3H), 4.97 (s, 2H), 4.70 (s, 2H), 4.67 – 4.57 (m, 2H), 4.53 (dt, J = 9.9, 6.3, 3.5 Hz, 2H), 4.38 – 4.28 (m, 2H), 4.18 (qd, J = 7.5, 3.8 Hz, 2H), 3.66 (dd, J = 4.7, 2.1 Hz, 1H), 3.57 (s, 3H), 3.56 (s, 3H), 0.91 (s, 10H), 0.12 (s, 6H).

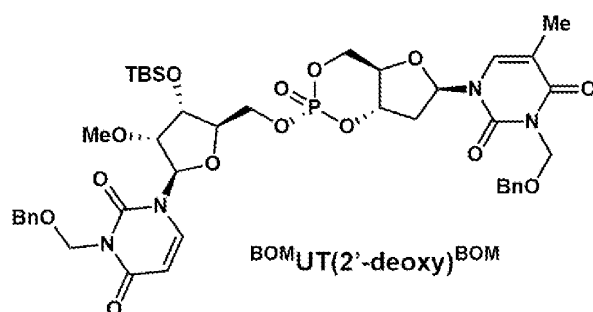
[0225] ^{31}P NMR (203 MHz, $CDCl_3$) δ -4.06, -6.54.

[0226] ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.57, 155.06, 153.17, 150.87, 150.28, 138.06, 137.86, 137.56, 128.65, 128.35, 127.75, 127.73, 127.38, 120.32, 102.05, 91.52, 88.88, 83.32, 81.41, 81.34, 80.45, 80.39, 78.48, 78.45, 77.24, 72.32, 71.06, 71.01, 70.29, 69.73, 69.66, 69.05, 66.21, 66.16, 59.57, 58.49, 25.72, 25.67, 18.10, -4.65, -4.91.

[0227] HR-MS (Q-TOF, ESI) calculated for $C_{49}H_{60}N_7O_{12}PSi$, $[M+Na]^+$: 998.388, found 998.3887.

- 84 -

h. 3-((benzyloxy)methyl)-1-((2R,4aR,6R,7aS)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methoxy)-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-5-methylpyrimidine-2,4(1H,3H)-dione



[0228] The compound was synthesized by following the general procedure GP10 using 3-OTBS-U-BOM (270 mg, 0.61 mmol, 1 equiv.), LiOtBu (54 mg, 0.67 mmol, 1.1 equiv.), T-BOM-Cl-CP (330 mg, 0.67 mmol, 1.1 equiv.), and Toluene:ACN (1:1, 6 mL). Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, MeOH:DCM gradient (0% to 5%), desired product was isolated as white solid. Yield = 500 mg, 91%, dr = 5:1.

[0229] ¹H NMR (500 MHz, CDCl₃) δ 7.61 – 7.55 (m, 1H), 7.43 – 7.29 (m, 10H), 6.92 (d, *J* = 1.4 Hz, 1H), 6.28 (dd, *J* = 8.7, 2.7 Hz, 1H), 4.99 – 4.82 (m, 1H), 4.73 (d, *J* = 7.2 Hz, 4H), 4.67 – 4.47 (m, 4H), 4.40 – 4.29 (m, 1H), 4.22 – 4.11 (m, 2H), 4.00 – 3.84 (m, 1H), 3.73 (q, *J* = 1.9 Hz, 1H), 3.59 (s, 3H), 2.68 – 2.40 (m, 2H), 2.04 – 1.88 (m, 4H), 0.94 (s, 12H), 0.14 (d, *J* = 5.4 Hz, 8H).

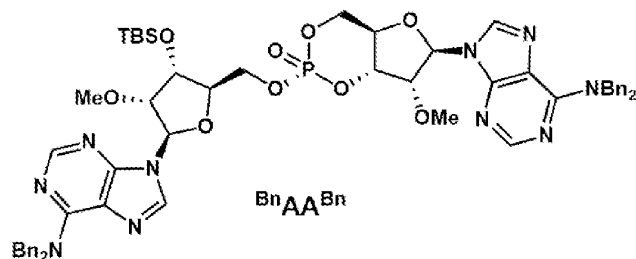
[0230] ³¹P NMR (162 MHz, CDCl₃) δ -3.64, -6.68.

[0231] ¹³C NMR (126 MHz, CDCl₃) δ 163.02, 162.61, 150.89, 150.67, 138.42, 138.01, 137.95, 133.83, 128.48, 128.45, 127.90, 127.86, 127.81, 127.77, 111.85, 102.01, 89.68, 86.08, 83.23, 81.41, 81.34, 77.36, 77.33, 77.30, 74.20, 74.15, 72.53, 72.46, 70.85, 70.40, 69.58, 69.52, 69.28, 66.84, 66.79, 58.61, 35.57, 35.50, 25.84, 25.78, 18.22, 13.42, -4.46, -4.80.

[0232] HR-MS (Q-TOF, ESI) calculated for Chemical Formula: C₄₂H₅₅N₄O₁₄PSi, [M+Na⁺]: 921.3114, found 921.3110.

- 85 -

i. AA-Bn2-dimer -- (2R,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-3-((tert-butyltrimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methoxy)-6-(6-(dibenzylamino)-9H-purin-9-yl)-7-methoxytetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinine 2-oxide



[0233] The compound was synthesized by following general procedure GP10 using 3-OTBS-A-Bn2 (400 mg, 0.69 mmol, 1 equiv.), LiOtBu (67 mg, 0.83 mmol, 1.2 equiv.), ABn2-Cl-CP (452 mg, 0.83 mmol, 1.2 equiv.), and Toluene:ACN (1:1, 7 mL). Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, MeOH:DCM gradient (0% to 5%), desired product was isolated as white solid. Yield = 650 mg, 86%, dr = 14:1.

[0234] ^{31}P NMR (162 MHz, CDCl_3) δ -4.02, -6.62.

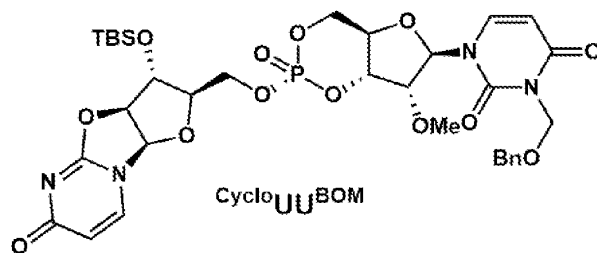
[0235] ^1H NMR (400 MHz, CDCl_3) δ 8.46 (s, 1H), 8.41 (s, 1H), 8.00 (s, 1H), 7.60 (s, 1H), 7.38 – 7.20 (m, 25H), 6.15 (d, J = 3.9 Hz, 1H), 5.87 (s, 1H), 5.61 (dd, J = 9.5, 5.1 Hz, 2H), 5.50 (s, 4H), 5.01 (s, 5H), 4.78 (t, J = 5.3 Hz, 1H), 4.62 – 4.24 (m, 10H), 3.56 (s, 3H), 3.55 (s, 3H), 1.00 (s, 9H), 0.22 (d, J = 1.3 Hz, 6H).

[0236] ^{13}C NMR (101 MHz, CDCl_3) δ 155.17, 155.14, 153.19, 152.92, 150.84, 150.37, 137.69, 137.66, 128.74, 128.69, 128.65, 127.96, 127.47, 127.42, 120.42, 120.39, 91.55, 87.33, 82.51, 82.31, 82.23, 80.56, 80.48, 78.65, 78.61, 77.36, 70.85, 70.77, 70.42, 69.61, 69.53, 66.77, 66.72, 59.57, 58.78, 51.04, 49.43, 25.90, 18.29, -4.55, -4.70.

[0237] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{56}\text{H}_{65}\text{N}_{10}\text{O}_9\text{PSi}$, $[\text{M}+\text{Na}^+]$: 1103.4335, found 1103.433.

- 86 -

j. 3-((benzyloxy)methyl)-1-(((2R,4aR,6R,7R,7aR)-2-(((2R,3R,3aS,9aR)-3-((tert-butyl)dimethylsilyl)oxy)-6-oxo-2,3,3a,9a-tetrahydro-6H-furo[2',3':4,5]oxazolo[3,2-a]pyrimidin-2-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



[0238] The compound was synthesized by following general procedure GP10 using 5'-OH-Cyclo-U (200 mg, 0.59 mmol, 1 equiv.), LiOtBu (52 mg, 0.65 mmol, 1.1 equiv.), U-BOM-Cl-CP (296 mg, 0.65 mmol, 1.1 equiv.), and Toluene:ACN (1:1, 9 mL). Column conditions: Reverse phase C-18 column, Next-Gen automated system, wet loading by dissolving in minimum amount of DMSO, Water:ACN gradient (10% to 100%), desired product was isolated as white solid. Yield = 230 mg, 51%, dr >99:1.

[0239] ^{31}P NMR (203 MHz, CD_3CN) δ -5.19.

[0240] ^{31}P NMR (203 MHz, CDCl_3) δ -4.64.

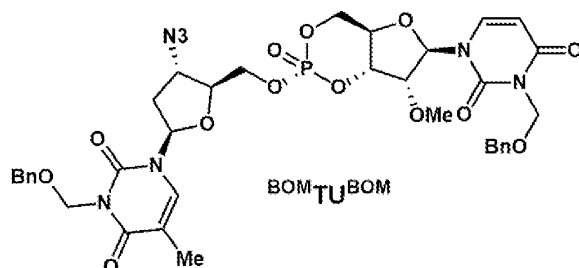
[0241] ^1H NMR (500 MHz, CD_3CN) δ 7.54 (d, J = 7.4 Hz, 1H), 7.41 – 7.25 (m, 6H), 6.23 (d, J = 5.8 Hz, 1H), 5.90 (d, J = 7.5 Hz, 1H), 5.71 (d, J = 8.1 Hz, 2H), 5.37 (s, 2H), 5.20 (dd, J = 5.8, 1.6 Hz, 1H), 4.64 (s, 2H), 4.63 – 4.58 (m, 1H), 4.57 (ddd, J = 3.6, 1.6, 0.6 Hz, 1H), 4.39 (dt, J = 10.3, 9.1 Hz, 1H), 4.29 (dtd, J = 5.4, 3.7, 1.7 Hz, 1H), 4.23 (td, J = 10.3, 5.7 Hz, 1H), 4.15 – 4.02 (m, 2H), 3.98 (ddd, J = 11.8, 6.8, 5.3 Hz, 1H), 3.52 (s, 3H), 2.13 (s, 4H), 0.93 (s, 8H), 0.20 (s, 3H), 0.17 (s, 3H).

[0242] ^{13}C NMR (126 MHz, CD_3CN) δ 172.57, 163.53, 161.05, 151.93, 141.24, 139.63, 136.85, 129.46, 128.74, 128.70, 110.64, 102.86, 94.76, 90.99, 90.10, 86.94, 86.87, 81.21, 81.15, 79.10, 79.06, 77.56, 72.77, 71.47, 71.26, 71.19, 70.67, 70.61, 67.64, 67.59, 59.60, 26.14, 18.74, -4.56, -4.65.

[0243] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{33}\text{H}_{43}\text{N}_4\text{O}_{13}\text{PSi}$, $[\text{M}+\text{Na}^+]$: 785.2226, found 785.2221.

- 87 -

k. 1-((2R,4S,5S)-4-azido-5-((((2R,4aR,6R,7R,7aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)tetrahydrofuran-2-yl)-3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione



[0244] The compound was synthesized by following general procedure GP10 using 2'-N₃ (300 mg, 0.77 mmol, 1 equiv.), LiOtBu (74 mg, 0.92 mmol, 1.2 equiv.), U-BOM-Cl-CP (426 mg, 0.93 mmol, 1.2 equiv.), and Toluene:ACN (1:1, 8 mL). Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, hexanes: ethyl acetate gradient (0% to 100%), desired product eluted at 100% ethyl acetate and was isolated as white solid. Yield = 520 mg, 83%, dr = 30:1.

[0245] ³¹P NMR (203 MHz, CDCl₃) δ -3.76, -6.49.

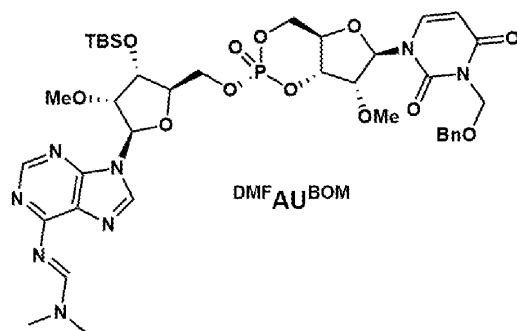
[0246] ¹H NMR (500 MHz, CDCl₃) δ 7.40 – 7.23 (m, 11H), 7.11 (d, *J* = 8.2 Hz, 1H), 6.18 (t, *J* = 6.4 Hz, 1H), 5.78 (d, *J* = 8.1 Hz, 1H), 5.50 (d, *J* = 13.7 Hz, 3H), 5.46 (d, *J* = 1.8 Hz, 2H), 4.78 (ddd, *J* = 10.1, 5.1, 1.3 Hz, 1H), 4.70 (d, *J* = 2.3 Hz, 4H), 4.67 – 4.58 (m, 1H), 4.54 – 4.43 (m, 2H), 4.43 – 4.31 (m, 2H), 4.27 (td, *J* = 10.3, 5.4 Hz, 1H), 4.11 (t, *J* = 6.2 Hz, 1H), 4.05 (dq, *J* = 5.5, 2.8 Hz, 1H), 3.56 (s, 3H), 2.49 (ddd, *J* = 14.0, 6.5, 4.8 Hz, 1H), 2.33 (ddd, *J* = 13.9, 7.5, 6.3 Hz, 1H), 1.96 (s, 3H).

[0247] ¹³C NMR (126 MHz, CDCl₃) δ 163.41, 162.13, 150.91, 150.37, 139.35, 138.04, 137.90, 134.06, 128.51, 128.45, 128.03, 127.95, 127.87, 127.83, 127.79, 127.73, 127.71, 127.70, 127.68, 110.84, 102.95, 95.33, 85.98, 82.28, 82.23, 80.26, 80.20, 78.13, 78.09, 77.41, 77.16, 76.91, 72.66, 72.64, 72.43, 70.84, 70.79, 70.69, 70.60, 69.50, 69.44, 67.86, 67.81, 60.09, 59.43, 37.73, 13.32.

[0248] HR-MS (Q-TOF, ESI) calculated for C₃₆H₄₀N₇O₁₃P, [M+H⁺]: 810.2494, found 810.2495.

- 88 -

1. (E)-N'-(9-(((2R,3R,4R,5R)-5-((((2R,4aR,6R,7R,7aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-4-((tert-butyl dimethylsilyl)oxy)-3-methoxytetrahydrofuran-2-yl)-9H-purin-6-yl)-N,N-dimethylformimidamide



[0249] The compound was synthesized by following general procedure GP10 using 3-OTBS-DMF-A-Bn2 (275 mg, 0.61 mmol, 1 equiv.), LiOtBu (54 mg, 0.67 mmol, 1.1 equiv.), U-BOM-Cl-CP (308 mg, 0.67 mmol, 1.1 equiv.), and in Toluene:ACN (1:1). Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM-MeOH gradient (0% to 5%), desired product was isolated as white solid. Yield = 460 mg, 86%, dr = 15:1. Mass was confirmed via LC-MS.

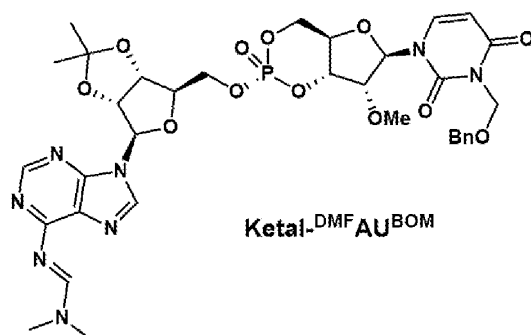
[0250] ^{31}P NMR (162 MHz, CDCl_3) δ -3.87, -6.91.

[0251] ^1H NMR (400 MHz, CDCl_3) δ 8.98 (s, 1H), 8.55 (d, J = 1.1 Hz, 1H), 8.05 (s, 1H), 7.38 – 7.22 (m, 7H), 6.05 (d, J = 3.4 Hz, 1H), 5.73 (dd, J = 8.2, 1.1 Hz, 1H), 5.48 – 5.39 (m, 2H), 5.37 (s, 1H), 4.79 (t, J = 5.6 Hz, 1H), 4.74 – 4.63 (m, 3H), 4.55 – 4.44 (m, 2H), 4.41 – 4.18 (m, 4H), 4.06 (d, J = 5.1 Hz, 1H), 3.97 (td, J = 10.2, 5.8 Hz, 1H), 3.49 (dd, J = 5.5, 1.1 Hz, 6H), 3.21 (dd, J = 17.4, 1.2 Hz, 6H), 0.95 (d, J = 1.1 Hz, 9H), 0.18 (d, J = 5.6 Hz, 6H).

[0252] ^{13}C NMR (101 MHz, CDCl_3) δ 162.32, 160.03, 158.62, 153.08, 151.27, 150.33, 141.00, 140.56, 137.90, 128.49, 128.44, 127.93, 127.74, 126.62, 102.54, 95.87, 87.59, 82.39, 81.83, 81.75, 79.90, 79.83, 78.29, 78.25, 77.36, 72.61, 70.52, 70.35, 70.32, 70.27, 69.06, 68.98, 66.41, 66.35, 59.39, 58.87, 41.46, 35.23, 25.88, 18.25, -4.57, -4.81.

- 89 -

m. (E)-N'-((9-((3aR,4R,6R,6aR)-6-((((2R,4aR,6R,7R,7aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purin-6-yl)-N,N-dimethylformimidamide



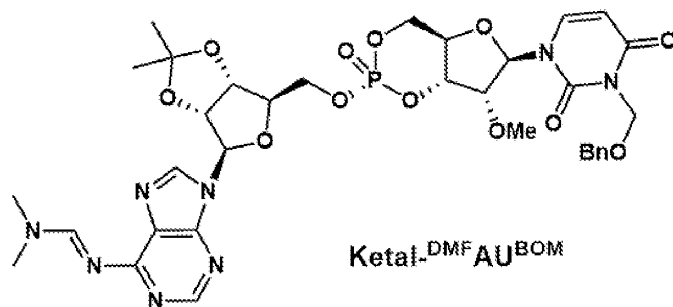
[0253] The compound was synthesized by following general procedure GP10 using KETAL-DMF-A-5'OH (215 mg, 0.59 mmol, 1 equiv.), LiOtBu (52 mg, 0.65 mmol, 1.1 equiv.), U-BOM-Cl-CP (299 mg, 0.65 mmol, 1.1 equiv.), and in Toluene:ACN (1:1). Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:MeOH gradient (0% to 10%), desired product was isolated as white solid. Yield = 82%, dr = 23:1. Mass was confirmed via LC-MS.

[0254] ³¹P NMR (162 MHz, CDCl₃) δ -4.24, -6.93.

[0255] ¹H NMR (400 MHz, CDCl₃) δ 9.02 – 8.99 (m, 1H), 8.60 (s, 1H), 8.04 (s, 1H), 7.39 – 7.29 (m, 5H), 7.19 (d, *J* = 8.2 Hz, 1H), 6.18 (d, *J* = 2.3 Hz, 1H), 5.77 (d, *J* = 8.1 Hz, 1H), 5.55 – 5.48 (m, 1H), 5.46 (d, *J* = 1.3 Hz, 2H), 5.38 (d, *J* = 1.0 Hz, 1H), 5.14 (dd, *J* = 6.2, 3.0 Hz, 1H), 4.71 (s, 2H), 4.59 – 4.27 (m, 5H), 4.04 (d, *J* = 5.1 Hz, 1H), 3.94 (td, *J* = 10.3, 5.6 Hz, 1H), 3.51 (s, 3H), 3.27 (d, *J* = 0.6 Hz, 3H), 3.22 (s, 3H), 1.65 (s, 3H), 1.43 (s, 3H).

- 90 -

n. (E)-N'-(1-((3aR,4R,6R,6aR)-6-((((2R,4aR,6R,7R,7aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-N,N-dimethylformimidamide



[0256] The compound was synthesized by following general procedure GP10 with modification using dmfc-5'-OH (250 mg, 0.74 mmol, 1 equiv.), LiOtBu (65 mg, 0.81 mmol, 1.1 equiv.), 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (U-BOM-Cl-CP, 373 mg, 0.81 mmol, 1.1 equiv.), and in Toluene:ACN (1:5). Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:MeOH gradient (0% to 10%), desired product was isolated as pale-white solid. Yield = 280 mg, 50%, dr = 14:1. Mass was confirmed via LC-MS.

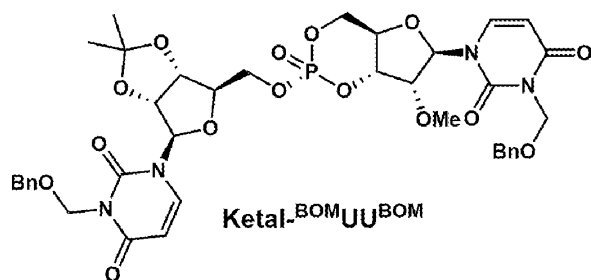
[0257] ³¹P NMR (162 MHz, CDCl₃) δ -3.94, -6.65.

[0258] ¹H NMR (400 MHz, CDCl₃) δ 8.75 (s, 1H), 7.75 (s, 1H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.39 – 7.21 (m, 8H), 6.08 (d, *J* = 7.2 Hz, 1H), 5.80 (d, *J* = 8.2 Hz, 1H), 5.69 (s, 1H), 5.58 (d, *J* = 1.6 Hz, 1H), 5.53 – 5.41 (m, 2H), 5.17 (dd, *J* = 6.3, 1.6 Hz, 1H), 4.95 (dd, *J* = 6.5, 3.1 Hz, 1H), 4.69 (s, 3H), 4.64 – 4.39 (m, 5H), 4.26 (td, *J* = 10.2, 5.6 Hz, 1H), 4.00 (d, *J* = 5.1 Hz, 1H), 3.55 (s, 3H), 3.33 (d, *J* = 13.6 Hz, 7H), 3.18 (s, 3H), 3.15 – 3.08 (m, 3H), 2.84 (s, 1H), 1.56 (s, 3H), 1.35 (s, 3H).

[0259] ¹³C NMR (101 MHz, CDCl₃) δ 172.40, 162.37, 159.02, 157.08, 156.12, 150.55, 144.57, 139.32, 137.96, 128.49, 127.91, 127.82, 127.77, 114.15, 103.19, 102.88, 98.00, 94.07, 87.01, 86.95, 85.05, 81.72, 80.48, 80.40, 77.98, 77.94, 77.36, 72.56, 70.73, 70.67, 70.59, 69.48, 69.40, 69.31, 69.25, 59.35, 46.60, 41.84, 39.27, 35.70, 35.56, 27.16, 25.33.

- 91 -

o. 3-((benzyloxy)methyl)-1-((2R,4aR,6R,7R,7aR)-2-(((3aR,4R,6R,6aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



[0260] The compound was synthesized by following general procedure GP10 using Ketal-U-BOM (300 mg, 0.74 mmol, 1 equiv.), LiOtBu (65 mg, 0.82 mmol, 1.1 equiv.), U-BOM-Cl-CP (374 mg, 0.82 mmol, 1.1 equiv.), and in Toluene:ACN (1:1, 8 mL). Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:MeOH gradient (0% to 5%), desired product was isolated as white solid. Yield = 550 mg, 90%, dr = 15:1.

[0261] ³¹P NMR (203 MHz, CDCl₃) δ -3.92, -6.77.

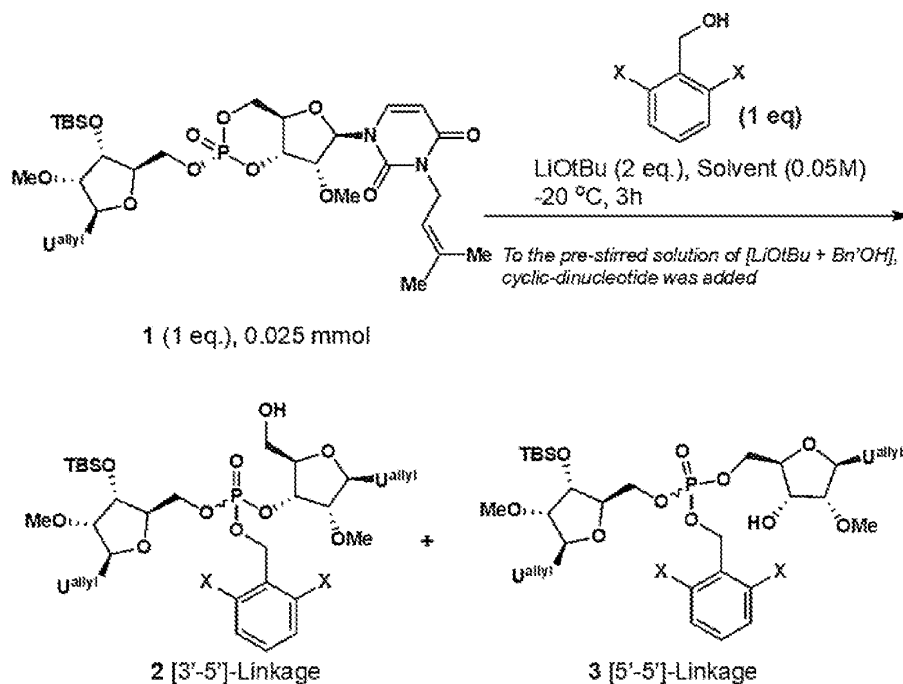
[0262] ¹H NMR (500 MHz, CDCl₃) δ 7.43 – 7.29 (m, 10H), 7.10 (d, *J* = 8.1 Hz, 1H), 5.83 – 5.75 (m, 2H), 5.72 (d, *J* = 2.2 Hz, 1H), 5.59 – 5.43 (m, 5H), 4.92 (ddd, *J* = 23.6, 6.5, 2.5 Hz, 2H), 4.78 – 4.67 (m, 5H), 4.50 – 4.36 (m, 4H), 4.35 – 4.21 (m, 1H), 4.10 (d, *J* = 5.1 Hz, 1H), 3.58 (s, 3H), 1.61 (s, 3H), 1.39 (s, 3H).

[0263] ¹³C NMR (126 MHz, CDCl₃) δ 162.48, 162.05, 150.82, 150.25, 140.40, 139.25, 137.84, 137.81, 128.38, 128.36, 127.82, 127.77, 127.67, 127.66, 127.61, 114.63, 102.76, 102.26, 102.20, 95.24, 95.20, 85.49, 85.43, 84.60, 80.61, 80.14, 80.08, 78.00, 77.96, 77.23, 72.53, 72.43, 70.64, 70.59, 70.47, 70.45, 69.21, 69.15, 68.44, 68.40, 59.39, 27.13, 25.29.

[0264] HR-MS (Q-TOF, ESI) calculated for C₃₈H₄₃N₄O₁₅P, [M+Na⁺]:849.2355, found 849.236.

Example 7. Regioselective Ring-Opening of Cyclic Phosphate of Dinucleosides

a. General procedure (GP12) for regioselective ring opening reaction of cyclic dinucleotides



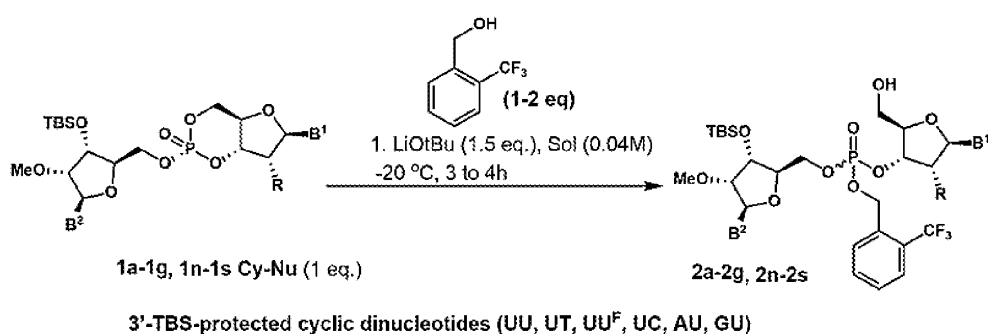
[0265] In a nitrogen-filled glovebox, an oven-dried reaction tube containing a Teflon-coated magnetic stir bar was charged with LiOtBu (0.05 mmol, 1.5 - 2 equiv.), a substituted benzyl alcohol nucleophile (BnOH) (0.025 mmol, 1.0 equiv.) and 0.25 mL of solvent, and then taken outside the glovebox. The mixture was left stirring for 20 minutes at RT and cooled to -20 °C over a period of 10 minutes. Then, a solution of cyclic dinucleotide (**1**) (0.025 mmol, 1 equiv.) in solvent (0.25 mL) was added in one portion at -20 °C and left stirring at that temperature for 3-4 hours. The reaction was quenched with a solution of acetic acid (0.05 mmol, 2.0 equiv.) in MeCN (0.5 mL) at -20 °C. Upon stirring for 5 minutes, a solution of internal standard, PO(OPh)₃ (0.025 mmol, 1.0 equiv.) in MeCN (0.5 mL) was added and NMR yields and regioselectivities were recorded. Results for various BnOH and solvents are shown in Table 3:

Table 3 - Ring-Opening of Cyclic Phosphate of Dinucleosides

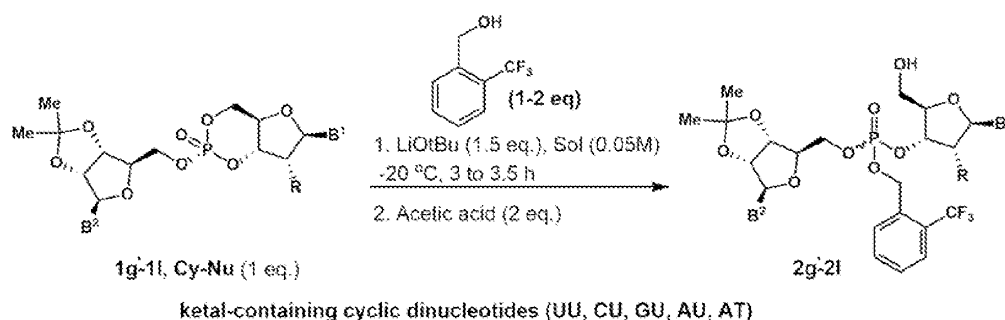
Entry	Alcohol	dr of 1	Solvent	Yield	2-[3-5]	3-[5-5]	S
1	2-Cl-BnOH	2:1	MeCN	97%	70%	27%	2.6
2	2-Ph-BnOH	2:1	MeCN	81%	62%	19%	3.3
3	2,6-Cl ₂ -BnOH	2:1	MeCN	99%	70%	29%	2.4
4	2,4,6-Me ₃ -BnOH	2:1	MeCN	97%	64%	33%	3.3
5	2-MeO-BnOH	4:1	MeCN	99%	70%	29%	2.4
6	2-Cl-BnOH	4:1	MeCN	89%	70%	19%	3.7
7	2-CF ₃ -BnOH	4:1	MeCN	92%	74%	18%	4.1
8	2,4-Cl ₂ -BnOH	4:1	MeCN	85%	71%	14%	5.1
9	2-Cl-BnOH	4:1	MTBE	86%	72%	14%	5.1
10	2-Cl-BnOH	4:1	Toluene	90%	75%	15%	5.0
11	2-CF ₃ -BnOH	4:1	Toluene	89%	71%	18%	4.0
12	2-Cl-BnOH	5:1	Toluene	88%	74%	14%	5.3
13	2-CF ₃ -BnOH	5:1	Toluene	86%	75%	11%	6.9
14	2,6-F ₂ -BnOH	5:1	Toluene	93%	74%	19%	3.9
15	1-NaphthylCH ₂ OH	5:1	Toluene	89%	76%	13%	5.9
16*	2-CF ₃ -BnOH	20:1	Toluene	92%	86%	6%	14

(* - BOM as protecting group on *N*-uridine; S denotes the ratio of [3'-5']-linkage over [5'-5']-linkage; yields and selectivities are determined via ³¹P-NMR)

b. General procedure (GP13) for regioselective ring opening reaction of cyclic dinucleotides

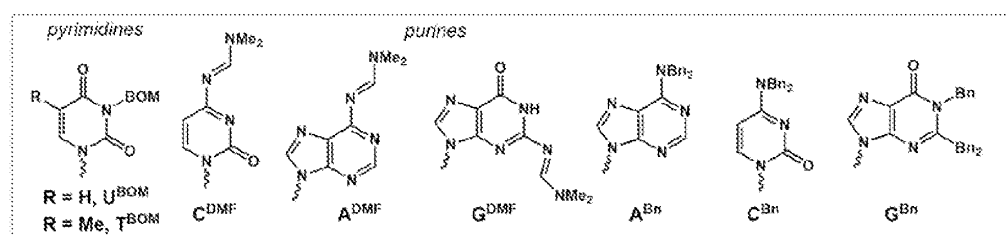


- 94 -



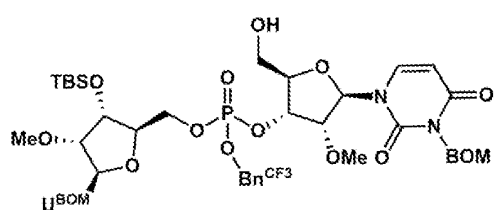
Unless otherwise noted, reaction condition: 1.5 eq LiOtBu, 1 eq. CF₃BnOH, 1 eq (0.025mmol) 1-Cy-Nu at -20 °C, 3h. S denotes the ratio of [3'-5']-linkage (**2**) over [5'-5']-linkage (**3**).

Conv., yields and selectivities are determined via ³¹P-NMR by using (PhO)₃PO as an internal standard

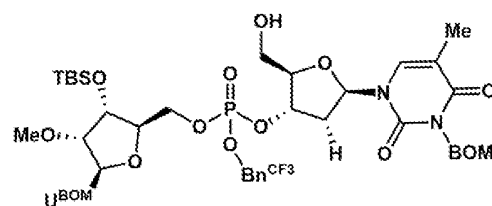


[0266] In a nitrogen-filled glovebox, an oven-dried reaction tube containing a Teflon-coated magnetic stir bar was charged with LiOtBu (0.037 mmol, 1.5 equiv.), 2-CF₃BnOH (as the substituted benzyl alcohol nucleophile, BnOH) (0.05 mmol, 2.0 equiv., 0.25 mL of stock solution in anhydrous toluene (0.2M)) and taken outside the glovebox. The mixture was left stirring for 20 minutes at room temperature and cooled to -20 to -25 °C over a period of 10 minutes. Then, a solution of protected cyclic dinucleotide (0.025 mmol, 1 equiv.) in toluene (0.35 mL) was added in one portion at -20 to -25 °C and left stirring at that temperature for 3-4 hours. The reaction was quenched with a solution of acetic acid (0.05 mmol, 2.0 equiv.) in MeCN(0.5 mL) at -20 °C. Upon stirring for 5 minutes, a solution of internal standard, PO(OPh)₃ (0.025 mmol, 1.0 equiv.) in MeCN(0.5 mL) was added and NMR yields were recorded. Resulting dinucleotides with their yield and diastereomeric ratio (dr) were as follows (S denotes the ratio of [3'-5']-linkage over [5'-5']-linkage; yields and selectivity are determined via ³¹P-NMR, using PO(OPh)₃ as internal standard).

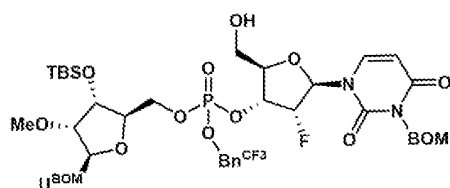
- 95 -



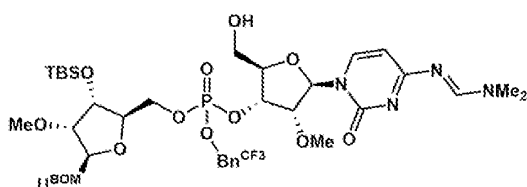
2a (from **1a**, dr 20:1)
 2 eq. CF_3BnOH , Tol (0.04M)
 Yield **92%**, [3-5] **86%**
 [5-5] 6%, S = 14.33



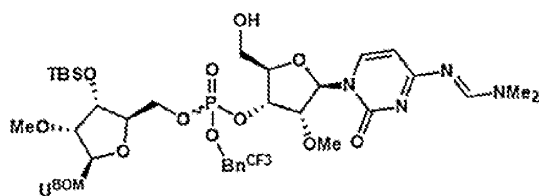
2b (from **1b**, dr 20:1)
 Tol (0.035M)
 Yield **97%**, [3-5] **92%**
 [5-5] 5%, S = 18.5



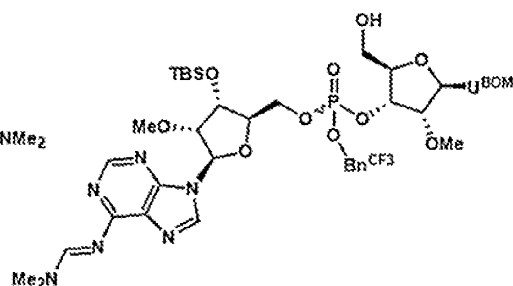
2c (from **1c**, dr 20:1)
 1.1 eq. LiOtBu , Tol (0.04M)
 Yield **90%**, [3-5] **73%**
 [5-5] 17%, S = 4.3



2d (from **1d**, dr 1:1)
 MeCN:Tol (1:1, 0.05M), 5h, Conv. 88%
 Yield **82%**, [3-5] **70%**
 [5-5] 12%, S = 5.83

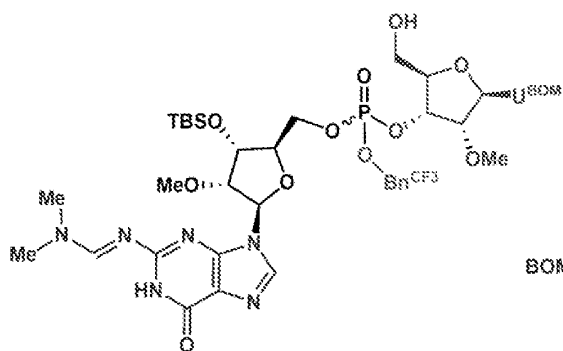


2d' (from **1d**, dr 1:1)
 Yield **82%**, [3-5] **70%**
 [5-5] 12%, S = 6

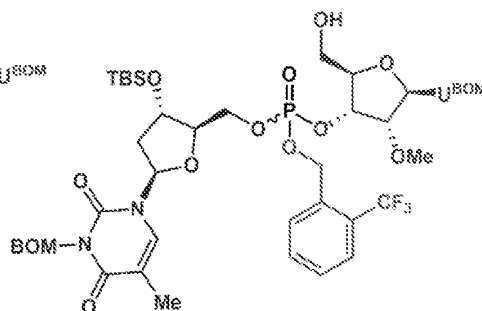


2e, (from **1e**, dr 34:1)
 Yield **100%**, [3-5] **90%**
 [5-5] 10%, S = 9

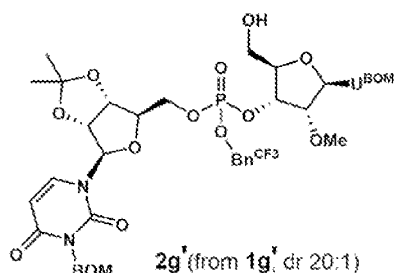
- 96 -



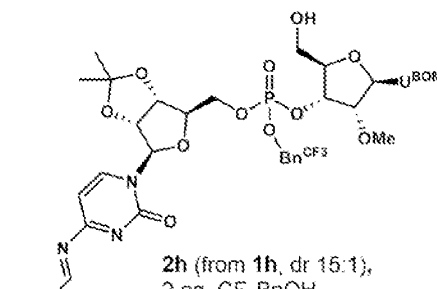
2f, (from **1f**, dr 6:1)
Yield 88%, [3-5] 75%,
[5-5] 13%, S = 6



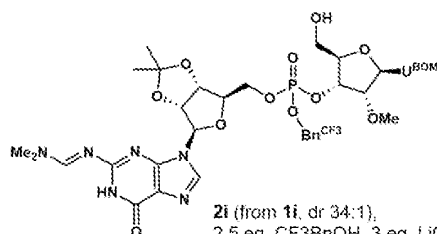
2g, (from **1g**, dr 30:1)
98% yield, [3-5] 94%,
[5-5] 4%, S = 23



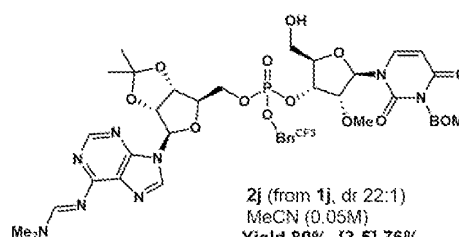
2g* (from **1g***, dr 20:1)
2 eq. CF₃BnOH, Tol (0.04M)
Yield 93%, [3-5] 88%
[5-5] 5%, S = 17.6



2h (from **1h**, dr 15:1),
2 eq. CF₃BnOH,
Tol:MeCN (3:1, 0.04M), Conv. 95%
Yield 84%, [3-5] 80%
[5-5] 4%, S = 20

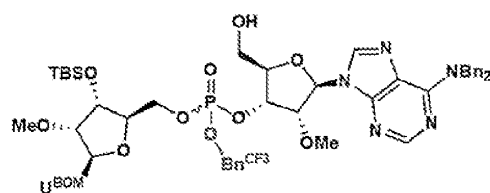
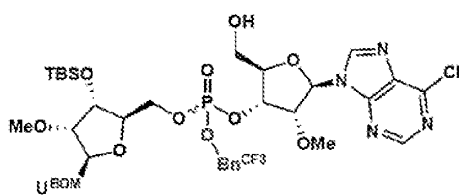
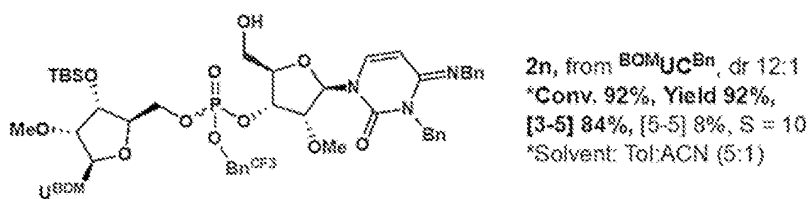
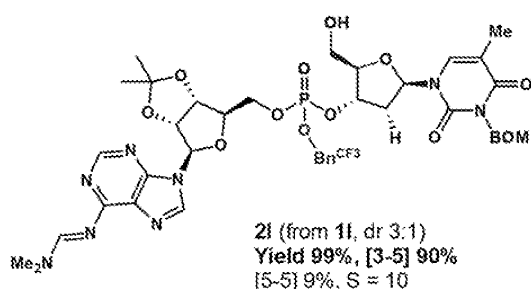
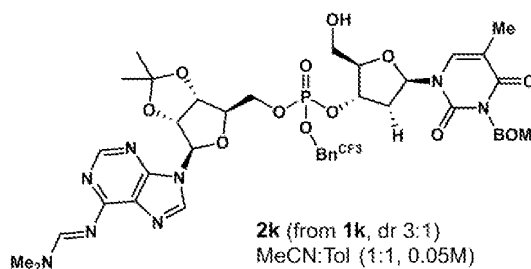


2i (from **1i**, dr 34:1),
2.5 eq. CF₃BnOH, 3 eq. LiOtBu
Tot:MeCN (1:1, 0.04M), Conv. 94%
Yield 91%, [3-5] 79%
[5-5] 12%, S = 6.6

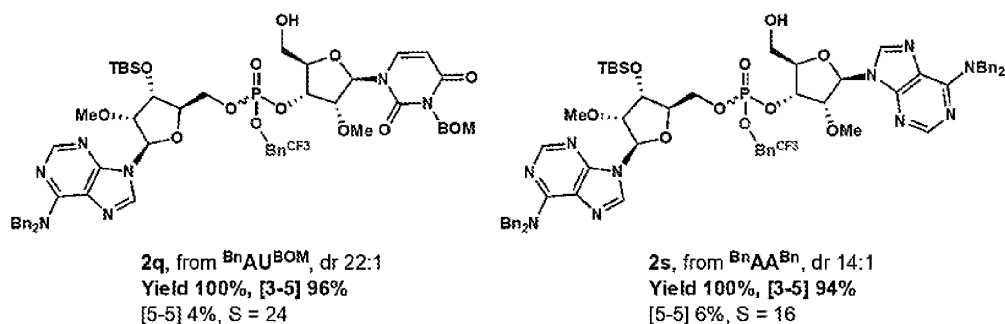


2j (from **1j**, dr 22:1)
MeCN (0.05M)
Yield 89%, [3-5] 76%
[5-5] 13%, S = 5.8

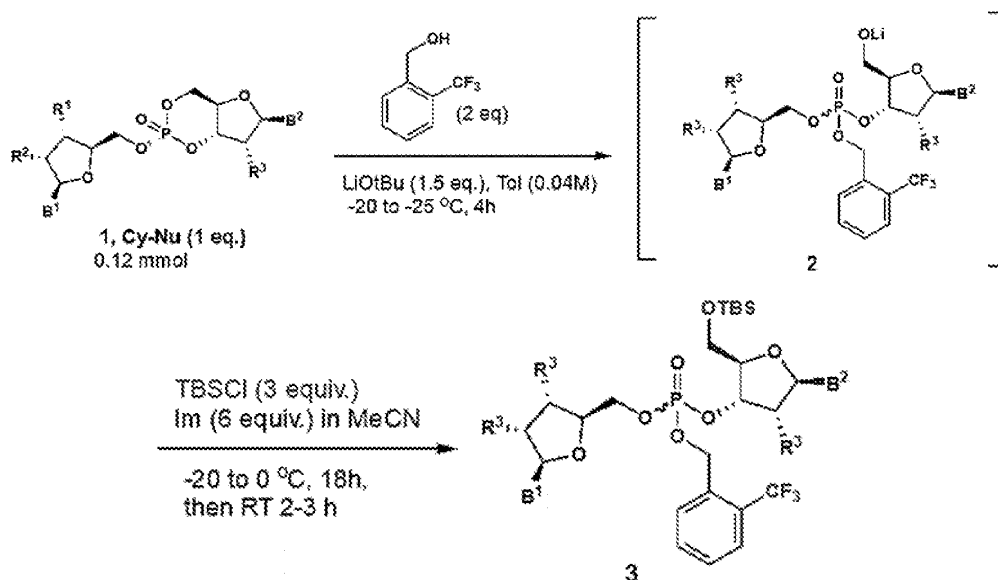
- 97 -



- 98 -



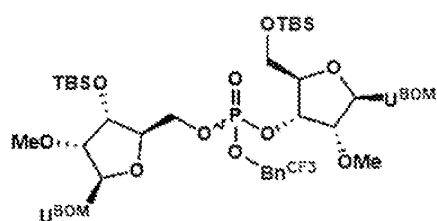
c. General procedure (GP14) for regioselective ring opening reaction of cyclic dinucleotides followed by trapping with TBSCl



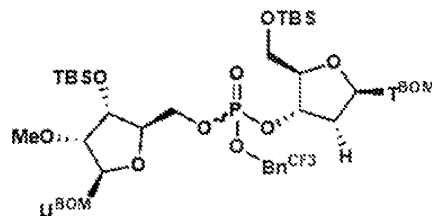
[0267] Step I: In a nitrogen-filled glovebox, an oven-dried reaction tube containing a Teflon-coated magnetic stir bar was charged with LiOtBu (0.18 mmol, 1.5 equiv.), 2- CF_3BnOH (as the substituted benzyl alcohol nucleophile, BnOH) (0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)) and taken outside the glovebox. The mixture was stirred at room temperature for 20 minutes and then cooled to -20 to -25 °C over a period of 10 minutes. Then, a solution of protected cyclic dinucleotide (0.12 mmol, 1 equiv.) in toluene (2 mL) was added in one portion at -20 °C and left stirring at that temperature for 4 hours.

- 99 -

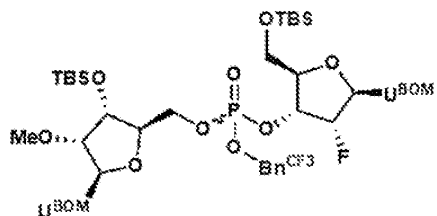
[0268] Step II: In 4-mL vial, a solution of TBSCl (0.36 mmol, 3 equiv.) and Imidazole (0.72 mmol, 6 equiv.) in 0.5 mL MeCN was prepared and subjected to the reaction tube of Step I with syringe in one portion. The resulting mixture was stirred for 1 hour at -20 °C and slowly warmed up to 0 °C, and stirred overnight (16-18 hours). The reaction was further warmed up to room temperature and monitored via LC-MS to ensure >90% conversion. Upon completion, a few drops of methanol were added to quench excess of TBS-Imidazolium salts. The reaction was diluted with dichloromethane and transferred to round bottom flask. The crude mixture was evaporated to dryness with aid of rotavapor and purified via Combi-Flash NextGen 300 automated chromatography system by using silica gel column with DCM-EtOAc(1% MeOH) gradient. Resulting cyclic dinucleotides with their yield and diastereomeric ratio (dr) were as follows:



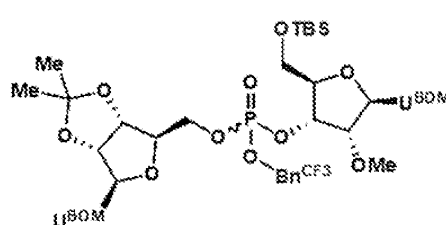
3a, UU-BOM,
75% yield, dr 28:1



3b, UT-BOM,
79% yield, dr 32:1

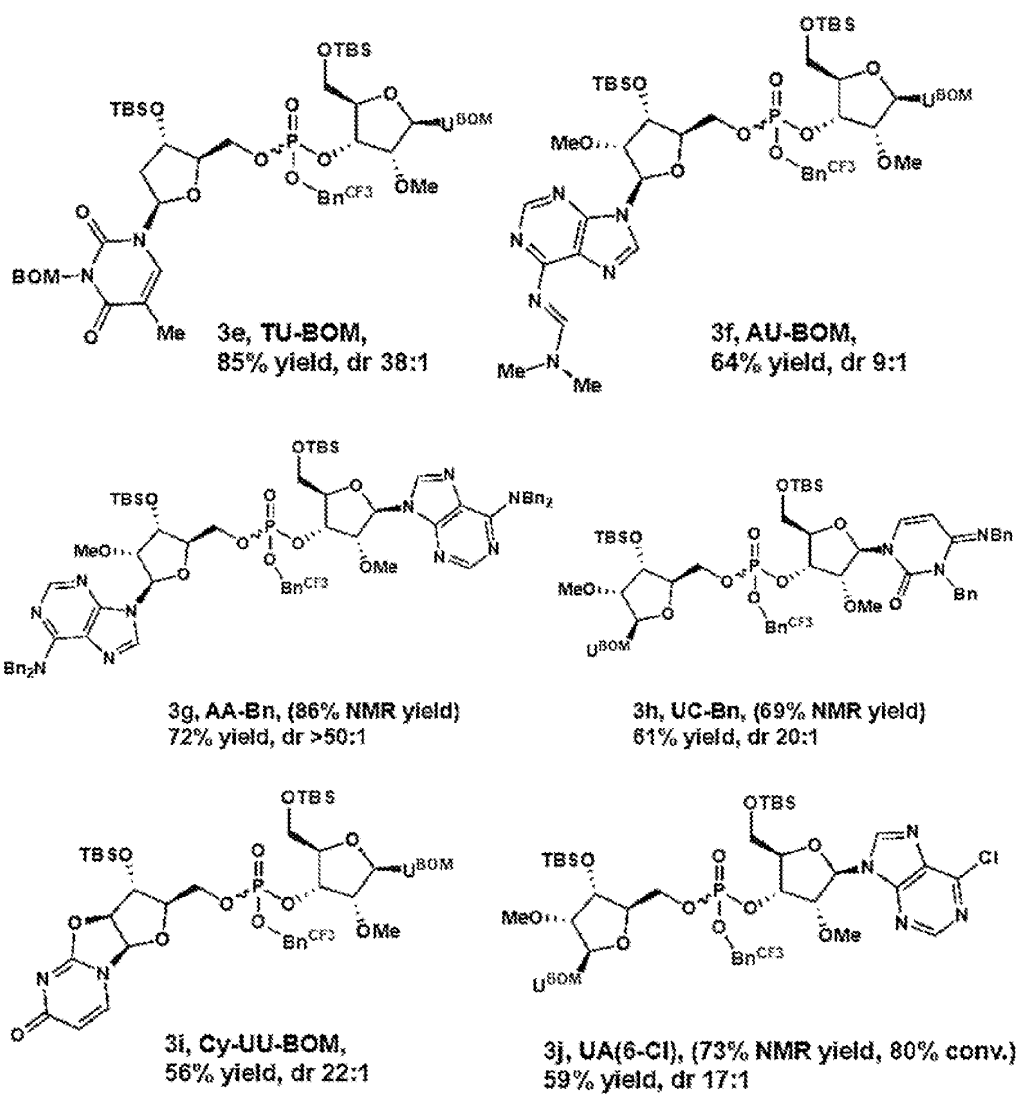


3c, UU^F-BOM,
76% yield, dr 35:1



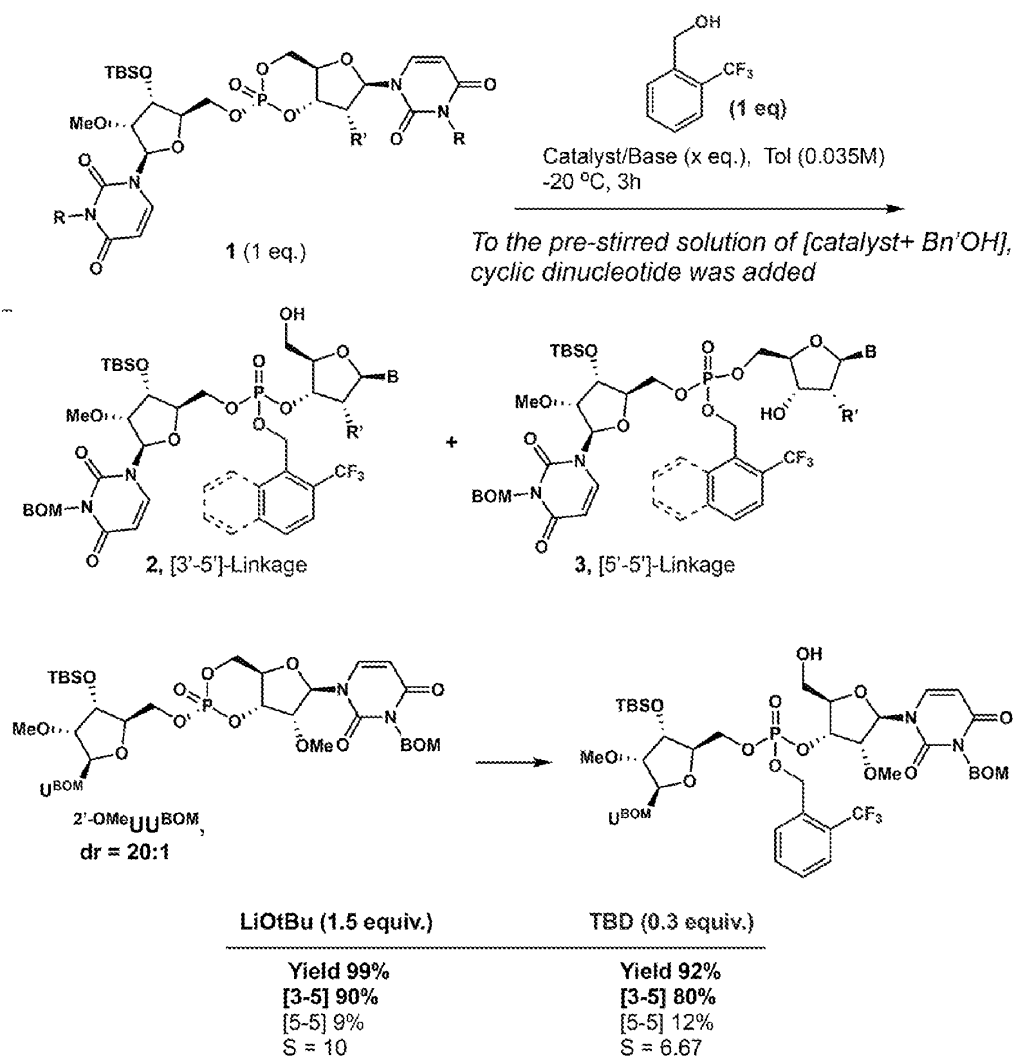
3d, ket-UU-BOM,
79% yield, dr 11:1

- 100 -

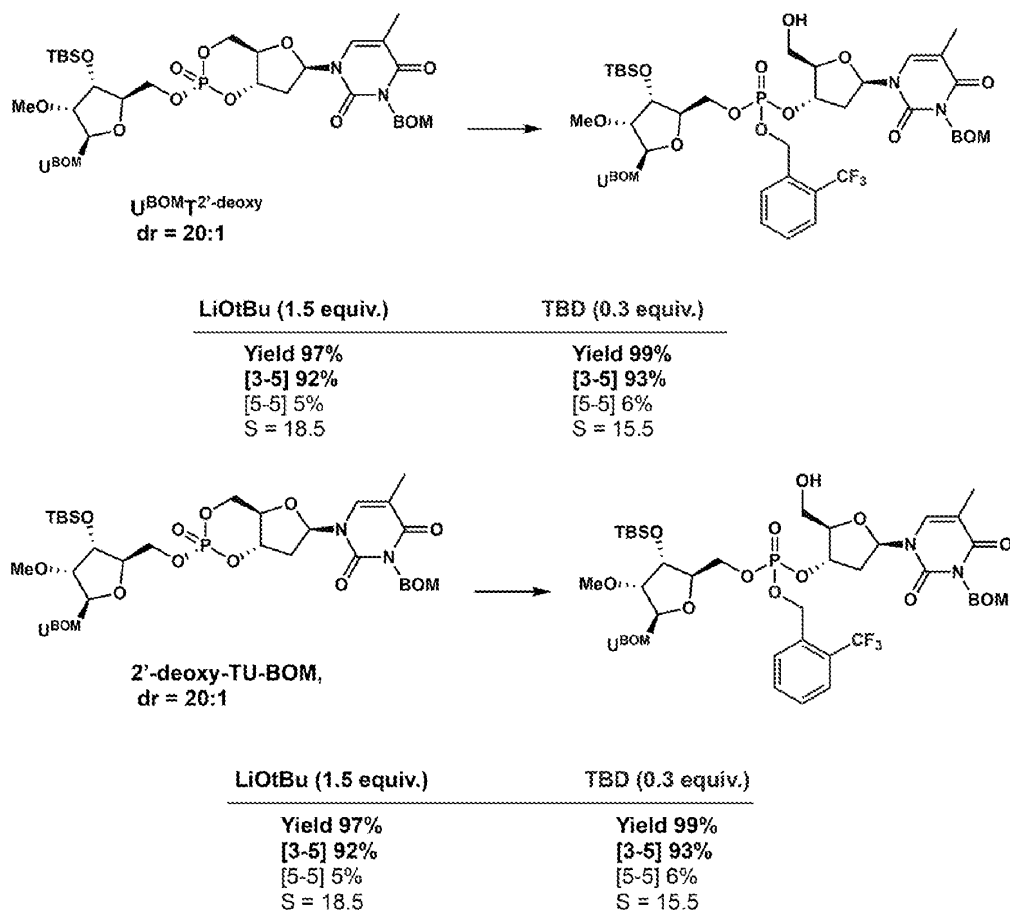


- 101 -

d. General procedure for regioselective ring opening reaction of cyclic dinucleotides with TBD catalyst

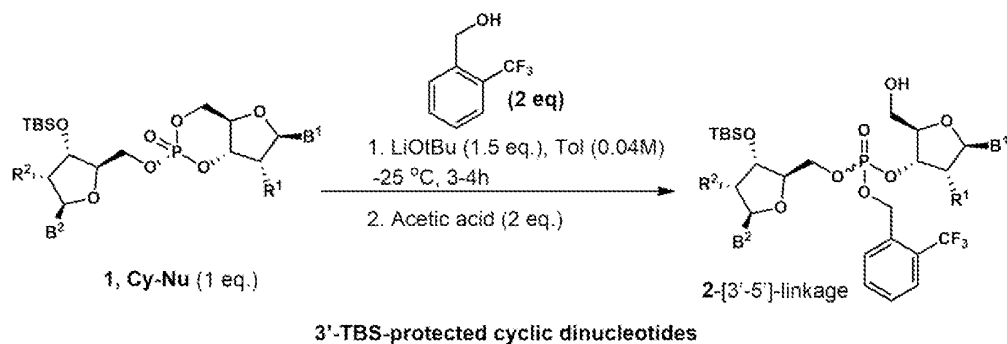


- 102 -



[0269] In a nitrogen-filled glovebox, an oven-dried reaction tube containing a Teflon-coated magnetic stir bar was charged with LiOtBu (0.18 mmol, 1.5 equiv.), 2-CF₃BnOH (as the substituted benzyl alcohol nucleophile, BnOH) (0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)) and taken outside the glovebox. The mixture was stirred at room temperature for 20 minutes and then cooled to -20 to -25 °C over a period of 10 minutes. Then, a solution of protected cyclic dinucleotide (0.12 mmol, 1 equiv.) in toluene (2 mL) was added in one portion at -20 °C and left stirring at that temperature for 4 hours.

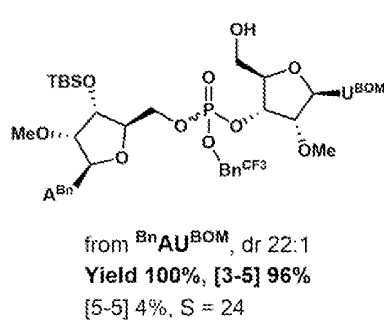
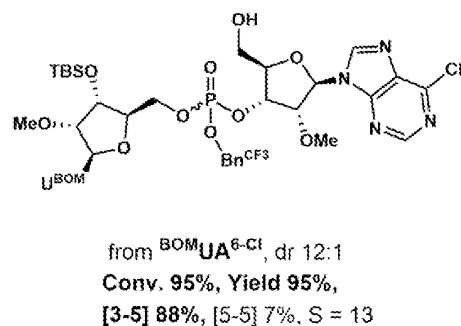
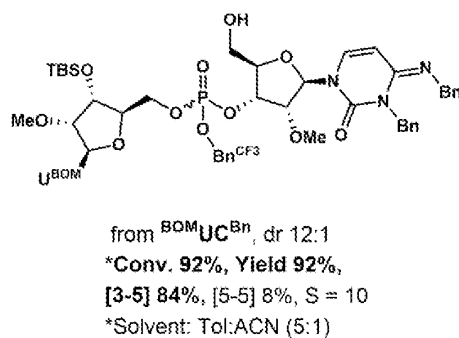
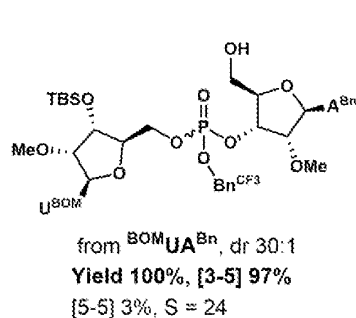
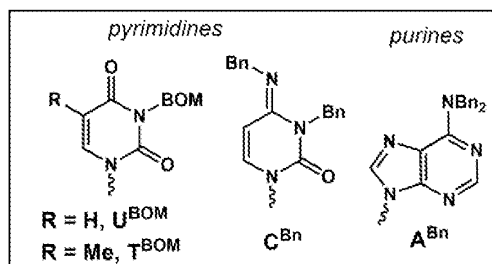
e. General procedure for regioselective ring opening reaction of cyclic dinucleotides



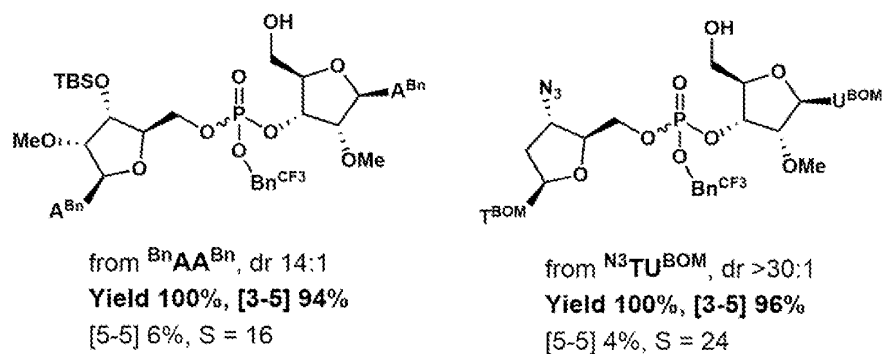
Reaction condition: **1-Cy-Nu**, 1 eq (0.025mmol) 1.5 eq LiOtBu, 2 eq. 2- CF_3BnOH , at $-20\text{ }^\circ\text{C}$, 4h.

S denotes the ratio of [3-5]-linkage (**2**) over [5-5]-linkage (**2'**).

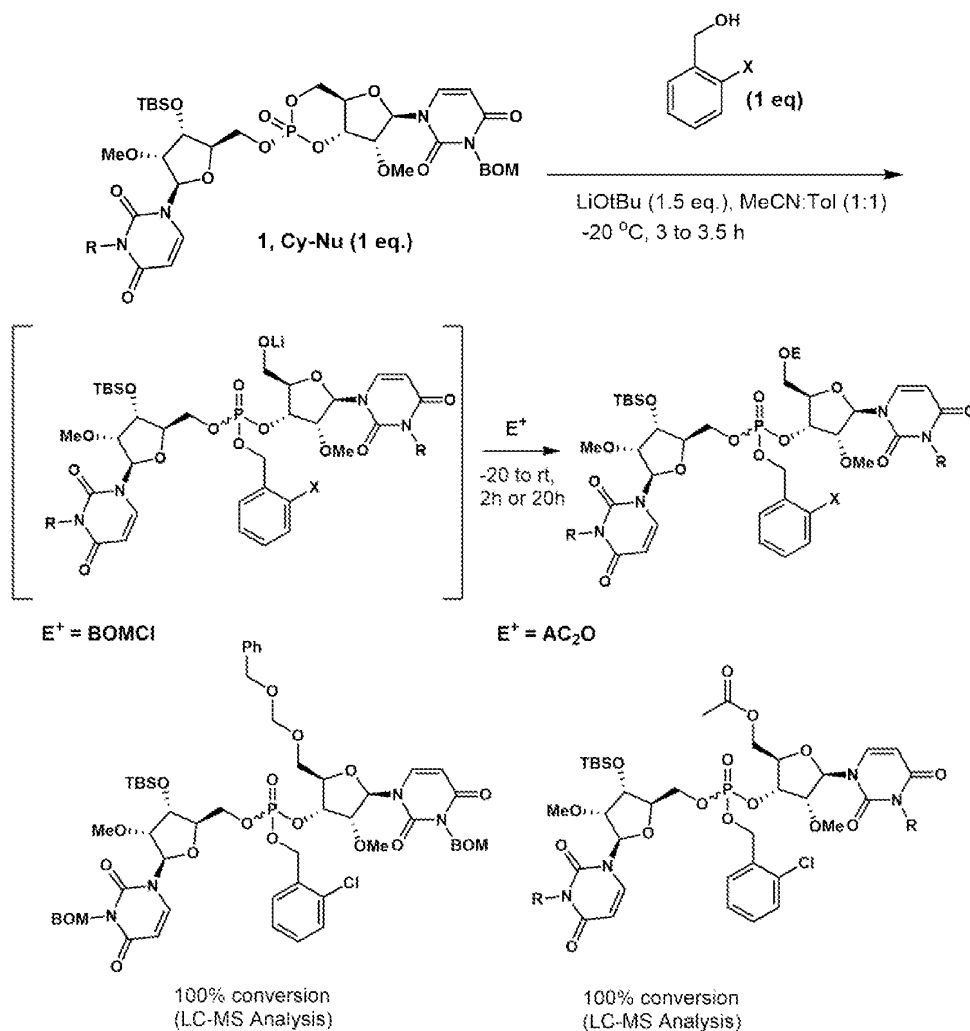
Conv., yields and selectivities are determined via ^{31}P -NMR by using $(\text{PhO})_3\text{PO}$ as an internal standard.



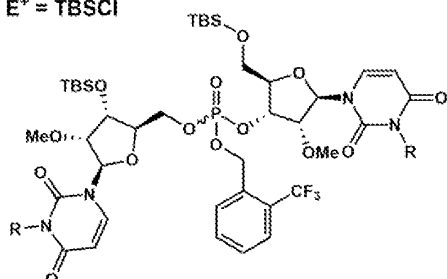
- 104 -



f. General procedure for regioselective ring opening reaction of cyclic dinucleotides



- 105 -

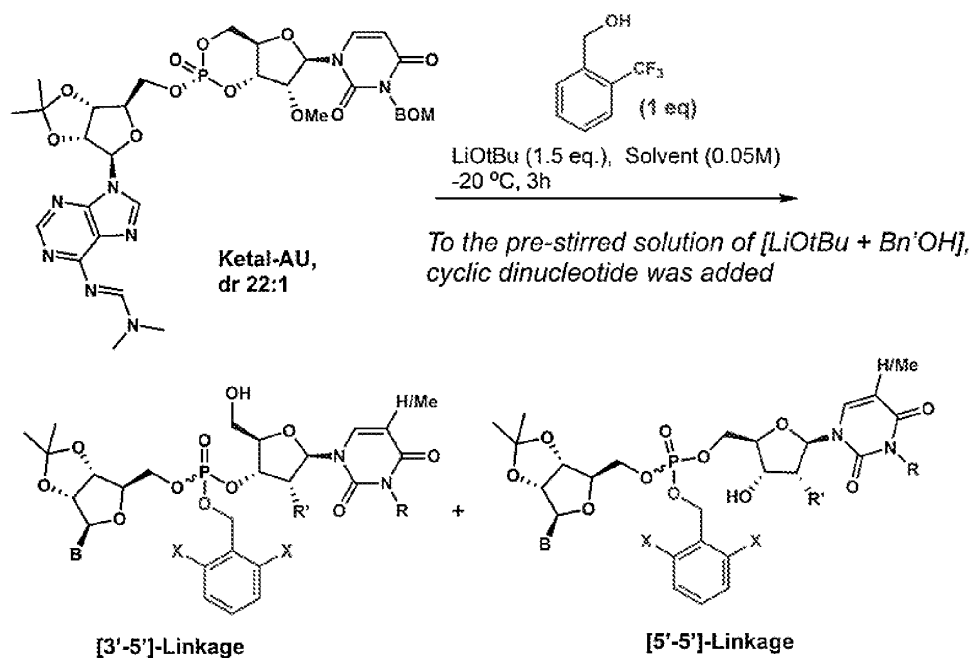
 $E^+ = \text{TBSCl}$ 

With 1.5 eq TBSOTf, 60% conv.
(TBSOTf has acidic impurities)
3 eq. TBSOTf, deprotection of existing TBS-group

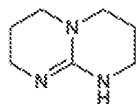
3 eq. TBSOTf + 6 eq. Imidazole
90% conversion based on HPLC,
71% NMR yield

No reaction without imidazole, with 3 eq. TBSCl+ 6 eq. Imidazole, 90% conv. via HPLC,
75% NMR yield, [5-5']-linkages remained unreacted.

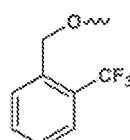
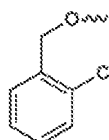
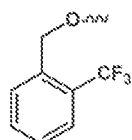
g. General procedure for regioselective ring opening reaction of cyclic dinucleotides



- 106 -



(0.3 equiv. of TBD)

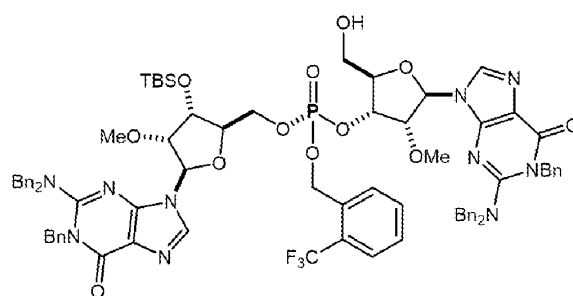
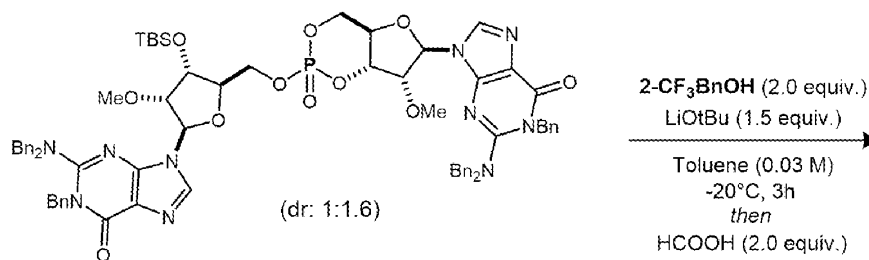


MeCN:Tol (1:1)
 Conv. 90%
 Yield 75%
 [3-5] 60%
 [5-5] 15%
 S = 4.0

MeCN:Tol (1:1)
 Conv. 100%
 Yield 100%
 [3-5] 83%
 [5-5] 17%
 S = 4.9

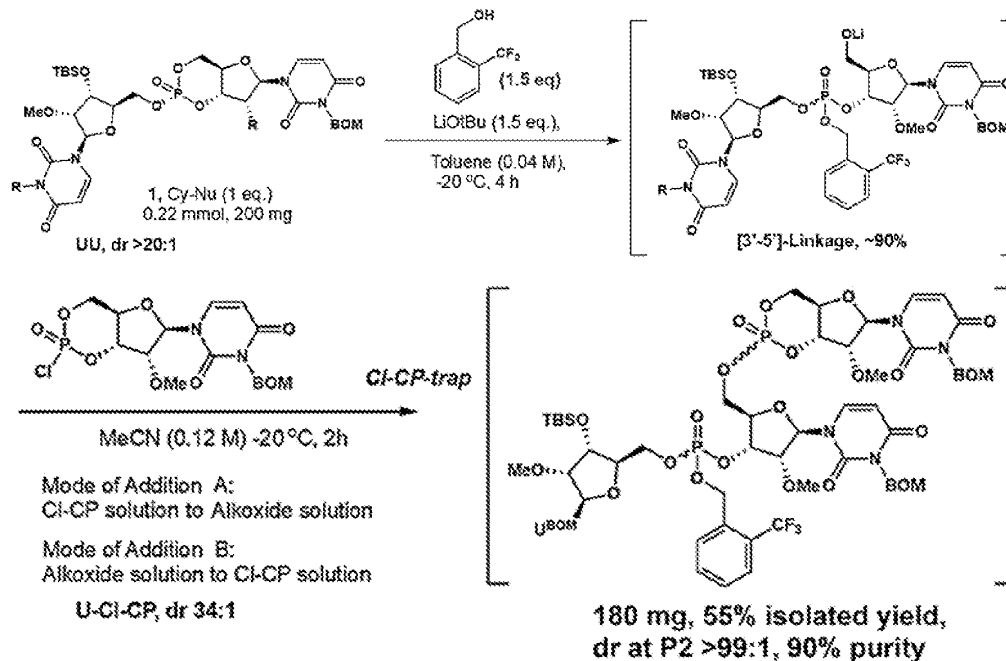
MeCN
 Conv. 100%
 Yield 89%
 [3-5] 76%
 [5-5] 13%
 S = 5.8

h. Guanosine dimer ring-opening



86% Isolated Yield

3'5' : 5'5' = 1:1.9

Example 8. Synthesis of cyclic trimer**a. General procedure (GP15) for one-pot synthesis of cyclic trimer**

[0270] **Step I:** In a nitrogen-filled glovebox, an oven-dried reaction tube A containing a Teflon-coated magnetic stir bar was charged with 2-CF₃BnOH (as a substituted benzyl alcohol nucleophile, BnOH) (0.18 mmol, 1.5 equiv.) and LiOtBu (0.18 mmol, 1.5 equiv.), dissolved in anhydrous toluene (1 mL) and taken outside the glovebox. The mixture was stirred at room temperature for 30 minutes and then cooled to -25 °C. Then, a solution of protected cyclic dinucleotide (0.12 mmol, 1 equiv.) in toluene (2 mL) was added in one portion at -25 °C and left stirring at that temperature for 4 hours.

[0271] **Step II:** Another reaction tube B was charged with Cl-CP (0.18 mmol, 1.5 equiv.) in solvent (*e.g.*, MeCN (0.2 mL)) and the reaction tube was taken outside of the glove box. The reaction tube was kept at -25 °C for 10 minutes and the pre-stirred alkoxide solution from reaction tube A was transferred to reaction tube B dropwise with the aid of a syringe. Alternatively, the pre-stirred Cl-CP solution from reaction tube B was transferred to reaction tube A dropwise with the aid of a syringe. The reactions were stirred at -25 °C for 18 hours. The reactions were diluted with dichloromethane and transferred to round bottom flask. The crude mixtures were evaporated to dryness with

- 108 -

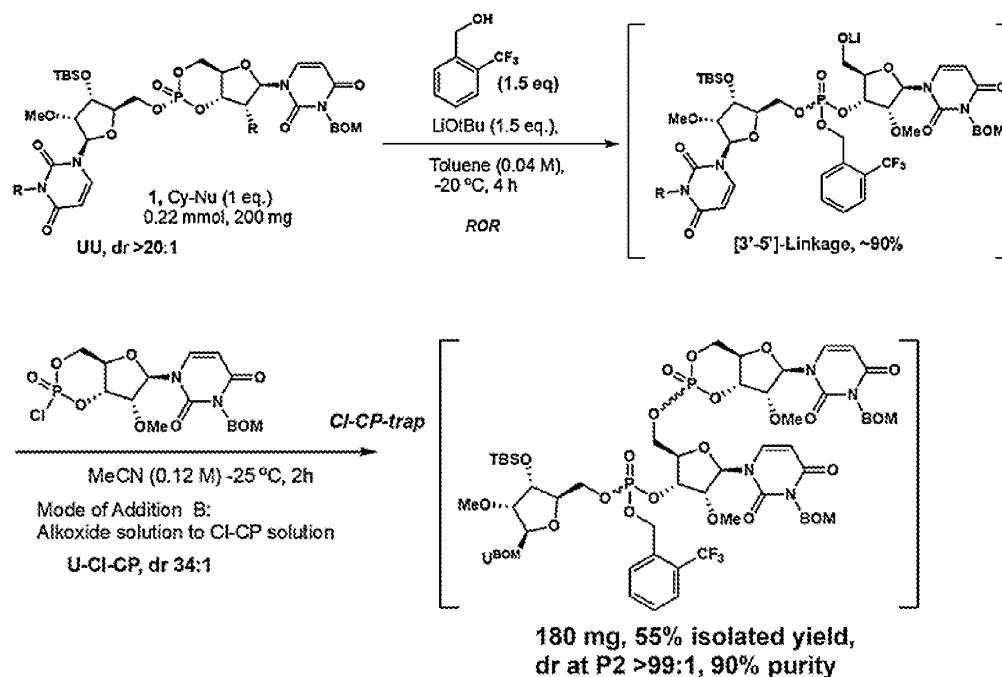
aid of rotavapor and purified via column chromatography by using DCM-EtOAc (1% MeOH) gradient.

[0272] Several different reaction conditions and substrate concentrations were studied for the synthesis of the cyclic trimers. Results are shown in Table 4:

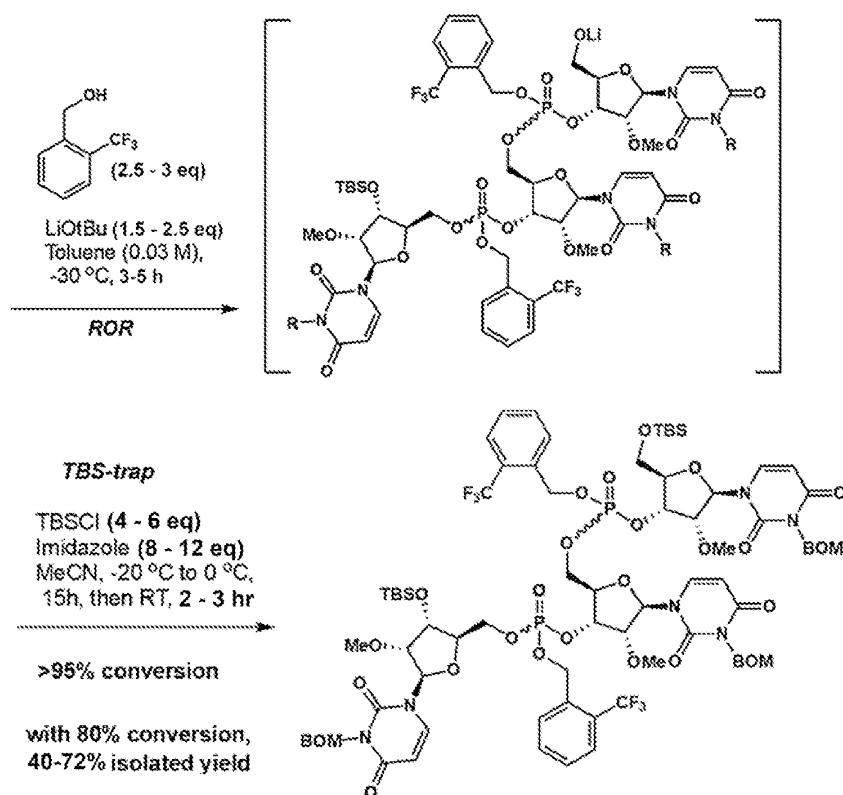
Table 4 - Reaction conditions and substrate concentrations for synthesis of cyclic trimers

dr of 1: Cy-Nu	Cl-CP (equiv.)	Solvent for Cl-CP Sol.	Mode of addition	Yield (major [3-5]-Cy-trimer)	dr (major [3-5]-Cy-trimer)
20:1	1.5	Tol:MeCN (0.2 mL)	A	91%	2.7:1
20:1	1.5	MeCN (0.2 mL)	A	84%	3:1
20:1	1.5	Toluene (0.3 mL)	B	66%	2:1
20:1	1.0	MeCN (0.2 mL)	B	74%	3.8:1
20:1	1.5	MeCN (0.2 mL)	B	85%	4.3:1
20:1*	1.5	MeCN (0.2 mL)	B	90%	5:1

b. General procedure (GP16) for one-pot synthesis of trinucleotide blockmers



- 109 -



[0273] Step I: In a nitrogen-filled glovebox, an oven-dried reaction tube A containing a Teflon-coated magnetic stir bar was charged with 2- CF_3BnOH (as a substituted benzyl alcohol nucleophile, BnOH) (0.18 mmol, 1.5 equiv.) and LiOtBu (0.18 mmol, 1.5 equiv.), dissolved in anhydrous toluene (0.18M, 1 mL) and taken outside the glovebox. The mixture was stirred at room temperature for 30 minutes and then cooled to $-25\text{ }^\circ\text{C}$. Then, a solution of protected cyclic dinucleotide (0.12 mmol, 1 equiv.) in toluene (2 mL) was added in one portion at $-25\text{ }^\circ\text{C}$ and left stirring at that temperature for 4 hours.

[0274] Step II: Another reaction tube B was charged with Cl-CP (0.18 mmol, 1.5 equiv.) in MeCN (0.6 mL) and the reaction tube was taken outside of the glove box. The reaction tube was kept at $-25\text{ }^\circ\text{C}$ for 10 minutes and the pre-stirred alkoxide solution from reaction tube A was transferred to reaction tube B dropwise with the aid of a syringe. The reaction was stirred at $-25\text{ }^\circ\text{C}$ for 18 hours.

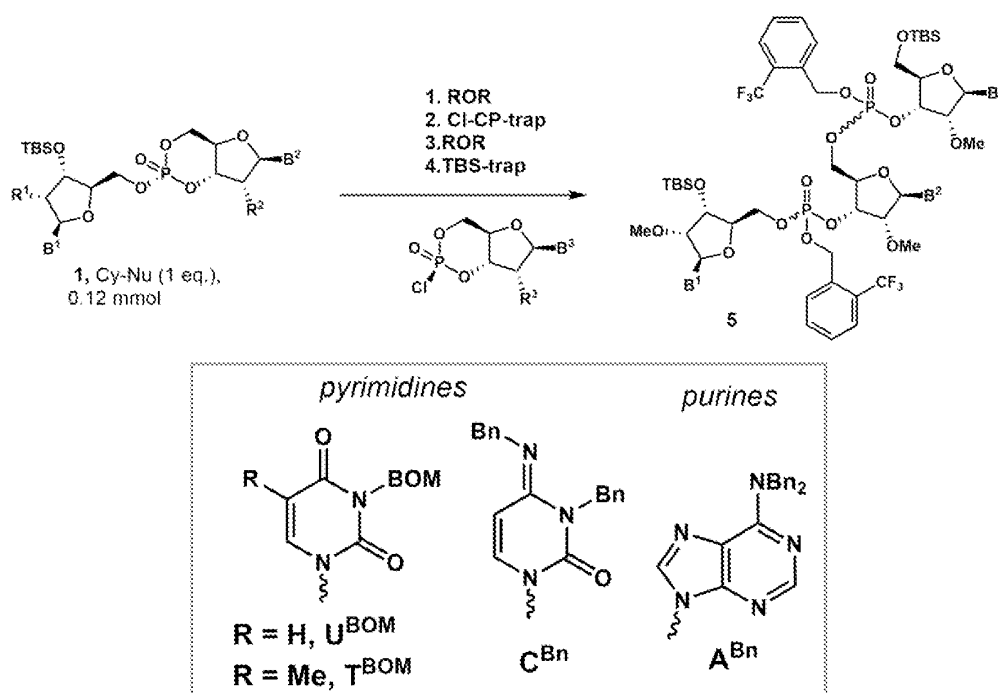
[0275] Step III: Reaction tube C was charged with 2- CF_3BnOH (as a substituted benzyl alcohol nucleophile, BnOH) (0.36 mmol, 3 equiv.) and LiOtBu (0.30 mmol, 2.5

- 110 -

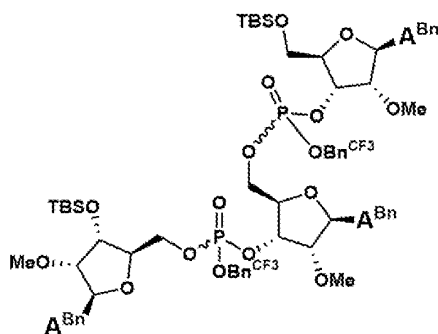
equiv.) in 1 mL of toluene and stirred for 30 minutes before adding to reaction tube B dropwise at -30 °C. The reaction was stirred for 5 hours at -30 °C.

[0276] Step IV: To the reaction **tube C**, a solution of TBSCl (0.72 mmol, 6 equiv.) and Imidazole (1.44 mmol, 12 equiv.) in 1 mL MeCN was added to one portion. The resulting mixture was stirred for 1 hour at -30 °C and slowly warmed up to 0 °C, stirred overnight (16-18 hours). The reaction was further warmed up to room temperature and monitored via LC-MS to ensure >90% conversion. Upon completion, a few drops of methanol were added to quench excess of TBS-Imidazolium salts. The reaction was diluted with dichloromethane and transferred to round bottom flask. The crude mixture was evaporated to dryness with aid of rotavapor and purified via column chromatography by using DCM-EtOAc(1% MeOH) gradient. The crude product was loaded on silica gel 10g column by dissolving in minimum amount of DCM.

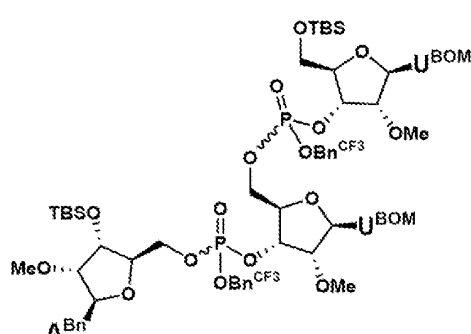
c. General procedure for 4-step one-pot synthesis of trinucleotide blockmers



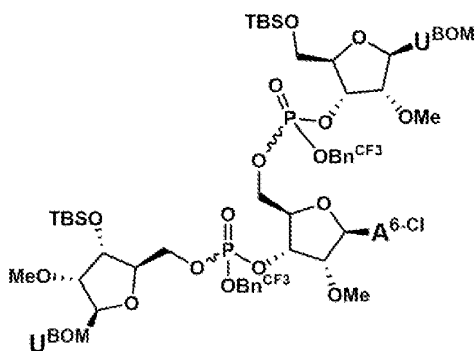
- 111 -



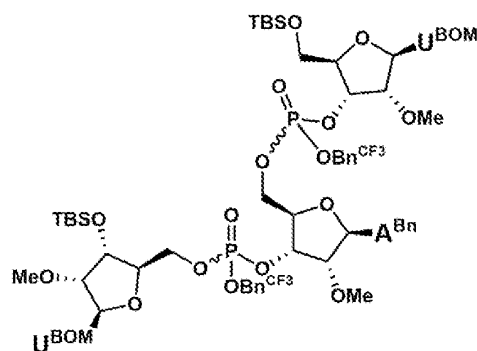
5c, AAA (Lys),
method A, 40% yield,
method B, 47% yield



5d, AUU (Leu),
method A, 26% yield
method B, 46% yield (NMR yield 73%)



5e, UA⁶ClU,
method A, 43% yield



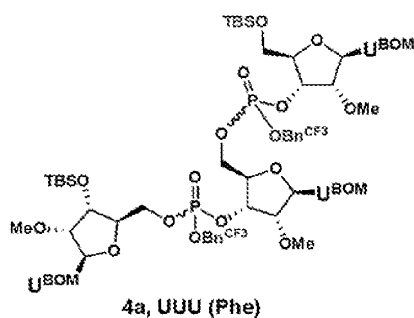
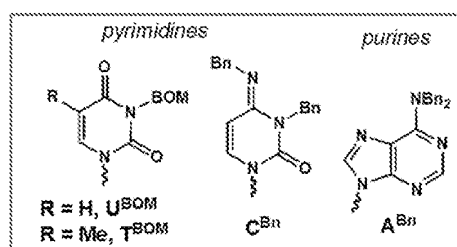
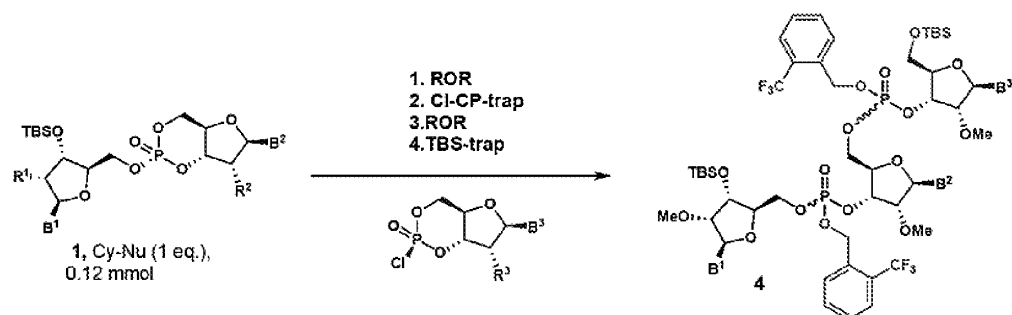
5f, UAU (Tyr)
method A, 44% yield
(NMR yield 70%)

[0277] Isolated yields are reported for one-pot, 4 steps sequence. NMR yields are reported based on ³¹P NMR by using (PhO)₃PO as an internal standard. Method A: compound was isolated via column chromatography on silica gel by using DCM:EtOAc (2% MeOH) gradient, and compound was isolated via C-18 reverse phase column.

d. Isolation of trimer blockmer

[0278] Crude trimer blockmer product was purified via reverse phase column-chromatography on automated system. Resulting trinucleotide blockmers with their yield were as follows:

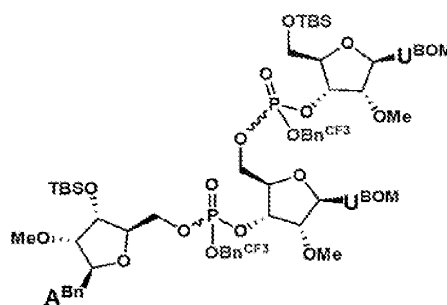
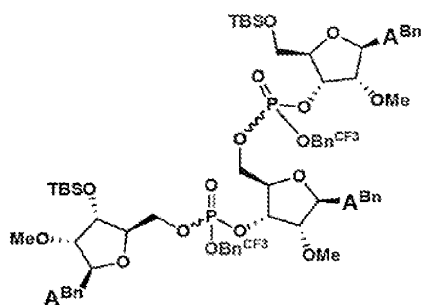
- 112 -



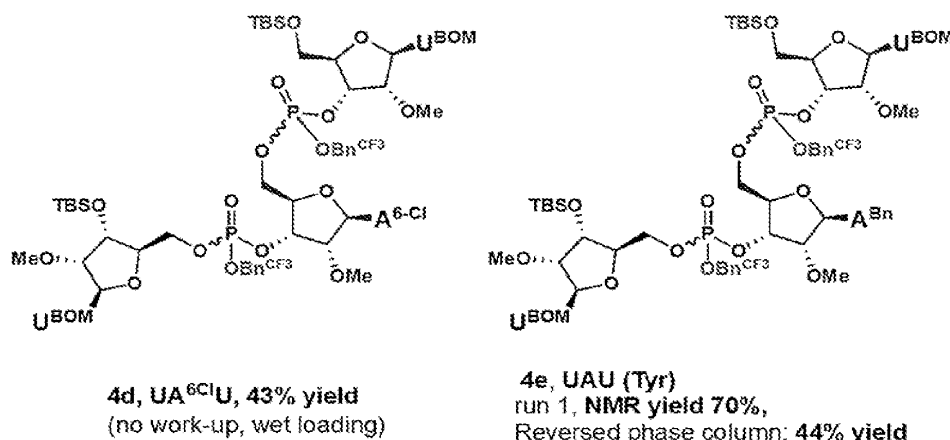
1. UUU, (no work-up), isolated via silica gel column chromatography, wet loading, **48% yield**

2. UUU, (aqueous work-up), isolated via silica gel column chromatography, dry loading, decomposition to dimer with BOM-deprotection + TBS-deprotection of 5'-O of trimer

3. UUU, **NMR yield 76%** (no work-up), isolated via C-18, reverse phase column chromatography, **45% yield**



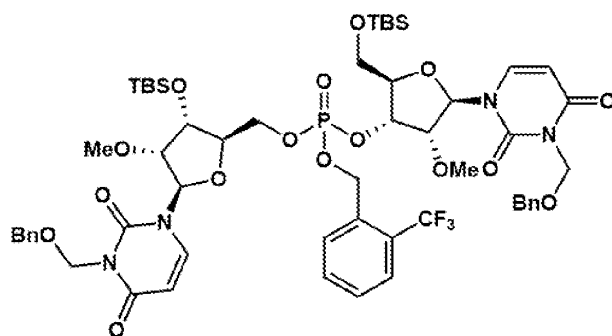
- 113 -



[0279] With reversed phase column chromatography, the compounds do not decompose but separation is not base line to base line. Addition of 0.1% buffer solution of NH₄OC(O)H or NH₄OAc in water can help in separation.

Example 9. Synthesis of open-ringed TBS-protected-dimers

a. (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyl)dimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl
(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(((tert-butyl)dimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) (2-
(trifluoromethyl)benzyl) phosphate



[0280] The compound was synthesized by following GP14 (2-steps, one-pot sequence) using UU-Cy-Nu (117 mg, 0.12 mmol) in 2 mL Toluene:ACN (5:1), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (49 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column

- 114 -

conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, Hexanes: Ethyl acetate (0% to 100%), desired product was isolated as white solid. Yield = 84 mg, 61%, dr = 29:1.

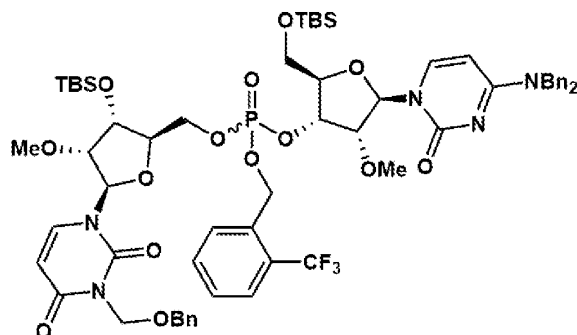
[0281] ³¹P NMR (162 MHz, CDCl₃) δ -1.91 (major), -2.49 (minor).

[0282] ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, *J* = 8.2 Hz, 1H), 7.56 (dd, *J* = 7.8, 4.7 Hz, 2H), 7.45 (dd, *J* = 25.5, 8.0 Hz, 2H), 7.35 (t, *J* = 7.7 Hz, 1H), 7.30 – 7.11 (m, 10H), 5.93 (d, *J* = 3.7 Hz, 1H), 5.75 (d, *J* = 2.2 Hz, 1H), 5.61 (d, *J* = 8.2 Hz, 1H), 5.54 (d, *J* = 8.2 Hz, 1H), 5.36 (qd, *J* = 9.7, 7.4 Hz, 4H), 5.27 – 5.14 (m, 3H), 4.77 (q, *J* = 5.5 Hz, 1H), 4.59 (d, *J* = 3.2 Hz, 4H), 4.28 (ddd, *J* = 11.7, 6.1, 2.2 Hz, 1H), 4.20 – 4.10 (m, 2H), 4.02 (qd, *J* = 7.5, 3.6 Hz, 2H), 3.90 (dd, *J* = 11.9, 1.9 Hz, 1H), 3.80 (t, *J* = 4.3 Hz, 1H), 3.70 (dd, *J* = 11.9, 1.7 Hz, 1H), 3.53 (dd, *J* = 4.7, 2.2 Hz, 1H), 3.43 (s, 3H), 3.36 (s, 3H), 0.83 (s, 9H), 0.78 (s, 9H), 0.03 (s, 6H), -0.03 (d, *J* = 10.0 Hz, 6H).

[0283] ¹³C NMR (101 MHz, CDCl₃) δ 162.62, 162.49, 151.06, 150.91, 138.23, 138.06, 137.98, 137.96, 133.61, 132.52, 129.65, 129.04, 128.45, 128.44, 128.08, 127.86, 127.82, 126.40, 126.34, 125.53, 102.20, 102.13, 89.27, 87.24, 83.30, 82.92, 82.83, 82.62, 81.59, 81.51, 73.79, 73.74, 72.39, 70.40, 70.38, 69.46, 66.17, 65.97, 65.92, 61.54, 58.58, 58.57, 25.99, 25.74, 18.47, 18.17, -4.55, -4.88, -5.45.

[0284] HR-MS (Q-TOF, ESI) calculated for C₅₆H₇₆F₃N₄O₁₆PSi₂, [M+Na⁺]: 1227.4377, found 1227.4368.

b. ((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl
((2R,3R,4R,5R)-2-(((tert-butyldimethylsilyl)oxy)methyl)-5-(4-(dibenzylamino)-2-oxopyrimidin-1(2H)-yl)-4-methoxytetrahydrofuran-3-yl) (2-(trifluoromethyl)benzyl)
phosphate



- 115 -

[0285] The compound was synthesized by following GP14 (2-steps, one-pot sequence) using UC-Cy-Nu (117 mg, 0.12 mmol) in 2 mL Toluene:ACN (5:1), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (49 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM: Ethyl acetate (2% MeOH) gradient (0% to 100%), desired product was isolated as white solid. Yield = 84 mg, 61%, dr = 20:1.

[0286] ³¹P NMR (203 MHz, CDCl₃) δ -1.91.

[0287] ¹⁹F NMR (471 MHz, CDCl₃) δ -59.76.

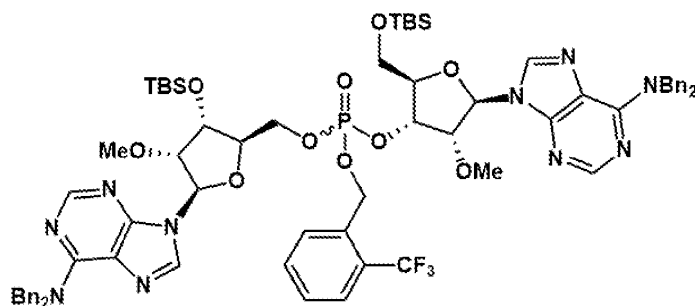
[0288] ¹H NMR (500 MHz, CDCl₃) δ 7.92 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 8.1 Hz, 2H), 7.50 (dd, *J* = 19.6, 7.9 Hz, 2H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.34 – 7.16 (m, 16H), 7.09 (s, 1H), 6.03 (d, *J* = 2.0 Hz, 1H), 5.80 (d, *J* = 2.3 Hz, 1H), 5.67 (d, *J* = 7.8 Hz, 1H), 5.57 (d, *J* = 8.2 Hz, 1H), 5.45 – 5.35 (m, 2H), 5.28 (d, *J* = 6.4 Hz, 2H), 4.98 (q, *J* = 14.2 Hz, 2H), 4.80 (ddd, *J* = 7.4, 6.1, 4.9 Hz, 1H), 4.64 (s, 2H), 4.43 (s, 2H), 4.31 (ddd, *J* = 11.6, 5.9, 2.0 Hz, 1H), 4.22 – 4.14 (m, 2H), 4.10 – 4.01 (m, 2H), 3.99 (dd, *J* = 12.0, 2.1 Hz, 1H), 3.94 (dd, *J* = 4.9, 2.1 Hz, 1H), 3.76 – 3.69 (m, 1H), 3.61 – 3.51 (m, 2H), 3.50 (s, 3H), 3.46 (s, 3H), 0.82 (s, 9H), 0.78 (s, 9H).

[0289] ¹³C NMR (126 MHz, CDCl₃) δ 164.28, 162.53, 155.41, 150.97, 141.16, 138.08, 137.99, 137.21, 136.23, 133.66, 132.56, 129.46, 129.18, 128.96, 128.71, 128.46, 127.84, 127.67, 126.50, 126.35, 126.31, 125.28, 123.10, 102.13, 91.85, 89.04, 88.14, 83.35, 82.53, 81.91, 81.83, 81.68, 81.62, 77.36, 72.92, 72.88, 72.40, 70.37, 69.40, 66.06, 65.80, 65.75, 60.80, 58.56, 58.50, 50.81, 50.31, 25.95, 25.88, 25.75, 18.41, 18.18, -4.56, -4.88, -5.43, -5.46.

[0290] HR-MS (Q-TOF, ESI) calculated for C₆₂H₈₁F₃N₅O₁₄PSi₂, [M+Na⁺]:1286.4900, found 1286.4894.

- 116 -

c. ((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methyl ((2R,3R,4R,5R)-2-(((tert-butyldimethylsilyl)oxy)methyl)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-3-yl) (2-(trifluoromethyl)benzyl) phosphate



**3g, AA-Bn, (86% NMR yield)
72% yield, dr >50:1**

[0291] The compound was synthesized by following GP14 (2-steps, one-pot sequence) using UU-Cy-Nu (130 mg, 0.12 mmol) in 2 mL Toluene, LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and Imidazole (49 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM: Ethyl acetate (1% MeOH) gradient (0% to 100%), desired product was isolated as white solid. Yield = 120 mg, 72%, dr = 50:1.

[0292] ¹⁹F NMR (471 MHz, CDCl₃) δ -59.86.

[0293] ³¹P NMR (203 MHz, CDCl₃) δ -1.77.

[0294] ¹H NMR (500 MHz, CDCl₃) δ 8.38 (s, 1H), 8.36 (s, 1H), 7.96 (s, 1H), 7.93 (s, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 7.7 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.37 – 7.21 (m, 25H), 6.17 (d, *J* = 6.6 Hz, 1H), 6.10 (d, *J* = 4.1 Hz, 1H), 5.49 (s, 3H), 5.36 (d, *J* = 6.3 Hz, 2H), 5.16 (ddd, *J* = 7.2, 4.7, 2.5 Hz, 1H), 4.96 (s, 3H), 4.59 (qd, *J* = 4.9, 2.6 Hz, 2H), 4.46 (ddd, *J* = 10.9, 5.8, 3.8 Hz, 1H), 4.38 (ddd, *J* = 13.4, 5.4, 3.5 Hz, 2H), 4.34 – 4.23 (m, 2H), 3.88 – 3.75 (m, 2H), 3.49 (s, 3H), 3.40 (s, 3H), 0.93 (s, 9H), 0.89 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H).

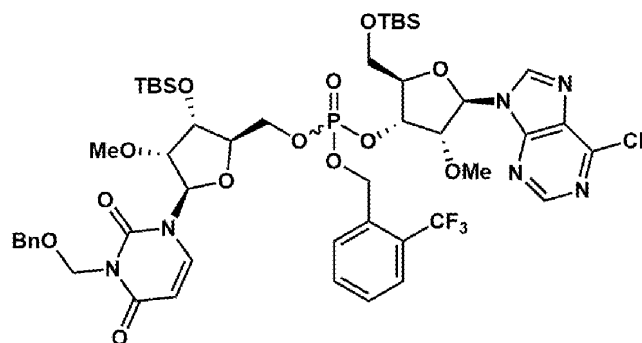
[0295] ¹³C NMR (126 MHz, CDCl₃) δ 155.08, 155.05, 152.93, 152.81, 151.30, 150.76, 137.90, 137.48, 136.94, 134.08, 132.39, 129.41, 128.71, 128.53, 128.07, 128.02,

- 117 -

127.39, 126.08, 126.04, 125.29, 123.11, 120.46, 120.17, 87.48, 85.43, 84.14, 84.09, 82.75, 82.68, 82.58, 82.08, 82.05, 77.36, 75.70, 75.66, 70.53, 66.68, 65.92, 62.66, 58.86, 58.71, 26.10, 25.86, 25.84, 18.46, 18.26, -4.55, -4.74, -5.25, -5.42.

[0296] HR-MS (Q-TOF, ESI) calculated for $C_{70}H_{86}F_3N_{10}O_{10}PSi_2$ $[M+Na^+]$: 1393.5649, Found 1393.5642.

d. ((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl
((2R,3R,4R,5R)-2-(((tert-butyldimethylsilyl)oxy)methyl)-5-(6-chloro-9H-purin-9-yl)-4-methoxytetrahydrofuran-3-yl) (2-(trifluoromethyl)benzyl) phosphate



[0297] The compound was synthesized by following GP14 (2-steps, one-pot sequence) using UA(6Cl)-Cy-Nu (101 mg, 0.12 mmol) in Toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (50 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM: ethyl acetate (1% MeOH) gradient (0% to 100%), desired product was isolated as white solid. Yield = 80 mg, 59%, dr = 17:1.

[0298] ³¹P NMR (203 MHz, CDCl₃) δ -1.71, -2.12.

[0299] ¹⁹F NMR (471 MHz, CDCl₃) δ -59.70.

[0300] ¹H NMR (500 MHz, CDCl₃) δ 8.75 (s, 1H), 8.47 (s, 1H), 7.66 (dd, *J* = 8.1, 2.2 Hz, 2H), 7.61 – 7.53 (m, 2H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.38 – 7.29 (m, 4H), 7.29 – 7.22 (m, 2H), 6.24 (d, *J* = 6.2 Hz, 1H), 5.87 (d, *J* = 2.1 Hz, 1H), 5.69 (d, *J* = 8.2 Hz, 1H), 5.50 – 5.30 (m, 4H), 5.12 (ddd, *J* = 7.2, 4.7, 2.7 Hz, 1H), 4.69 (s, 2H), 4.51 – 4.39 (m, 3H), 4.27 (ddd, *J* = 11.6, 5.2, 2.9 Hz, 1H), 4.19 – 4.08 (m, 3H), 3.97 (dd, *J* = 11.6, 2.8 Hz, 1H),

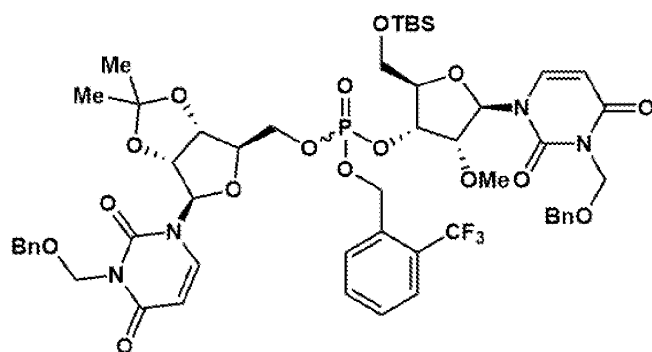
- 118 -

3.86 (dd, $J = 11.6, 2.4$ Hz, 1H), 3.65 (dd, $J = 4.4, 2.1$ Hz, 1H), 3.54 (s, 3H), 3.38 (s, 3H), 0.95 (s, 9H), 0.93 – 0.85 (m, 10H), 0.18 – 0.05 (m, 12H).

[0301] ^{13}C NMR (126 MHz, CDCl_3) δ 162.51, 152.43, 151.70, 151.40, 150.92, 143.39, 138.06, 137.94, 133.62, 133.55, 132.49, 132.21, 129.68, 129.06, 128.46, 128.12, 127.86, 127.82, 126.45, 126.41, 126.37, 126.32, 123.08, 102.19, 89.37, 85.94, 84.67, 84.63, 83.35, 83.15, 83.12, 81.54, 81.47, 77.36, 75.60, 75.55, 72.42, 70.38, 69.45, 66.26, 65.91, 65.87, 62.66, 60.53, 58.90, 58.60, 26.12, 26.08, 25.75, 25.74, 18.53, 18.20, -4.52, -4.84, -5.23, -5.36.

[0302] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{49}\text{H}_{67}\text{ClF}_3\text{N}_6\text{O}_{13}\text{PSi}_2$, $[\text{M}+\text{Na}^+]$: 1149.3575, found 1149.3568.

e. ((3aR,4R,6R,6aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl ((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyl)dimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl) (2-(trifluoromethyl)benzyl) phosphate



[0303] The compound was synthesized by following GP14 (2-steps, one-pot sequence) using katal-UU-Cy-Nu (100 mg, 0.12 mmol, dr 10:1) in Toluene (1.6 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2- CF_3BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (50 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, hexanes: ethyl acetate gradient (0% to 100%), desired product was isolated as white solid. Yield = 107mg, 79%, dr = >99:1

[0304] ^{31}P NMR (203 MHz, CDCl_3) δ -2.26, -2.31.

- 119 -

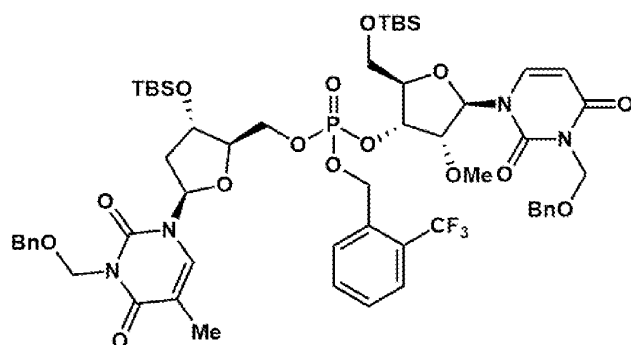
[0305] ^{19}F NMR (471 MHz, CDCl_3) δ -59.73, -59.85.

[0306] ^1H NMR (500 MHz, CDCl_3) δ 7.87 (d, J = 8.2 Hz, 1H), 7.66 (t, J = 8.0 Hz, 2H), 7.59 – 7.53 (m, 1H), 7.43 (q, J = 7.2 Hz, 1H), 7.39 – 7.23 (m, 12H), 7.16 (d, J = 8.1 Hz, 1H), 6.05 (d, J = 4.0 Hz, 1H), 5.73 – 5.64 (m, 2H), 5.61 (d, J = 2.0 Hz, 1H), 5.49 (d, J = 9.8 Hz, 1H), 5.48 – 5.42 (m, 2H), 5.37 (d, J = 9.8 Hz, 1H), 5.32 (d, J = 6.5 Hz, 2H), 4.93 – 4.78 (m, 4H), 4.70 (s, 2H), 4.67 (s, 2H), 4.38 – 4.26 (m, 4H), 3.97 (dd, J = 11.8, 1.9 Hz, 1H), 3.92 (t, J = 4.4 Hz, 1H), 3.80 (dd, J = 11.9, 1.7 Hz, 1H), 3.46 (s, 3H), 1.55 (s, 3H), 1.34 (s, 3H), 0.92 (s, 9H), 0.12 (s, 6H).

[0307] ^{13}C NMR (126 MHz, CDCl_3) δ 162.65, 162.47, 151.11, 150.83, 140.82, 138.30, 137.99, 137.96, 133.86, 133.80, 132.47, 129.43, 128.78, 128.44, 127.86, 127.85, 127.83, 127.75, 126.24, 126.19, 114.79, 102.45, 102.20, 95.72, 87.20, 85.96, 85.91, 84.56, 84.53, 83.04, 82.98, 82.67, 82.65, 80.91, 77.36, 73.79, 73.75, 72.47, 72.37, 70.44, 70.41, 67.70, 67.66, 66.05, 66.01, 65.98, 61.71, 58.60, 27.21, 26.00, 25.37, 18.46, -5.47.

[0308] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{52}\text{H}_{64}\text{F}_3\text{N}_4\text{O}_{16}\text{PSi}$, $[\text{M}+\text{Na}^+]$: 1139.3668, found 1139.3661.

f. (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl
(((2R,3S,5R)-5-(3-((benzyloxy)methyl)-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methyl) (2-
(trifluoromethyl)benzyl) phosphate



[0309] The compound was synthesized by following GP14 (2-steps, one-pot sequence) using UT-Cy-Nu (110 mg, 0.12 mmol, dr 5:1) in Toluene (1.6 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2- CF_3BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and

- 120 -

imidazole (50 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, hexanes: ethyl acetate gradient (0% to 100%), desired product was isolated as white solid. Yield = 112 mg, 85%, dr = >99:1.

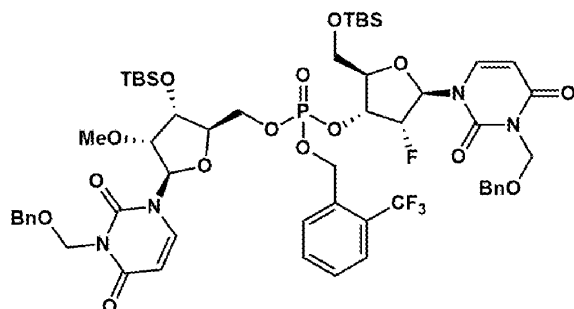
[0310] ³¹P NMR (203 MHz, CDCl₃) δ -1.97, -2.42.

[0311] ¹⁹F NMR (471 MHz, CDCl₃) δ -59.77.

[0312] ¹H NMR (500 MHz, CDCl₃) δ 7.87 (d, *J* = 8.2 Hz, 1H), 7.65 (dd, *J* = 7.8, 3.7 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.40 – 7.35 (m, 4H), 7.35 – 7.27 (m, 6H), 7.25 (s, 1H), 6.31 (dd, *J* = 7.2, 6.2 Hz, 1H), 6.05 (d, *J* = 3.9 Hz, 1H), 5.71 (d, *J* = 8.2 Hz, 1H), 5.52 – 5.42 (m, 4H), 5.34 (d, *J* = 6.5 Hz, 2H), 4.87 (q, *J* = 5.3 Hz, 1H), 4.70 (d, *J* = 7.3 Hz, 4H), 4.35 (dt, *J* = 7.0, 3.6 Hz, 1H), 4.28 (dt, *J* = 5.6, 1.8 Hz, 1H), 4.24 (dd, *J* = 6.2, 3.5 Hz, 2H), 4.00 (dt, *J* = 8.8, 2.1 Hz, 2H), 3.94 (t, *J* = 4.3 Hz, 1H), 3.81 (dd, *J* = 11.9, 1.7 Hz, 1H), 3.47 (s, 3H), 2.25 (ddd, *J* = 13.5, 6.2, 3.6 Hz, 1H), 2.01 (dt, *J* = 13.6, 6.9 Hz, 1H), 1.86 (d, *J* = 1.2 Hz, 3H), 0.93 (s, 9H), 0.88 (s, 10H), 0.13 (s, 6H), 0.07 (d, *J* = 4.7 Hz, 6H).

[0313] HR-MS (Q-TOF, ESI) calculated for C₅₆H₇₆F₃N₄O₁₅PSi₂, [M+Na⁺]: 1211.4427, found 1211.4438.

g. (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyl)dimethylsilyl)oxy)methyl)-4-fluorotetrahydrofuran-3-yl
(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyl)dimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) (2-
(trifluoromethyl)benzyl) phosphate



[0314] The titled compound was synthesized by following GP14 (2-steps, one-pot sequence) using F-UU-Cy-Nu (108 mg, 0.12 mmol, dr 5:1) in Toluene (2 mL), LiOtBu (11.5 mg, 0.14 mmol, 1.2 equiv.), and 2-CF₃BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL

- 121 -

stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (50 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, hexanes: ethyl acetate gradient (0% to 100%), desired product was isolated as white solid. Yield = 97 mg, 75%, dr = >99:1

[0315] ³¹P NMR (203 MHz, CDCl₃) δ -2.03, -2.07.

[0316] ¹⁹F NMR (471 MHz, CDCl₃) δ -59.64, -202.82 (dt, *J* = 51.4, 15.3 Hz).

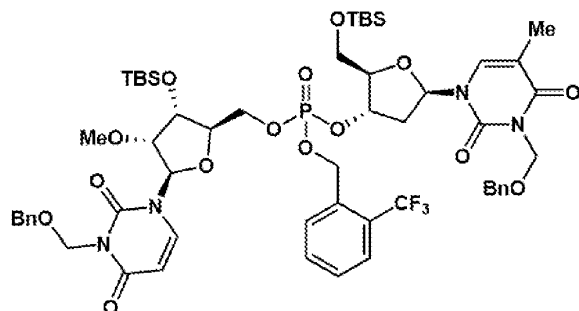
[0317] ¹H NMR (500 MHz, CDCl₃) δ 7.74 (d, *J* = 8.2 Hz, 1H), 7.69 – 7.54 (m, 3H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.39 – 7.22 (m, 11H), 6.09 (dd, *J* = 15.2, 2.6 Hz, 1H), 5.84 (d, *J* = 2.1 Hz, 1H), 5.71 (d, *J* = 8.2 Hz, 1H), 5.65 (d, *J* = 8.2 Hz, 1H), 5.51 – 5.41 (m, 4H), 5.34 – 5.27 (m, 3H), 5.00 – 4.86 (m, 2H), 4.70 (d, *J* = 3.6 Hz, 4H), 4.40 (ddd, *J* = 11.6, 6.2, 2.3 Hz, 1H), 4.31 – 4.21 (m, 2H), 4.17 – 4.00 (m, 3H), 3.83 (dd, *J* = 12.0, 1.9 Hz, 1H), 3.64 (dd, *J* = 4.9, 2.2 Hz, 1H), 3.53 (s, 3H), 0.91 (s, 9H), 0.89 (s, 9H), 0.12 (d, *J* = 1.3 Hz, 6H), 0.09 (s, 3H), 0.06 (s, 3H).

[0318] ¹³C NMR (126 MHz, CDCl₃) δ 162.49, 162.39, 150.88, 150.84, 138.05, 137.98, 137.95, 133.23, 133.16, 132.58, 129.98, 129.22, 128.44, 127.85, 127.82, 127.78, 126.51, 126.47, 126.42, 102.48, 102.13, 92.37, 92.35, 90.82, 90.80, 89.38, 88.08, 87.82, 83.28, 82.31, 82.24, 81.45, 81.39, 72.80, 72.76, 72.68, 72.64, 72.51, 72.39, 70.47, 70.37, 69.43, 66.50, 66.22, 66.17, 60.95, 58.58, 25.97, 25.72, 18.48, 18.17, -4.55, -4.92, -5.44, -5.46.

[0319] HR-MS (Q-TOF, ESI) calculated for C₅₅H₇₃F₄N₄O₁₅PSi₂, [M+Na⁺]: 1215.4177, found 1215.4171.

- 122 -

h. ((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl ((2R,3S,5R)-5-(3-((benzyloxy)methyl)-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl) (2-(trifluoromethyl)benzyl) phosphate



[0320] The compound was synthesized by following GP-14 (2-steps, one-pot sequence) using UT-Cy-Nu (110 mg, 0.12 mmol) in Toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (50 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, hexanes: ethyl acetate gradient (0% to 100%), desired product was isolated as white solid. Yield = 115 mg, 79%, dr = >99:1.

[0321] ³¹P NMR (203 MHz, CDCl₃) δ -1.88.

[0322] ¹⁹F NMR (471 MHz, CDCl₃) δ -59.55.

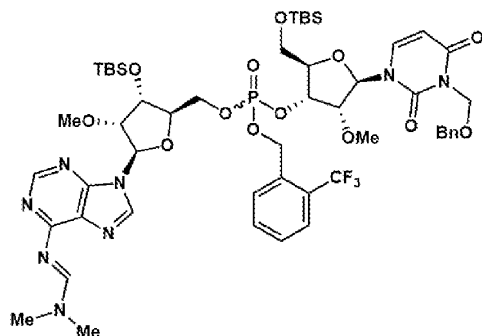
[0323] ¹H NMR (500 MHz, CDCl₃) δ 7.74 – 7.69 (m, 1H), 7.69 – 7.58 (m, 2H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.43 (q, *J* = 1.2 Hz, 1H), 7.41 – 7.37 (m, 4H), 7.37 – 7.28 (m, 5H), 7.28 – 7.23 (m, 2H), 6.37 (dd, *J* = 9.1, 5.2 Hz, 1H), 5.89 (d, *J* = 2.1 Hz, 1H), 5.72 (d, *J* = 8.2 Hz, 1H), 5.53 – 5.44 (m, 4H), 5.32 (qd, *J* = 12.7, 7.0 Hz, 2H), 5.01 (t, *J* = 5.8 Hz, 1H), 4.71 (s, 4H), 4.42 (ddd, *J* = 11.6, 6.0, 2.2 Hz, 1H), 4.29 (q, *J* = 1.9 Hz, 1H), 4.25 (ddd, *J* = 11.6, 5.3, 3.1 Hz, 1H), 4.20 – 4.11 (m, 2H), 3.90 (dd, *J* = 11.5, 2.1 Hz, 1H), 3.85 (dd, *J* = 11.5, 2.2 Hz, 1H), 3.68 (dd, *J* = 4.6, 2.1 Hz, 1H), 3.56 (s, 3H), 2.53 – 2.46 (m, 1H), 2.06 (dddd, *J* = 15.3, 9.1, 5.6, 2.1 Hz, 1H), 1.93 (d, *J* = 1.2 Hz, 3H), 0.92 (d, *J* = 0.7 Hz, 19H), 0.13 (d, *J* = 1.1 Hz, 6H), 0.12 (d, *J* = 4.3 Hz, 6H).

- 123 -

[0324] ^{13}C NMR (126 MHz, CDCl_3) δ 163.46, 162.48, 150.97, 150.88, 138.10, 137.94, 133.65, 133.40, 133.33, 132.57, 130.03, 129.19, 128.41, 128.37, 128.26, 127.80, 127.75, 127.72, 126.48, 126.44, 126.39, 110.57, 102.12, 89.40, 85.96, 85.92, 85.40, 83.27, 81.45, 81.38, 79.70, 79.66, 72.36, 72.32, 70.67, 70.36, 69.45, 66.21, 65.97, 65.93, 63.38, 58.55, 39.39, 39.35, 25.97, 25.72, 18.35, 18.16, 13.31, -4.54, -4.87, -5.33, -5.46.

[0325] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{56}\text{H}_{76}\text{F}_3\text{N}_4\text{O}_{15}\text{PSi}_2$, $[\text{M}+\text{Na}^+]$: 1211.4427, found 1211.4420.

i. (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl
(((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(6-(((E)-
(dimethylamino)methylene)amino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-
yl)methyl) (2-(trifluoromethyl)benzyl) phosphate



[0326] The compound was synthesized by following GP-14 (2-steps, one-pot sequence) using N-dmf-3-TBS-AU-BOM-Cy-Nu (105 mg, 0.12 mmol) in Toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2- CF_3BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (50 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:MeOH gradient (0% to 5%), desired product was isolated as white solid. Yield = 90 mg, 64%, dr = 10:1.

[0327] ^{31}P NMR (162 MHz, CDCl_3) δ -2.10, -2.33.

[0328] ^{19}F NMR (471 MHz, CDCl_3) δ -59.82.

[0329] ^1H NMR (400 MHz, CDCl_3) δ 8.93 (s, 1H), 8.48 (s, 1H), 8.01 (s, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.63 (d, J = 7.9 Hz, 2H), 7.56 – 7.46 (m, 1H), 7.45 – 7.20 (m, 8H), 6.05

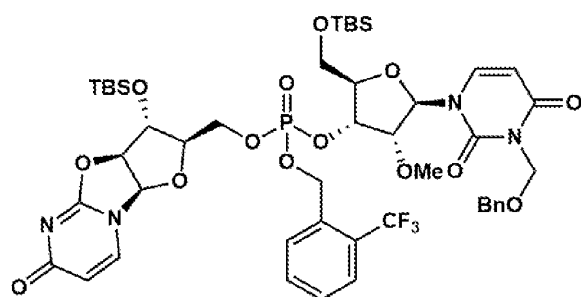
- 124 -

– 5.99 (m, 2H), 5.69 (d, $J = 8.2$ Hz, 1H), 5.47 (q, $J = 9.8$ Hz, 2H), 5.36 – 5.27 (m, 2H), 4.85 (q, $J = 5.3$ Hz, 1H), 4.71 (s, 2H), 4.57 (q, $J = 5.0$ Hz, 1H), 4.45 – 4.36 (m, 2H), 4.33 – 4.20 (m, 3H), 3.94 – 3.85 (m, 2H), 3.74 (dd, $J = 12.0, 1.7$ Hz, 1H), 3.45 (s, 3H), 3.40 (s, 3H), 3.24 (s, 3H), 3.18 (s, 3H), 0.91 (s, 10H), 0.90 (s, 8H), 0.10 (d, $J = 6.8$ Hz, 7H), 0.08 (d, $J = 1.0$ Hz, 6H).

[0330] ^{13}C NMR (101 MHz, CDCl_3) δ 162.70, 159.93, 158.36, 158.31, 152.84, 151.30, 151.09, 140.79, 138.44, 138.02, 133.89, 133.80, 132.42, 129.51, 128.65, 128.42, 127.87, 127.80, 127.56, 126.89, 126.18, 126.13, 126.07, 102.10, 87.63, 87.26, 82.99, 82.92, 82.80, 82.74, 82.72, 82.20, 73.73, 73.68, 72.36, 70.67, 70.40, 67.03, 66.97, 65.93, 61.61, 58.65, 58.58, 41.39, 35.26, 25.98, 25.82, 18.43, 18.23, –4.58, –4.82, –5.48, –5.50.

[0331] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{63}\text{H}_{81}\text{F}_3\text{N}_7\text{O}_{13}\text{PSi}_2$, $[\text{M}+\text{Na}^+]$: 1310.5013, found 1310.5008.

j. Cyclo-UU-BOM-TBS-TRAP



[0332] The compound was synthesized by following GP-14 (2-steps, one-pot sequence) using cyclo-UU-Cy-Nu (90 mg, 0.12 mmol) in Toluene:ACN (5:1, 2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2- CF_3BnOH [43 mg, 0.24 mmol, 2.0 equiv., 1 mL stock solution in anhydrous toluene, (0.24M)] for step 1 and TBSCl (55 mg, 0.36 mmol, 3 equiv.) and imidazole (50 mg, 0.72 mmol, 6 equiv.) in 0.5 mL MeCN for step 2. Column conditions: SiO2-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, hexanes: ethyl acetate gradient (0% to 100%), desired product was isolated as white solid. Yield = 70 mg, 56%, dr = >99:1.

[0333] ^{31}P NMR (162 MHz, CDCl_3) δ –2.50.

[0334] ^{19}F NMR (376 MHz, CDCl_3) δ –59.62.

[0335] ^1H NMR (400 MHz, CDCl_3) δ 7.87 (d, $J = 8.2$ Hz, 1H), 7.66 (dq, $J = 15.0, 7.7$ Hz, 3H), 7.46 (t, $J = 7.6$ Hz, 1H), 7.42 – 7.26 (m, 7H), 6.12 (dd, $J = 19.4, 5.3$ Hz, 2H),

- 125 -

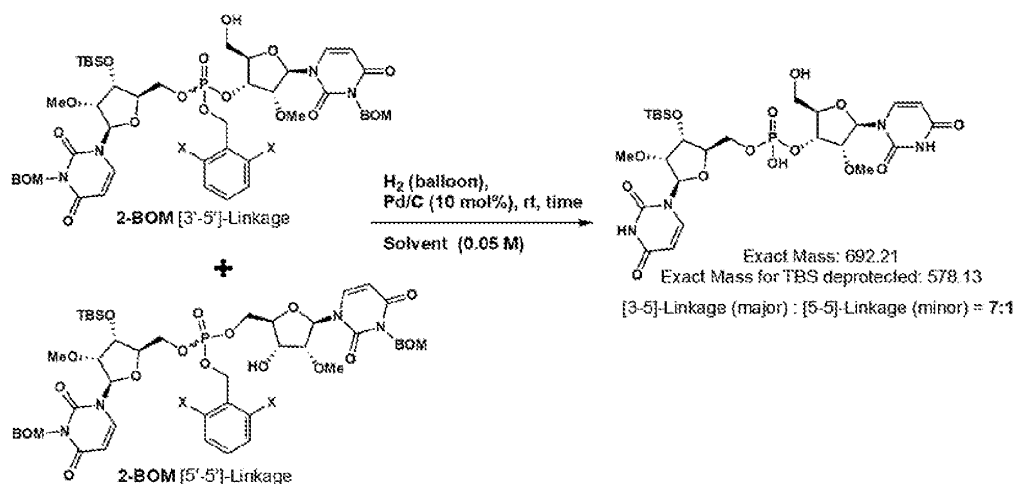
6.04 (d, $J = 7.5$ Hz, 1H), 5.72 (d, $J = 8.1$ Hz, 1H), 5.48 (q, $J = 9.7$ Hz, 2H), 5.36 – 5.27 (m, 2H), 5.12 – 5.06 (m, 1H), 4.88 – 4.80 (m, 1H), 4.69 (s, 2H), 4.56 – 4.50 (m, 1H), 4.36 – 4.26 (m, 2H), 4.07 – 3.82 (m, 5H), 3.40 (d, $J = 2.1$ Hz, 3H), 0.92 (s, 10H), 0.89 (s, 9H), 0.13 (dd, $J = 10.4, 8.5$ Hz, 12H).

[0336] ^{13}C NMR (101 MHz, CDCl_3) δ 171.16, 162.54, 159.36, 151.11, 138.38, 137.85, 134.33, 133.52, 133.43, 132.59, 129.85, 128.78, 128.33, 127.76, 127.72, 126.07, 126.02, 110.85, 102.22, 89.82, 88.77, 86.73, 86.52, 86.45, 83.33, 83.26, 82.51, 82.48, 76.42, 74.70, 74.64, 72.23, 70.31, 66.14, 65.90, 65.85, 62.10, 58.50, 25.90, 25.54, 18.32, 17.87, -4.93, -5.08, -5.55, -5.58.

[0337] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{47}\text{H}_{64}\text{F}_3\text{N}_4\text{O}_{14}\text{PSi}_2$, $[\text{M}+\text{Na}^+]$: 1075.3539, found 1075.3505.

Example 10. Removal of protecting groups via hydrogenation

a. General procedure (GP17) for removal of protecting groups via hydrogenation



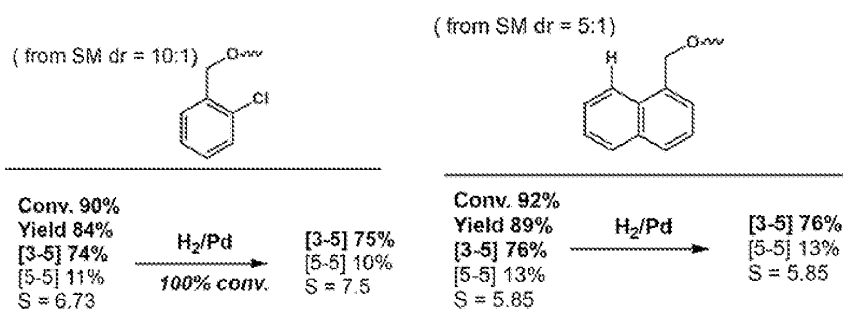
[0338] An oven-dried reaction tube containing a Teflon-coated magnetic stir bar was charged with BOM- and benzyl protected [3'-5'] and [5'-5'] dinucleotide linkages (1 equiv.) and methanol (10:1, 0.05M) and HCO_2H (0.5%). To it was added 10% Pd-C (10%) under N_2 . H_2 gas was introduced to the reaction mixture using a double-folded balloon. Reaction was monitored by LC-MS. Upon completion, the reaction mixture was filtered through a Celite pad and washed with MeOH. All volatiles were evaporated in

vacuo. A solution of internal standard, PO(OPh)₃ (0.025 mmol, 1.0 equiv.) in MeCN (0.5 mL) was added and NMR yields were recorded.

[0339] Results for a study of the rate of protecting group (PG) hydrogenation are shown in Table 5 (with BOM as the nucleoside protecting group):

Table 5 - Rate of Protecting Group (PG) hydrogenation

Entry	Benzyl PG	Solvent	Time	Results
1	2-CF ₃ -Bn	MeOH/THF	2h	incomplete deprotection of both Bn and BOM group
2	2-CF ₃ -Bn	MeOH/THF	18h	complete deprotection of Bn and incomplete deprotection of BOM
3	1-Naphthalene-CH ₂	MeOH/THF	2h	complete deprotection of Bn and incomplete deprotection of BOM
4	1-Naphthalene-CH ₂	MeOH/THF	18h	complete deprotection of Bn and incomplete deprotection of BOM
5	2-Cl-Bn	MeOH	3h	complete deprotection of Bn and BOM



b. Deprotection of dinucleotide blockmer, AA

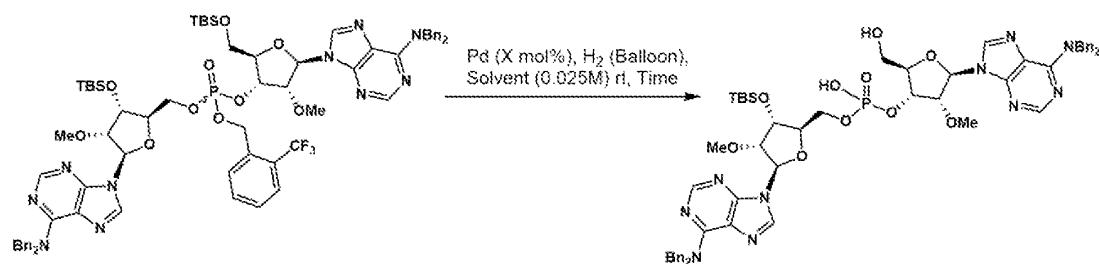
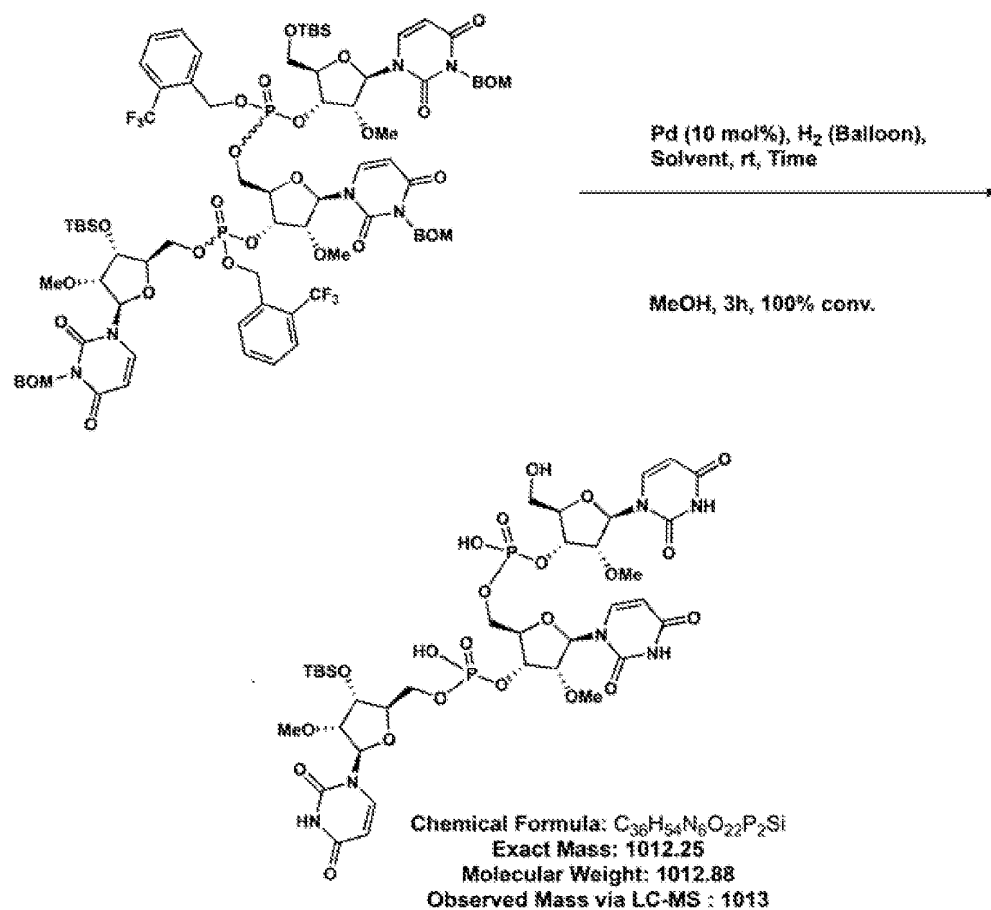


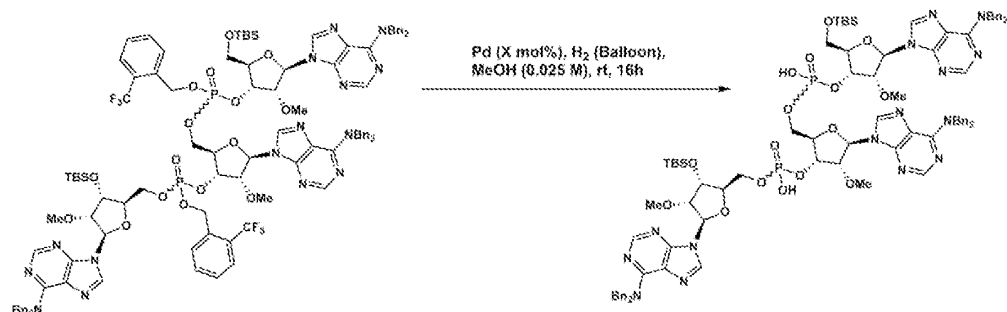
Table 6 - Deprotection of dinucleotide blockmer

Entry	Pd (x mol%)	Solvent/Additives	Remark
1	10%	MeOH	No significant conv.
2	70%	MeOH	3h, 50% conv., two products
3	100%	IPA:H ₂ O (10:1) HCOOH (10 mol%)	2h, 20% conv.
4	100%	IPA:H ₂ O (10:1) AcOH (10 mol%)	2h, ~20% conv.

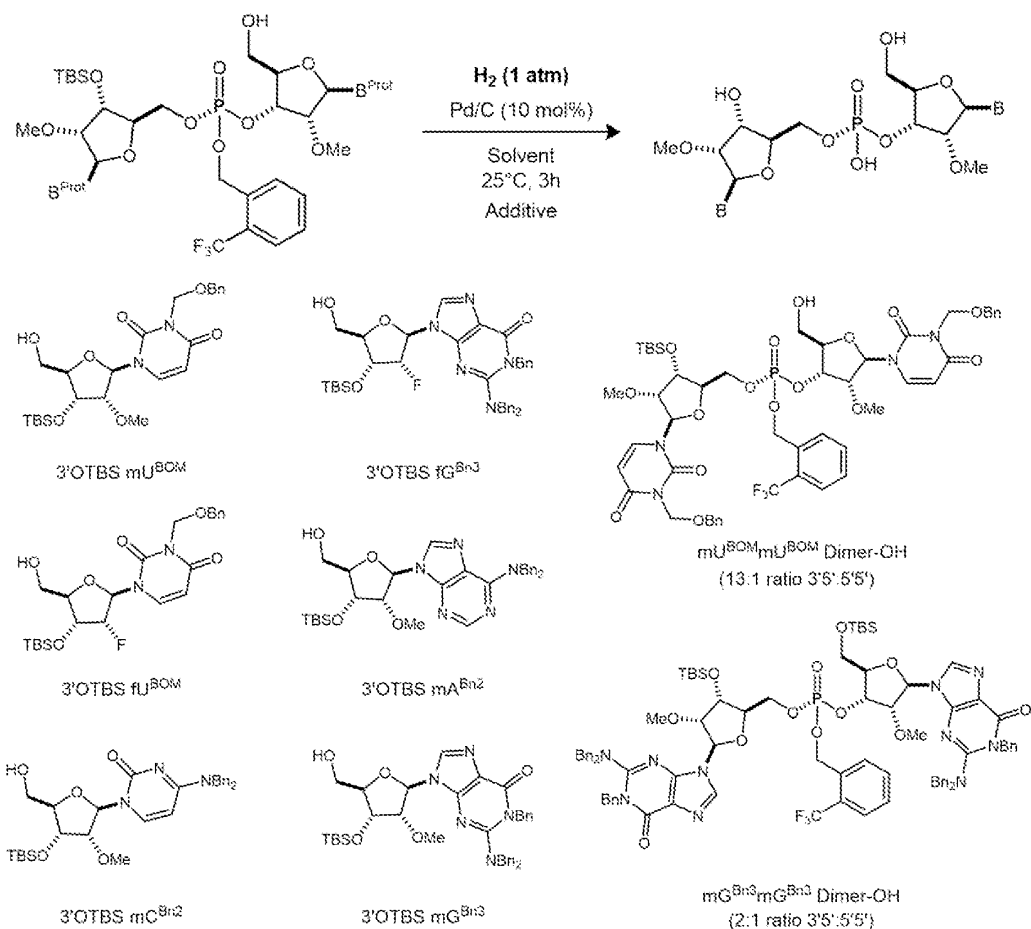
Conversion based on LC-MS and 31P-NMR analysis

c. Deprotection of trinucleotide blockmer, UUU

- 128 -

d. Deprotection of trinucleotide blockmer, AAA

Entry	Pd (x mol%)	Remark
1	10 mol%	No reaction
2	80 mol%	70% Conv. based on ¹⁹ F NMR non-selective deprotection

e. Deprotection Screen of Novel Nucleosides and dimers

- 129 -

Screening set up:

[0340] In an N₂ filled glovebox solid nucleosides were added to doseheads for dispensing. Nucleotides with syrup consistencies were dispensed as stock solutions in acetonitrile, thereafter the acetonitrile was evaporated under a stream of N₂.

[0341] Solid substrates were added to doseheads and dispensed on the Quantos (5 mg basis, with the dinucleotide 3'-5' and 5'-5' mixtures adjusted such that 5 mg of the desired isomer was dispensed). Pd/C (Johnson Matthey JM 10R39) was dispensed at 10 mol% loading on the Quantos. Thereafter solvents were added to the reaction wells neat, or in the case of mixtures the solvents were pre-mixed first then dispensed from Falcon tubes to the appropriate vials using a single channel pipette. The plate was fitted with a slitted mat to which cross-slits had been added with an Exacto-style flat-bladed tool and the plate was removed from the glove box and placed in the CAT96 apparatus, which was then sealed. The CAT96 was programmed to run for 3 hours at 30 psi H₂ atmosphere at RT, then the program was run with a fixed stir rate of 300 rpm.

Preparation of analytical samples:

[0342] The plate was unsealed and a 20 µL aliquot from each vial was taken then added to an appropriately labeled 0.45 µm filter vial using a multichannel pipette. The samples were filtered into a 96-well collection plate and 10 µL sample aliquots from each well was added to the appropriate corresponding well in a 96-well labeled UPLC sample plate using a multi-channel pipette.

[0343] 20 mL of a 2.5 mM stock solution of internal standard IS-01 (13.6 mg) using MeOH was prepared. 400 µL of the internal standard stock solution was added to each filter vial using a multi-channel pipette and the filter vial tops were inserted to filter the samples. The samples were analyzed using LCMS (the OA high pH fast method at 2.0 µL injection volume). Each sample was given a lot number reflecting its position in the reaction plates and directly correlated with that position on the analytical plates, e.g., CAX-D01021-012-XXX where XXX included A01-H12.

[0344] Screening Results are shown in Table 7A and Table 7B (X = Mixture of partial deprotected species; 0 = Mixture of full and partial deprotected product; + = 95+% conversion to fully deprotected product)

- 130 -

Table 7A – Screening Results with No Additive

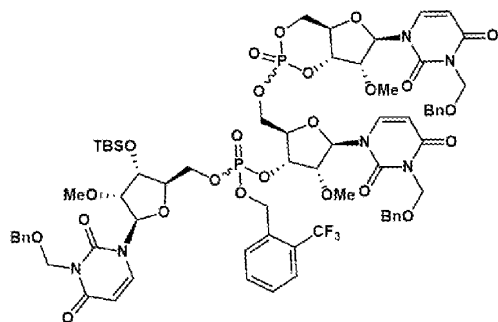
Nucleoside	EtOH	IPA	THF	EtOAc	10:1 EtOH:H ₂ O	10:1 IPA:H ₂ O
3' OTBS mU ^{BOM}	+	+	+	+	+	+
3' OTBS fG ^{Bn3}	X	X	X	X	X	X
3' OTBS fU ^{BOM}	+	+	0	+	+	0
3' OTBS mA ^{Bn2}	X	X	X	X	X	X
3' OTBS mC ^{Bn2}	X	X	X	X	X	X
3' OTBS mG ^{Bn3}	X	X	X	X	X	X
mU ^{BOM} mU ^{BOM} Dimer-OH	0	0	0	0	0	0
mG ^{Bn3} mG ^{Bn3} Dimer-OH	0	0	0	0	0	0

Table 7B – Screening Results with 0.5 mol% Formic Acid Additive

Nucleoside	EtOH	IPA	THF	EtOAc	10:1 EtOH:H ₂ O	10:1 IPA:H ₂ O
3' OTBS mU ^{BOM}	+	+	+	+	+	+
3' OTBS fG ^{Bn3}	X	X	X	X	X	X
3' OTBS fU ^{BOM}	0	0	0	+	+	+
3' OTBS mA ^{Bn2}	X	X	X	X	X	X
3' OTBS mC ^{Bn2}	X	X	X	X	X	X
3' OTBS mG ^{Bn3}	X	X	X	X	X	X
mU ^{BOM} mU ^{BOM} Dimer-OH	0	0	0	0	0	0
mG ^{Bn3} mG ^{Bn3} Dimer-OH	0	0	0	0	0	0

Example 11. Synthesis of cyclic trimers**Cyclic trimer: UUU**

a. (2R,3R,4R,5R)-5-(3-(((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-((((4aR,6R,7R,7aR)-6-(3-(((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl (((2R,3R,4R,5R)-5-(3-(((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyl)dimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) (2-(trifluoromethyl)benzyl) phosphate



[0345] The compound was synthesized by following GP-15 (2-steps, one-pot sequence) using UU-Cy-Nu (200 mg, 0.22 mmol) in Toluene (3 mL), LiOtBu (26 mg, 0.33 mmol, 1.5 equiv.), and 2-CF₃BnOH [58 mg, 0.33 mmol, 1.5 equiv., 2 mL stock solution in anhydrous toluene, (0.33M)] for step 1 and U-BOM-Cl-CP (160 mg, 0.35 mmol, 1.6 equiv.) in 1.2 mL MeCN for step 2. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:MeCN (2% iPrOH) gradient (0% to 80%). Fractions were analyzed via LC-MS and the fractions containing desired product was pooled, concentrated to afford the titled compound as white solid with 90% purity. Overall yield (2 steps) = 180 mg, 57%.

[0346] ³¹P NMR (203 MHz, CDCl₃) δ -1.87, -4.01.

[0347] ¹⁹F NMR (471 MHz, CDCl₃) δ -59.70.

[0348] ¹H NMR (500 MHz, CDCl₃) δ 7.67 (dd, *J* = 11.6, 7.8 Hz, 2H), 7.60 (q, *J* = 7.5 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.52 – 7.41 (m, 3H), 7.40 – 7.27 (m, 15H), 7.11 (d, *J* = 8.2 Hz, 1H), 5.93 (d, *J* = 3.5 Hz, 1H), 5.91 – 5.86 (m, 1H), 5.86 – 5.74 (m, 3H), 5.66 (d, *J* = 8.2 Hz, 1H), 5.55 – 5.40 (m, 8H), 5.35 (d, *J* = 6.5 Hz, 2H), 4.86 (q, *J* = 6.1 Hz, 1H), 4.78 (dd, *J* = 10.0, 5.1 Hz, 1H), 4.74 – 4.59 (m, 8H), 4.52 – 4.43 (m, 3H), 4.42 (dd, *J* =

- 132 -

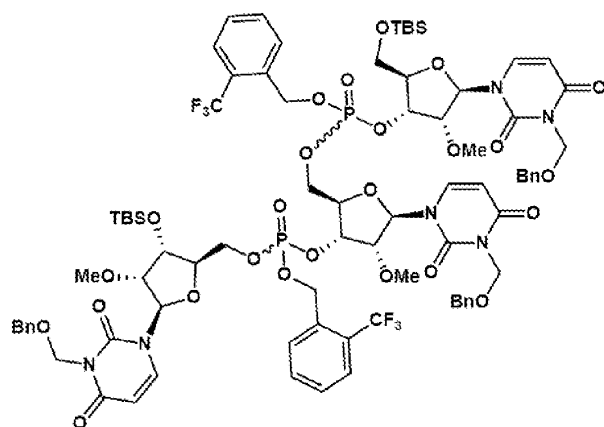
9.4, 2.9 Hz, 1H), 4.38 (dt, $J = 6.1, 2.8$ Hz, 1H), 4.27 (ddd, $J = 10.5, 9.0, 5.4$ Hz, 2H), 4.18 – 4.06 (m, 4H), 4.00 (dd, $J = 5.2, 3.5$ Hz, 1H), 3.67 (dd, $J = 4.4, 2.3$ Hz, 1H), 3.56 (s, 3H), 3.52 (s, 3H), 3.48 (s, 3H), 0.89 (s, 9H), 0.08 (d, $J = 11.2$ Hz, 6H).

[0349] ^{13}C NMR (126 MHz, CDCl_3) δ 162.58, 162.45, 162.17, 151.00, 150.96, 150.39, 139.62, 138.38, 138.02, 137.96, 137.92, 132.60, 129.62, 129.11, 128.51, 128.48, 127.95, 127.90, 127.89, 127.87, 127.84, 127.75, 126.43, 102.97, 102.68, 102.09, 95.60, 89.41, 88.52, 83.24, 81.64, 81.47, 80.39, 80.22, 80.16, 78.09, 73.18, 72.66, 72.47, 72.44, 70.84, 70.78, 70.61, 70.47, 70.41, 69.55, 66.34, 59.46, 58.72, 58.58, 53.57, 31.07, 18.20, -4.54, -4.85.

[0350] HR-MS (Q-TOF, ESI) calculated for $\text{C}_{68}\text{H}_{81}\text{F}_3\text{N}_6\text{O}_{24}\text{P}_2\text{Si}$, $[\text{M}+\text{H}^+]$: 1513.4572, found 1513.4565.

TBS-trap-UUU-BOM-[3'-5']-trimer-blockmer

b. (2R,3R,4R,5R)-5-(3-(((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-((((((2R,3R,4R,5R)-5-(3-(((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyl dimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl)oxy)((2-(trifluoromethyl)benzyl)oxy)phosphoryl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl (((2R,3R,4R,5R)-5-(3-(((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(((tert-butyl dimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) (2-(trifluoromethyl)benzyl) phosphate



[0351] The compound was synthesized by following GP-16 (4-steps, one-pot sequence) using UU-Cy-Nu (110 mg, 0.12 mmol) in toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2- CF_3BnOH [32 mg, 0.18 mmol, 1.5 equiv., 1 mL stock

- 133 -

solution in anhydrous toluene, (0.18M)] for step 1 and U-BOM-Cl-CP (88 mg, 0.19 mmol, 1.6 equiv.) in 0.6 mL MeCN for step 2. LiOtBu (24 mg, 0.30 mmol, 2.5 equiv.), and 2-CF₃BnOH [64 mg, 0.36 mmol, 3 equiv., 1 mL stock solution in anhydrous toluene, (0.36M)] for step 3 and TBSCl (109 mg, 0.72 mmol, 6 equiv.) and imidazole (98 mg, 1.44 mmol, 12 equiv.) in 1 mL MeCN for step 4. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:Ethyl acetate (2% MeOH) gradient (0% to 80%). Fractions were analyzed via LC-MS and the fractions containing desired product was pooled, concentrated to afford the titled compound as white solid with 90% purity. Overall yield (4 steps-sequence) = 106 mg, 49%.

[0352] ³¹P NMR (203 MHz, CDCl₃) δ -1.66, -2.20.

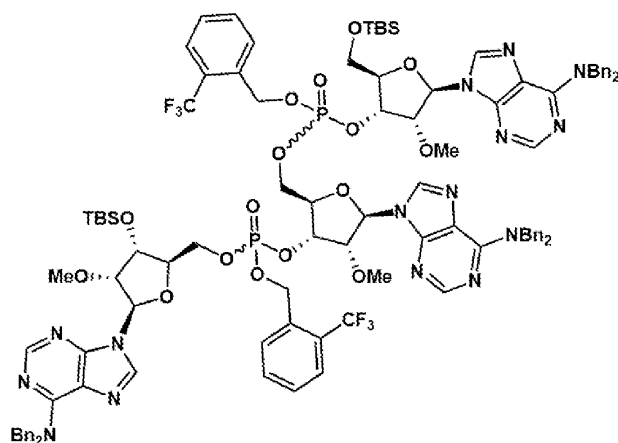
[0353] ¹H NMR (500 MHz, CDCl₃) δ 7.86 (d, *J* = 8.2 Hz, 1H), 7.65 (dd, *J* = 11.7, 7.7 Hz, 5H), 7.58 (td, *J* = 7.8, 4.5 Hz, 3H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.47 – 7.40 (m, 4H), 7.38 – 7.28 (m, 14H), 7.28 – 7.23 (m, 6H), 6.06 (d, *J* = 4.3 Hz, 1H), 5.92 (d, *J* = 4.2 Hz, 1H), 5.83 (d, *J* = 2.2 Hz, 1H), 5.74 – 5.61 (m, 4H), 5.52 – 5.29 (m, 12H), 4.90 – 4.81 (m, 2H), 4.69 (d, *J* = 4.9 Hz, 7H), 4.47 – 4.18 (m, 7H), 4.17 – 4.05 (m, 3H), 3.98 (dd, *J* = 11.9, 2.0 Hz, 1H), 3.91 – 3.87 (m, 2H), 3.82 (dd, *J* = 11.9, 1.7 Hz, 1H), 3.66 – 3.61 (m, 1H), 3.52 (s, 3H), 3.43 (s, 3H), 3.42 (s, 3H), 0.93 (s, 9H), 0.88 (s, 9H), 0.13 (s, 6H), 0.09 (s, 3H), 0.06 (s, 3H).

[0354] ¹³C NMR (126 MHz, CDCl₃) δ 162.61, 162.50, 162.30, 151.12, 151.02, 150.89, 138.26, 138.22, 137.97, 137.96, 137.90, 133.42, 132.64, 132.59, 130.12, 129.64, 129.24, 129.14, 128.46, 128.45, 127.87, 127.85, 127.83, 126.48, 126.44, 126.40, 102.72, 102.32, 102.11, 89.45, 88.31, 87.02, 83.23, 83.11, 83.05, 82.61, 81.54, 81.48, 81.33, 81.31, 80.60, 74.35, 74.31, 73.46, 73.42, 72.43, 72.39, 70.46, 70.43, 70.38, 69.46, 66.52, 66.33, 66.16, 66.12, 65.97, 61.85, 58.72, 58.62, 58.56, 25.99, 25.73, 18.46, 18.17, -4.56, -4.88, -5.46.

[0355] HR-MS (Q-TOF, ESI) calculated for C₈₂H₁₀₂F₆N₆O₂₅P₂Si₂, [M+Na⁺]: 1825.5705, found 1825.5695.

- 134 -

c. ((2R,3R,4R,5R)-3-((tert-butyl dimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methyl ((2R,3R,4R,5R)-2-((((((2R,3R,4R,5R)-2-(((tert-butyl dimethylsilyl)oxy)methyl)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-3-yl)oxy)((2-(trifluoromethyl)benzyl)oxy)phosphoryl)oxy)methyl)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-3-yl) (2-(trifluoromethyl)benzyl) phosphate



[0356] The compound was synthesized by following GP-16 (4-steps, one-pot sequence) using AA-Bn₂-Cy-Nu (130 mg, 0.12 mmol) in toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [32 mg, 0.18 mmol, 1.5 equiv., 1 mL stock solution in anhydrous toluene, (0.18M)] for step 1 and A-Bn₂-Cl-CP (104 mg, 0.19 mmol, 1.6 equiv.) in 0.6 mL MeCN for step 2. LiOtBu (24 mg, 0.30 mmol, 2.5 equiv.), and 2-CF₃BnOH [64 mg, 0.36 mmol, 3 equiv., 1 mL stock solution in anhydrous toluene, (0.36M)] for step 3 and TBSCl (109 mg, 0.72 mmol, 6 equiv.) and imidazole (98 mg, 1.44 mmol, 12 equiv.) in 1 mL MeCN for step 4. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:Ethyl acetate (2% MeOH) gradient (0% to 80%). Fractions were analyzed via LC-MS and the fractions containing desired product was pooled, concentrated to afford the titled compound as white solid with 90% purity. Overall yield (4 steps-sequence) = 100 mg, 40%. Mass confirmed by LC-MS.

[0357] ³¹P NMR (203 MHz, CD₃CN) δ -1.90, -2.28.

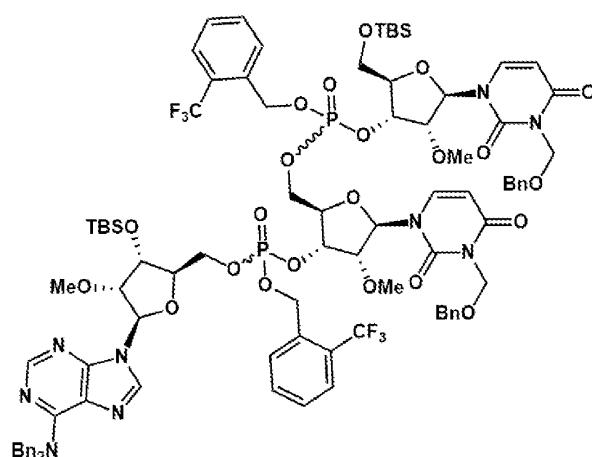
[0358] ¹⁹F NMR (471 MHz, CD₃CN) δ -59.96, -60.01.

- 135 -

[0359] ^1H NMR (500 MHz, CD_3CN) δ 8.26 (d, $J = 2.9$ Hz, 1H), 8.24 (s, 2H), 8.07 (s, 1H), 8.02 (d, $J = 4.8$ Hz, 1H), 8.00 (s, 1H), 7.66 (ddd, $J = 10.3, 5.9, 2.3$ Hz, 4H), 7.53 (q, $J = 8.1$ Hz, 2H), 7.42 (dt, $J = 15.1, 7.6$ Hz, 2H), 7.31 – 7.12 (m, 35H), 6.08 (d, $J = 4.4$ Hz, 1H), 6.05 (d, $J = 6.5$ Hz, 1H), 6.01 (d, $J = 5.8$ Hz, 1H), 5.45 (s, 4H), 5.29 (t, $J = 7.8$ Hz, 6H), 5.13 (ddd, $J = 7.5, 4.8, 2.8$ Hz, 2H), 4.91 (s, 5H), 4.69 (dt, $J = 16.8, 5.0$ Hz, 3H), 4.43 – 4.30 (m, 7H), 4.19 (dd, $J = 7.1, 3.8$ Hz, 2H), 3.76 – 3.67 (m, 2H), 3.37 (s, 3H), 3.32 (s, 3H), 3.31 (s, 3H), 0.91 (s, 9H), 0.83 (s, 9H), 0.11 (d, $J = 3.7$ Hz, 6H), -0.00 (d, $J = 1.3$ Hz, 6H).

[0360] ^{13}C NMR (126 MHz, CD_3CN) δ 169.09, 155.37, 155.34, 155.31, 152.94, 152.89, 152.80, 151.58, 151.32, 151.26, 138.77, 138.68, 138.28, 134.48, 133.24, 130.58, 130.53, 129.46, 129.41, 129.06, 129.02, 128.99, 128.09, 128.03, 127.64, 127.61, 126.64, 126.59, 126.55, 125.96, 123.78, 120.59, 120.52, 120.32, 117.86, 87.36, 86.87, 85.87, 84.38, 83.37, 82.96, 81.98, 81.07, 75.72, 74.97, 70.92, 67.71, 67.09, 66.33, 62.93, 58.67, 58.53, 58.37, 31.77, 25.85, 25.81, 25.64, 18.50, 18.24, -4.93, -5.12, -5.67, -5.76.

d. (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-((((((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyl dimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl)oxy)((2-(trifluoromethyl)benzyl)oxy)phosphoryl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl (((2R,3R,4R,5R)-3-((tert-butyl dimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methyl) (2-(trifluoromethyl)benzyl) phosphate



- 136 -

[0361] The compound was synthesized by following GP-16 (4-steps, one-pot sequence) using Bn2-AU-Cy-Nu (120 mg, 0.12 mmol) in toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [32 mg, 0.18 mmol, 1.5 equiv., 1 mL stock solution in anhydrous toluene, (0.18M)] for step 1 and U-BOM-Cl-CP (88 mg, 0.19 mmol, 1.6 equiv.) in 0.6 mL MeCN for step 2. LiOtBu (24 mg, 0.30 mmol, 2.5 equiv.), and 2-CF₃BnOH [64 mg, 0.36 mmol, 3 equiv., 1 mL stock solution in anhydrous toluene, (0.36M)] for step 3 and TBSCl (109 mg, 0.72 mmol, 6 equiv.) and imidazole (98 mg, 1.44 mmol, 12 equiv.) in 1 mL MeCN for step 4. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:Ethyl acetate (2% MeOH) gradient (0% to 80%). Fractions were analyzed via LC-MS and the fractions containing desired product was pooled, concentrated to afford the titled compound as white solid with 90% purity. Overall yield (4 steps-sequence) = 60 mg, 26%.

[0362] Alternatively, the crude product was purified with reversed-phase C-18 column chromatography with the aid of Next-Gen automated system. The crude product was loaded on pre-packed 25g, C-18 column by dissolving in minimum amount of DMSO, Water:ACN gradient (0% to 100%). Fractions were analyzed via LC-MS and the fractions containing desired product was pooled, concentrated to afford the titled compound as white solid with 90% purity. Overall yield (4 steps-sequence) = 105 mg, 46%. Mass confirmed by LC-MS.

[0363] ³¹P NMR (203 MHz, CDCl₃) δ -1.85, -2.30.

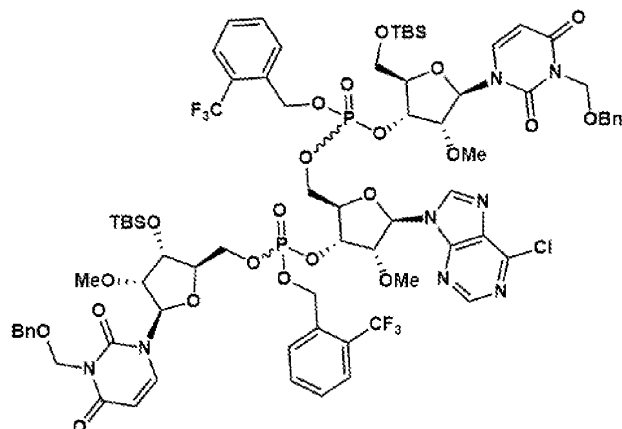
[0364] ¹H NMR (500 MHz, CDCl₃) δ 7.92 (d, *J* = 4.2 Hz, 1H), 7.90 – 7.84 (m, 1H), 7.62 (ddd, *J* = 17.2, 7.7, 1.3 Hz, 4H), 7.54 (dd, *J* = 8.3, 6.7 Hz, 1H), 7.50 – 7.24 (m, 26H), 6.07 (dd, *J* = 11.4, 4.0 Hz, 2H), 5.75 – 5.61 (m, 2H), 5.52 – 5.36 (m, 6H), 5.36 – 5.22 (m, 5H), 5.02 – 4.79 (m, 4H), 4.85 (ddt, *J* = 17.5, 7.1, 5.0 Hz, 2H), 4.74 – 4.63 (m, 5H), 4.37 – 4.19 (m, 7H), 4.00 – 3.92 (m, 1H), 3.92 – 3.78 (m, 3H), 3.47 (s, 3H), 3.43 (s, 3H), 3.39 (s, 3H), 0.91 (d, *J* = 2.7 Hz, 18H), 0.13 – 0.09 (m, 12H).

[0365] ¹³C NMR (126 MHz, CDCl₃) δ 162.62, 162.34, 155.06, 152.78, 151.10, 151.05, 151.03, 150.65, 138.31, 138.23, 137.98, 137.96, 137.92, 137.58, 133.64, 133.48, 133.42, 132.60, 132.46, 130.03, 129.51, 129.11, 128.75, 128.71, 128.69, 128.43, 127.98, 127.84, 127.81, 127.41, 126.39, 126.35, 126.22, 126.18, 126.13, 126.09, 125.27, 125.24, 123.09, 123.07, 120.41, 102.67, 102.27, 88.06, 87.58, 87.01, 83.12, 83.06, 82.65, 82.63,

- 137 -

82.59, 82.40, 82.34, 81.37, 81.34, 80.69, 80.64, 74.32, 74.28, 73.39, 73.36, 72.37, 70.50, 70.42, 67.03, 66.99, 66.42, 66.12, 66.08, 61.85, 58.74, 58.71, 58.68, 58.66, 58.61, 25.98, 25.81, 18.44, 18.39, 18.22, -4.58, -4.82, -5.46, -5.49.

e. (2R,3R,4R,5R)-2-((((((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl)oxy)((2-(trifluoromethyl)benzyl)oxy)phosphoryl)oxy)methyl)-5-(6-chloro-9H-purin-9-yl)-4-methoxytetrahydrofuran-3-yl (((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) (2-(trifluoromethyl)benzyl) phosphate



[0366] The compound was synthesized by following GP-16 (4-steps, one-pot sequence) using UA(6Cl)-Cy-Nu (101 mg, 0.12 mmol) in toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [32 mg, 0.18 mmol, 1.5 equiv., 1 mL stock solution in anhydrous toluene, (0.18M)] for step 1 and U-BOM-Cl-CP (89 mg, 0.19 mmol, 1.6 equiv.) in 0.6 mL MeCN for step 2. LiOtBu (24 mg, 0.30 mmol, 2.5 equiv.), and 2-CF₃BnOH [64 mg, 0.36 mmol, 3 equiv., 1 mL stock solution in anhydrous toluene, (0.36M)] for step 3 and TBSCl (109 mg, 0.72 mmol, 6 equiv.) and imidazole (98 mg, 1.44 mmol, 12 equiv.) in 1 mL MeCN for step 4. Column conditions: SiO₂-10g column, Next-Gen automated system, wet loading by dissolving in minimum amount of DCM, DCM:Ethyl acetate (2% MeOH) gradient (0% to 80%). Fractions were analyzed via LC-MS and the fractions containing desired product was pooled, concentrated to afford the

- 138 -

titled compound as white solid with 90% purity. Overall yield (4 steps-sequence) = 100 mg, 40%, dr 4:1. Mass confirmed by LC-MS.

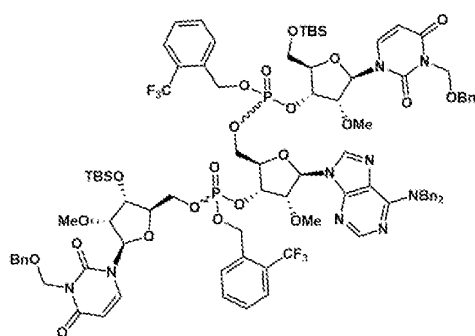
[0367] ^{31}P NMR (203 MHz, CDCl_3) δ -1.16 (P1, minor diastereomer), -1.51 (P1, major diastereomer), -2.36, (P2, major diastereomer), -2.67 (P2, minor diastereomer).

[0368] ^1H NMR (500 MHz, CDCl_3) for mixture of diastereomers: δ 8.69 (s, 1H), 8.32 (s, 1H), 7.88 – 7.78 (m, 2H), 7.65 (q, $J = 7.4$ Hz, 4H), 7.62 – 7.48 (m, 8H), 7.48 – 7.39 (m, 4H), 7.39 – 7.26 (m, 16H), 7.26 – 7.18 (m, 3H), 6.16 – 6.02 (m, 3H), 5.87 – 5.81 (m, 2H), 5.76 – 5.68 (m, 2H), 5.66 (dd, $J = 8.2, 1.6$ Hz, 1H), 5.57 – 5.25 (m, 15H), 5.18 (tdd, $J = 7.8, 4.8, 2.7$ Hz, 2H), 4.94 – 4.83 (m, 2H), 4.73 – 4.62 (m, 8H), 4.56 – 4.48 (m, 2H), 4.48 – 4.32 (m, 5H), 4.32 – 4.23 (m, 3H), 4.18 – 4.03 (m, 6H), 4.02 – 3.89 (m, 3H), 3.81 (dd, $J = 11.9, 1.7$ Hz, 1H), 3.66 (p, $J = 2.5$ Hz, 2H), 3.52 (s, 6H), 3.44 (s, 4H), 3.34 (s, 4H), 0.89 (d, $J = 3.6$ Hz, 27H), 0.16 – 0.04 (m, 18H).

[0369] ^{13}C NMR (126 MHz, CDCl_3) for mixture of diastereomers δ 162.61, 162.46, 152.33, 152.31, 151.69, 151.65, 151.42, 151.17, 151.11, 150.88, 150.86, 144.05, 144.01, 138.35, 138.26, 138.22, 138.20, 137.96, 137.89, 137.76, 133.41, 132.51, 132.44, 132.41, 129.80, 129.78, 129.57, 129.10, 129.04, 128.97, 128.94, 128.42, 128.41, 127.83, 127.78, 126.34, 125.23, 123.05, 120.87, 102.46, 102.28, 102.09, 102.04, 89.59, 89.47, 88.28, 87.02, 86.75, 86.32, 83.26, 83.22, 83.13, 83.07, 82.88, 82.59, 82.57, 82.13, 82.08, 82.03, 81.49, 81.43, 81.00, 80.93, 80.90, 75.08, 74.67, 74.63, 74.49, 74.45, 72.41, 72.38, 72.33, 70.40, 70.36, 69.46, 69.44, 68.25, 66.37, 66.32, 66.09, 66.05, 62.07, 61.89, 60.49, 59.02, 58.95, 58.91, 58.86, 58.61, 58.55, 25.96, 25.93, 25.71, 25.70, 21.16, 18.41, 18.16, 14.31, -4.56, -4.90, -5.50, -5.54, -5.57.

- 139 -

f. (2R,3R,4R,5R)-2-((((((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl)oxy)((2-(trifluoromethyl)benzyl)oxy)phosphoryl)oxy)methyl)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-3-yl (((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) (2-(trifluoromethyl)benzyl) phosphate



[0370] The compound was synthesized by following GP-16 (4-steps, one-pot sequence) using UA-Bn2-Cy-Nu (120 mg, 0.12 mmol) in toluene (2 mL), LiOtBu (15 mg, 0.18 mmol, 1.5 equiv.), and 2-CF₃BnOH [32 mg, 0.18 mmol, 1.5 equiv., 1 mL stock solution in anhydrous toluene, (0.18M)] for step 1 and U-BOM-Cl-CP (88 mg, 0.19 mmol, 1.6 equiv.) in 0.6 mL MeCN for step 2. LiOtBu (24 mg, 0.30 mmol, 2.5 equiv.), and 2-CF₃BnOH [64 mg, 0.36 mmol, 3 equiv., 1 mL stock solution in anhydrous toluene, (0.36M)] for step 3 and TBSCl (109 mg, 0.72 mmol, 6 equiv.) and imidazole (98 mg, 1.44 mmol, 12 equiv.) in 1 mL MeCN for step 4. The crude product was purified with reversed-phase C-18 column chromatography with the aid of Next-Gen automated system. The crude product was loaded on pre-packed 25g, C-18 column by dissolving in minimum amount of DMSO, Water:ACN gradient (0% to 100%). Fractions were analyzed via LC-MS and the fractions containing desired product was pooled, concentrated to afford the titled compound as white solid with 90% purity. Overall yield (4 steps-sequence) = 100 mg, 44%. Mass confirmed by LC-MS.

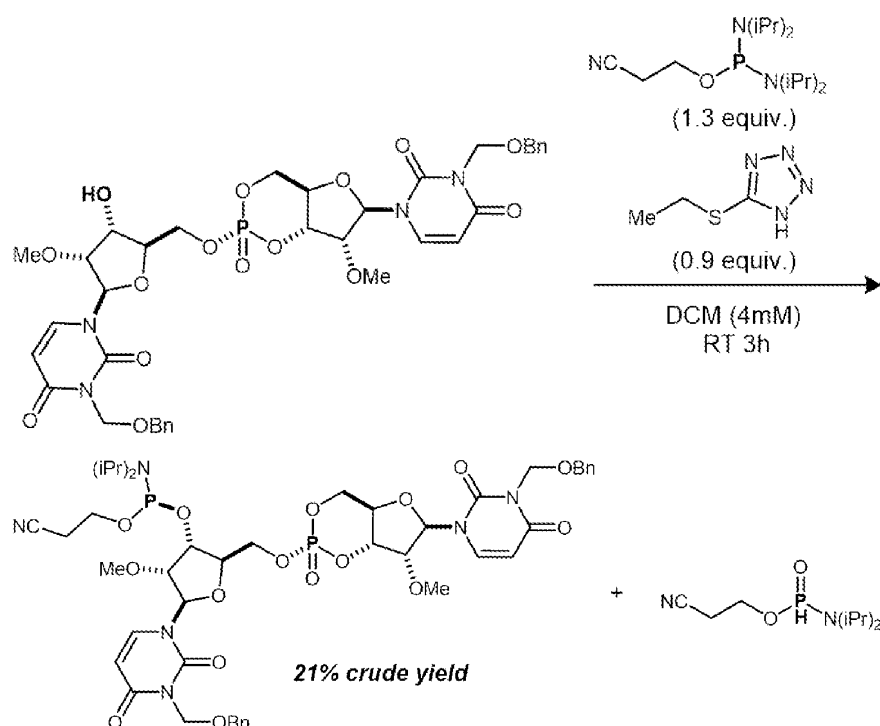
[0371] ³¹P NMR (203 MHz, CD₃CN) δ -1.95, -2.21.

[0372] ¹⁹F NMR (471 MHz, CD₃CN) δ -59.86, -59.99.

- 140 -

[0373] ^1H NMR (500 MHz, CD_3CN) δ 8.26 (s, 1H), 8.06 (s, 1H), 7.78 (dd, $J = 8.3, 2.8$ Hz, 1H), 7.66 (ddd, $J = 30.5, 14.5, 7.6$ Hz, 5H), 7.57 – 7.41 (m, 4H), 7.35 – 7.16 (m, 21H), 6.08 (d, $J = 4.4$ Hz, 1H), 6.00 (d, $J = 5.2$ Hz, 1H), 5.85 (d, $J = 4.9$ Hz, 1H), 5.72 – 5.65 (m, 1H), 5.61 (dd, $J = 8.1, 5.0$ Hz, 1H), 5.45 (s, 1H), 5.39 – 5.31 (m, 6H), 5.28 (dd, $J = 17.2, 8.8$ Hz, 3H), 4.95 – 4.87 (m, 2H), 4.67 – 4.53 (m, 5H), 4.40 – 4.22 (m, 7H), 4.18 (q, $J = 4.8$ Hz, 1H), 4.00 – 3.85 (m, 3H), 3.81 (dd, $J = 11.9, 2.2$ Hz, 1H), 3.41 – 3.31 (m, 9H), 0.93 – 0.86 (m, 18H), 0.13 – 0.08 (m, 12H).

Example 12. Synthesis of Cyclic Nucleotide Amidite



Procedure A

[0374] In an N_2 filled glovebox, a vial (Vessel A) was charged with 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-hydroxy-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (0.200g, 250 μmol , 1.0 eq.), 3-((bis(diisopropylamino)phosphaneyl)oxy)propanenitrile (103 μL , 325 μmol , 1.3 eq.) and a stir bar. The reaction in Vessel A was sealed then removed from the glovebox and DCM

- 141 -

(4 mL) was added to the vessel then allowed to cool to 0 °C over the course of 10 minutes.

[0375] In a separate vial (Vessel B) outside of the glovebox, 5-(ethylthio)-1H-tetrazole (29.3 mg, 225 μ mol, 0.9 eq.) was added as a solid, and Vessel B is then purged and sparged 5 times with N₂. Thereafter DCM (1.2 mL, 47 mM total) was added to Vessel B and the solution in Vessel B was then transferred to Vessel A dropwise at 0 °C. The reaction in Vessel A was warmed to room temperatures and stirred for 3 hours or until starting material was consumed. Vessel A was then opened to air and the solvent was evaporated. The compounds was purified by column chromatography using 100% ethyl acetate. 0.138g of product mixture was isolated with 2-cyanoethyl N,N-diisopropylphosphonamidate as a light-yellow oil. Actual amount of product in this mixture was 60mg, 60 μ mol, 24% yield. 2-cyanoethyl N,N-diisopropylphosphonamidate (hydrolyzed 2-Cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite hydrolyzed) at 14ppm was observes as difficult to separate from the product.

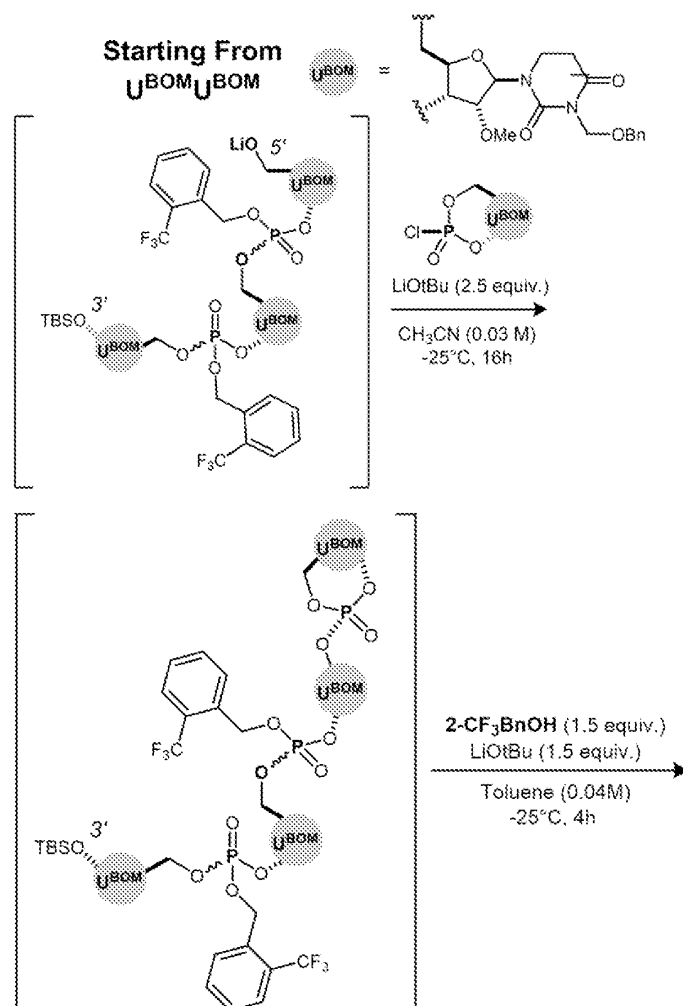
[0376] ³¹P NMR (203 MHz, CD₃CN) δ -1.11.

Procedure B

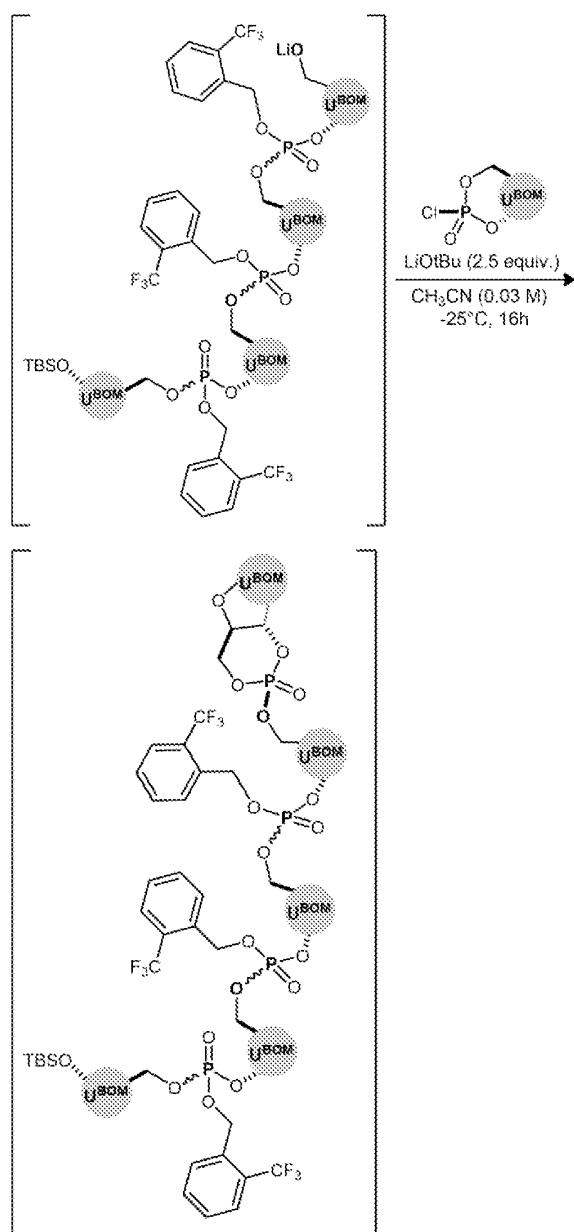
[0377] A heat dried round bottom flask (Vessel A) was charged with 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-hydroxy-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (0.200 g, 250 μ mol, 1.0 eq.), 3-((bis(diisopropylamino)phosphaneyl)oxy)propanenitrile (103 μ L, 325 μ mol, 1.3 eq.), a dry pack of sieves, and a stir bar was added to the reaction vessel. Thereafter Vessel A was sealed, then purged and sparged 5x with N₂. Anhydrous DCM (4 mL) was then added to Vessel A and allowed to cool to 0 °C over the course of 10 minutes.

[0378] In a separate vial (Vessel b), 5-(ethylthio)-1H-tetrazole (29.3 mg, 225 μ mol, 0.9 eq.) was added as a solid, then purged and sparged 5x with N₂. Thereafter, DCM (1.2 mL, 47 mM total) was added to Vessel B and the solution in Vessel B was then transferred to Vessel A dropwise at 0 °C. Vessel A was warmed to room temperature and stirred for 3 hours or until starting material was consumed. Reaction progress was monitored by LCMS. Vessel A was then opened to air and the solvent was evaporated. The compound was purified by column chromatography using 100% ethyl acetate.

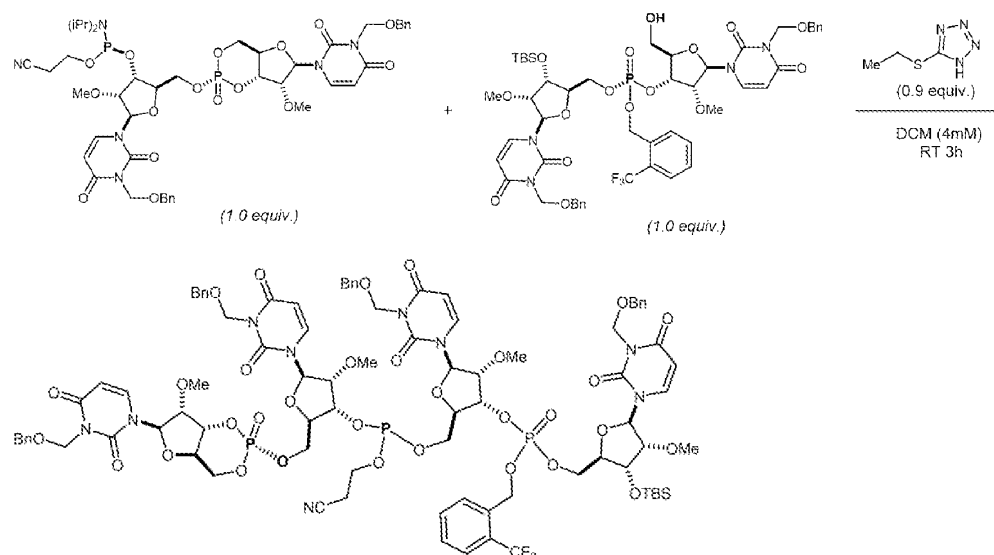
- 142 -

Example 13. Synthesis of cyclic tetramer**a. Linear Synthesis of Fragments in Solution Phase**

- 143 -



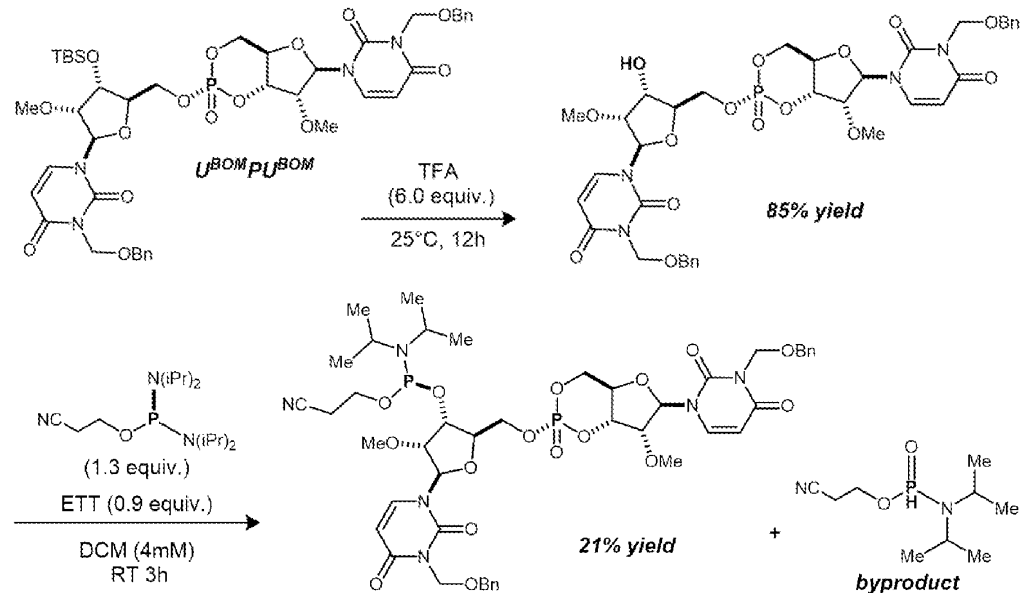
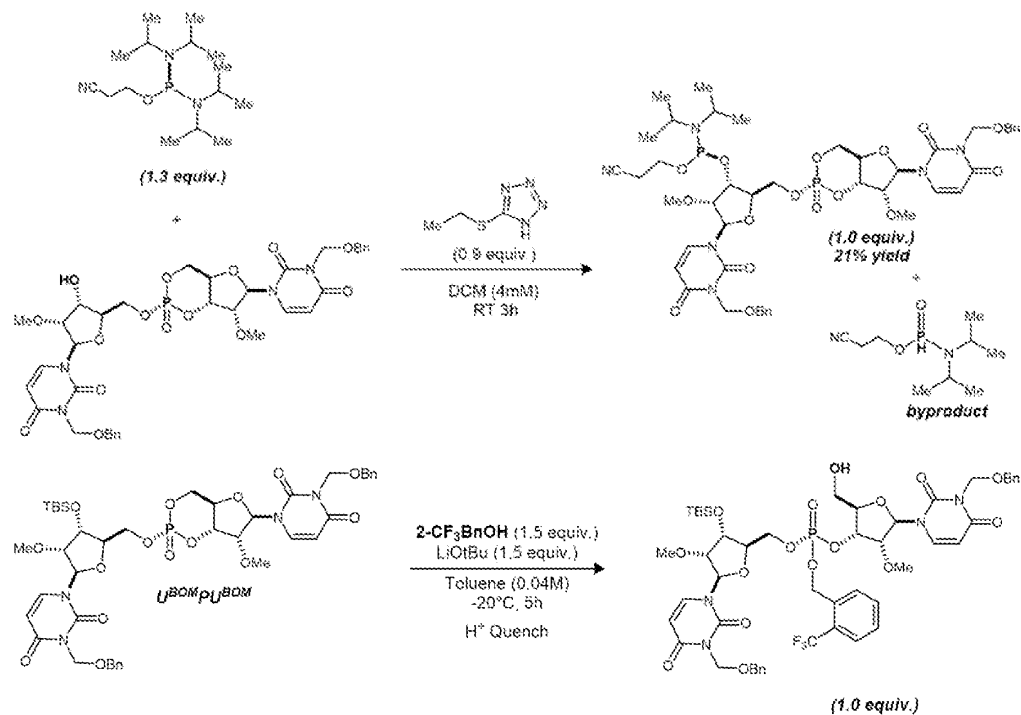
- 144 -

b. Synthesis via solution phase amidite chemistry using convergent dimer coupling

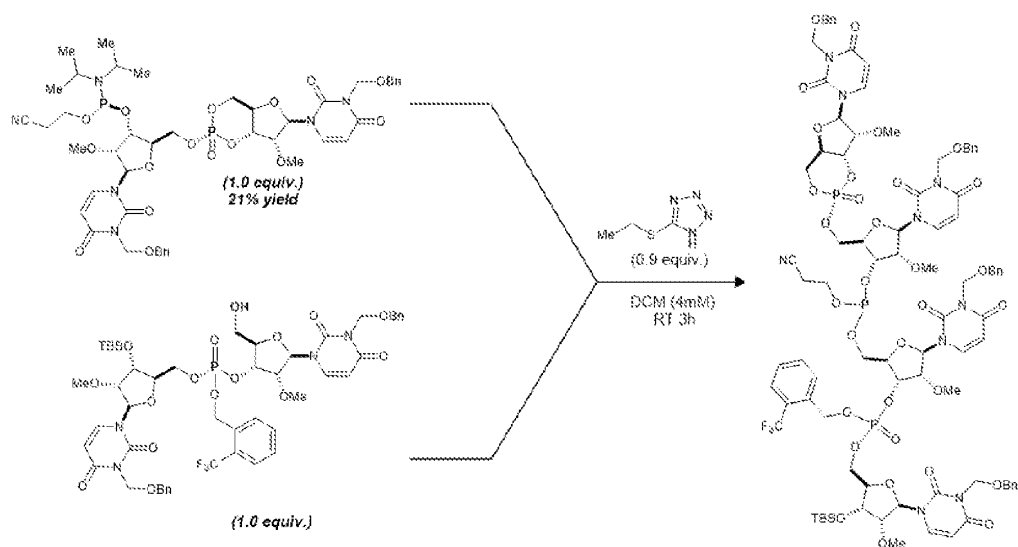
[0379] To a vial (Vessel A) was added (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl (((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) (2-(trifluoromethyl)benzyl) phosphate (22 mg, 20 μ mol, 1.0 eq.), a stirbar, a pack of sieves, and (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((4aR,6R,7R,7aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl (2-cyanoethyl) (20 mg, 20 μ mol, 1.0 equiv.). Reaction Vessel A was then sealed then purged and sparged 5x with N₂. Anhydrous DCM (4 mL) was added to Vessel A and the reaction was allowed to cool to 0 °C over the course of 10 minutes.

[0380] To a separate vial (Vessel B) was added 5-(ethylthio)-1H-tetrazole (2.3 mg, 18 μ mol, 0.9 equiv.), and the vessel was sealed. Vessel B was then purged and sparged 5x with N₂. Then DCM (1 mL, 4 mmol total) was added to Vessel B. The contents of Vessel B were then transferred to Vessel A dropwise at 0 °C. After the addition, Vessel A was warmed to room temperature and stirred for 3 hours or until starting material was consumed. Reaction progress was monitored by LCMS.

- 145 -

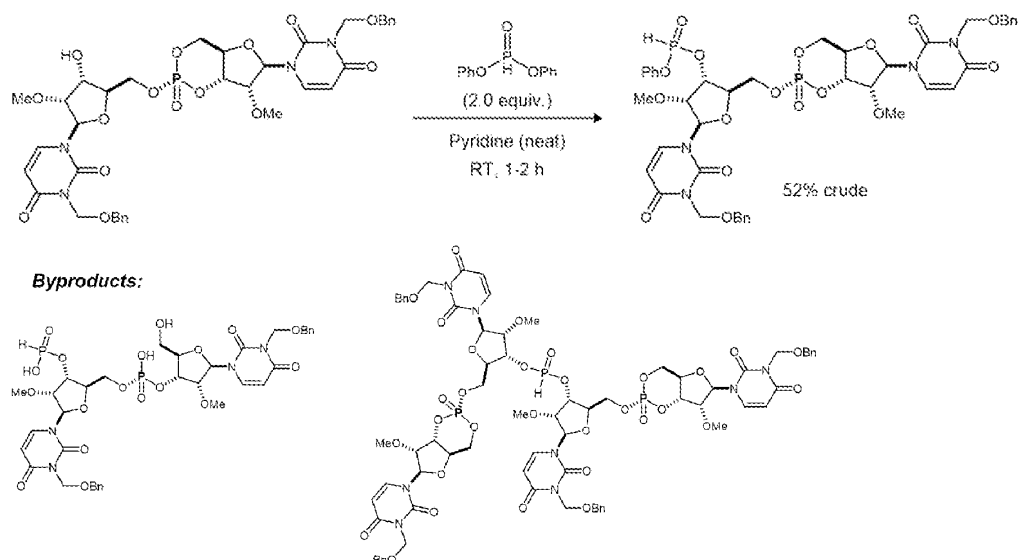
c. Amidite synthesis using cyclic dinucleotidesd. Convergent synthesis of longer fragments in solution phase

- 146 -



[0381] Process provided >95% conversion, with ~22% crude yield of tetramer products confirmed via LC integration.

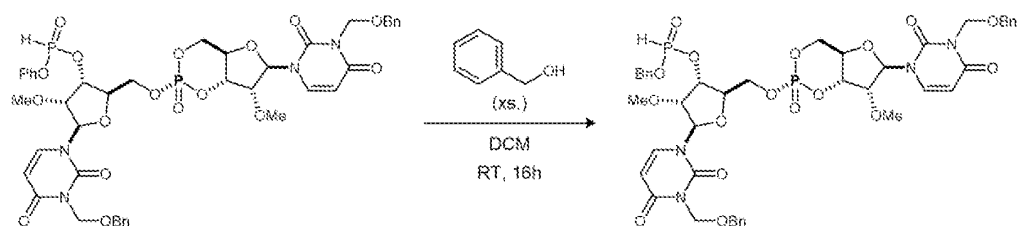
e. Longmer Synthesis



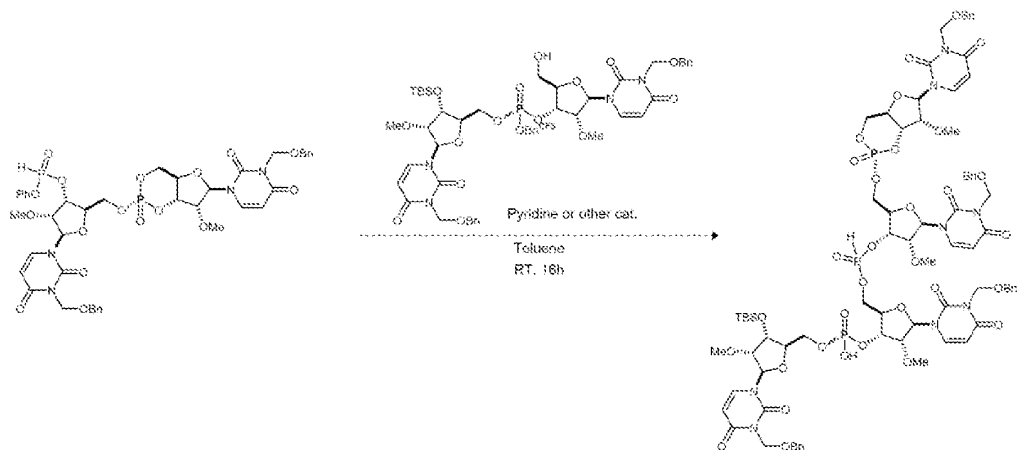
[0382] A 8 mL vial was charged with 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-hydroxy-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (150 mg, 1 Eq, 187 μ mol) while stirring with a stir bar, then purged and sparged 3 times with N₂. Anhydrous

- 147 -

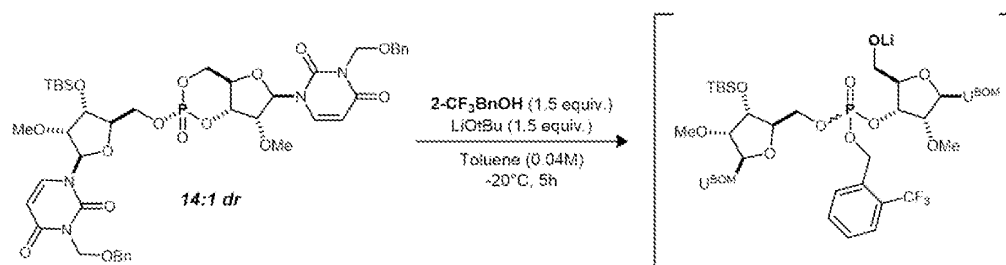
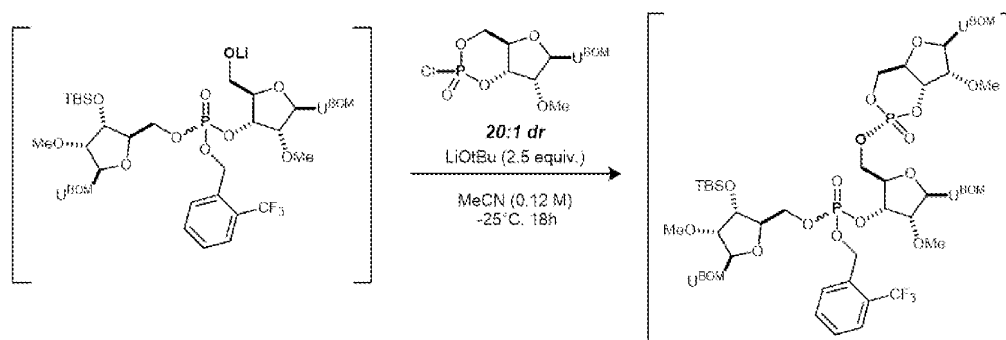
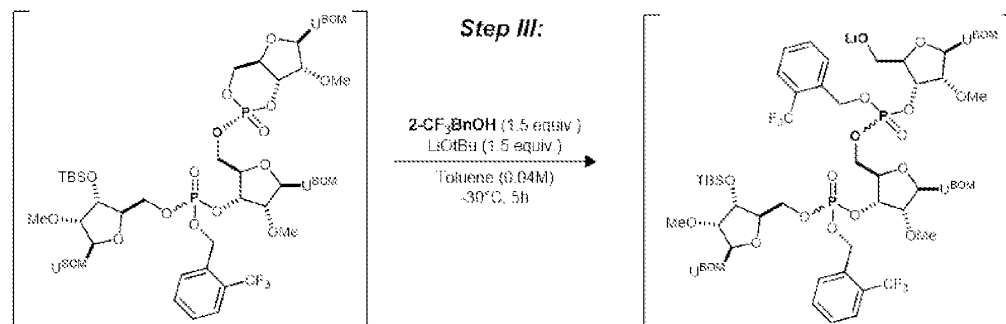
pyridine (1.48 g, 1.52 mL, 100 Eq, 18.7 mmol) was then added to the vessel. Diphenyl phosphonate (65.8 mg, 53.8 μ L, 1.5 Eq, 281 μ mol) was added and the reaction was stirred at room temperature, then monitored by LCMS. Upon reaction completion, a crude yield of 52% was calculated from LCMS. The contents of the reaction were then used in subsequence reaction steps.



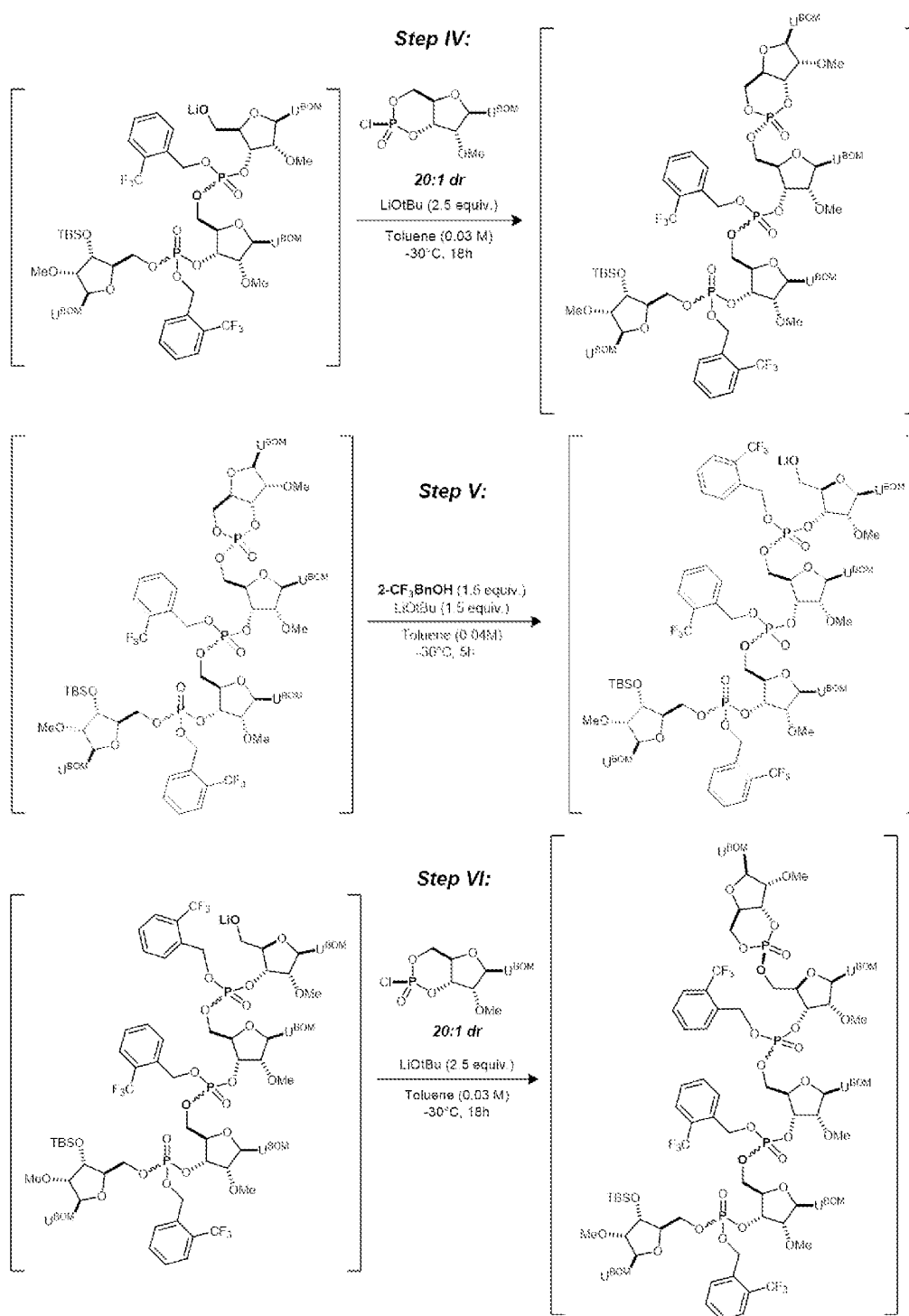
[0383] A 0.25 mL aliquot of the reaction product containing (2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((4aR,6R,7R,7aR)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-4-methoxytetrahydrofuran-3-yl phenyl phosphonate was obtained and concentrated by rotovap to remove the majority of remaining pyridine. 0.5 mL anhydrous DCM was then added to dissolve the crude residue and an excess of benzyl alcohol (15 μ L) was added to the mixture. The reaction was allowed to stir at RT overnight. After 18 hours, the starting material was fully consumed to product. A crude yield of 43% was observed by LCMS.



- 148 -

Example 14. Synthesis of cyclic pentamer using one-pot solution phase chemistry**Step I:****Step II:****Step III:**

- 149 -



- 150 -

[0384] Step I: A 50 mL flask (Vessel A) containing a stir bar was dried under vacuum using a heat gun 2x. The vessel was then purged and sparged with N₂ 3x. Upon the cooling of the flask, (2-(trifluoromethyl)phenyl)methanol (22 uL, 0.16 mmol, 1.5 equiv.) was added under N₂. Thereafter LitBuO (13 mg, 0.16 mmol, 1.5 equiv.) was weighed into a separate vial then purged and sparged 3x with N₂, and then Toluene (0.9 mL) was added to this vial. The solution in the vial was added to Vessel A and the reaction was stirred at room temperature for 30 min, then cooled to -25 °C.

[0385] To a separate vial was added 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (99 mg, 0.11 mmol, 1.0 equiv.)), then purged and sparged 3x with N₂. Toluene (1.8 mL) was added to this vial and the resulting solution of was then added to Vessel A at -25 °C in one portion. Reaction Vessel A was stirred for 4 hours at this temperature.

[0386] Step II: A separate vial was charged with 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (74 mg, 0.16 mmol, 1.5 equiv.) then sealed. This vial was purged and sparged 3x with N₂ then dry acetonitrile (0.53 mL) was added. The resulting solution was then added to Vessel A dropwise at -25 °C. Reaction Vessel was allowed to stir at -25 °C for 18 hours, after which a reaction aliquot was taken for analysis to observe reaction completion.

[0387] Step III: Chiller temperature was dropped to -25 °C. A 50mL RBF (Vessel B) containing a stir bar was dried under vacuum using a heat gun 2x. The vessel was then purged and sparged with N₂ 3x. Upon the cooling of the flask (2-(trifluoromethyl)phenyl)methanol (22 uL, 0.16 mmol, 1.5 equiv.) was added under N₂. Thereafter LitBuO (13 mg, 0.16 mmol, 1.5 equiv.) was weighed into a vial then purged and sparged 3x with N₂ and Toluene (0.9 mL) was added to this vial. After 30 minutes the contents of Vessel B were added to Vessel A at -30 °C. The reaction was then stirred for 5 hours at -25 °C. After this time a reaction aliquot was taken for analysis to observe reaction completion.

- 151 -

[0388] Step IV: A 100 mL flask (Vessel C) containing a stir bar was dried under vacuum using a heat gun 2x. Upon the cooling of Vessel C, the flask was charged with 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (89 mg, 0.19 mmol, 1.8 equiv.) and the vessel was sealed. Reaction Vessel C was purged and sparged 3x with N₂ and then anhydrous acetonitrile (0.53 mL) was added to the vessel. Vessel C was then cooled to -25 °C for 10 minutes. The contents from reaction Vessel A were then transferred to reaction Vessel C dropwise, then stirred at -25 °C for 18 hours. After 18 hours a reaction aliquot was taken to observe reaction status.

[0389] Step V: A vial containing a stir bar was dried under vacuum using a heat gun 2x. Upon the cooling of the vial, LitBuO (22 mg, 0.27 mmol, 2.5 equiv.) was weighed into the vial then purged and sparged 3x with N₂. Toluene (0.9 mL) was added to this vial followed by (2-(trifluoromethyl)phenyl)methanol (43 uL, 0.32 mmol, 3.0 equiv.) and the solution was stirred for 30 minutes. After this time the contents of the vial were added to Vessel C at -25 °C. The reaction was stirred for 4 hours at -25 °C. An aliquot was taken from Vessel C to monitor reaction progress.

[0390] Step VI: A 100 mL flask (Vessel D) containing a stir bar was dried under vacuum using a heat gun 2x. Upon the cooling of Vessel D, the flask was charged with 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (89 mg, 0.19 mmol, 1.8 equiv.) and the vessel was sealed. Reaction Vessel D was purged and sparged 3x with N₂ and anhydrous acetonitrile (0.53 mL) was added to the vessel. Vessel D was then cooled to -25 °C for 10 minutes. The contents from reaction Vessel C were then transferred to reaction Vessel D dropwise then stirred at -25 °C for 18 hours. After 18 hours a reaction aliquot was taken to observe reaction status.

Example 15. Templated enzymatic ligation using trimers to make ninemers

[0391] FIG. 1A and FIG. 1B present an example of an enzyme ligation reaction of shortmers to produce a ninemer. Each reaction was be run with a reaction control to ensure enzyme activity.

- 152 -

a. Reaction set up procedure:

[0392] Two LCMS vials were used as reaction vessels. Reaction Vessel A was the control reaction with no enzyme and Reaction Vessel B was the enzyme reaction. Each Vessel was charged with the solutions of HO-UUU-OH (32.51 mM), Phos-CCC-OH (25.97 mM), 9mer Guide (25.02 mM), Tris-HCL pH 7.5 (1000 mM), MgCl₂ solution (1000 mM), KCl solution (3000 mM), ATP (100 mM), DTT (100 mM) and nuclease free water respectively. Lastly to vessel B.

[0393] Reaction vessel A was charged with: HO-UUU-OH (6.2 uL, 1.0 equiv. of a 32.51 mM solution), Phos-CCC-OH (15.4 uL, 2.0 equiv. of a 25.97 mM solution), 9mer Guide (8.0 uL, 1.0 equiv. of a 25.02 mM solution), Tris-HCL pH 7.5 (25.0 uL, 125 equiv. of a 1000 mM solution), MgCl₂ solution (5.0 uL, 25 equiv. of a 1000 mM solution), KCl solution (16.7 uL, 250 equiv. of a 3000 mM solution), ATP (10.0 uL, 5.0 equiv. of a 100 mM solution), DTT (5.0 uL, 2.4 equiv. of a 100 mM solution) and nuclease free water (408.7 uL) respectively.

[0394] Reaction vessel B was charged with: HO-UUU-OH (6.2 uL, 1.0 equiv. of a 32.51 mM solution), Phos-CCC-OH (15.4 uL, 2.0 equiv. of a 25.97 mM solution), 9mer Guide (8.0 uL, 1.0 equiv. of a 25.02 mM solution), Tris-HCL pH 7.5 (25.0 uL, 125 equiv. of a 1000 mM solution), MgCl₂ solution (5.0 uL, 25 equiv. of a 1000 mM solution), KCl solution (16.7 uL, 250 equiv. of a 3000 mM solution), ATP (10.0 uL, 5.0 equiv. of a 100 mM solution), DTT (5.0 uL, 2.4 equiv. of a 100 mM solution) and nuclease free water (408.7 uL) respectively. Lastly, Codexis 3.007 enzyme (2.1 uL, 0.0625 equiv. of a 5.91 mM solution) was added.

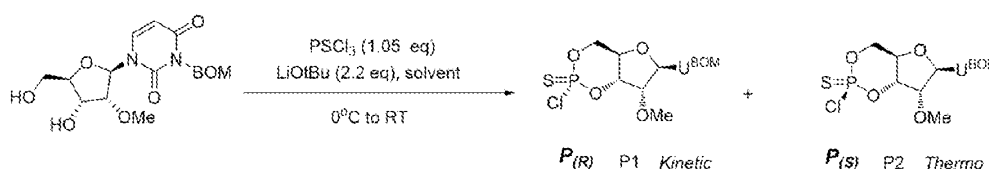
[0395] Both vessels were then added to a thermomixer and heated at 37C and agitated at 350 rpm for 18 hours.

b. Reaction quench procedure:

[0396] After 18 hours, 25 uL of reaction aliquots were taken from reaction vessel A and reaction vessel B and added to separate LCMS vials. Thereafter EDTA (125 uL of a 26.7 mM solution) was added to each aliquot to quench the reaction (aim to use 5:1 ratio for aliquots). This solution was then analyzed by LCMS, with results showing evidence of both the enzyme ligation reaction and the control reaction.

[0397] Crude yield was determined by using a denaturing UPLC method to integrate 9mer guide as the internal standard and compare the ratio of product to 9mer guide.

[0398]

Example 16. Synthesis of cyclic-phosphorothioate nucleosides**a. Synthesis of Cl-cyclic phosphorothioate with PSCl₃*****Procedure 1***

[0399] A stirred solution of thiophosphoryl chloride (1.05 mmol, 1.05 eq) and N-protected nucleoside diol BOM-U (1 mmol, 1.0 eq) in DCM (5 mL) was prepared. The solution was charged with a 2 mL solution of lithium tert-butoxide in 1 M THF (2.05 mmol, 2.05 eq) over a period of 5 minutes under Schlenk condition at 0 °C. After the addition, the mixture was warmed to ambient temperature overnight. An aliquot of crude product mixture was concentrated to monitor by LCMS and NMR for conversion, which observed two diastereomers P1 and P2 by P NMR with dr = 1.7:1 to 1.3:1.

[0400] NMR data of the two isomers: ³¹P NMR (162 MHz, CDCl₃) δ 61.23 (P1), 57.83 (P2). ¹H NMR (400 MHz, CDCl₃) P1, δ 7.40 – 7.27 (m, 5H), 7.11 (dd, J = 8.1, 0.8 Hz, 1H), 5.78 (dd, J = 8.2, 0.8 Hz, 1H), 5.46 (d, J = 1.0 Hz, 2H), 5.42 (s, 1H), 4.97 – 4.88 (m, 1H), 4.86 – 4.66 (m, 2H), 4.71 (s, 2H), 4.64 – 4.46 (m, 1H), 4.22 – 4.13 (m, 1H), 3.61 (d, J = 0.8 Hz, 3H). ¹H NMR (400 MHz, CDCl₃) P2, δ 7.42 – 7.30 (m, 5H), 7.18 – 7.09 (m, 1H), 5.82 (d, J = 8.1 Hz, 1H), 5.49 (d, J = 3.5 Hz, 2H), 5.40 – 5.35 (m, 1H), 4.86 (dt, J = 9.9, 5.0 Hz, 1H), 4.74 (s, 2H), 4.71 – 4.62 (m, 1H), 4.53 (ddd, J = 10.8, 9.6, 3.0 Hz, 1H), 4.27 (dd, J = 8.8, 4.9 Hz, 2H), 3.62 (s, 3H).

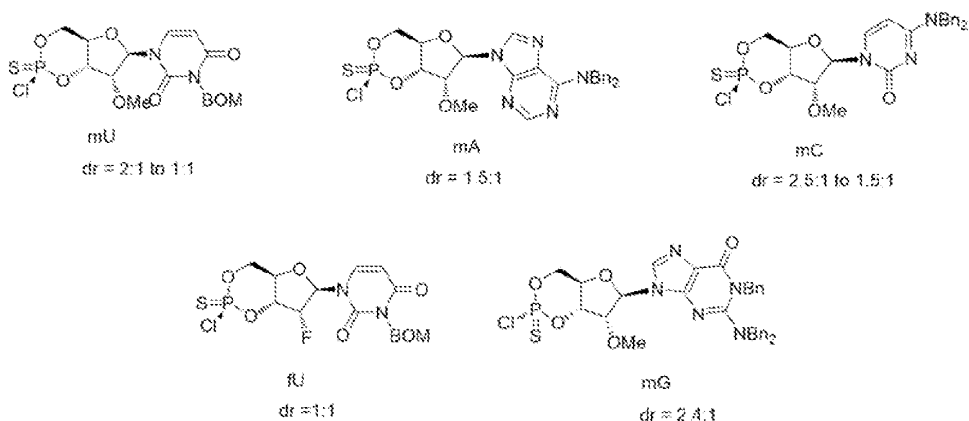
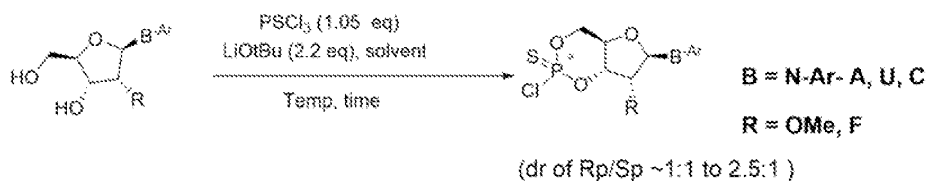
[0401] **Purification method (a):** Crude product was precipitated with 0.1 mL MTBE at 0 °C while stirring. The filtrate was concentrated and vacuumed for NMR and analysis.

[0402] **Purification method (b):** Crude product was concentrated by rotavap and then purified by wet loading (dissolved in DCM) to silica gel column EA/Hex 0-100% to give two diastereomers P1 and P2 with 40% combined isolation yield.

[0403] Reaction conditions and Cr d.r. results are shown in Table 8 [Cr dr is P(R):P(S) by P NMR of crude reaction. 4a and 4b are two time points of same batch. Reaction 1 used purification method (a). Reaction 2-4 used purification method (b)].

Table 8 - Synthesis of Cl-cyclic phosphorothioate

Reaction	Solvent	Total concentration	Temp	Scale	Cr d.r.	Conversion	Iso yield
1	DCM	0.15 M	0 °C to RT	0.53 mmol	13:1	26%	20%
2	DCM/THF 1:1	0.25 M	0 °C to RT	0.5 mmol	1.2:1	NA	NA
3	DCM/THF 2:1	0.17 M	0 °C to RT	0.5 mmol	0.9:1	NA	NA
4	DCM/THF 5:2	0.15 M	0 °C, 1.5 h	1.0 mmol	1.3:1	99%	41%
5	DCM/THF 5:2	0.15 M	-20 °C, 20 h	0.13 mmol	NA	23%	NA
6	DCM/THF 5:2	0.15 M	0 °C, 20 h	0.5 mmol	1.8:1	97%	41%
7	DCM/THF 5:2	0.15 M	0 °C, 1 h	2.64 mmol	1.7:1	97%	56%
8	DCM/THF 5:2	0.15 M	0 °C to RT	3.0 mmol	1:2:1	99%	52%

Procedure 2

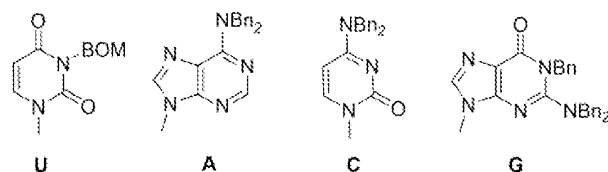
[0404] To a stirring solution of thiophosphoryl chloride (1.05 eq) and N-protected nucleoside diol N-Ar protected nucleobase (1.0 eq) in DCM (x mL), was added a charged solution of lithium *tert*-butoxide in 1 M THF or MeTHF solvent (2.05 eq) over a period

- 155 -

of 5 minutes under Schlenk condition at 0 to -20 °C in ice bath or cryocooler. Conversion and dr of crude reaction mixture was monitored by LCMS and NMR after 1-20 hours.

[0405] ^{31}P NMR (162 MHz, CDCl_3), mA: δ 60.82 (Rp), 58.44 (Sp). mC: δ 61.50 (Rp), 58.90 (Sp). fU: δ 60.35 (Rp), 57.42 (Sp). mG: 61.86 (Rp), 57.32 (Sp). fA LCMS

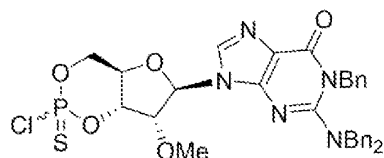
Scope of Nucleobases



Entry	Nucleo Base	Rxn Base	Temp	Cr. Yield	isol. Yield	Rp/Sp	Scale	Purity
1	mU	LiOtBu	0 °C to RT	91 %	85 %	~1:1	10 mmol	>90%
2	fU	LiOtBu	-20 °C	40%	26%	1:1	2 mmol	83%
3	mC	LiOtBu	-20 °C	40%	30 %	2.5:1	1 mmol	69%/87%
4	mA	LiOtBu	-12 to -20 °C	70%	65%	1.5:1	2 mmol	75%/95%
5	mG	LiOtBu	-20 °C	74%	54%	2.4:1	10 mmol	80%
6	fA	LiOtBu	-20 °C	28%	ND	~1:1	1 mmol	NA

b. Synthesis of Cl-cyclic-2'-methoxy-G-phosphorothioate

(1-benzyl-9-((4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-sulfitotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-2-(dibenzylamino)-1,9-dihydro-6H-purin-6-one)



mG

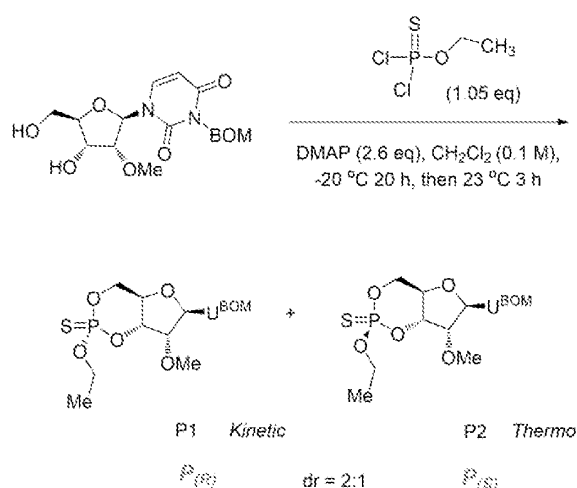
[0406] Under schlenk line N2, 1-benzyl-2-(dibenzylamino)-9-((2R,3R,4R,5R)-4-hydroxy-5-(hydroxymethyl)-3-methoxytetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (5.677 g, 1 Eq, 10.00 mmol) dissolved in DCM (50 mL) at RT then slowly cooled to -20 °C over 90 minutes, then freshly distilled phosphorothioyl trichloride (1.778 g, 1.06 mL, 1.05 Eq, 10.50 mmol) was added by syringe needle dropwise, at 1:50pm followed by addition of THF (9.3 mL) 2-methylpropan-2-ol, lithium salt (2.2 M in THF) (1.662 g, 2.05 Eq, 20.50 mmol) by syringe needle over 10min. after 30 min, ~50 uL crude sample was collected by syringe for analysis, crude PNMR show two main product isomer peaks dr ~ 2.4:1. Starting material was mostly consumed after 30 minutes (with 1% remained

- 156 -

detected on UV, integration determined by uncorrected LCMS) and other small peaks of impurities earlier eluted than product. Reaction was stopped after 1 h. Crude reaction solution was concentrated to remove most of solvent before redissolved in DCM then loaded on silica gel column (40g), which eluted with EA/Hex (0-80%) by combi-flash system, affording 3.56g product as light beige to white solid (isolated yield 54%, dr 2.2:1).

[0407] ^{13}C NMR (101 MHz, CDCl_3) δ 157.50, 145.65, 138.96, 136.14, 135.61, 135.50, 128.78, 128.76, 128.73, 128.68, 128.21, 128.02, 127.91, 127.73, 127.46, 126.45, 126.39, 91.10, 90.81, 79.86, 78.80, 78.73, 77.36, 77.25, 77.04, 76.73, 71.73, 71.61, 69.27, 69.13, 59.64, 59.62, 55.59, 55.50, 48.29

c. Synthesis of alkoxy-cyclic phosphorothioate via PSORCl_2



[0408] N-protected nucleoside diol BOM-U (75.7 mg, 1 eq, 0.2 mmol) and DMAP (63.5 mg, 2.6 eq, 520 μmol) in 1.8 mL DCM (2 mL) were added O-ethyl phosphorodichloridothioate (37.6 mg, 1.05 eq, 210 μmol) in 0.2 mL DCM slowly under schlenk line at $-20\text{ }^\circ\text{C}$ stirring for 20 hour with the aid of a cryocooler. The reaction was raised to room temperature and stirred for 3 hours. Crude product was concentrated and redissolved in 0.5 mL DCM for loading onto a 5g biotage silica gel column, then purified with elute EA/Hex 0-100%, and eluted at $\sim 30\%$ EA/Hex to give two diastereomers P1 and P2 (dr of P1:P2 = 2:1) with isolate yield 40%.

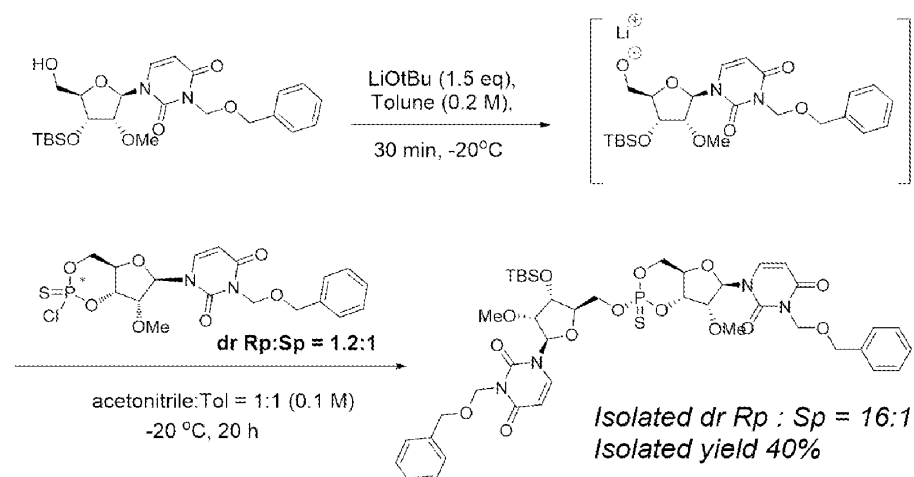
[0409] NMR data of the two isomers: ^{31}P NMR (162 MHz, CDCl_3) δ 66.52 (P1), 61.80 (P2). ^1H NMR (400 MHz, CDCl_3) P1, δ 7.42 – 7.27 (m, 6H), 7.18 (d, $J = 8.2\text{ Hz}$,

- 157 -

1H), 5.82 (d, $J = 8.2$ Hz, 1H), 5.69 (t, $J = 0.9$ Hz, 1H), 5.51 (s, 2H), 4.74 (s, 2H), 4.69 – 4.47 (m, 3H), 4.44 – 4.24 (m, 3H), 4.01 (d, $J = 4.9$ Hz, 1H), 3.62 (s, 3H).

Example 17. Synthesis of cyclic thioate dinucleotides via cyclic chlorophosphorothioate

a. Cyclic U*U (Procedure 1)



[0410] Solution A: Under nitrogen atmosphere via the Schlenk-line technique, a 20 mL oven dried reaction tube A equipped with a magnetic stir bar, was charged with LiOtBu (48.7 mg, 1.5 eq, 609 μ mol). The stock of solution of 3'-TBS-5'-OH nucleoside (200 mg, 1 eq, 406 μ mol) in toluene (2 mL) was added to the reaction tube A, put and kept it at -20 °C with aid of a cryocooler, stirred for 30 minutes.

[0411] Solution B: Cyclic chlorophosphorothioate monomer PS-Cl UBOM (dr Rp:Sp=1.2:1, 231 mg, 1.0 eq, 487 μ mol) was dissolved in acetonitrile (2 mL) in an oven dried flask at -20 °C with cyrocooler under Schlenk-line condition. solution A was added into B over 5 minutes by syringe needle dropwise, stirred for 20 hour. Aliquot of 50 μ L was collected by syringe and concentrated for ^{31}P NMR and LCMS analysis to confirm reaction completion and dr (Rp:Sp = 13:1) of crude reaction. Crude reaction solution was concentrated to remove most of solvent before loading on silica gel column (5 g), which eluted with EA/Hex (0-60%) by combi-flash system, affording 150 mg major isomer "Rp" as white solid (isolated yield 40%, dr 16:1).

[0412] ^{31}P NMR (162 MHz, CDCl_3) δ 66.20 (Rp, less polar), 62.00 (Sp, more polar).

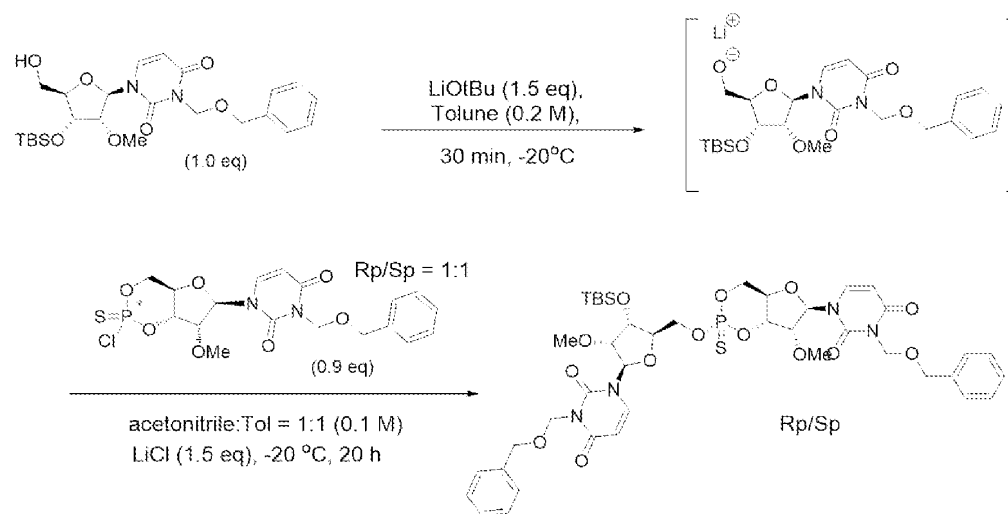
- 158 -

[0413] ^1H NMR (400 MHz, CDCl_3) δ 7.73 (dd, $J = 8.2, 5.5$ Hz, 1H), 7.44 – 7.25 (m, 10H), 7.13 (s, 1H), 5.93 (d, $J = 1.8$ Hz, 1H), 5.90 – 5.78 (m, 2H), 5.64 – 5.44 (m, 5H), 4.74 (d, $J = 1.5$ Hz, 4H), 4.71 – 4.50 (m, 4H), 4.47 – 4.38 (m, 1H), 4.30 – 4.06 (m, 5H), 3.68 (q, $J = 2.0$ Hz, 1H), 3.64 – 3.51 (m, 6H), 0.93 (d, $J = 2.7$ Hz, 9H), 0.17 – 0.09 (m, 6H).

[0414] ^{13}C NMR (101 MHz, CDCl_3) δ 171.16, 162.62, 162.08, 150.86, 150.28, 138.70, 138.21, 137.86, 128.38, 128.35, 127.85, 127.75, 127.73, 127.62, 102.80, 101.88, 94.29, 88.80, 83.39, 81.34, 81.24, 80.43, 80.34, 77.35, 77.03, 76.71, 72.54, 72.31, 71.21, 71.15, 70.47, 70.26, 69.08, 69.00, 66.40, 66.35, 60.41, 59.24, 58.43, 25.68, 21.07, 18.08, 14.22, -4.61, -4.86.

[0415] HRMS (ESI+): m/z calcd. for $\text{C}_{42}\text{H}_{65}\text{N}_4\text{O}_{14}\text{PSi}[\text{M}+\text{H}]^+$: 930.29; found 930.31 (Rp), 930.33 (Sp).

b. Cyc U*U (Procedure 2)



[0416] Solution A: Under nitrogen atmosphere via the Schlenk-line technique, a 20 mL reaction flask A equipped with a magnetic stir bar, was charged with LiOtBu (96.1 mg, 1.5 Eq, 1.20 mmol) cooled to -20°C with aid of a cryocooler. The stock of solution of 3'-TBS-5'-OH nucleoside (394 mg, 1 Eq, 800 μmol) in toluene (4 mL) was added to the reaction tube A via syringe needle slowly, stirred for 30 minutes at -20°C with aid of a cryocooler, stirred for 30 minutes.

[0417] Solution B: Cyclic chlorophosphorothioate monomer PS-Cl UBOM (dr $\text{Rp:Sp}=1:1$, 500 mg, 70% Wt, 0.921 Eq, 737 μmol) was dissolved in acetonitrile (4 mL) at

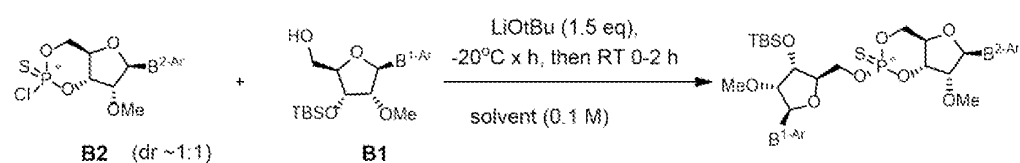
- 159 -

-20 °C with cyrocooler under Schlenk-line condition. solution A was added into B over 5 minutes by syringe needle dropwise, stirred for 20 hour, 50 uL was concentrated to test P NMR and LCMS to confirm reaction completion and product dr (Rp:Sp = 27:1) of crude reaction.

[0418] Reactant cyclic chloro-phosphorothioate monomer PS-Cl UBOM used in procedure 2 is not purified by silica gel column chromatography. The synthesis and quenching method is as follows.

[0419] Under schlenk line N₂, diol-U-BOM (3.784 g, 1 Eq, 10.00 mmol) and phosphorothioyl trichloride (1.778 g, 1.06 mL, 1.05 Eq, 10.50 mmol) dissolved in DCM (48 mL) at 0 °C stirred for 20 minutes, followed by addition of THF (20 mL)lithium 2-methylpropan-2-olate (1 M THF solution) (1.601 g, 2.0 Eq, 20.00 mmol) by syringe and needle, warm from 0 °C to 23 °C over 4 hours. Reaction completed and was concentrated by rotovap. The condensed crude product solution was slowly added mixture of Heptane/MTBE/EA = 50 mL/50 mL/ 30 mL to partially precipitate salt impurity and other insoluble byproduct. The filtrate solution was concentrated and vacuumed to give light brown solid 5.6 g. Based on theoretical yield 4.75 g and actual mass 5.6 g after precipitation, there could be ~0.80 g mixture of LiOH, LiOtBu and LiCl = 10-15 mmol ~ 1-1.5 eq. 10 mmol calculated based on MW 80 of LiOtBu.

c. Two-step addition (General)



[0420] Solution A: Under nitrogen atmosphere via the Schlenk-line technique, a 20 mL reaction tube A equipped with a magnetic stir bar, was charged with LiOtBu (1.5 eq). The stock of solution of 3'-TBS-5'-OH nucleoside (1 eq) in toluene (0.2 M) was added to the reaction tube A, put and kept it at -20 °C with aid of a cryocooler, stirred for 30 minutes.

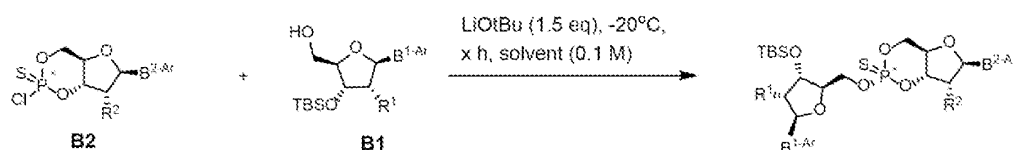
[0421] Solution B: Cyclic chlorophosphorothioate monomer (dr Rp:Sp~1:1, 1.0 eq) was dissolved in acetonitrile (0.2 M) in an oven dried flask at -20 °C with cyrocooler under Schlenk-line condition. Solution A was added into B over 5 minutes by syringe needle dropwise, stirred for 20 hours. Aliquot of 50 uL was collected by syringe and

- 160 -

concentrated for ^{31}P NMR and LCMS analysis to confirm reaction completion and dr of crude reaction. Crude reaction solution was concentrated to remove most of solvent and dissolved in minimum DCM before loading on silica gel column, which eluted with EA/Hex (0-60%) by combi-flash system, affording major isomer "Rp" as white solid, dr ~ 4:1 to 20:1.

Table 9 - Two-step addition

entry	B2 eq	B1 eq	isol yield	LCMS y%	cr Rp/Sp	Uncovered B1	Uncovered B2
1	0.9eq mU	1.1eq mU	35%	55%	27:1	37%	8%
2	1.2eq mU	1eq mU	40%	63%	13:1	12%	25%
3	1.6 eq mU	mU	33%	80%	5:1	0	20%
4	1.1eq mU	1eq mU	80%	85%	4:1	2.6%	12%
5*	1.1eq mU	1eq mU	85%	94%	6:1	1%	2%
6	1.1eq mA	1eq mA	30%	52%	14:1	24%	24%

d. One-pot addition

[0422] B1 5'-OH-3'TBS-monomer (1 eq) and B2 Cl-PS-cyclic monomer (1.2 Eq, 120 μmol) were mix in a heat gun vacuum dried glass vessel at room temp followed by fully dissolving in anhydrous DCM (0.1 M) then cooled to -20C under N₂ with aid of cryocooler. Lithium 2-methylpropan-2-olate (2.2 M in THF) (1.5 Eq) was added into the reaction vessel by syringe needle dropwise over 5min. Then kept the temperature and stirred for 1-20 hours under N₂ until reaction completed. LCMS was used to monitor conversion. After 1 h, Aliquot of 50 uL was collected by syringe and concentrated for ^{31}P NMR and LCMS analysis to confirm reaction completion as 82% crude yield and cr dr (Rp:Sp) of crude reaction. Crude reaction solution was concentrated to remove most of solvent and dissolved in minimum DCM before loading on silica gel column, which eluted with EA/Hex (0-60%) by combi-flash system, affording 90 mg major isomer "Rp" as white solid (isolated yield 82%, dr 10:1).

Table 10 – One-pot addition

Entry	B2 eq	B1 eq	solvent	dr of B2	isol yield	LCMS y%	cr dr Rp/Sp	Unconverted B1	Unconverted B2
*1	1.1eq mU	1eq mU	Tol	1:1	85%	94%	6:1	1%	2%
2	1.3eq mG	1eq mA	DCM	2.4:1	62%	78%	10:1	13%	None
3	1.1eq mU	1eq mA	DCM	1:1	81%	83%	10:1	3.7%	8.1%
*4	1.1eq mC	1eq mU	Tol	2.5:1	/	<50%	NA	50%	40%
5	1.1eq mA	1eq mU	DCM	1.5:1	36%	41%	21:1	3.5%	7.1%
6	1.1eq mU	1eq fU	DCM	1:1	60%	61%	17:1	12.6%	18.2%
7	1.1eq fU	1eq mA	DCM	1:1.2	35%	38%	1.2:1	4.4%	5.0%
8	1.1eq mA	1eq mA	DCM	1.5:1	30%	52%	14:1	24%	24%
9	1.3eq mG	1eq mG	DCM	2.2:1	/	62%	12:1	7%	7%
10	1.4eq mG	1eq mU	DCM	2.2:1	46%	58%	>10:1	1.3%	12%

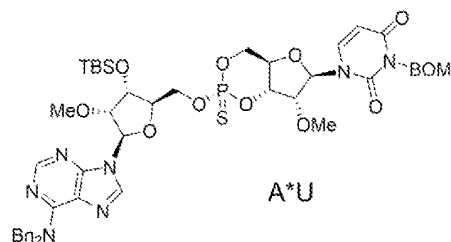
e. Cyclic PS mAmU

[0423] B1 5'-OH-3'TBS mA ((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methanol (57.6 mg, 1 Eq, 100 μ mol) and B2 Cl-PS-cyclic mU monomer 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-sulfidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (57.0 mg, 1.2 Eq, 120 μ mol) were mix in a heat gun vacuum dried glass vessel at room temp followed by fully dissolving in anhydrous DCM (2 mL) then cooled to -20 °C under N₂ with aid of cryocooler. Lithium 2-methylpropan-2-olate (2.2 M in THF) (12.0 mg, 68 μ L, 1.5 Eq, 150 μ mol) was added into the reaction vessel by syringe needle dropwise over 5min. Then kept the temperature and stirred for 2 hrs under N₂ until reaction completed. LCMS was used to monitor conversion. After 1 h, Aliquot of 50 μ L was collected by syringe and concentrated for 31P NMR and LCMS analysis to confirm reaction completion as 82% crude yield and cr dr (Rp:Sp = 10:1) of crude reaction. Crude reaction solution was concentrated to remove most of solvent and dissolved in minimum DCM before loading on silica gel column (5g), which eluted with EA/Hex (0-60%) by combi-flash system, affording 90 mg major isomer "Rp" as white solid (isolated yield 82%, dr 10:1). ³¹P NMR (162 MHz, CDCl₃) Rp 66.40, Sp isomer not confirmed due to trace amount detected may get confirmation after scale-up.

- 162 -

Example 18. Spectra of dinucleotides

a. 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-3-((tert-butyl)dimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-sulfitotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione

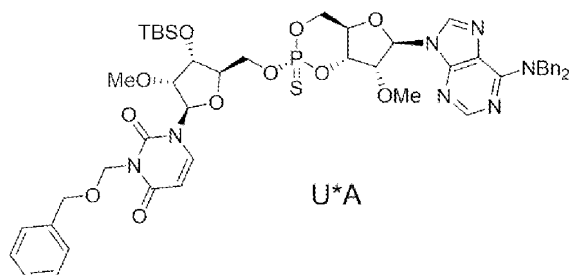


[0424] cr dr=17:1, purified dr > 30:1

[0425] ^{31}P NMR (162 MHz, CDCl_3) δ 66.27 (Rp)

[0426] ^1H NMR (400 MHz, CDCl_3) δ 8.51 (s, 1H), 8.04 (s, 1H), 7.37 (t, $J = 5.6$ Hz, 4H), 7.35 – 7.27 (m, 32H), 6.16 (d, $J = 4.2$ Hz, 2H), 5.75 (d, $J = 8.1$ Hz, 2H), 5.57 (s, 2H), 5.47 (s, 3H), 4.76 – 4.64 (m, 6H), 4.49 – 4.38 (m, 5H), 4.32 – 4.24 (m, 3H), 4.06 (d, $J = 4.9$ Hz, 2H), 3.64 – 3.50 (m, 8H), 2.07 (s, 2H), 0.98 (s, 9H), 0.19 (t, $J = 1.2$ Hz, 6H).

b. 3-((benzyloxy)methyl)-1-((2R,3R,4R,5R)-4-(((2S,4aR,6R,7R,7aR)-6-(6-(dibenzylamino)-9H-purin-9-yl)-7-methoxy-2-sulfitotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione

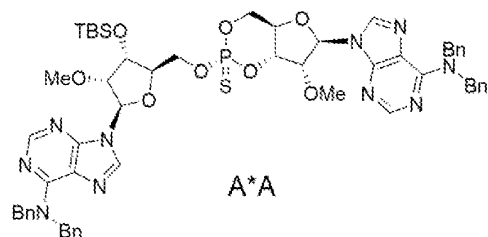


[0427] cr dr=29:1, purified dr = 20:1

[0428] ^{31}P NMR (162 MHz, CDCl_3) δ 65.97 (Rp),

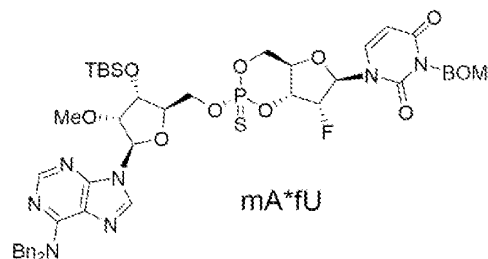
- 163 -

c. (2S,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methoxy)-6-(6-(dibenzylamino)-9H-purin-9-yl)-7-methoxytetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinine 2-sulfide



[0429] ^{31}P NMR (162 MHz, CDCl_3) δ 66.18 (Rp) cr dr = 14:1, 25:1

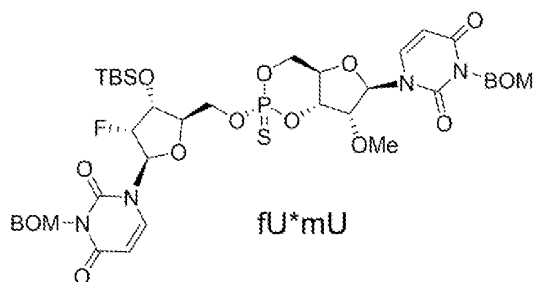
d. 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-fluoro-2-sulfidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



[0430] ^{31}P NMR (162 MHz, CDCl_3) δ 66.03 (Rp), 61.49(Sp).

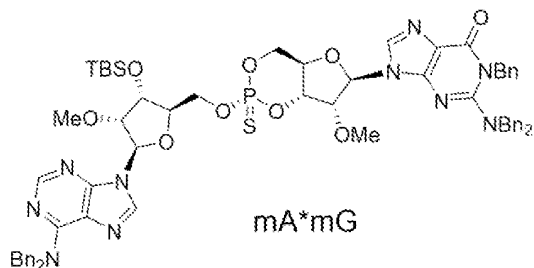
- 164 -

e. 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyl)dimethylsilyl)oxy)-4-fluorotetrahydrofuran-2-yl)methoxy)-7-methoxy-2-sulfidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione



[0431] ^{31}P NMR (162 MHz, CDCl_3) δ 66.36 (Rp)

f. 1-benzyl-9-((2S,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-3-((tert-butyl)dimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-sulfidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-2-(dibenzylamino)-1,9-dihydro-6H-purin-6-one



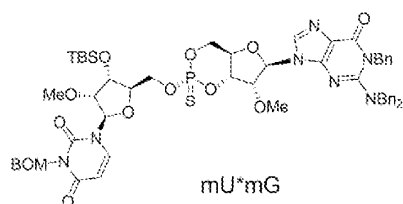
[0432] ^{31}P NMR (162 MHz, CDCl_3) δ 66.21(Rp), 62.05(Sp). Yield 62%. ^1H NMR (400 MHz, CDCl_3) δ 8.43 (s, 1H), 8.01 (s, 1H), 7.58 (s, 1H), 7.36 – 7.19 (m, 21H), 7.13 (dt, J = 7.3, 2.9 Hz, 6H), 6.18 (d, J = 4.0 Hz, 1H), 5.67 (d, J = 15.5 Hz, 1H), 5.61 (s, 2H), 5.32 (s, 1H), 5.23 (ddd, J = 9.5, 5.1, 2.8 Hz, 1H), 4.72 – 4.60 (m, 2H), 4.50 – 4.42 (m, 2H), 4.42 – 4.37 (m, 3H), 4.34 – 4.10 (m, 6H), 3.94 (d, J = 5.1 Hz, 1H), 3.54 (s, 3H), 3.35 (s, 3H), 0.98 (s, 9H), 0.20 (s, 6H).

[0433] ^{13}C NMR (100 MHz, CDCl_3) δ 158.20, 157.51, 154.96, 152.72, 150.71, 145.82, 138.93, 137.71, 137.39, 136.23, 136.14, 136.05, 128.66, 128.60, 128.01, 127.87, 127.82, 127.55, 127.35, 127.33, 126.42, 126.36, 122.31, 120.20, 91.02, 87.12, 82.65,

- 165 -

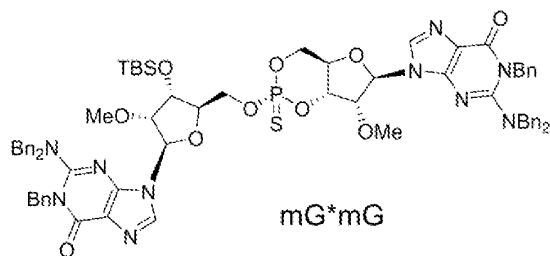
82.41, 82.32, 80.17, 80.08, 77.97, 77.92, 77.37, 77.25, 77.05, 76.73, 70.88, 70.82, 70.43, 69.27, 69.19, 67.42, 59.30, 58.62, 56.06, 55.77, 53.45, 48.11, 25.78, 25.78, 25.78, 18.17, - 4.64, -4.76.

g. 1-((2R,3R,4R,5R)-5-((((2S,4aR,6R,7R,7aR)-6-(1-benzyl-2-(dibenzylamino)-6-oxo-1,6-dihydro-9H-purin-9-yl)-7-methoxy-2-sulfidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-3-methoxytetrahydrofuran-2-yl)-3-((benzyloxy)methyl)pyrimidine-2,4(1H,3H)-dione



[0434] ^{31}P NMR (162 MHz, CDCl_3) δ 65.72, 61.88. ^1H NMR (400 MHz, CDCl_3) δ 7.80 – 7.71 (m, 1H), 7.70 (s, 1H), 7.43 – 7.30 (m, 5H), 7.28 (dd, $J = 6.4, 3.7$ Hz, 1H), 7.18 – 7.10 (m, 5H), 7.05 (dd, $J = 6.6, 3.0$ Hz, 1H), 5.93 (d, $J = 1.6$ Hz, 1H), 5.82 (d, $J = 8.2$ Hz, 1H), 5.68 (d, $J = 14.2$ Hz, 2H), 5.62 (s, 1H), 5.60 – 5.45 (m, 2H), 5.32 (s, 2H), 5.31 – 5.26 (m, 1H), 4.73 (s, 2H), 4.64 (dd, $J = 11.4, 6.6$ Hz, 1H), 4.52 (ddd, $J = 22.9, 9.7, 4.9$ Hz, 1H), 4.44 (s, 1H), 4.40 (s, 2H), 4.38 – 4.31 (m, 1H), 4.27 (d, $J = 14.9$ Hz, 3H), 4.24 – 4.10 (m, 5H), 4.01 (d, $J = 5.2$ Hz, 1H), 3.68 (q, $J = 1.7$ Hz, 1H), 3.61 (s, 3H), 3.46 (s, 1H), 3.40 (s, 2H), 0.94 (s, 9H), 0.15 (d, $J = 2.1$ Hz, 6H).

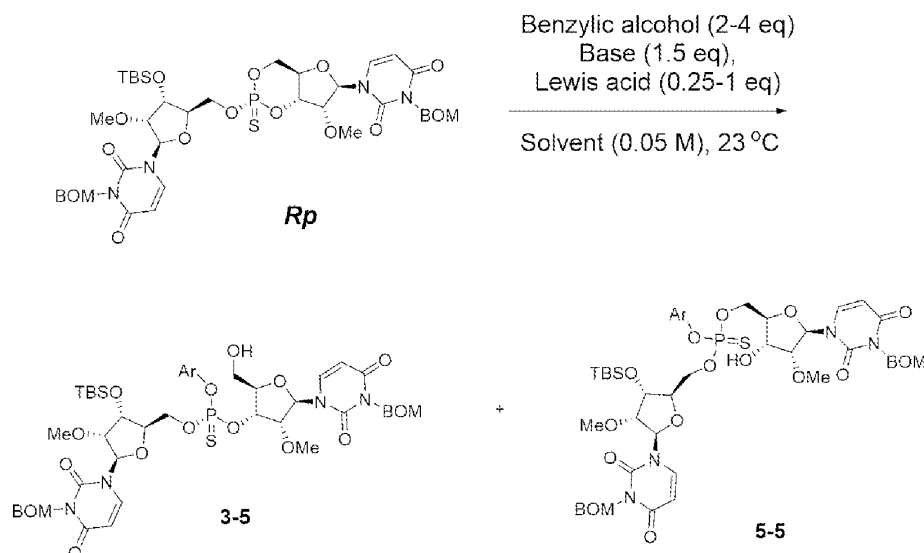
h. 1-benzyl-9-((2S,4aR,6R,7R,7aR)-2-((((2R,3R,4R,5R)-5-(1-benzyl-2-(dibenzylamino)-6-oxo-1,6-dihydro-9H-purin-9-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-sulfidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-2-(dibenzylamino)-1,9-dihydro-6H-purin-6-one



[0435] ^{31}P NMR (162 MHz, CDCl_3) δ 66.73 (Rp)

Example 19. Nucleophilic Ring Opening of Cyclic Dinucleotide thioate at P with Benzylic Alkoxides

a. Scheme 1. General catalysis condition procedure for U*U



[0436] Solution A: Cuprous chloride (CuCl) (0.25-1 Eq) (green powder) was added to a heat gun dried vial under N₂, and then dissolved with solvent-1 (0.05 M) at room temperature followed by addition of base (1.5-3 Eq), 10 minutes later added phenylmethanol (2 Eq) or other benzylic alcohol (e.g., CF₃BnOH) stirring for 20 minutes at RT under N₂.

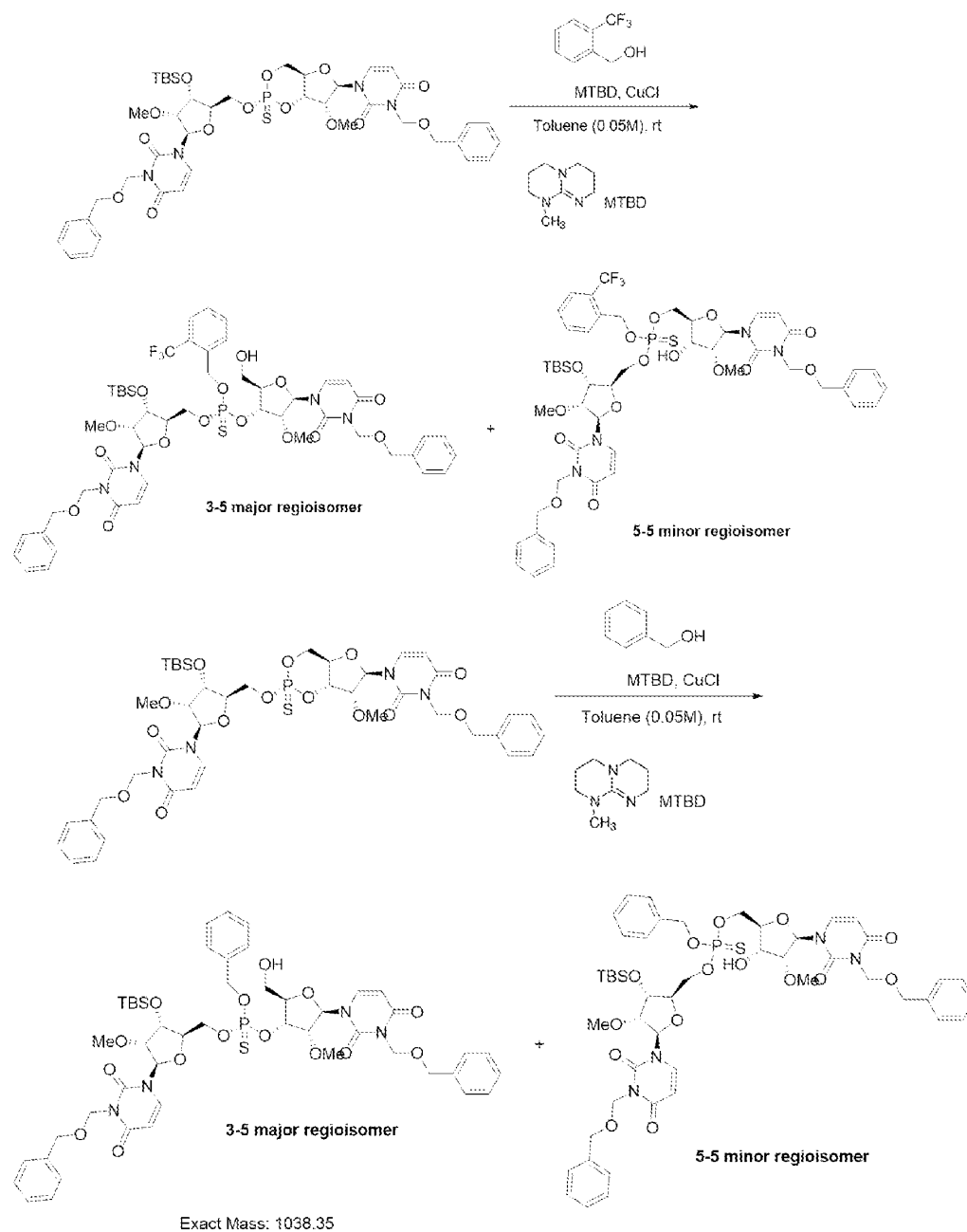
[0437] Solution B: 13:45 3-((benzyloxy)methyl)-1-((2*S*,4*aR*,6*R*,7*R*,7*aR*)-2-(((2*R*,3*R*,4*R*,5*R*)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-sulfidotetrahydro-4*H*-furo[3,2-*d*][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1*H*,3*H*)-dione (93 mg, 1 Eq, 0.10 mmol) was dissolved in Toluene-2 (1.3 mL) under schlenk line at 23 °C, followed by addition of solution A dropwise into solution B by syringe needle over 5 minutes. Reaction completed in 20 hours upon monitoring by LCMS.

[0438] The ring opening reaction was screened in 34 reactions using cyc U*U with different dr (8:1 or 13:1) to optimize the conditions. From these 34 reactions run in 1 screening, the conditions were improved: MTBD (1.5 eq), BnOH or 2-CF₃-BnOH (2 eq), CuCl (0.25 eq) with toluene, MTBE, and acetonitrile as solvent and for t = 20-40 h (Conv. of SM 95%, [S]~5:1 from [S]<1:1).

- 167 -

[0439] A second round of screening was executed to further optimize the process using cyc U*U, dr=4:1, 2-CF₃-BnOH, various Lewis acids, bases and solvents combinations.

b. Scheme 2. Detailed procedure for U*U

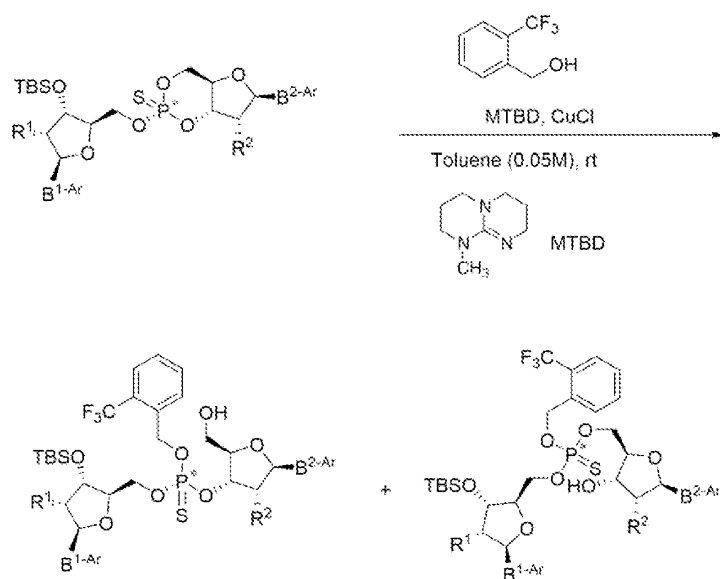


- 168 -

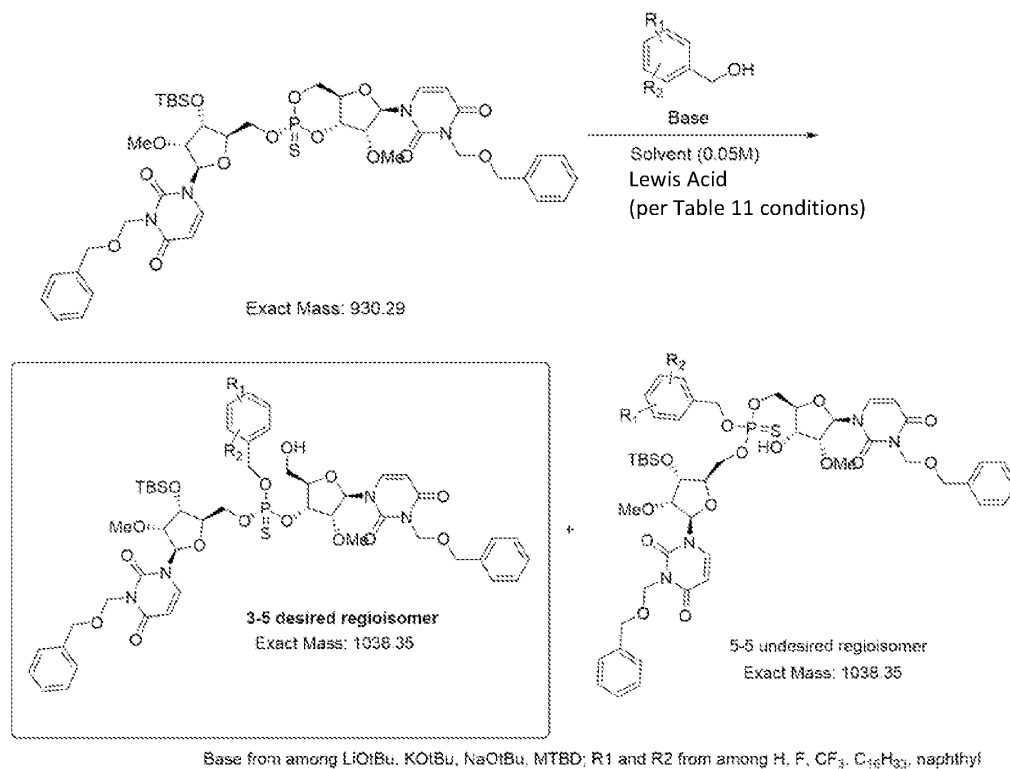
[0440] Solution A: Cuprous chloride (CuCl) (2.5 mg, 0.64 μ L, 0.25 Eq, 25 μ mol) (green powder) was added to a heat gun dried vial under N₂, and then dissolved with Toluene-1 (0.7 mL) at room temperature followed by addition of mTBD (1-methyl-2,3,4,6,7,8-hexahydro-1H-pyrimido[1,2-a]pyrimidine) (23 mg, 22 μ L, 1.5 Eq, 0.15 mmol), 10 minutes later added phenylmethanol (22 mg, 21 μ L, 2 Eq, 0.20 mmol) or other benzylic alcohol (e.g. CF₃BnOH) stirring for 20min at RT under N₂.

[0441] Solution B: 13:45 3-((benzyloxy)methyl)-1-((2S,4aR,6R,7R,7aR)-2-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methoxy)-7-methoxy-2-sulfidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (93 mg, 1 Eq, 0.10 mmol) was dissolved in Toluene-2 (1.3 mL) under schlenk line at 23 °C, followed by addition of solution A dropwise into solution B by syringe needle over 5 minutes. Reaction completed in 20 hours upon monitoring by LCMS.

c. General library scope procedure

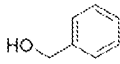
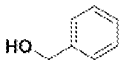
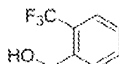
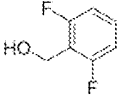
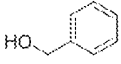
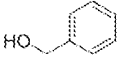
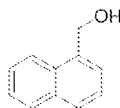
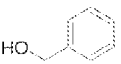
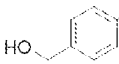
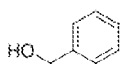
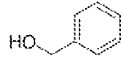
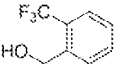
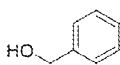
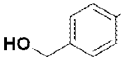
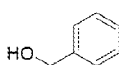
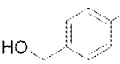
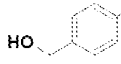
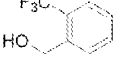


- 169 -

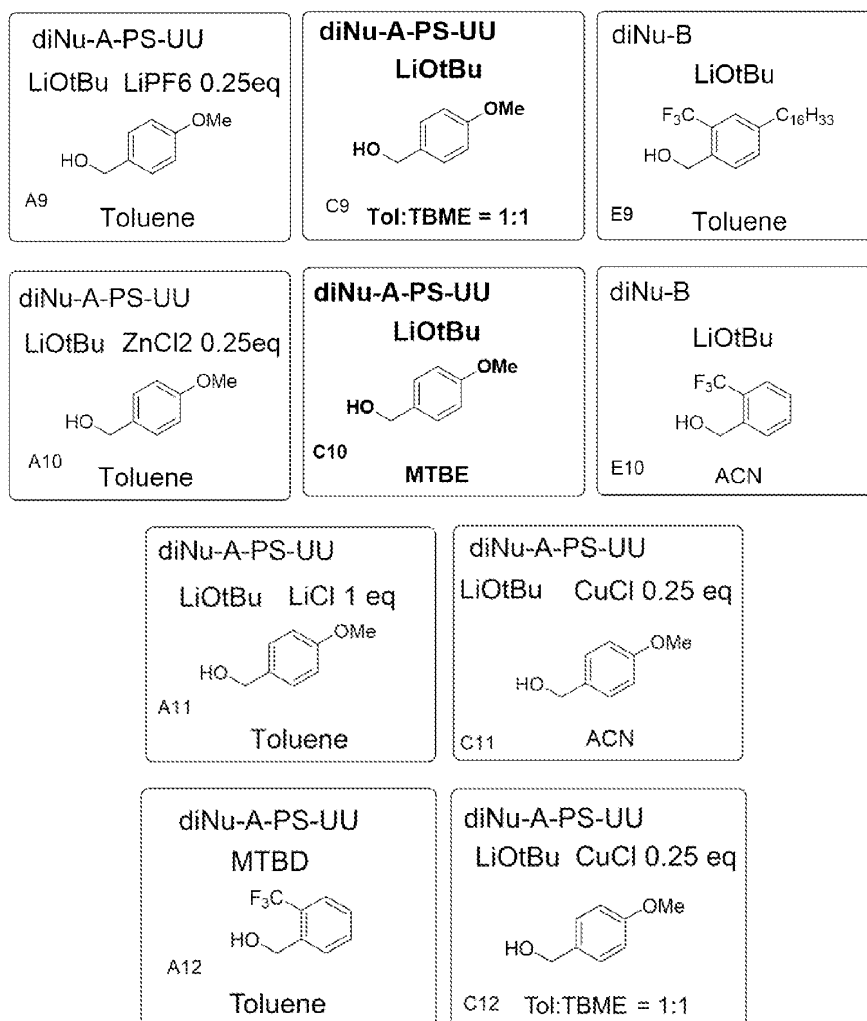
d. First Screening -Plate Design:

diNu-A-PS-UU LiOtBu F₃C HO-CH₂-C₆H₄ A1 Toluene	diNu-A-PS-UU MTBD HO-CH₂-C₆H₄-OMe C1 Toluene	diNu-A-PS-UU LiCl 0.25eq LiOtBu HO-CH₂-C₆H₄-OMe E1 ACN:Tol = 1:1
diNu-A-PS-UU LiOtBu HO-CH₂-C₆H₄-OMe A2 Toluene	diNu-A-PS-UU MTBD CuCl 0.25eq HO-CH₂-C₆H₄ C2 Toluene	diNu-A-PS-UU LiOtBu CuCl 0.25 eq HO-CH₂-C₆H₄-OMe E2 ACN:Tol = 1:1

- 170 -

diNu-A-PS-UU LiOtBu  A3 Toluene	diNu-A-PS-UU MTBD CuCl 1 eq  C3 Toluene	diNu-A-PS-UU LiOtBu 3eq  E3 Toluene
diNu-A-PS-UU LiOtBu  A4 Toluene	diNu-A-PS-UU NaOtBu  C4 Toluene	diNu-A-PS-UU LiOtBu 2.5 eq  E4 Toluene
diNu-A-PS-UU LiOtBu  A5 Toluene	diNu-A-PS-UU LiOtBu 3 eq  C5 Toluene	diNu-A-PS-UU LiOtBu 2.5 eq  E5 Toluene
diNu-A-PS-UU LiOtBu CuCl 0.25 eq  A6 Toluene	diNu-A-PS-UU KOtBu  C6 Toluene	diNu-A-PS-UU LiOtBu 2.5 eq  E6 Toluene
diNu-A-PS-UU LiOtBu CuCl 1 eq  A7 Toluene	diNu-A-PS-UU LiOtBu  C7 ACN:Tol = 1:1	diNu-B LiOtBu  E7 Toluene
diNu-A-PS-UU LiOtBu LiCl 0.25eq  A8 Toluene	diNu-A-PS-UU LiOtBu  C8 ACN	diNu-B LiOtBu  E8 Toluene

- 171 -



Procedure for First Screening

[0442] In a glove box container, each cyclic dinucleotide substrate was dispensed as a stock solution to appropriate reaction vials according to the Plate Design, in rows A and C (using toluene), and E and G (using CAN) with a manual pipette. The toluene stock solutions were the blown down under a stream of nitrogen. Solid bases and additives were dispensed to the appropriate alkoxide forming vials in rows B, D, F of a 96-vial plate using the Quantos.

[0443] Stock solutions of the alkoxides were prepared by: (i) adding the appropriate solvents to the solid bases/additives in the appropriate vials using a manual pipette; (ii)

- 172 -

adding solid alcohols (weighed in the glove box) to the appropriate vials; and (iii) adding the liquid MTBD base to the appropriate vials using a manual pipette. Appropriate solvents were then added to the substrate vials in rows A, C, E. The plate was sealed using the appropriate screw pattern and stirred at RT in the glove box at 800 rpm, allowing at least 30 minutes for the benzylic alkoxides to form and the substrate mixtures to redissolve from time of final addition to alkoxide vials.

[0444] The plate was unsealed and the solutions of alkoxide (one per reaction vial) were then transferred to the corresponding reaction vials (dropwise, ~10 seconds per addition) using a manual pipette. The plate was sealed again using the appropriate screw pattern and stirred at RT in the glove box at 800 rpm.

[0445] The plate was unsealed at 2, 4, 20, 27, and 66 hours and sampled as follows: (1) 20-60 mL of a 2.5 mM stock solution of internal standard TPP (triphenyl phosphate) using CAN; (2) 20 μ L sample aliquot from each well was added to a 0.45 μ m filter vial using a single-channel pipette; (3) 400 μ L of the internal standard stock solution was added to each well using a multi-channel pipette; (4) the samples were analyzed using UPLC high pH_NoMeOH method - Dreadnought (0.5 μ L injection volume); and (5) each sample was given a lot number reflecting its position in the reaction and analytical plates (CAX-D00157-067-XXX where XXX runs from A01 to E10). The solvent in various wells went dry on several occasions. In those cases, 100 μ L of the appropriate solvent was added to reconstitute the reactions. Before the final sample prep, 100 μ L of the appropriate solvent was once again added to all reaction wells.

[0446] Screening conditions and results are shown in Table 11 (Baseline Reaction Conditions: room temperature, toluene (0.05 M), reaction base (1.5 eq), ArOH (2 eq)). [s] depicts ratio of [3-5]:[5-5] regio-isomer products.

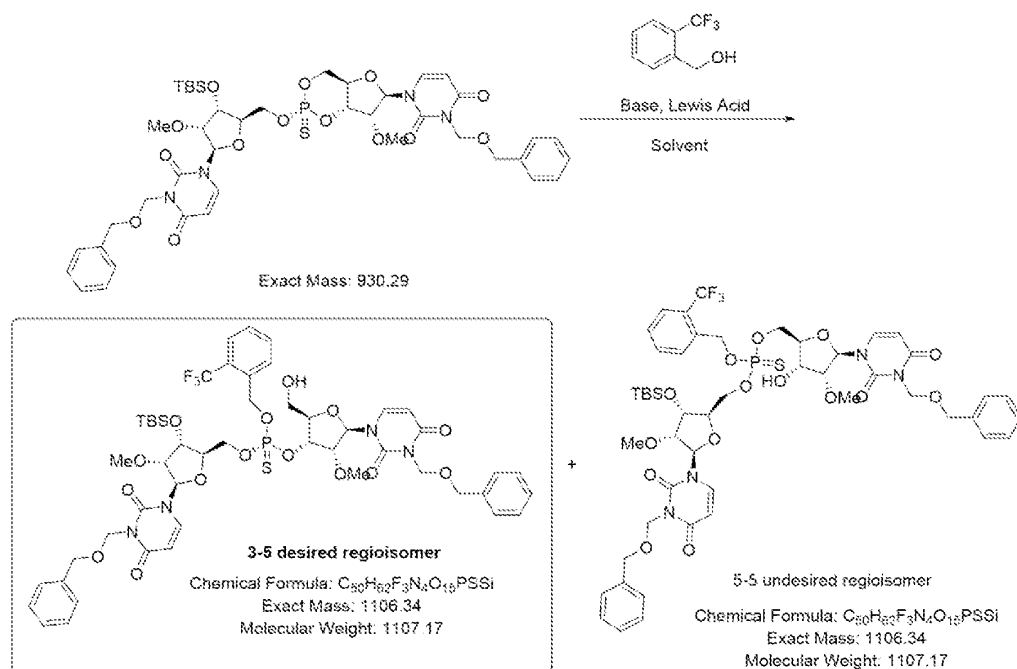
Table 11 – Screening conditions and results

Entry	Alcohol	c	dr	Lewis acid	Base	Solvent	Yield	2a-[3'-5']	2a'-[5'-5']	S
1	2-CF ₃ -BnOH	13:1		None	3 eq LiOtBu	Tol:MeCN (1:1)	95%	47%	47%	1
2	BnOH	8:1		CuCl (0.25 eq)	mTBD	Toluene	90%	74%	16%	4.5
3	BnOH	8:1		None	mTBD	Toluene	90%	54%	36%	1.5
4	2-CF ₃ -BnOH	8:1		CuCl (0.25 eq)	mTBD	Toluene	70%	56%	14%	4
5	2-CF ₃ -BnOH	8:1		CuCl (1 eq)	mTBD	Toluene	99%	74%	25%	3
6	BnOH	13:1		CuCl (1 eq)	mTBD	Toluene	89%	67%	22%	3

- 173 -

7	BnOH	8:1	CuCl (2 eq)	mTBD	Toluene	60%	45%	14.7%	3
8	BnOH	13:1	None	NaOtBu	Toluene				
9	BnOH	13:1	None	KOtBu	Toluene				
10	p-MeO-BnOH	13:1	CuCl (0.25 eq)	LiOtBu	Toluene				
11	p-MeO-BnOH	13:1	LiCl (0.25 eq)	LiOtBu	Toluene				
12	p-MeO-BnOH	13:1	LiCl (1 eq)	LiOtBu	Toluene				NA
13	p-MeO-BnOH	13:1	ZnCl ₂ (0.25 eq)	LiOtBu	Toluene				
14	p-MeO-BnOH	13:1	LiPF ₆ (0.25 eq)	LiOtBu	Toluene				
15	2,6-F ₂ -BnOH	13:1	None	LiOtBu	Toluene				
16	1-NaphthylCH ₂ OH	13:1	None	LiOtBu	Toluene				
17	BnOH	8:1	None	LiOtBu	Toluene	<0.5%	NA	NA	NA
18	2-CF ₃ -BnOH	13:1	None	LiOtBu	Toluene	6.6%	3.8%	2.7%	1.4%

ii. Second Screening -



Procedure for Second Screening

[0447] Cyclic dinucleotide substrates were dispensed as a stock solution to appropriate reaction vials in rows A and C (using toluene), and E and G (using ACN) with a manual pipette. The stock solutions were blown down under a stream of N₂. Solid bases and

- 174 -

additives were then dispensed to the appropriate alkoxide forming vials in rows B, D, F and H of a 1 mL × 96 vial plate using the Quantos.

[0448] Stock solutions of the alkoxides were prepared and stirred for 20 minutes. The substrates were dissolved in the appropriate solvent and stirred for 20 minutes. After this time the alkoxide solutions from B were added to A, dropwise, and the same process was followed for D to C, F to E and H to G. The plate was sealed using the appropriate screw pattern and stirred at RT in the glove box at 800 rpm, allowing at least 30 minutes for the benzylic alkoxides to form and the substrate mixtures to redissolve from the time of final addition to alkoxide vials.

[0449] The plate was unsealed and the solutions of alkoxide (one per reaction vial) were then transferred to the corresponding reaction vials (dropwise, ~10 seconds per addition) using a manual pipette. The plate was again sealed using the appropriate screw pattern and stirred at RT in the glove box at 800 rpm. The plate was unsealed at 2 and 20 hours for sampling and analysis.

Plate Observations

[0450] Species were observed and identified in UPLC during reactions: (i) Retention times were determined using the XBRIDGE POS SCAN LONG method (260 nm); and (ii) Waters XBridge C18, 2.5 µm, 3.0 × 75 mm @ 40 °C; A: 5mM AmFormate in water; B: ACN; Gradient 0-95%B in 13.50 min Hold at 95%B 2.25 min. Total run time 15.75 min; Flow Rate 0.8 mL/min.

[0451] The screen resulted in varying conversion to DP 3'-5' and in a majority of cases 5'-5' DP was the major isomer. Two (2) timepoints were taken: 2 and 20 HR (the latter being the endpoint). The 1st timepoint at 2 h, which likely allowed interconversion of 3'-5' (kinetic product) to the 5'-5' (thermodynamic product). The 2nd timepoint at 20 h, in some cases saw more interconversion of 3'-5' (kinetic product) to the 5'-5' (thermodynamic product). No internal standard was used in the reaction.

[0452] FIG. 2A-2F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 2HR; PhMe (Toluene). FIG. 3A-3F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 2HR; ACN. FIG. 4A-4F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 20HR; PhMe (Toluene). FIG. 5A-5F show the results for Dinucleotide Screen - Plate Key Spotfire Output Overlay 20HR; ACN.

- 175 -

[0453] Screening conditions and results using toluene are shown in Table 12 (Baseline Reaction Conditions: room temperature, toluene (0.05 M), reaction base (1.5 eq), Lewis acid (0.25 eq)). [s] depicts ratio of [3-5]:[5-5] regio-isomer products. This screen was conducted using starting material with dr of 4:1.

Table 12 – Screening conditions and results (Toluene)

Entry	Lewis acid	Base	Solvent	Yield	2a-[3'-5']	2a'-[5'-5']	S
1	CuCl	MTBD	Toluene	99%	26%	74%	0.35
2	CuCl ₂	MTBD	Toluene	15%	12%	3%	4.00
3	CuBr	MTBD	Toluene	99%	20%	80%	0.25
4	Cu(I)acetate	MTBD	Toluene	100%	21%	79%	0.27
5	Cu(OAc) ₂	MTBD	Toluene	73%	62%	11%	5.64
6	(CH ₃ CN) ₄ Cu+BF ₄ -	MTBD	Toluene	100%	15%	85%	0.18
7	LiCl	MTBD	Toluene	8%	5%	3%	1.67
8	Zn(OTf) ₂	MTBD	Toluene	18%	15%	3%	5.00
9	MgBr ₂	MTBD	Toluene	5%	3%	2%	1.50
10	CuCl	TBD	Toluene	100%	33%	67%	0.49
11	CuCl ₂	TBD	Toluene	93%	59%	34%	1.74
12	CuBr	TBD	Toluene	98%	43%	56%	0.77
13	Cu(I)acetate	TBD	Toluene	99%	14%	86%	0.16
14	Cu(OAc) ₂	TBD	Toluene	99%	36%	63%	0.57
15	(CH ₃ CN) ₄ Cu+BF ₄ -	TBD	Toluene	100%	14%	86%	0.16
16	LiCl	TBD	Toluene	96%	51%	45%	1.13
17	Zn(OTf) ₂	TBD	Toluene	5%	4%	1%	4.00
18	MgBr ₂	TBD	Toluene	95%	56%	39%	1.44

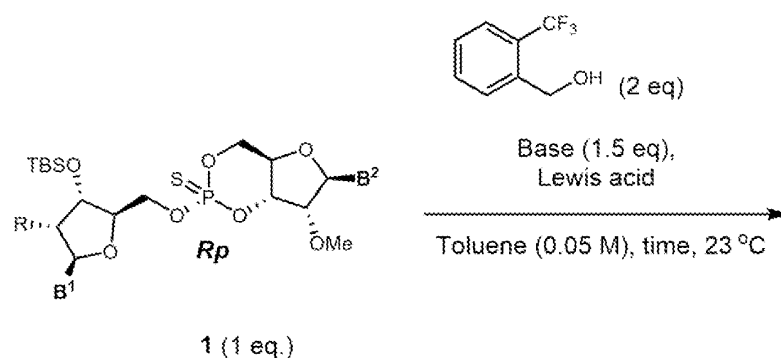
[0454] Screening conditions and results using ACN are shown in Table 13 (Baseline Reaction Conditions: room temperature, toluene (0.05 M), reaction base (1.5 eq), Lewis acid (0.25 eq)). [s] depicts ratio of [3-5]:[5-5] regio-isomer products. This screen was conducted using starting material with dr of 4:1.

- 176 -

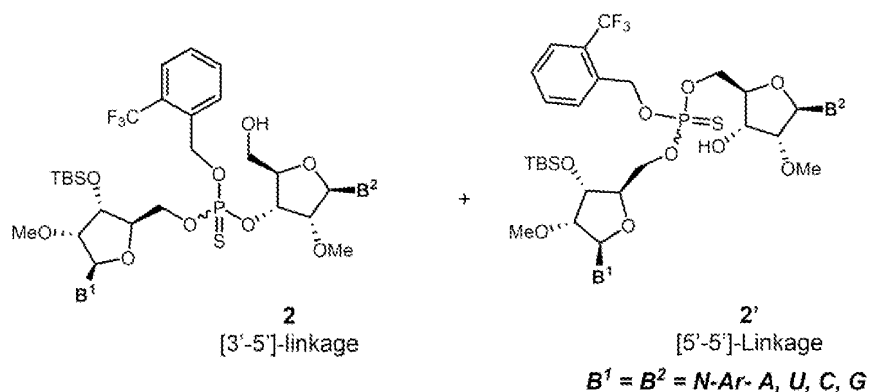
Table 13 – Screening conditions and results (ACN)

Entry	Lewis acid	Base	Solvent	Yield	2a-[3'-5']	2a'-[5'-5']	S
1	CuCl	MTBD	ACN	96%	8%	88%	0.09
2	CuCl ₂	MTBD	ACN	78%	49%	29%	1.68
3	CuBr	MTBD	ACN	97%	9%	89%	0.10
4	Cu(I)acetate	MTBD	ACN	94%	13%	81%	0.16
5	Cu(OAc) ₂	MTBD	ACN	53%	36%	17%	2.12
6	(CH ₃ CN) ₄ Cu+BF ₄ -	MTBD	ACN	97%	8%	89%	0.90
7	LiCl	MTBD	ACN	28%	13%	15%	0.87
8	Zn(OTf) ₂	MTBD	ACN	10%	7%	3%	2.33
9	MgBr ₂	MTBD	ACN	4%	2%	2%	1.0
10	CuCl	TBD	ACN	87%	12%	75%	0.16
11	CuCl ₂	TBD	ACN	13%	44%	21%	2.09
12	CuBr	TBD	ACN	90%	14%	76%	0.18
13	Cu(I)acetate	TBD	ACN	87%	9%	78%	0.11
14	Cu(OAc) ₂	TBD	ACN	93%	10%	83%	0.12
15	(CH ₃ CN) ₄ Cu+BF ₄ -	TBD	ACN	92%	18%	74%	0.24
16	LiCl	TBD	ACN	86%	33%	53%	0.62
17	Zn(OTf) ₂	TBD	ACN	8%	5%	3%	1.67
18	MgBr ₂	TBD	ACN	82%	36%	46%	0.78

iii. Nucleobase Combinations Screening -



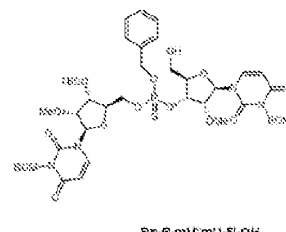
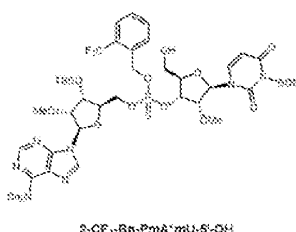
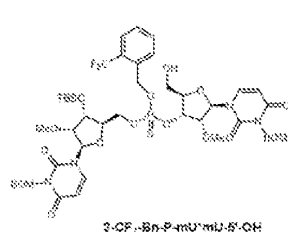
- 177 -



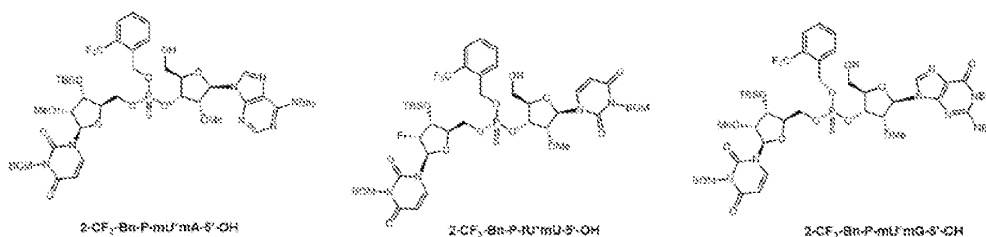
[0455] Screening conditions and results are shown in Table 14 (Baseline Reaction Conditions: room temperature, toluene (0.05 M), reaction base (1.5 eq), Lewis acid (0.25 eq)). [s] depicts ratio of [3-5]:[5-5] regio-isomer products.

Table 14 – Screening conditions and results

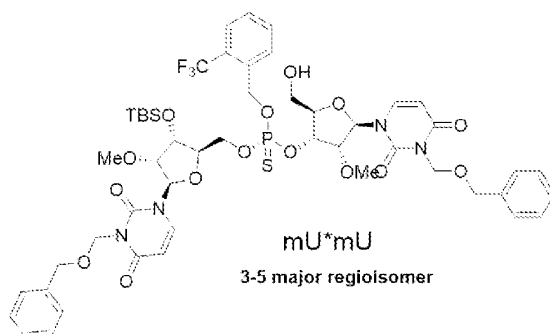
Entry	B ¹ , B ²	dr of 1	Lewis acid	Base	Time	Yield	2a-[3'-5']	2a'-[5'-5']	S
1	mU, mU	8:1	No	LiOTBu	20h	<1%	NA	NA	NA
2	mU, mU	6:1	CuCl (1 eq)	MTBD	45min	54.7%	46%	8.7%	5.3
3	mU, mU	6:1	CuCl (1 eq)	MTBD	22h	55%	43%	12.1%	3.5
4	mU, mU	8:1	CuCl(0.25 eq)	MTBD	44 h	70%	56%	14%	4
5	mU, mU	4:1	CuCl(0.25 eq)	MTBD	40 h	46%	39%	6.9%	5.5
6*	mU, mU	4:1	CuCl(0.25 eq)	MTBD	20 h	38%	30%	8.2%	3.7
7*	mU, mU	4:1	CuCl(0.25 eq)	MTBD	20 h	51%	36%	14.7	2.4
8	mA, mU	10:1	CuCl (1 eq)	MTBD	4h	95%	76%	19%	4
9	mU, mA	21:1	CuCl (0.5 eq)	MTBD	20h	75%	60%	15%	4
10	mU, mG	20:1	CuCl (0.25 eq)	MTBD	70 h	34%	22%	12%	2
11	mU, mG	20:1	CuCl (0.25 eq)	MTBD	5 h	6.5%	5.8%	0.7%	8
12	fU, mU	17:1	CuCl (0.25 eq)	MTBD	20 h	29.7%	22%	2.7%	8



- 178 -

**Example 20. Spectra of ring-opened dinucleotides**

a. O-((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl) O-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) O-(2-(trifluoromethyl)benzyl) phosphorothioate



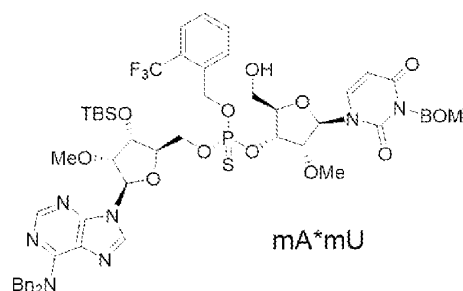
[0456] [3'-3'] ³¹P NMR (162 MHz, CDCl₃) δ 68.11 (Rp), 67.23 (Sp)

[0457] [5'-5'] ³¹P NMR (162 MHz, CDCl₃) δ 68.57 (Rp), 68.28 (Sp)

[0458] DP Exact mass: 1106.34 (1107.35 MH⁺), Measured mass: 1107.46 MH⁺

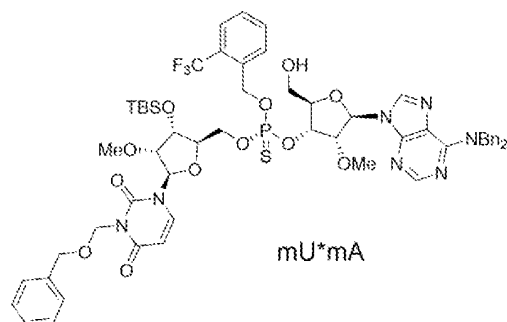
- 179 -

b. O-((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl) O-(((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(6-(dibenzylamino)-9H-purin-9-yl)-4-methoxytetrahydrofuran-2-yl)methyl) O-(2-(trifluoromethyl)benzyl) phosphorothioate



[0459] ^{31}P NMR (162 MHz, CDCl_3): [3'-5'], δ 67.92 (Rp), 67.61 (Sp); [5'-5'], 68.95

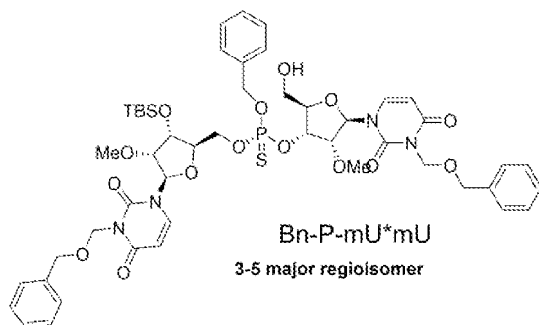
c. O-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) O-((2R,3R,4R,5R)-5-(6-(dibenzylamino)-9H-purin-9-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl) O-(2-(trifluoromethyl)benzyl) phosphorothioate



[0460] ^{31}P NMR (162 MHz, CDCl_3): [3'-5'], δ 67.66 (Rp), 67.52 (Sp). [5'-5'], 68.75

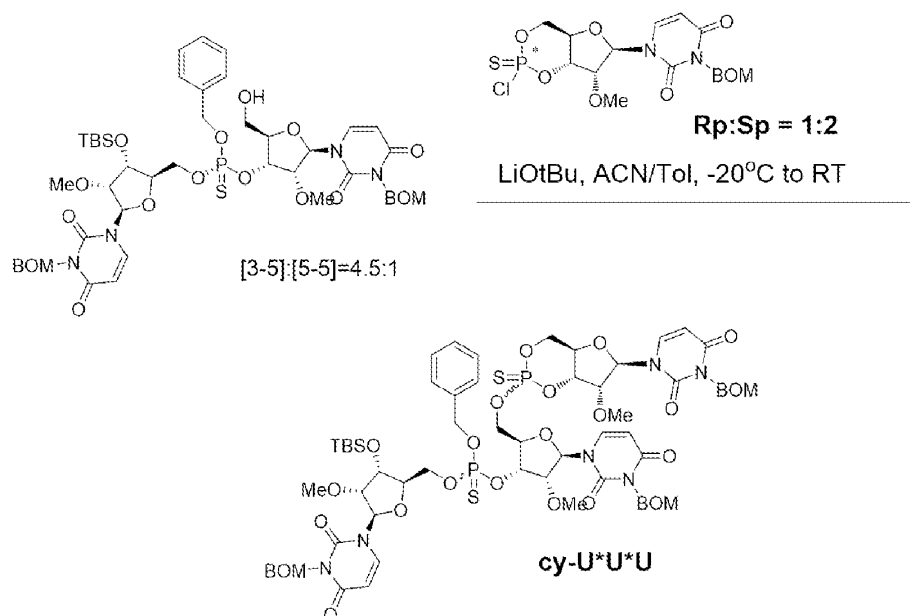
- 180 -

d. O-benzyl O-((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl) O-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) phosphorothioate



[0461] ^{31}P NMR (162 MHz, CDCl_3): [3'-5'], δ 68.38 (Rp), 67.73 (Sp). [5'-5'], 68.94

**Example 21. Synthesis of Trimer Phosphorothioate from Ring Open
Dinucleotide Thioate**



[0462] Under Schlenk line condition, reaction tube A (heat gun dried) was charged with Lithium 2-methyl-2-propanolate (6.0 mg, 7.0 μ L, 1.5 Eq, 75 μ mol) and stirring bar under N₂, followed addition of stock solution of O-benzyl O-((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl) O-(((2R,3R,4R,5R)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-((tert-butyldimethylsilyl)oxy)-4-methoxytetrahydrofuran-2-yl)methyl) phosphorothioate (0.10 g, 50% Wt, 1 Eq, 50 μ mol) in Toluene (1 mL) at -20 °C for 10 minutes.

[0463] Another reaction tube B was charged with Cl-CP (0.18 mmol, 1.5 equiv.) 3-((benzyloxy)methyl)-1-((4aR,6R,7R,7aR)-2-chloro-7-methoxy-2-sulfitotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)pyrimidine-2,4(1H,3H)-dione (36 mg, 1.5 Eq, 75 μ mol) in MeCN (0.6 mL). The reaction tube was kept at -25 °C for 10 minutes and the pre-stirred alkoxide solution from reaction tube A was transferred to reaction tube B dropwise with the aid of a syringe. The reaction was stirred at -20 °C for 18 hours, then warmed to room temperature and stirred for 4 hr. LCMS show desired product peaks of main product and isomers by MH⁺ 1477.3 Based on the uncorrected UPLC reaction crude yield is about (below screen shot of LCMS, assumed newly formed dr ~

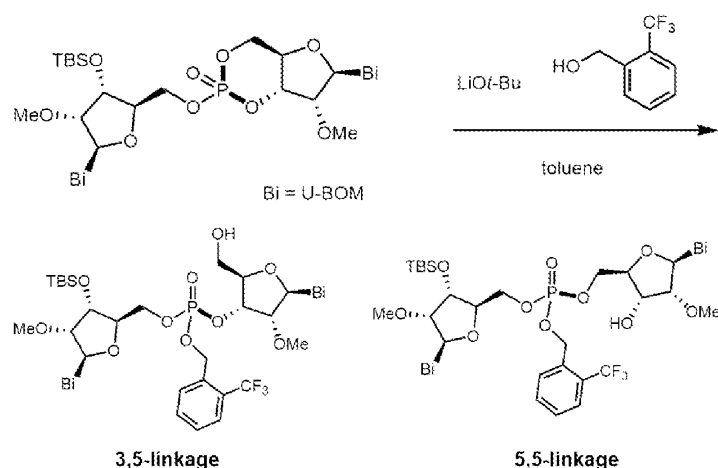
- 182 -

56:25:12:3:2:1.6 defined by retention time and characterization features of cyclic dinucleotide), large amount of PS-Cl remained unreacted. LCMS showed the starting material and 6 product isomers separated, with MS-ID confirmed by EIC (not HRMS).

Example 22. Solution-phase synthesis of oligonucleotides using flow technology

[0464] A general overview of a solution-phase method for oligonucleotide synthesis is presented in FIG. 6.

[0465] A general overview of a continuous-flow oligonucleotide synthesis system is presented in FIG. 7. FIG. 7 shows a glass microreactor ("200 μ l flow reactor"), but any of the types of reactors suggested herein as known in the art can be used.



[0466] A study was completed to compare the efficacy and efficiency of using a batch system or a flow system for oligonucleotide synthesis according to the present disclosure. Twelve test reactions were completed with flow systems using different temperatures (-20°C , 0°C , ambient temperature ($\sim 20\text{--}25^{\circ}\text{C}$), and 45°C) and different residence times (30, 40, or 50 minutes). Solvent (toluene) and concentrations were fixed from the batch procedure. Analysis by ^{31}P NMR and LCMS revealed that performing the reaction at ambient temperature with 50 minutes of residence time allows full conversion of the reaction with the same selectivity ratio as in a batch with cryogenic conditions.

[0467] A solution of LiOtBu (0.148 M, 1 equiv.) and 2- CF_3BnOH (0.2 M) in toluene [Solution A] was prepared, bubbled with nitrogen, and kept as a stock solution in a 100 ml glass bottle. A solution of cyclic dinucleotide in toluene (0.07 M) [Solution B] was prepared, bubbled with nitrogen, and kept as stock solution in a 100 ml glass bottle. Methanol was used as quench, bubbled with nitrogen, and kept in a third 100 ml glass

bottle. All solutions were connected to a nitrogen line saturated with toluene (solutions A and B) or methanol, accordingly.

[0468] Each bottle was connected through a 1/16" O.D. tubing to a VICI M6HP pump. Solutions A and B were fed into a MR-LAB-MS flow reactor of 200 μ L volume (from Little Things Factory). The temperature was controlled by the Polar Bear device (from Uniqsis Ltd.) and kept at 22 °C.

[0469] At the outlet of the reactor a PEEK-tee was used to mix and quench the reaction mixture with methanol. This line was connected to a fraction collector to automatically discard or collect fractions of the quenched reaction.

[0470] The first step was to flush the reactor with toluene to ensure complete cleanliness of the reactor and tubing. Then, after the pumps and lines were primed with the reagents and quench solutions, the three pumps were set to flowrates of 1.7 μ L/min for solution A, 2.3 μ L/min for solution B (yielding 50 min residence time), and 6.6 μ L/min for methanol. During the first three hours of the experiment, the reaction outlet mixture was discarded (unsteady operation). When the steady-state conditions were reached, two fractions were collected: the first fraction corresponds to 2.5 hours of collection time with a theoretical mass of 26.7 mg product, and a second fraction of 2 hour corresponding to a theoretical value of 21.4 mg product.

[0471] The two fractions were combined, volatiles were evaporated under vacuum and the obtained mass was 48 mg. The crude was analyzed by LCMS to determine the selectivity ratio between isomers and its purity.

[0472] Table 15 summarizes certain details for comparison.

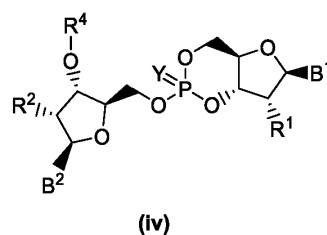
Table 15

	Batch	Flow
Isomer Control	85:15	85:15
Cryogenic Conditions	-20 °C (Required)	Ambient Temperature
Reaction Time	4-6 hours (27 mg scale)	45-60 minutes
Reproducibility	Medium	High
Safety Hazards	Medium	Low

CLAIMS

We claim:

1. A method for preparing a dinucleotide of Formula (iv) or a salt or solvate thereof,



wherein:

Y is O or S;

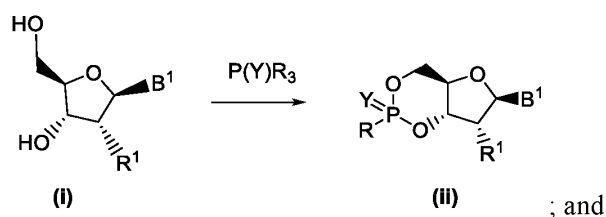
R¹ and R² are independently chosen from hydrogen, hydroxy, halogen, N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy; optionally wherein R¹ and R² are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

B¹ and B² are nucleobases, which may optionally be protected with one or more protecting groups; and

R⁴ is hydrogen or a protecting group;

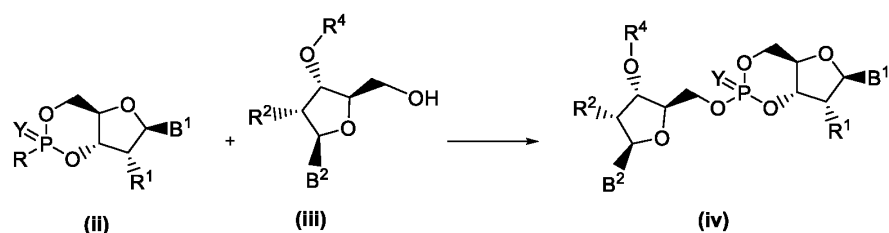
the method comprising:

(a) reacting a nucleoside of Formula (i) with P(Y)R₃, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii)



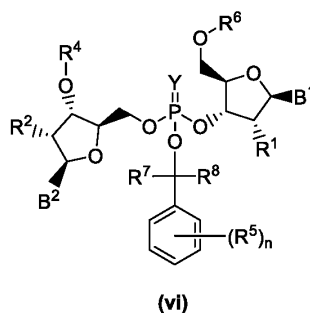
(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R⁴ is a protecting group:

- 185 -



wherein the method further comprises optionally removing one or more of the protecting groups.

2. A method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof,



wherein:

Y is O or S;

R^1 and R^2 are independently chosen from hydrogen, hydroxy, halogen, N_3 , (C_1 - C_{20}) alkyl, and (C_1 - C_{20}) alkoxy; optionally wherein R^1 and R^2 are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

B^1 and B^2 are nucleobases, which may optionally be protected;

R^4 is hydrogen or a protecting group;

n is chosen from 1, 2, 3, 4, and 5;

each R^5 is independently a substituent on the phenyl ring of a benzyl protecting group;

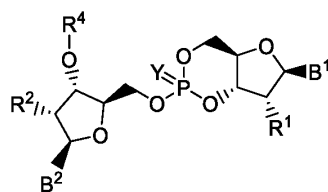
R^6 is hydrogen or a protecting group; and

R^7 and R^8 are independently selected from hydrogen, (C_1 - C_{20}) alkyl and aryl;

the method comprising:

- (i) providing the cyclic phosphorus dinucleotide of Formula (iv) of claim 1, wherein R^4 is a protecting group:

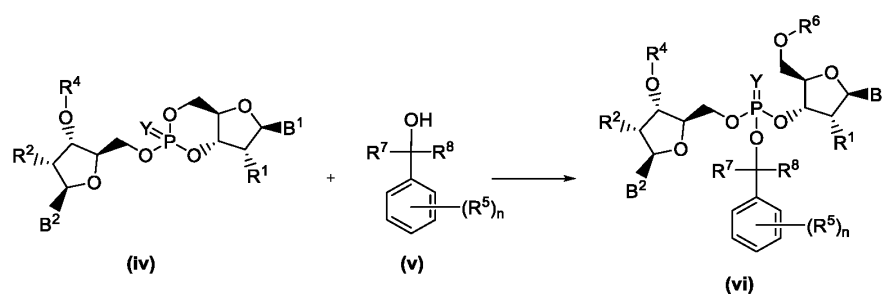
- 186 -



(iv)

; and

(ii) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):



(iv)

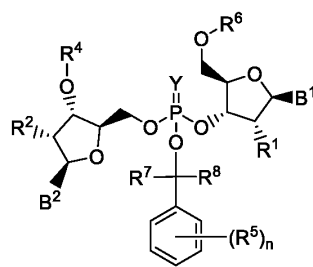
(v)

(vi)

wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl;

wherein the method further comprises optionally removing one or more of the protecting groups.

3. A method for preparing a dinucleotide of Formula (vi) or a salt or solvate thereof,



(vi)

;

wherein:

Y is O or S;

R^1 and R^2 are independently chosen from hydrogen, hydroxy, halogen, N₃, (C₁-

- 187 -

C₂₀) alkyl, and (C₁-C₂₀) alkoxy; optionally wherein R¹ and R² are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

B¹ and B² are nucleobases, which may optionally be protected;

R⁴ is hydrogen or a protecting group;

n is chosen from 1, 2, 3, 4, and 5;

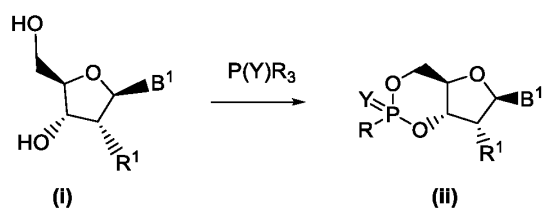
each R⁵ is independently a substituent on the phenyl ring of the benzyl protecting group;

R⁶ is hydrogen or a protecting group; and

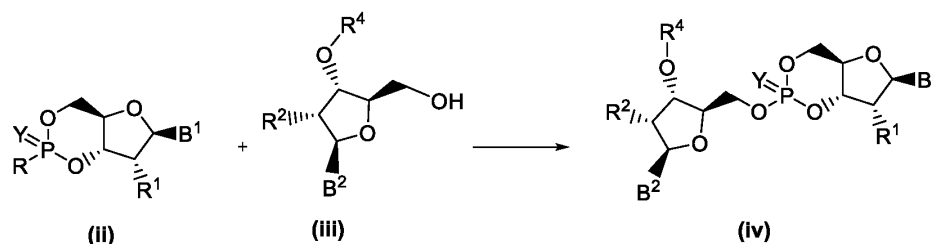
R⁷ and R⁸ are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl;

the method comprising:

(a) reacting a nucleoside of Formula (i) with P(Y)R₃, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl; R is Cl, Br, or (C₁-C₆) alkoxy, thereby forming a cyclic phosphorous nucleoside of Formula (ii):



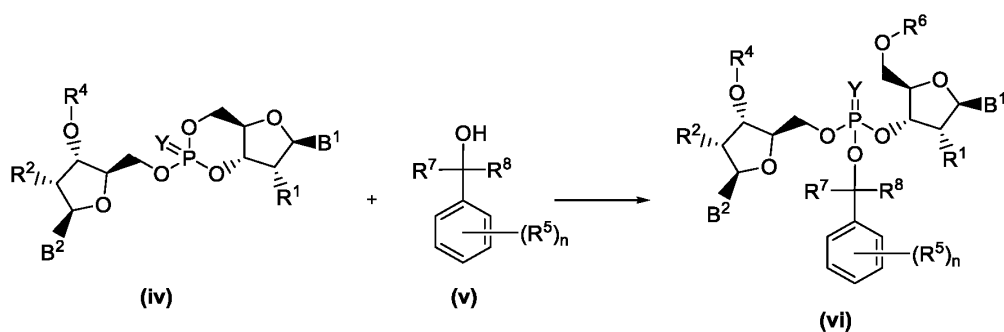
(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R⁴ is a protecting group:



; and

(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):

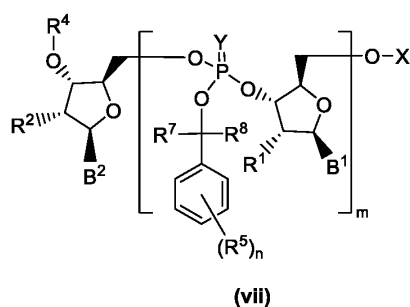
- 188 -



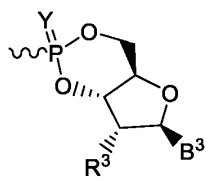
wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl;

wherein the method further comprises optionally removing one or more of the protecting groups.

4. A method for preparing an oligonucleotide of Formula (vii):



wherein:



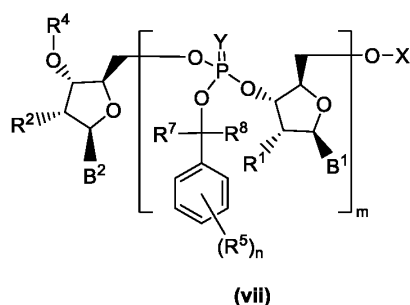
X is hydrogen, a protecting group, or
each Y is independently O or S;

R^1 , R^2 , and R^3 are independently chosen from hydrogen, hydroxy, halogen, N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy; optionally wherein R^1 , R^2 , and R^3 are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

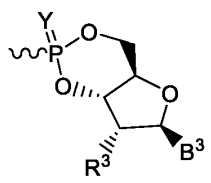
- 189 -

R^4 is H or a protecting group;
 n is chosen from 1, 2, 3, 4, and 5;
 each R^5 is independently a substituent on the phenyl ring of the benzyl protecting group;
 each R^7 and R^8 is independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl;
 B^1 , B^2 , and B^3 are nucleobases, which may optionally be protected; and
 m is chosen from integers ranging from 1 to 18;
 the method comprising:
 (i) providing the dinucleotide of Formula (vi) from claim 2 or claim 3; and
 (ii) repeating the reactions of steps (a), (b), and (c) from claim 3 one or more times
 to obtain an oligonucleotide of Formula (vii);
 wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is
 reacted with the 5' terminal nucleoside of the dinucleotide or oligonucleotide;
 wherein the method further comprises optionally removing one or more of the
 protecting groups.

5. A method for preparing an oligonucleotide of Formula (vii):



wherein:



X is hydrogen, a protecting group, or
 each Y is independently O or S;
 R^1 , R^2 , and R^3 are independently chosen from hydrogen, hydroxy, halogen, N₃,
 (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy; optionally wherein R^1 , R^2 , and R^3 are

- 190 -

independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

R^4 is H or a protecting group;

n is chosen from 1, 2, 3, 4, and 5;

each R^5 is independently a substituent on the phenyl ring of the benzyl protecting group;

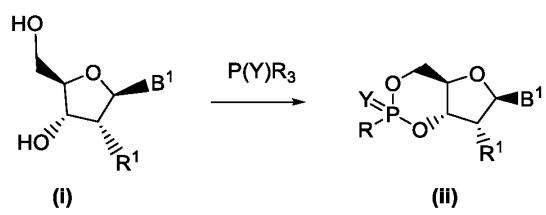
each R^7 and R^8 is independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl;

B^1 , B^2 , and B^3 are nucleobases, which may optionally be protected; and

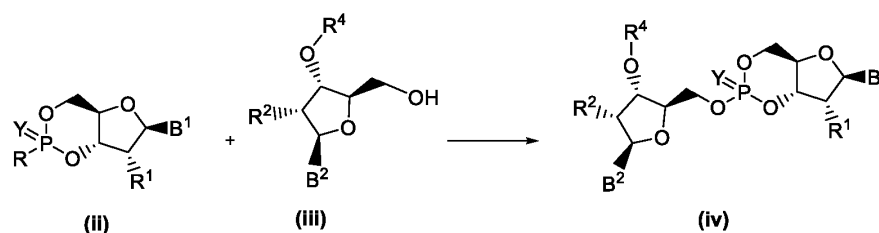
m is chosen from integers ranging from 1 to 18;

the method comprising:

(a) reacting a nucleoside of Formula (i) with $P(Y)R_3$, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):

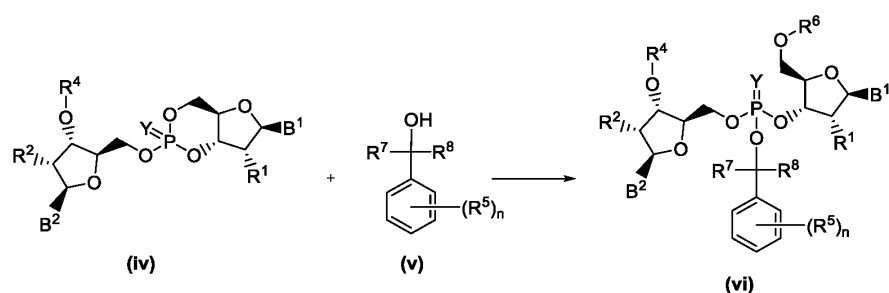


(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:



(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):

- 191 -



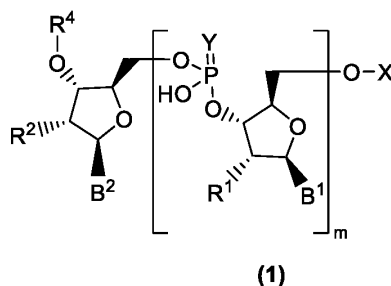
wherein: each R^5 is independently a substituent on the phenyl ring of the benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl; and

(d) repeating the reactions of steps (a), (b), and (c) one or more times to obtain an oligonucleotide of Formula (vii);

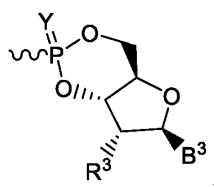
wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the dinucleotide or oligonucleotide;

wherein the method further comprises optionally removing one or more of the protecting groups.

6. A method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof,



wherein:



X is hydrogen, a protecting group, or
each Y is independently O or S;

R^1 , R^2 , and R^3 are independently chosen from hydrogen, hydroxy, halogen, N_3 ,

- 192 -

(C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy; optionally wherein R¹, R², and R³ are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

B¹, B², and B³ are nucleobases, which may optionally be protected;

R⁴ is hydrogen or a protecting group; and

m is chosen from integers ranging from 0 to 18;

the method comprising:

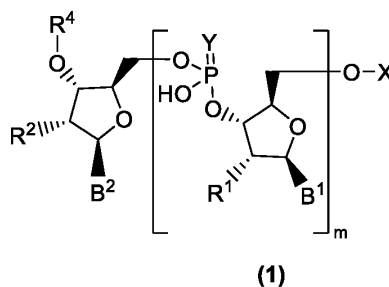
(i) providing the dinucleotide of Formula (vi) from claim 2 or claim 3, or the oligonucleotide of Formula (vii) from claim 4 or claim 5;

(ii) hydrogenating the dinucleotide of Formula (vi) or the oligonucleotide of Formula (vii); and

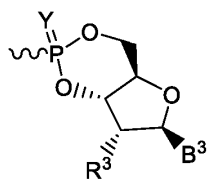
(iii) optionally removing one or more of the protecting groups;

wherein the method produces the oligonucleotide of Formula (1) or a salt or solvate thereof.

7. A method for preparing an oligonucleotide of Formula (1) or a salt or solvate thereof,



wherein:



X is hydrogen, a protecting group, or

each Y is independently O or S;

R¹, R², and R³ are independently chosen from hydrogen, hydroxy, halogen, N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy; optionally wherein R¹, R², and R³ are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl

- 193 -

(MOE);

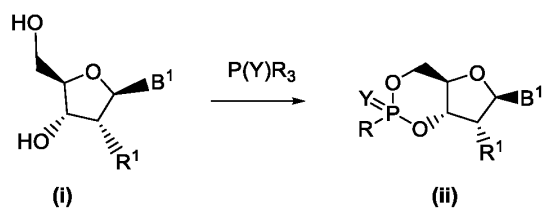
B^1 , B^2 , and B^3 are nucleobases, which may optionally be protected;

R^4 is hydrogen or a protecting group; and

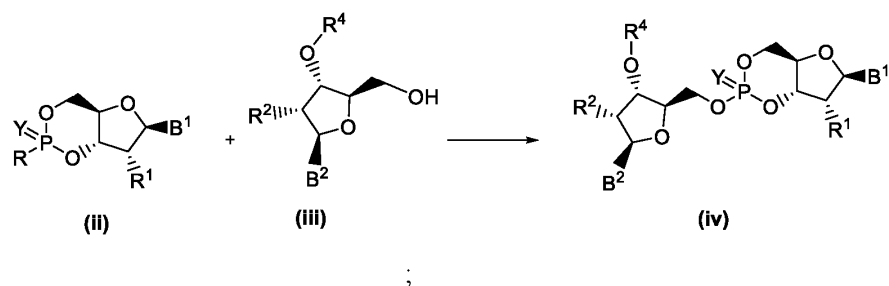
m is chosen from integers ranging from 0 to 18;

the method comprising:

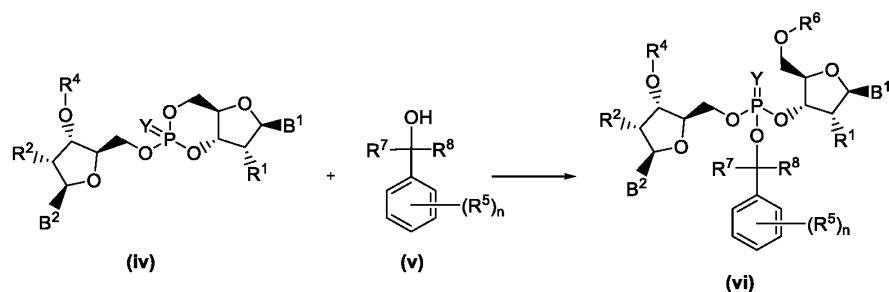
(a) reacting a nucleoside of Formula (i) with $P(Y)R_3$, wherein Y is O or S, and wherein each R is independently selected from Cl, Br, (C_1-C_6) alkoxy, (C_6-C_{12}) aryloxy, S- (C_1-C_6) alkyl, O-aryl, and S-aryl, thereby forming a cyclic phosphorous nucleoside of Formula (ii):



(b) reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii) to form a cyclic phosphorus dinucleotide of Formula (iv), wherein R^4 is a protecting group:



(c) reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) to form a dinucleotide of Formula (vi):

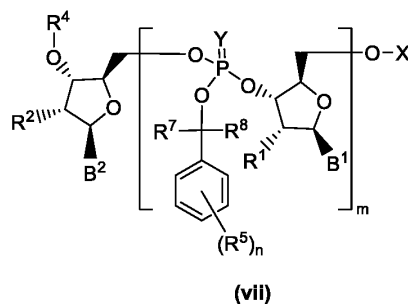


wherein: each R^5 is independently a substituent on the phenyl ring of the

- 194 -

benzyl alcohol nucleophile; n is chosen from 1, 2, 3, 4, and 5; R^6 is hydrogen or a protecting group; and R^7 and R^8 are independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl;

(d) optionally, repeating the reactions of steps (a), (b), and (c) one or more times to obtain an oligonucleotide of Formula (vii):



wherein, in repeating step (b), the cyclic phosphorous nucleoside of Formula (ii) is reacted with the 5' terminal nucleoside of the oligonucleotide;

(e) hydrogenating the product of step (c) or step (d); and

(f) optionally removing one or more of the protecting groups;

wherein the method produces the oligonucleotide of Formula (1) or a salt or solvate thereof.

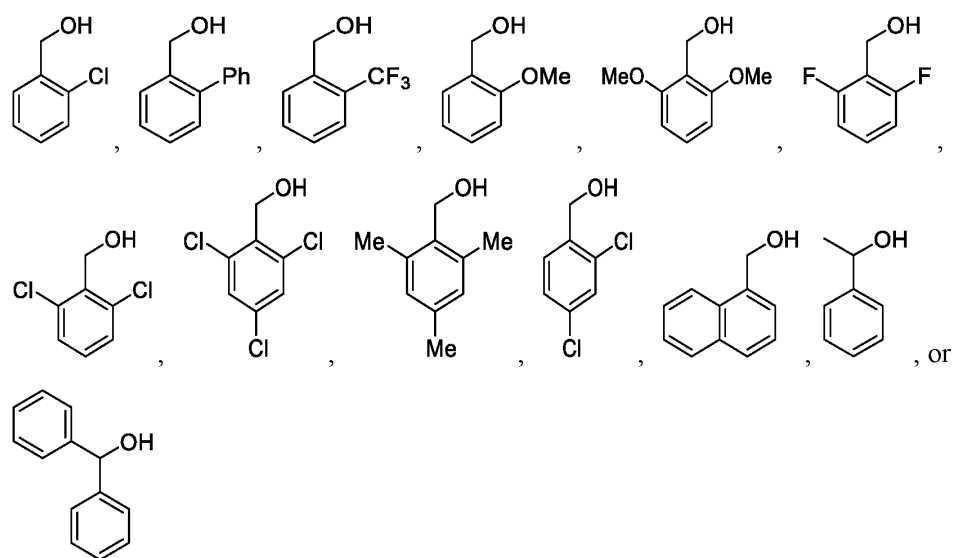
8. The method according to any one of claims 2 to 7, wherein the cyclic phosphorus dinucleotide of Formula (iv) is obtained as a mixture of stereoisomers, and wherein the stereoisomers are separated prior to the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v).
9. The method according to any one of claims 2 to 8, wherein each instance of R^5 is an ortho- or para- substitution on the benzyl group; optionally wherein each instance of R^5 is an ortho- substitution on the benzyl group.
10. The method according to any one of claims 2 to 9, wherein each instance of R^5 is independently chosen from halogen, (C_1-C_{20}) alkyl, (C_1-C_{20}) alkoxy, trifluoromethyl, and phenyl; optionally wherein each R^5 is independently selected from phenyl,

- 195 -

methyl, methoxy, fluoro, chloro, and CF_3 ; optionally wherein R^5 is selected from chloro and CF_3 .

11. The method according to any one of claims 2 to 8, wherein R^5 is a fused phenyl ring forming a naphthyl ring system.

12. The method according to any one of claims 2 to 8, wherein the substituted benzyl alcohol nucleophile of Formula (v) is:



13. The method according to any one of claims 2 to 12, wherein the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) is carried out in a solvent system comprising:

tetrahydrofuran (THF); 2-methyltetrahydrofuran (2Me-THF); diethyl ether; 1,4-dioxane; dimethylsulfoxide (DMSO); N-methylpyrrolidone (NMP); dimethylacetamide (DMA); dichloromethane (DCM); acetonitrile (MeCN); methyl tert-butyl ether (MTBE); tertAmyl alcohol; or toluene.

14. The method according to any one of claims 2 to 13, wherein the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v) is carried out in the presence of a base chosen from: n-

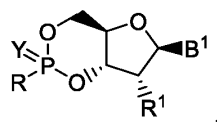
- 196 -

butyllithium (n-BuLi); lithium tertbutoxide (LiOtBu); lithium bis(trimethylsilyl)amide (LiHMDS); potassium bis(trimethylsilyl)amide (KHMDs); lithium tetramethylpiperidide (LiTMP); or Li-R_Z, where R_Z is alkyl or aryl; sodium t-butoxide (NaOtBu) + lithium chloride (LiCl); potassium t-butoxide (KOtBu) + lithium chloride (LiCl); triethylamine (Et₃N); Diazabicycloundecene (DBU); 4-Dimethylaminopyridine (DMAP); S-collidine; and triazabicyclodecene (TBD).

15. The method according to claim 14, wherein the base is pre-mixed with the substituted benzyl alcohol nucleophile of Formula (v) prior to the step of reacting the cyclic phosphorus dinucleotide of Formula (iv) with a substituted benzyl alcohol nucleophile of Formula (v).
16. The method according to claim 14 or claim 15, wherein the base is lithium tertbutoxide.
17. The method according to any one of claims 2 to 16, wherein the dinucleotide of Formula (vi) is isolated by reacting with a silyl chloride.
18. The method of any one of claims 1 to 17, wherein the cyclic phosphorus chloride nucleoside of Formula (ii) is obtained as a mixture of stereoisomers, and wherein the stereoisomers are separated prior to the step of reacting the cyclic phosphorous nucleoside of Formula (ii) with a nucleoside of Formula (iii).
19. The method of any one of claims 1 to 18, wherein R² is an alkoxy group linked to the adjacent O to form a ketal protecting group with R₄.
20. The method of any one of claims 1 to 19, wherein R⁴ is chosen from H, a silyl group, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl (TBS, TBDMS), t-butyldiphenylsilyl, triisopropylsilyl, or a ketal protecting group formed with R².
21. The method of any one of claims 1 to 20, wherein each nucleobase is chosen from pyrimidines and purines.

- 197 -

22. The method of any one of claims 1 to 21, wherein each nucleobase is protected with one or more protecting groups independently chosen from dimethyl formamide (DMF), formamidine, DMF-formamidine, N-methylpyrrolidin-2-ylidenyl (PyA), pivaloyloxymethyl (POM), phthalimido, chloro, bromo, acyl, allyl, benzyl, tert-butyloxycarbonyl (Boc), and benzyloxymethyl (BOM).
23. The method of any one of claims 1 to 22, wherein the method is a liquid phase process.
24. The method of any one of claims 1 to 23, wherein the method is conducted using continuous flow.
25. The method of any one of claims 1 to 24, wherein the method is carried out in a flow reactor.
26. The method of claim 24 or claim 25, wherein the method is performed at ambient temperature.
27. The method of any one of claims 24-26, wherein the reaction time is decreased as compared to the same reaction in a batch reactor; optionally wherein the reaction time in the flow reactor is from 45-120 minutes; optionally from 45-60 minutes.
28. A dinucleotide or oligonucleotide produced by the method of any one of claims 1 to 25.
29. A cyclic phosphorous nucleoside of the following formula:

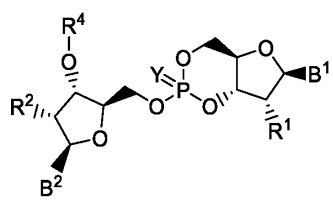


or a salt or solvate thereof, wherein: Y is O or S; each R is independently selected from Cl, Br, (C₁-C₆) alkoxy, (C₆-C₁₂) aryloxy, S-(C₁-C₆) alkyl, O-aryl, and S-aryl; R¹ is chosen from hydrogen, hydroxy, halogen, N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy

- 198 -

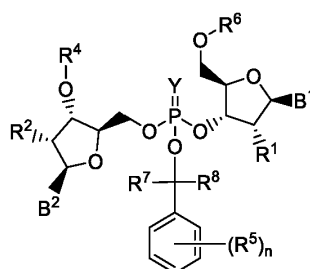
(optionally wherein R^1 is chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE)); and B^1 is nucleobase, which may be optionally protected.

30. A cyclic phosphorus dinucleotide of the following formula:



or a salt or solvate thereof, wherein: Y is O or S; R^1 and R^2 are independently chosen from hydrogen, hydroxy, halogen, N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (optionally wherein R^1 and R^2 are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE)); R^4 is H or a protecting group; and B^1 and B^2 are nucleobases, which may optionally be protected.

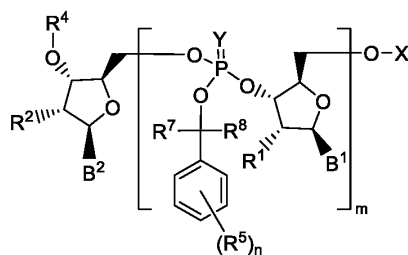
31. A dinucleotide of the following formula:



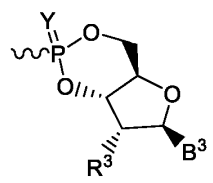
or a salt or solvate thereof, wherein: Y is O or S; R^1 and R^2 are independently chosen from hydrogen, hydroxy, halogen, N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy (optionally wherein R^1 and R^2 are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE)); R^4 is H or a protecting group; each R^5 is independently a substituent on the phenyl ring of the benzyl protecting group; each R^7 and R^8 is independently selected from hydrogen, (C_1-C_{20}) alkyl and aryl; B^1 and B^2 are nucleobases, which may optionally be protected; and n is chosen from 1, 2, 3, 4, and 5.

32. An oligonucleotide of the following formula:

- 199 -



or a salt or solvate thereof, wherein:



X is hydrogen, a protecting group, or

each Y is independently O or S;

R¹, R², and R³ are independently chosen from hydrogen, hydroxy, halogen, N₃, (C₁-C₂₀) alkyl, and (C₁-C₂₀) alkoxy; optionally wherein R¹, R², and R³ are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

R⁴ is H or a protecting group;

n is chosen from 1, 2, 3, 4, and 5;

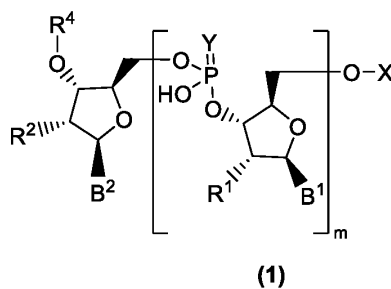
each R⁵ is independently a substituent on the phenyl ring of the benzyl protecting group;

each R⁷ and R⁸ is independently selected from hydrogen, (C₁-C₂₀) alkyl and aryl;

B¹, B², and B³ are nucleobases, which may optionally be protected; and

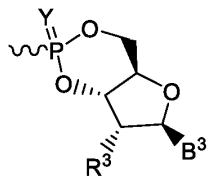
m is chosen from integers ranging from 1 to 18.

33. An oligonucleotide of the following formula:



- 200 -

or a salt or solvate thereof, wherein:



X is hydrogen, a protecting group, or

each Y is independently O or S;

R^1 , R^2 , and R^3 are independently chosen from hydrogen, hydroxy, halogen, N_3 , (C_1-C_{20}) alkyl, and (C_1-C_{20}) alkoxy; optionally wherein R^1 , R^2 , and R^3 are independently chosen from hydrogen, fluorine, methoxy, and -O-methoxyethyl (MOE);

B^1 , B^2 , and B^3 are nucleobases, which may optionally be protected;

R^4 is hydrogen or a protecting group; and

m is chosen from integers ranging from 0 to 18.

34. A solution comprising: (i) a nucleoside of claim 29; (ii) a dinucleotide of claim 28, claim 30, or claim 31; and/or (iii) an oligonucleotide of claim 28, claim 32, or claim 33.
35. Use of a dinucleotide or an oligonucleotide of any one of claims 28 and 30-34, in the synthesis of an oligonucleotide or polynucleotide.
36. Use of a dinucleotide or an oligonucleotide of any one of claims 28 and 30-34, as a blockmer amidite in solid phase, chemical ligation, or other synthesis method of an oligonucleotide or polynucleotide.
37. Use of a dinucleotide or an oligonucleotide of any one of claims 26 and 28-32, in an enzymatic ligation reaction for the synthesis of an oligonucleotide or polynucleotide.