

Cyclic Dinucleotide Analogue, Pharmaceutical Composition Thereof, and Application

[0001] This application claims the priority of Chinese patent application CN201811105973.6 filed on September 21, 2018, Chinese patent application CN201811276297.9 filed on October 30, 2018, Chinese patent application CN201910042984.2 filed on January 17, 2019 and Chinese patent application CN201910287528.4 filed on April 11, 2019. The contents of aforesaid Chinese patent applications are incorporated herein by reference in their entireties.

Technical Field

[0002] The present disclosure relates to a cyclic di-nucleotide analogue, a pharmaceutical composition thereof and a use thereof as STING agonist and for activation of the STING pathway.

Background

[0003] Stimulator of Interferon Genes (STING), also known as T1MEM173, MITA, MPYS and ERIS, is an important signaling molecule in innate immune signaling. The protein encoded by this gene contains a 5-stranded transmembrane structure and plays an important regulatory role in the immune response associated with viral or bacterial infection. STING is a pattern recognition receptor which detects exogenous nucleic acids in the cytoplasm and activates signal transduction pathways associated with type I interferon responses. In addition, it has been shown that STING is involved in the regulation of apoptotic signaling by interacting with the type II major histocompatibility complex (MHCII). Studies performed on human tumors with spontaneous T-cell infiltration have shown that CD8⁺ T-cell infiltration is closely associated with the transcriptional profile of type I interferons (Harlin *et al.*, *Cancer Res*, 2009; 69(7): OF1). Mechanistic studies carried out on mouse models have shown that T cell activation processes against tumor-associated antigens show abnormalities in experimental animals with defective type I interferon signaling (Diamond *et al.*, *J. Exp. Med.*, 2011; 208(10): 1989; Fuerte *et al.*, *J. Exp. Med.*, 2011; 208(10):2005). Further studies on tumor recognition by the innate immune system *in vivo* and on the signaling pathways involved in this process, such as tumor-triggered IFN expression mediated by antigen-presenting cells (APCs), have

revealed that the STING signaling pathway can be activated by cytoplasmic DNA, and that these exogenous nucleic acids can be recognized by cyclic-GMP-AMP synthetase (cGAS) and then catalyzes the generation of cyclized nucleic acids such as cyclic GMP-AMP (cGMP) which can act as endogenous ligands activating STING signaling (Sun et al, *Science*, 2013; 339(15): 786). Activated STING can subsequently induce autophosphorylation of TBK1 kinase and phosphorylation of interferon regulatory factor 3 (IRF-3), and phosphorylated IRF3 can further activate the gene transcription process of type I interferon and regulate the synthesis and secretion of type I interferon, which in turn induces an immune response. In summary, it has been shown that the STING signaling pathway plays an extremely important role in the tumor recognition process by the innate immune system, and the activation of this signaling pathway on antigen-presenting cells is directly related to the activation of T cells against tumor-associated antigens. Based on its role in tumor immune recognition, it can be expected that activation of STING signaling by drugs or other pharmacological approaches can enhance IFN expression and have a positive effect on tumor therapy. Therefore, the development of STING signaling agonists for the treatment of tumor diseases has become a hot research topic.

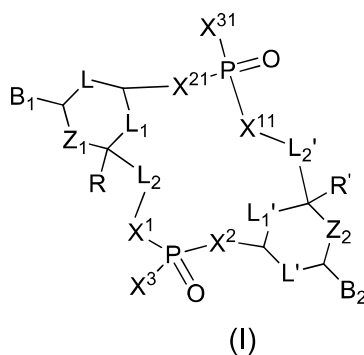
[0004] In addition, it has been shown that the stimulation of STING signaling pathway activation also contributes to antiviral responses. Loss of functional response at the cellular or organismal level demonstrates that viral load cannot be controlled in the absence of STING. Activation of the STING signaling pathway triggers immune response leading to anti-vascular and pro-inflammatory cytokines against the virus and mobilizes the innate and acquired immune systems. Thus, small molecule compounds with agonistic effects on the STING signaling pathway have potential for the treatment of chronic viral infections and could be used, for example, to treat HBV.

[0005] Several cyclic di-nucleotide analogs with agonistic effects on the STING signaling pathway have been disclosed (WO2014/093936, WO2014/189805, WO2014/189806, WO2016/120305, WO2016/145102, WO2017/027645, WO2017/075477, WO2017/093933, WO2017/123657, WO2017/123669, WO2017/161349, WO2017/186711, WO2018/009466, WO2018/009648, WO2018/009652, WO2018/ 045204, WO2018/060323, WO2018/065360, WO2018/098203, WO2018/100558), but at present, no STING agonist has been approved for marketing.

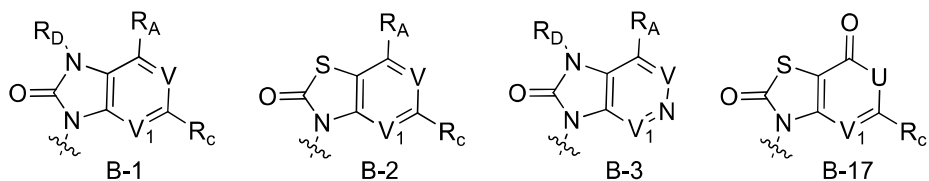
Content of the present disclosure

[0006] The technical problem to be solved in the present disclosure is to provide a novel cyclic di-nucleotide analogue, a pharmaceutical composition thereof and a use thereof. The cyclic di-nucleotide analogue of the present disclosure has good STING modulating effect and can effectively treat, alleviate and/or prevent various diseases caused by immunosuppression, such as tumors, infectious diseases, neurodegenerative diseases, psychiatric disorders or autoimmune diseases.

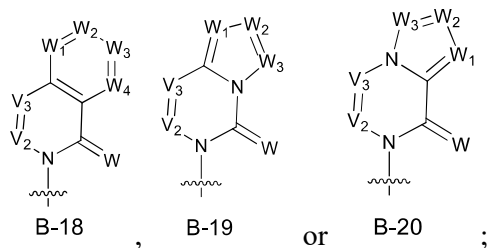
[0007] The present disclosure provides a cyclic di-nucleotide analogue (I), an isomer, a prodrug, a stable isotope derivative or a pharmaceutically acceptable salt thereof;



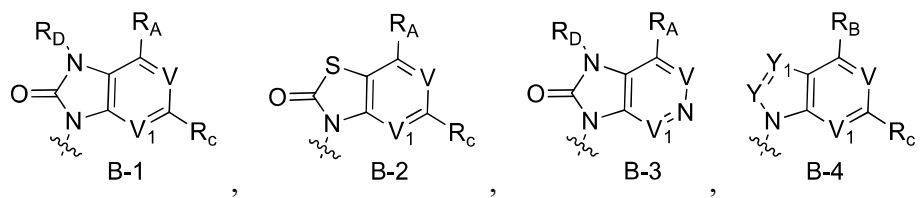
[0008] wherein, each of Z₁, Z₂ is independently O, S, SO₂, CH₂, CF₂ or Se;

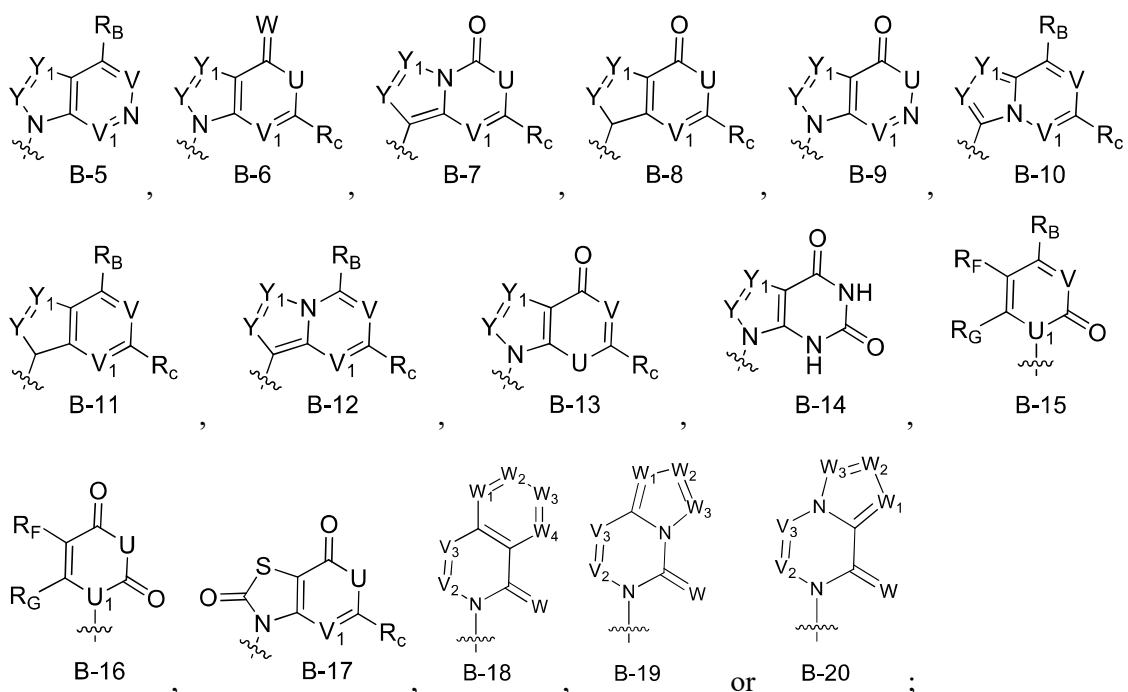


[0009] B₁ is



[0010] B₂ is





[0011] L and L₁ are each independently a connecting bond or CR¹R²;

[0012] L' and L₁' are each independently a connecting bond or CR¹¹R²¹;

[0013] L₂ is O, S or CR³R⁴; L₂' is O, S or CR³¹R⁴¹;

[0014] X¹ is O, S or CR⁵R⁶; X¹¹ is O, S or CR⁵¹R⁶¹;

[0015] X² is O, S, or CR⁷R⁸; and X²¹ is O, S, or CR⁷¹R⁸¹;

[0016] X³ and X³¹ are each independently OH, SH or BH₃⁻;

[0017] R and R' are each independently hydrogen, C₂₋₆ alkenyl, C₂₋₆ alkynyl or C₁₋₆ alkyl; the C₂₋₆ alkenyl, C₂₋₆ alkynyl or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido and cyano;

[0018] R¹ and R² are each independently hydrogen, halogen, cyano, hydroxyl, thiol, amino, azido, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkyl, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, halo-C₁₋₆ alkylthio, C₁₋₆ alkylamino, OC(O)R^a or OR^a; the C₂₋₆ alkenyl, C₂₋₆ alkynyl, or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido, and cyano;

[0019] R¹¹ and R²¹ are each independently hydrogen, halogen, cyano, hydroxyl, thiol, amino, azido, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkyl, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, halo-C₁₋₆ alkylthio, C₁₋₆ alkylamino, OC(O)R^a, or OR^a; the C₂₋₆ alkenyl, C₂₋₆ alkynyl, or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl,

amino, azido, and cyano;

[0020] alternatively, R^1 and R^2 together form carbonyl;

[0021] alternatively, R^{11} and R^{21} together form carbonyl;

[0022] alternatively, R is $-CH_2-$, R^1 is $-O-$, R and R^1 are interconnected to form heterocycloalkyl;

[0023] alternatively, R' is $-CH_2-$, R^{11} is $-O-$, R' and R^{11} are interconnected to form heterocycloalkyl;

[0024] R^3 and R^4 are each independently hydrogen, deuterium, halogen or C_{1-6} alkyl;

[0025] R^{31} and R^{41} are each independently hydrogen, deuterium, halogen or C_{1-6} alkyl;

[0026] R^5 and R^6 are each independently hydrogen, deuterium, halogen or C_{1-6} alkyl;

[0027] R^{51} and R^{61} are each independently hydrogen, deuterium, halogen or C_{1-6} alkyl;

[0028] R^7 and R^8 are each independently hydrogen, deuterium, halogen or C_{1-6} alkyl;

[0029] R^{71} and R^{81} are each independently hydrogen, deuterium, halogen or C_{1-6} alkyl;

[0030] Y and Y_1 are each independently CR_E or N ;

[0031] U is $CHRE'$ or NRD' ;

[0032] U_1 is CH or N ;

[0033] V , V_1 , V_2 and V_3 are each independently CR_E'' or N ;

[0034] W is O or S ;

[0035] W_1 , W_2 , W_3 and W_4 are each independently N or CR_F' ;

[0036] each of R_A , R_B , R_C , R_E , R_E' , R_E'' , R_F , R_F' and R_G is independently H , halogen, $-CN$, $-NO_2$, $-N_3$, R^c , $-SR^c$, $-OR^c$, $-OC(O)R^c$, $-OC(O)OR^c$, $-OC(O)NR^bR^c$, $-C(O)OR^c$, $-C(O)R^c$, $-C(O)NR^bR^c$, $-NR^bR^c$, $-NR^bC(O)R^c$, $-N(R^b)C(O)OR^c$, $-N(R^a)C(O)NR^bR^c$, $-NR^bS(O)_2R^c$, $-NR^bC(=NH)R^c$, $-NR^bC(=NR^c)NH_2$, $-S(O)_{1-2}R^c$, $-S(O)_2NR^bR^c$ or $-NR^aS(O)_2NR^bR^c$;

[0037] each of R_D and R_D' is independently H or R^c ;

[0038] each of R^a and R^b is independently H , C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} alkyl, halo- C_{1-6} alkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aryl- C_{1-6} alkyl, heteroaryl- C_{1-6} alkyl, cycloalkyl- C_{1-6} alkyl, or heterocycloalkyl- C_{1-6} alkyl;

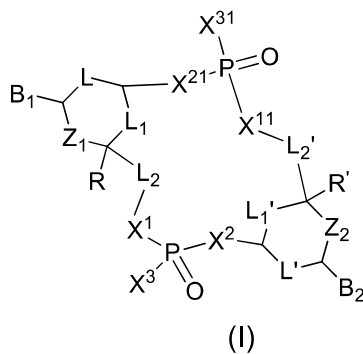
[0039] each R^c is independently H , substituted or unsubstituted C_{1-10} alkyl, substituted or unsubstituted C_{2-8} alkenyl, substituted or unsubstituted C_{2-8} alkynyl, substituted or unsubstituted C_{3-10} cycloalkyl, substituted or unsubstituted C_{6-10} aryl, substituted or

unsubstituted 3-10 membered heterocycloalkyl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₆₋₁₀ aryl-C₁₋₆ alkyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 5-10 membered heteroaryl-C₁₋₆ alkyl; the C₁₋₁₀ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₁₀ cycloalkyl, C₆₋₁₀ aryl, 3-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, or 5-10 membered heteroaryl-C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by one or more R^d;

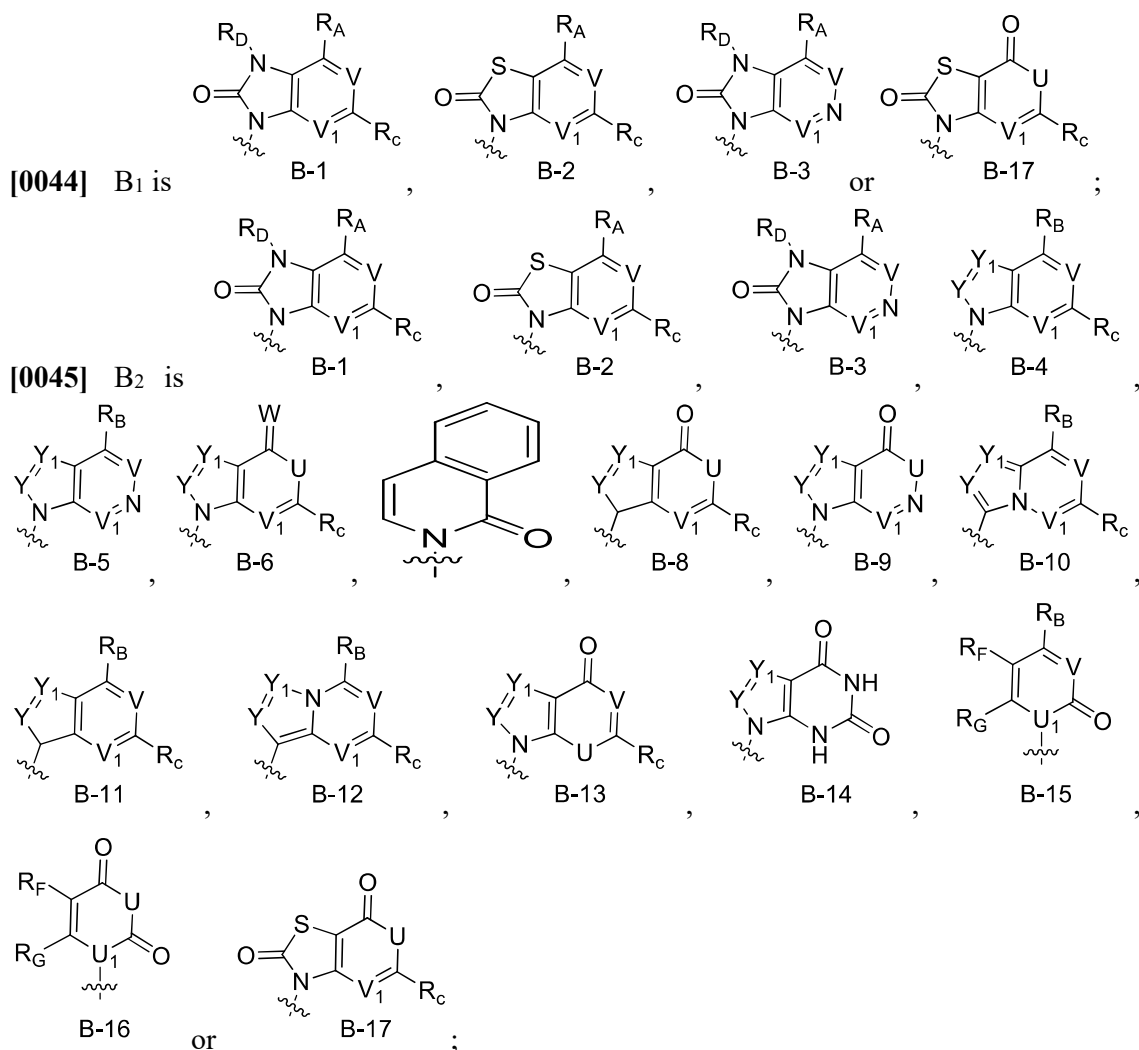
[0040] each R^d is independently halogen, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, C₁₋₆ alkyl, -CN, -N₃, -SR^e, -OR^e, -C(O)R^e, -NR^eR^{e'}, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, or substituted or unsubstituted 3-10 membered heterocycloalkyl; the C₆₋₁₀ aryl, 5-10 membered heteroaryl, C₃₋₁₀ cycloalkyl or 3-10 membered heterocycloalkyl is unsubstituted or selectively substituted at any position by one or more substituents selected from halogen, hydroxyl, cyano, amino, C₁₋₄ alkyl, halo-C₁₋₄ alkyl, C₁₋₄ alkoxy, C₁₋₄ alkylamino and halo-C₁₋₄ alkoxy;

[0041] each of R^e and R^{e'} is independently C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ alkyl, halo-C₁₋₆ alkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aryl-C₁₋₆ alkyl, heteroaryl-C₁₋₆ alkyl, cycloalkyl-C₁₋₆ alkyl, or heterocycloalkyl-C₁₋₆ alkyl.

[0042] In some embodiments, the cyclic di-nucleotide analogue (I), the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt, having the structural general formula of



[0043] wherein, Z₁, Z₂ are each independently O, S, SO₂, CH₂, CF₂ or Se;



or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido, and cyano;

[0054] R^{11} and R^{21} are each independently hydrogen, halogen, cyano, hydroxyl, thiol, amino, azido, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkyl, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, halo-C₁₋₆ alkylthio, C₁₋₆ alkylamino, OC(O)R^a or OR^a; the C₂₋₆ alkenyl, C₂₋₆ alkynyl, or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido, and cyano;

[0055] alternatively, R^1 and R^2 together form carbonyl;

[0056] alternatively, R^{11} and R^{21} together form carbonyl;

[0057] alternatively, R is -CH₂-, R^1 is -O-, R and R^1 are interconnected to form heterocycloalkyl;

[0058] alternatively, R' is -CH₂-, R^{11} is -O-, R' and R^{11} are interconnected to form heterocycloalkyl;

[0059] R^3 and R^4 are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;

[0060] R^{31} and R^{41} are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;

[0061] R^5 and R^6 are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;

[0062] R^{51} and R^{61} are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;

[0063] R^7 and R^8 are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;

[0064] R^{71} and R^{81} are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;

[0065] Y and Y₁ are each independently CR_E or N;

[0066] U is CHRE' or NR_D';

[0067] U₁ is CH or N;

[0068] V and V₁ are each independently CRE'' or N;

[0069] W is O or S;

[0070] each of R_A, R_B, R_C, R_E, R_E', R_E'', R_F and R_G is independently H, halogen, -CN, -NO₂, -N₃, R^c, -SR^c, -OR^c, -OC(O)R^c, -OC(O)OR^c, -OC(O)NR^bR^c, -C(O)OR^c, -C(O)R^c, -C(O)NR^bR^c, -NR^bR^c, -NR^bC(O)R^c, -N(R^b)C(O)OR^c, -N(R^a)C(O)NR^bR^c, -NR^bS(O)₂R^c, -NR^bC(=NH)R^c, -NR^bC(=NR^c)NH₂, -S(O)₁₋₂R^c, -S(O)₂NR^bR^c or -NR^aS(O)₂NR^bR^c;

[0071] each of R_D and R_D' is independently H or R^c;

[0072] each of R^a and R^b is independently H, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ alkyl, halo-C₁₋₆

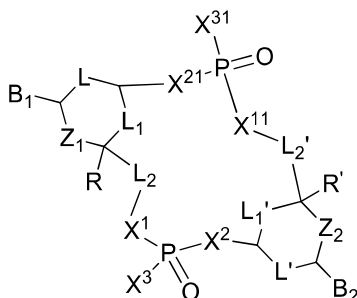
alkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aryl-C₁₋₆ alkyl, heteroaryl-C₁₋₆ alkyl, cycloalkyl-C₁₋₆ alkyl, or heterocycloalkyl-C₁₋₆ alkyl;

[0073] each R^c is independently H, substituted or unsubstituted C₁₋₁₀ alkyl, substituted or unsubstituted C₂₋₈ alkenyl, substituted or unsubstituted C₂₋₈ alkynyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 3-10 membered heterocycloalkyl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₆₋₁₀ aryl-C₁₋₆ alkyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 5-10 membered heteroaryl-C₁₋₆ alkyl; the C₁₋₁₀ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₁₀ cycloalkyl, C₆₋₁₀ aryl, 3-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, or 5-10 membered heteroaryl-C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by one or more R^d;

[0074] each R^d is independently halogen, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, C₁₋₆ alkyl, -CN, -N₃, -SR^e, -OR^e, -C(O)R^e, -NR^eR^{e'}, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, or substituted or unsubstituted 3-10 membered heterocycloalkyl; the C₆₋₁₀ aryl, 5-10 membered heteroaryl, C₃₋₁₀ cycloalkyl or 3-10 membered heterocycloalkyl is unsubstituted or selectively substituted at any position by one or more substituents selected from halogen, hydroxyl, cyano, amino, C₁₋₄ alkyl, halo-C₁₋₄ alkyl, C₁₋₄ alkoxy, C₁₋₄ alkylamino and halo-C₁₋₄ alkoxy;

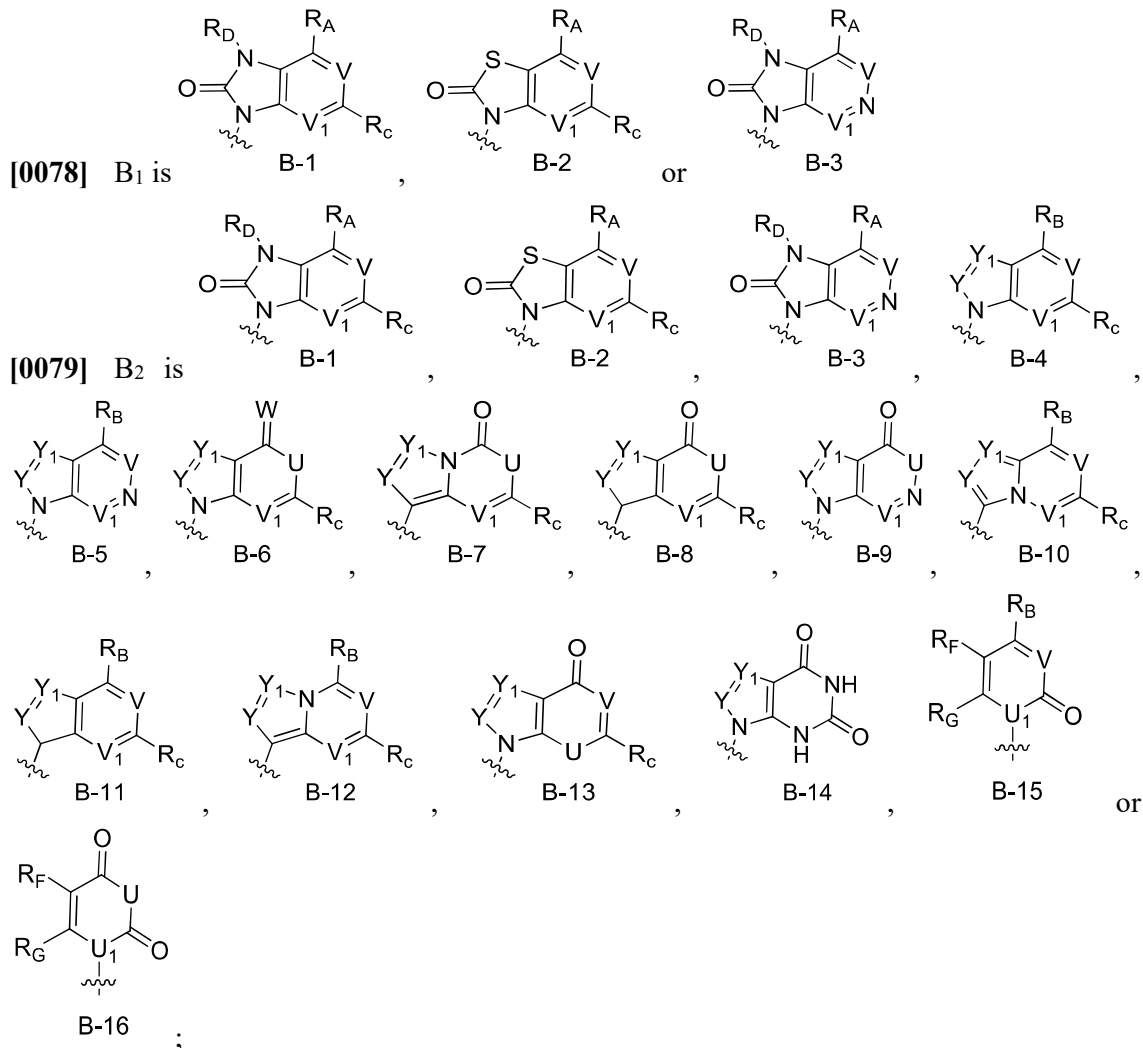
[0075] each of R^e and R^{e'} is independently C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ alkyl, halo-C₁₋₆ alkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aryl-C₁₋₆ alkyl, heteroaryl-C₁₋₆ alkyl, cycloalkyl-C₁₋₆ alkyl, or heterocycloalkyl-C₁₋₆ alkyl.

[0076] In some embodiments, the cyclic di-nucleotide analogue (I), the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof, having the structural general formula of



(I)

[0077] wherein, Z₁, Z₂ are each independently O, S, SO₂, CH₂, CF₂ or Se;



[0080] L and L₁ are each independently a connecting bond or CR¹R²;

[0081] L' and L₁' are each independently a connecting bond or CR¹¹R²¹;

[0082] L₂ is O, S or CR³R⁴; L₂' is O, S or CR³¹R⁴¹;

[0083] X¹ is O, S or CR⁵R⁶; X¹¹ is O, S or CR⁵¹R⁶¹;

- [0084]** X^2 is O, S or CR^7R^8 ; and X^{21} is O, S or $CR^{71}R^{81}$;
- [0085]** X^3 and X^{31} are each independently OH, SH or BH_3^- ;
- [0086]** R and R' are each independently hydrogen, C₂₋₆ alkenyl, C₂₋₆ alkynyl or C₁₋₆ alkyl; the C₂₋₆ alkenyl, C₂₋₆ alkynyl or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido and cyano;
- [0087]** R^1 and R^2 are each independently hydrogen, halogen, cyano, hydroxyl, thiol, amino, azido, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkyl, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, halo-C₁₋₆ alkylthio, C₁₋₆ alkylamino, $OC(O)R^a$, or OR^a ; the C₂₋₆ alkenyl, C₂₋₆ alkynyl, or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido, and cyano;
- [0088]** R^{11} and R^{21} are independently hydrogen, halogen, cyano, hydroxyl, thiol, amino, azido, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkyl, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, halo-C₁₋₆ alkylthio, C₁₋₆ alkylamino, $OC(O)R^a$, or OR^a ; the C₂₋₆ alkenyl, C₂₋₆ alkynyl, or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido, and cyano;
- [0089]** alternatively, R^1 and R^2 together form carbonyl;
- [0090]** alternatively, R^{11} and R^{21} together form carbonyl;
- [0091]** alternatively, R is -CH₂-, R^1 is -O-, R and R^1 are interconnected to form heterocycloalkyl;
- [0092]** alternatively, R' is -CH₂-, R^{11} is -O-, R' and R^{11} are interconnected to form heterocycloalkyl;
- [0093]** R^3 and R^4 are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;
- [0094]** R^{31} and R^{41} are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;
- [0095]** R^5 and R^6 are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;
- [0096]** R^{51} and R^{61} are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;
- [0097]** R^7 and R^8 are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;
- [0098]** R^{71} and R^{81} are each independently hydrogen, deuterium, halogen or C₁₋₆ alkyl;
- [0099]** Y and Y₁ are each independently CR_E or N;
- [0100]** U is CHRE' or NR_D';
- [0101]** U₁ is CH or N;

[0102] V and V₁ are each independently CRE" or N;

[0103] W is O or S;

[0104] each of R_A, R_B, R_C, R_E, R_{E'}, R_{E''}, R_F and R_G is independently H, halogen, -CN, -NO₂, -N₃, R^c, -SR^c, -OR^c, -OC(O)R^c, -OC(O)OR^c, -OC(O)NR^bR^c, -C(O)OR^c, -C(O)R^c, -C(O)NR^bR^c, -NR^bR^c, -NR^bC(O)R^c, -N(R^b)C(O)OR^c, -N(R^a)C(O)NR^bR^c, -NR^bS(O)₂R^c, -NR^bC(=NH)R^c, -NR^bC(=NR^c)NH₂, -S(O)₁₋₂R^c, -S(O)₂NR^bR^c or -NR^aS(O)₂NR^bR^c;

[0105] each of R_D and R_{D'} is independently H or R^c;

[0106] each of R^a and R^b is independently H, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ alkyl, halo-C₁₋₆ alkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aryl-C₁₋₆ alkyl, heteroaryl-C₁₋₆ alkyl, cycloalkyl-C₁₋₆ alkyl, or heterocycloalkyl-C₁₋₆ alkyl;

[0107] each R^c is independently H, substituted or unsubstituted C₁₋₁₀ alkyl, substituted or unsubstituted C₂₋₈ alkenyl, substituted or unsubstituted C₂₋₈ alkynyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 3-10 membered heterocycloalkyl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₆₋₁₀ aryl-C₁₋₆ alkyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 5-10 membered heteroaryl-C₁₋₆ alkyl; the C₁₋₁₀ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₁₀ cycloalkyl, C₆₋₁₀ aryl, 3-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, or 5-10 membered heteroaryl-C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by one or more R^d;

[0108] each R^d is independently halogen, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, C₁₋₆ alkyl, -CN, -N₃, -SR^c, -OR^c, -C(O)R^c, -NR^cR^{c'}, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, or substituted or unsubstituted 3-10 membered heterocycloalkyl; the C₆₋₁₀ aryl, 5-10 membered heteroaryl, C₃₋₁₀ cycloalkyl or 3-10 membered heterocycloalkyl is unsubstituted or selectively substituted at any position by one or more substituents selected from halogen, hydroxyl, cyano, amino, C₁₋₄ alkyl, halo-C₁₋₄ alkyl, C₁₋₄ alkoxy, C₁₋₄ alkylamino and halo-C₁₋₄ alkoxy;

[0109] each of R^c and R^{c'} is independently C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ alkyl, halo-C₁₋₆ alkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aryl-C₁₋₆ alkyl, heteroaryl-C₁₋₆ alkyl,

cycloalkyl-C₁₋₆ alkyl, or heterocycloalkyl-C₁₋₆ alkyl.

[0110] In some embodiments, each R^c is independently H, substituted or unsubstituted C₁₋₁₀ alkyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 3-10 membered heterocycloalkyl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₆₋₁₀ aryl-C₁₋₆ alkyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 5-10 membered heteroaryl-C₁₋₆ alkyl; the C₁₋₁₀ alkyl, C₃₋₁₀ cycloalkyl, C₆₋₁₀ aryl, 3-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, 3-10 membered heterocycloalkyl-C₁₋₆ alkyl or 5-10 membered heteroaryl-C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by one or more R^d; R^d is as defined above.

[0111] In the definition of R^c, the substituted or unsubstituted C₁₋₁₀ alkyl is preferably substituted or unsubstituted C₁₋₆ alkyl, such as methyl, ethyl, propyl, isopropyl, *tert*-butyl, *n*-butyl; wherein the methyl, ethyl, propyl, isopropyl, *tert*-butyl, or *n*-butyl is substituted or unsubstituted;

[0112] in the definition of R^c, the substituted or unsubstituted C₃₋₁₀ cycloalkyl is preferably cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 2,3-dihydro-1*H*-inden-1-yl, 2,3-dihydro-1*H*-inden-2-yl; wherein the cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 2,3-dihydro-1*H*-inden-1-yl, or 2,3-dihydro-1*H*-inden-2-yl is substituted or unsubstituted;

[0113] in the definition of R^c, the substituted or unsubstituted C₆₋₁₀ aryl is preferably substituted or unsubstituted phenyl;

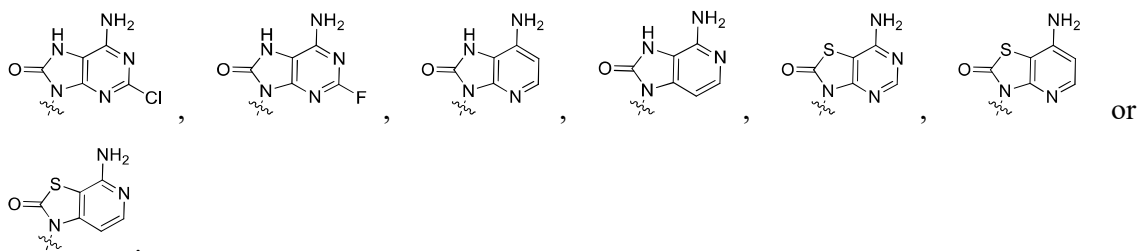
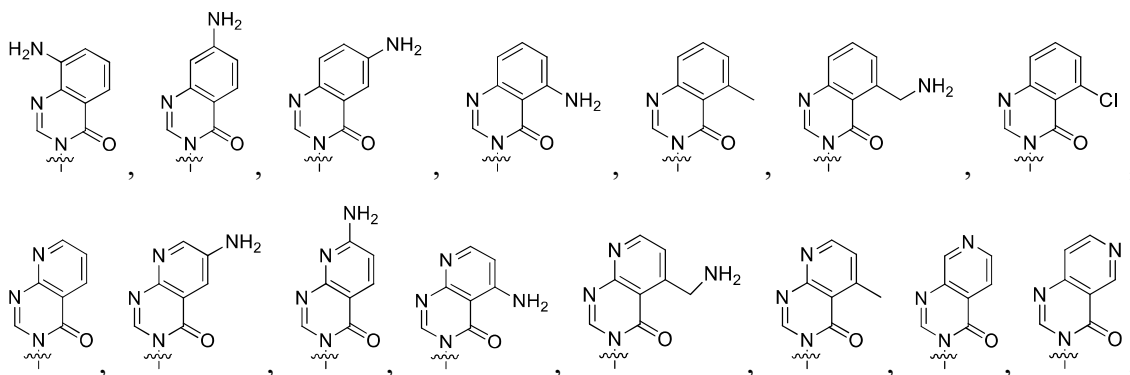
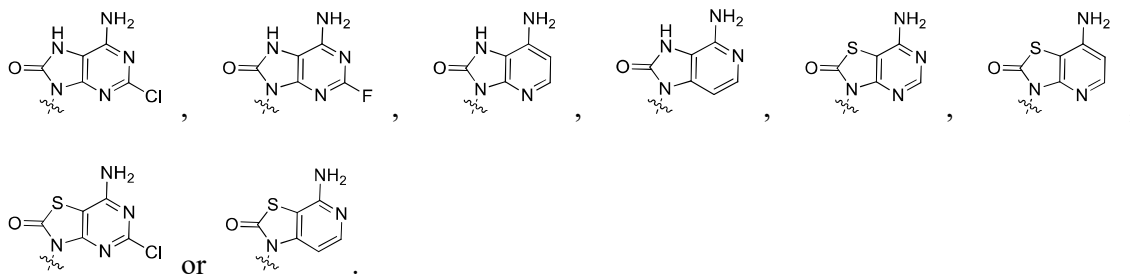
[0114] in the definition of R^c, the substituted or unsubstituted 3-10 membered heterocycloalkyl is preferably tetrahydrofuran-3-yl, tetrahydro-2*H*-pyran-3-yl, tetrahydro-2*H*-pyran-4-yl, pyrrolidin-3-yl, piperidin-3-yl, piperidin-4-yl; wherein the tetrahydrofuran-3-yl, tetrahydro-2*H*-pyran-3-yl, tetrahydro-2*H*-pyran-4-yl, pyrrolidin-3-yl, piperidin-3-yl, piperidin-4-yl is substituted or unsubstituted;

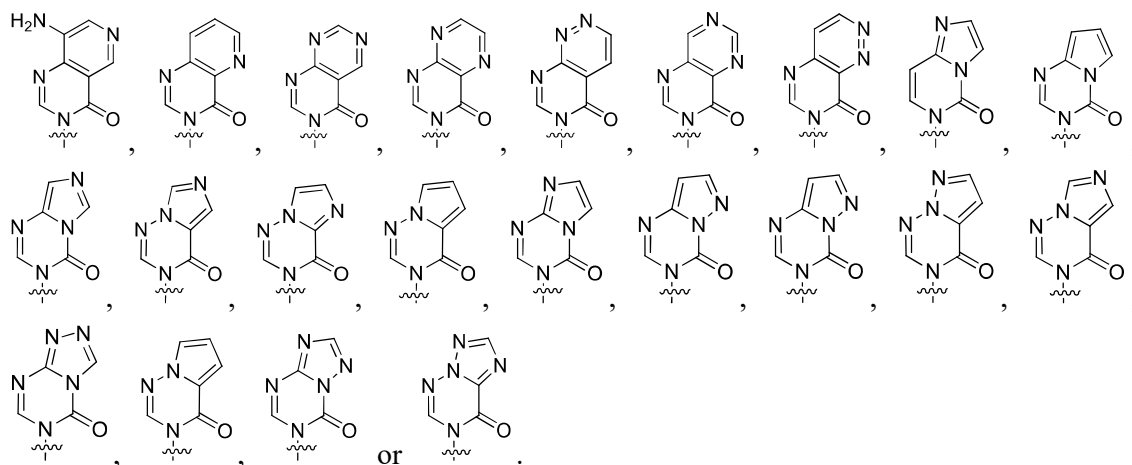
[0115] in the definition of R^c, the substituted or unsubstituted 5-10 membered heteroaryl is preferably substituted or unsubstituted 5-6-membered heteroaryl.

[0116] In some embodiments, each R^c is independently H, C₁₋₄ alkyl or halo-C₁₋₄ alkyl.

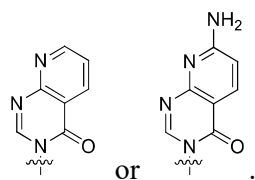
[0117] In some embodiments, each R^b is independently H or C₁₋₄ alkyl.

- [0118] In some embodiments, Z_1 is O.
- [0119] In some embodiments, Z_2 is O.
- [0120] In some embodiments, in the definition of B-4 to B-16, Y is N.
- [0121] In some embodiments, in the definition of B-4 to B-16, Y is CR_E , and R_E is preferably H, F, Cl, $-CF_3$, $-CH_3$, $-CN$, $-NH_2$.
- [0122] In some embodiments, in the definition of B-4 to B-16, Y_1 is N.
- [0123] In some embodiments, in the definition of B-4 to B-16, Y_1 is CR_E , and R_E is preferably H, F, Cl, $-CF_3$, $-CH_3$, $-CN$ or $-NH_2$.
- [0124] In some embodiments, in the definition of B-6 to B-9, B-13, B-16 to B-17, U is $NR_{D'}$ and $R_{D'}$ is H or $-CH_3$.
- [0125] In some embodiments, in the definition of B-1 to B-5, B-10 to B-13, B-15, V is N or CH.
- [0126] In some embodiments, in the definition of B-1 to B-12, B-17, V_1 is N or CH.
- [0127] In some embodiments, in the definition of B-1 to B-3, R_A is H, halogen, $-OR^c$, $-NR^bR^c$; wherein R^b and R^c are as defined above.
- [0128] In some embodiments, in the definition of B-4, B-5, B-10 to B-12, B-15, R_B is H, halogen, $-OR^c$, $-NR^bR^c$; wherein R^b and R^c are as defined above.
- [0129] In some embodiments, in the definition of B-1, B-2, B-4, B-6 to B-8, B-10 to B-13, B-17, the R_C is H, F, Cl, $-OR^c$, $-SR^c$, $-NR^bR^c$ or R^c ; wherein R^c is as defined above.
- [0130] In some embodiments, in the definition of B-15, B-16, R_G is H.
- [0131] In some embodiments, in the definition of B-15, B-16, R_F is H.
- [0132] In some embodiments, in the definition of B-18 to B-20, V_3 is N or CH.
- [0133] In some embodiments, in the definition of B-18 to B-20, V_2 is CH.
- [0134] In some embodiments, in the definition of B-18 to B-20, W_1 is N or CH.
- [0135] In some embodiments, in the definition of B-18 to B-20, W_2 is $CR_{F'}$; wherein $R_{F'}$ is H or $-NH_2$.
- [0136] In some embodiments, in the definition of B-18 to B-20, W_3 is CH.
- [0137] In some embodiments, in the definition of B-18, W_4 is CH.

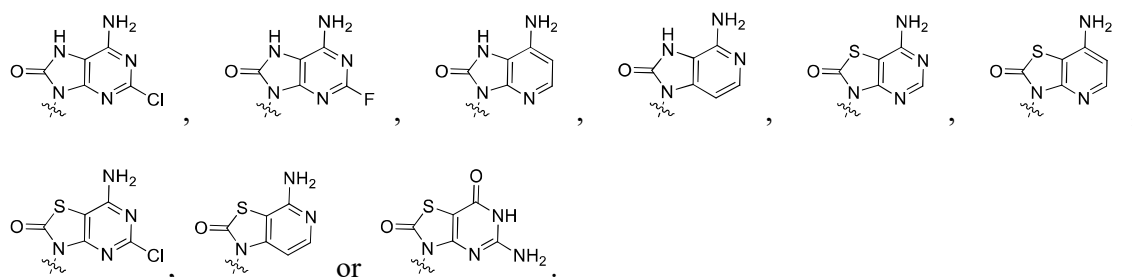
Nc1nc(Cl)n2c(=O)n(c2s1)C(=O)NNC1=NC2=C(N1)S(=O)(=O)C(=O)N2



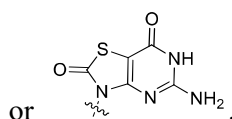
[0143] In some embodiments, B₁ is any of the following structures:



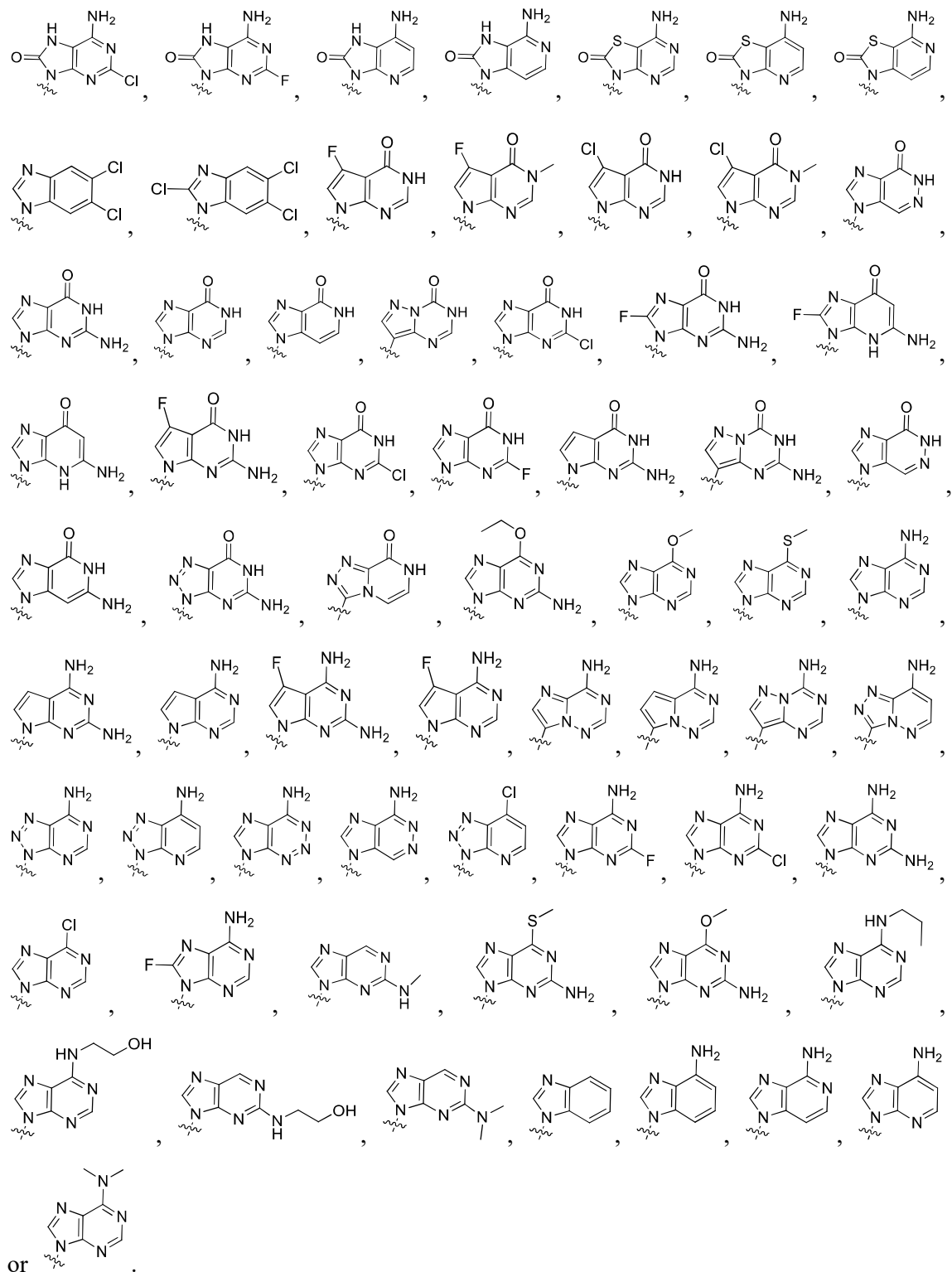
[0144] In some embodiments, B₁ is any of the following structures:



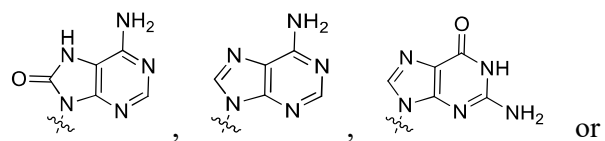
[0145] In some embodiments, B₁ is any of the following structures:

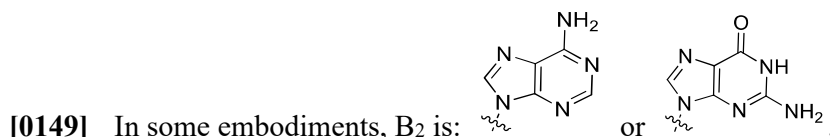
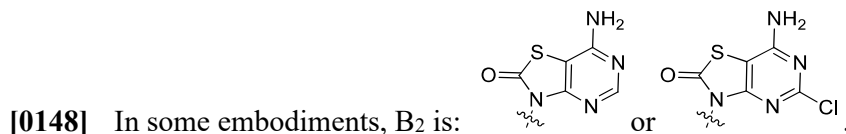
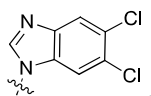


[0146] In some embodiments, B₂ is any of the following structures:



[0147] In some embodiments, B₂ is:





[0150] In some embodiments, when L is a connecting bond, then L₁ is CR¹R²; R¹ and R² are defined as previously described.

[0151] In some embodiments, when L is CR¹R², then L₁ is a connecting bond; R¹ and R² are defined as previously described.

[0152] In some embodiments, when L' is a connecting bond, then L₁' is CR¹¹R²¹; R¹¹ and R²¹ are defined as previously described.

[0153] In some embodiments, when L' is CR¹¹R²¹, then L₁' is a connecting bond; R¹¹ and R²¹ are defined as previously described.

[0154] In some embodiments, L₂ is CH₂.

[0155] In some embodiments, L₂' is CH₂.

[0156] In some embodiments, X¹ is O.

[0157] In some embodiments, X¹¹ is O.

[0158] In some embodiments, X² is O.

[0159] In some embodiments, X²¹ is O.

[0160] In some embodiments, R^a is C₁₋₄ alkyl or halo-C₁₋₄ alkyl.

[0161] In some embodiments, R^a is C₁₋₄ alkyl.

[0162] In some embodiments, R¹ and R² are each independently hydrogen, halogen, hydroxyl, or OR^a; R^a is defined as previously described.

[0163] In some embodiments, R¹¹ and R²¹ are each independently hydrogen, halogen, hydroxyl, or OR^a; R^a is defined as previously described.

[0164] In some embodiments, R¹ is hydrogen.

[0165] In some embodiments, R² is hydrogen, fluorine, hydroxyl or methoxy.

[0166] In some embodiments, R¹¹ is hydrogen.

[0167] In some embodiments, R²¹ is hydrogen, fluorine, hydroxyl or methoxy.

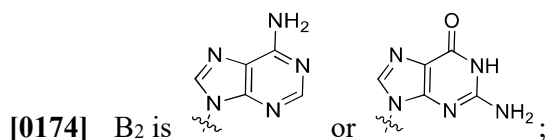
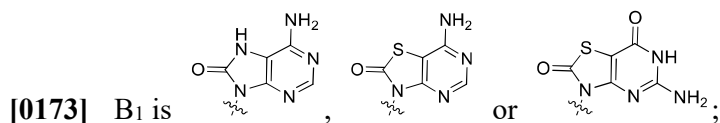
[0168] In some embodiments, R is hydrogen.

[0169] In some embodiments, R' is hydrogen.

[0170] In some embodiments, certain groups in the compound represented by Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof can be as defined below, and undescribed groups can be as defined in any of the above embodiments:

[0171] Z₁ is O;

[0172] Z₂ is O;



[0175] L and L₁ are each independently a connecting bond or CR¹R²;

[0176] L' and L₁' are each independently a connecting bond or CR¹¹R²¹; and, L, L₁, L' and L₁' are defined in the following combinations:

[0177] 1) L is a connecting bond, L₁ is CR¹R², L' is CR¹¹R²¹, L₁' is a connecting bond, or

[0178] 2) L is CR¹R², L₁ is a connecting bond, L' is a connecting bond, L₁' is CR¹¹R²¹;

[0179] L₂ is CH₂;

[0180] L₂' is CH₂;

[0181] X¹ is O;

[0182] X¹¹ is O;

[0183] X² is O;

[0184] X²¹ is O;

[0185] X³ and X³¹ are each independently OH or SH;

[0186] R and R' are each independently hydrogen;

[0187] R¹ and R² are each independently hydrogen, halogen, hydroxyl or OR^a;

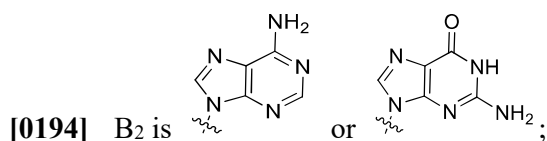
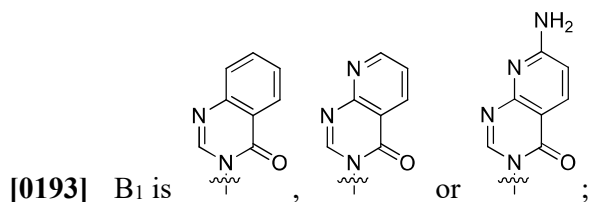
[0188] R¹¹ and R²¹ are each independently hydrogen, halogen, hydroxyl or OR^a;

[0189] each R^a is independently C₁₋₄ alkyl or halo-C₁₋₄ alkyl.

[0190] In some embodiments, certain groups in the compound represented by Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof can be as defined below, and undescribed groups can be as defined in any of the above embodiments:

[0191] Z₁ is O;

[0192] Z₂ is O;



[0195] L and L₁ are each independently a connecting bond or CR¹R²;

[0196] L' and L₁' are each independently a connecting bond or CR¹¹R²¹; and, L, L₁, L' and L₁' are as defined in the following combinations:

[0197] 1) L is a connecting bond, L₁ is CR¹R², L' is CR¹¹R²¹, L₁' is a connecting bond, or

[0198] 2) L is CR¹R², L₁ is a connecting bond, L' is a connecting bond and L₁' is CR¹¹R²¹;

[0199] L₂ is CH₂;

[0200] L₂' is CH₂;

[0201] X¹ is O;

[0202] X¹¹ is O;

[0203] X² is O;

[0204] X²¹ is O;

[0205] X³ and X³¹ are each independently OH or SH;

[0206] R and R' are each independently hydrogen;

[0207] R¹ and R² are each independently hydrogen, halogen, hydroxyl or OR^a;

[0208] R¹¹ and R²¹ are each independently hydrogen, halogen, hydroxyl or OR^a;

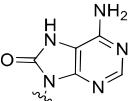
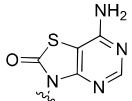
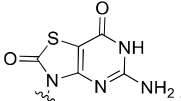
[0209] each R^a is independently C₁₋₄ alkyl or halo-C₁₋₄ alkyl.

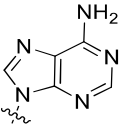
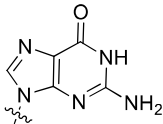
[0210] In some embodiments, certain groups in the compound represented by Formula I, the

isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof can be as defined below, and undescribed groups can be as defined in any of the above embodiments:

[0211] Z₁ is O;

[0212] Z₂ is O;

[0213] B₁ is ,  or ;

[0214] B₂ is  or ;

[0215] L and L₁ are each independently a connecting bond or CR¹R²;

[0216] L' and L₁' are each independently a connecting bond or CR¹¹R²¹; and, L, L₁, L' and L₁' are as defined in the following combinations:

[0217] 1) L is a connecting bond, L₁ is CR¹R², L' is CR¹¹R²¹, L₁' is a connecting bond, or

[0218] 2) L is CR¹R², L₁ is a connecting bond, L' is a connecting bond, L₁' is CR¹¹R²¹;

[0219] L₂ is CH₂;

[0220] L₂' is CH₂;

[0221] X¹ is O;

[0222] X¹¹ is O;

[0223] X² is O;

[0224] X²¹ is O;

[0225] X³ and X³¹ are each independently SH;

[0226] R and R' are each independently hydrogen;

[0227] R¹ is hydrogen;

[0228] R² is hydrogen, halogen, hydroxyl or OR^a;

[0229] R¹¹ is hydrogen;

[0230] R²¹ is hydrogen, halogen, hydroxyl or OR^a;

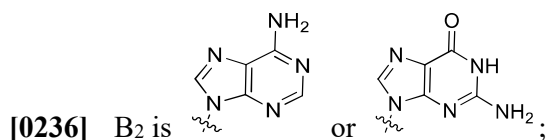
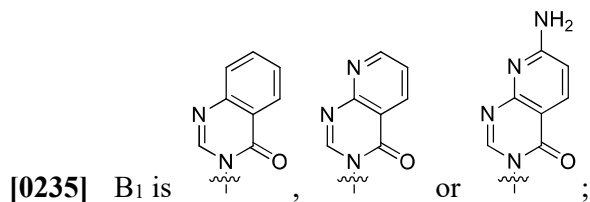
[0231] each R^a is independently C₁₋₄ alkyl.

[0232] In some embodiments, certain groups in the compound represented by Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof can be as

defined below, and undescribed groups can be as defined in any of the above embodiments:

[0233] Z_1 is O;

[0234] Z_2 is O;



[0237] L and L_1 are each independently a connecting bond or CR^1R^2 ;

[0238] L' and L_1' are each independently a connecting bond or $CR^{11}R^{21}$; and, L, L_1 , L' and L_1' are as defined in the following combinations:

[0239] 1) L is a connecting bond, L_1 is CR^1R^2 , L' is $CR^{11}R^{21}$, L_1' is a connecting bond, or

[0240] 2) L is CR^1R^2 , L_1 is a connecting bond, L' is a connecting bond and L_1' is $CR^{11}R^{21}$;

[0241] L_2 is CH_2 ;

[0242] L_2' is CH_2 ;

[0243] X^1 is O;

[0244] X^{11} is O;

[0245] X^2 is O;

[0246] X^{21} is O;

[0247] X^3 and X^{31} are SH;

[0248] R and R' are hydrogen;

[0249] R^1 is hydrogen;

[0250] R^2 is hydrogen, halogen, hydroxyl or OR^a ;

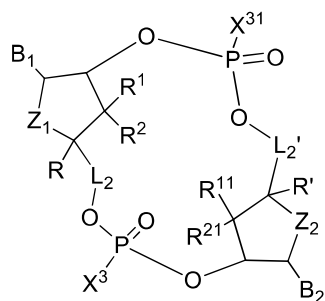
[0251] R^{11} is hydrogen;

[0252] R^{21} is hydrogen, halogen, hydroxyl or OR^a ;

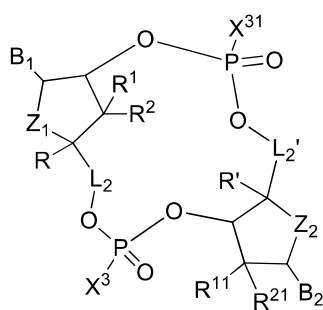
[0253] each R^a is independently C_{1-4} alkyl.

[0254] In some embodiments, the compound of Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof, is the compound of Formula II, III, IV

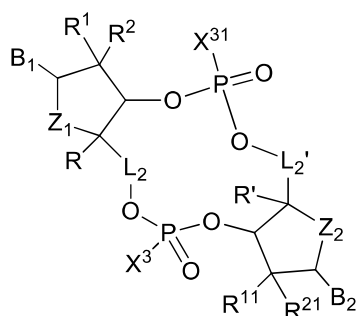
or V, an isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof:



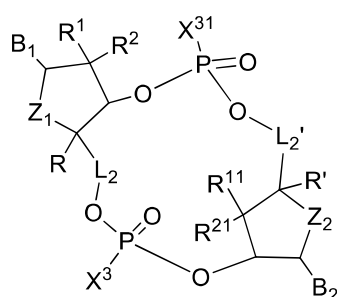
(II)



(III)



(IV)



(V)

[0255] wherein, B₁, B₂, Z₁, Z₂, R, R', R¹, R², R¹¹, R²¹, X³, X³¹, L₂ and L₂' are defined as described previously.

[0256] The combinations including any of the B₁, B₂, Z₁, Z₂, R, R', R¹, R², R¹¹, R²¹, X³, X³¹, L₂ and L₂' embodiments as described in Formula I are included in the scope of the Formula II, III, IV or V in the present disclosure.

[0257] All embodiments of Formula II, III, IV or V described below are included in the scope of the Formula II, III, IV or V in the present disclosure.

[0258] In some preferred embodiments of Formula II, III, IV or V, R¹ is H; R² is -OH, F, -N₃, -SCF₃ or -OCH₃.

[0259] In some preferred embodiments of Formula II, III, IV or V, R¹¹ is H; R²¹ is -OH, F, -N₃, -SCF₃ or -OCH₃.

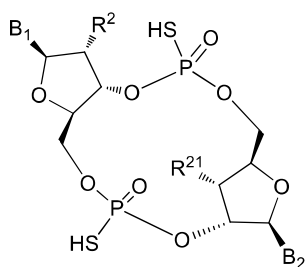
[0260] In some preferred embodiments of Formula II, III, IV or V, R is -CH₂-, R¹ is -O-, R and R¹ are interconnected to form heterocycloalkyl.

[0261] In some preferred embodiments of Formula II, III, IV or V, R' is -CH₂-, R¹¹ is -O-, R' and R¹¹ are interconnected to form heterocycloalkyl.

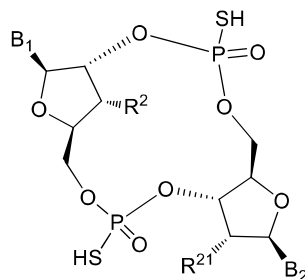
[0262] In some preferred embodiments of Formula II, III, IV or V, Z₁ is O; Z₂ is O.

[0263] In some preferred embodiments of Formula II, III, IV or V, L₂ is -CH₂-; L₂' is -CH₂-.

[0264] In some embodiments, the compound of Formula I, the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof, is preferably the compound of Formula VI or VII, an isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof:



(VI)



(VII)

[0265] wherein, B₁, B₂, R² and R²¹ are defined as previously described.

[0266] The combinations including any of the B₁, B₂, R² and R²¹ embodiments as described in Formula I are included in the scope of the Formula VI or VII in the present disclosure.

[0267] All embodiments of Formula VI or VII described below are included in the scope of the Formula VI or VII in the present disclosure.

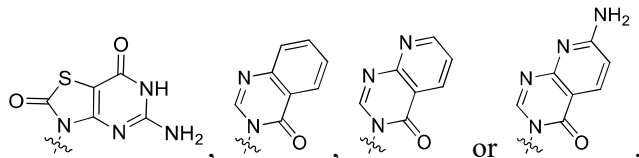
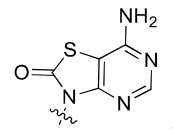
[0268] In some preferred embodiments of Formula VI or VII, R² is -OH.

[0269] In some preferred embodiments of Formula VI or VII, R² is F.

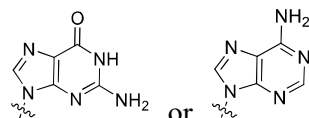
[0270] In some preferred embodiments of Formula VI or VII, R²¹ is -OH.

[0271] In some preferred embodiments of Formula VI or VII, R²¹ is F.

[0272] In some preferred embodiments of Formula VI or VII, B₁ is



[0273] In some preferred embodiments of Formula VI or VII, B₂ is

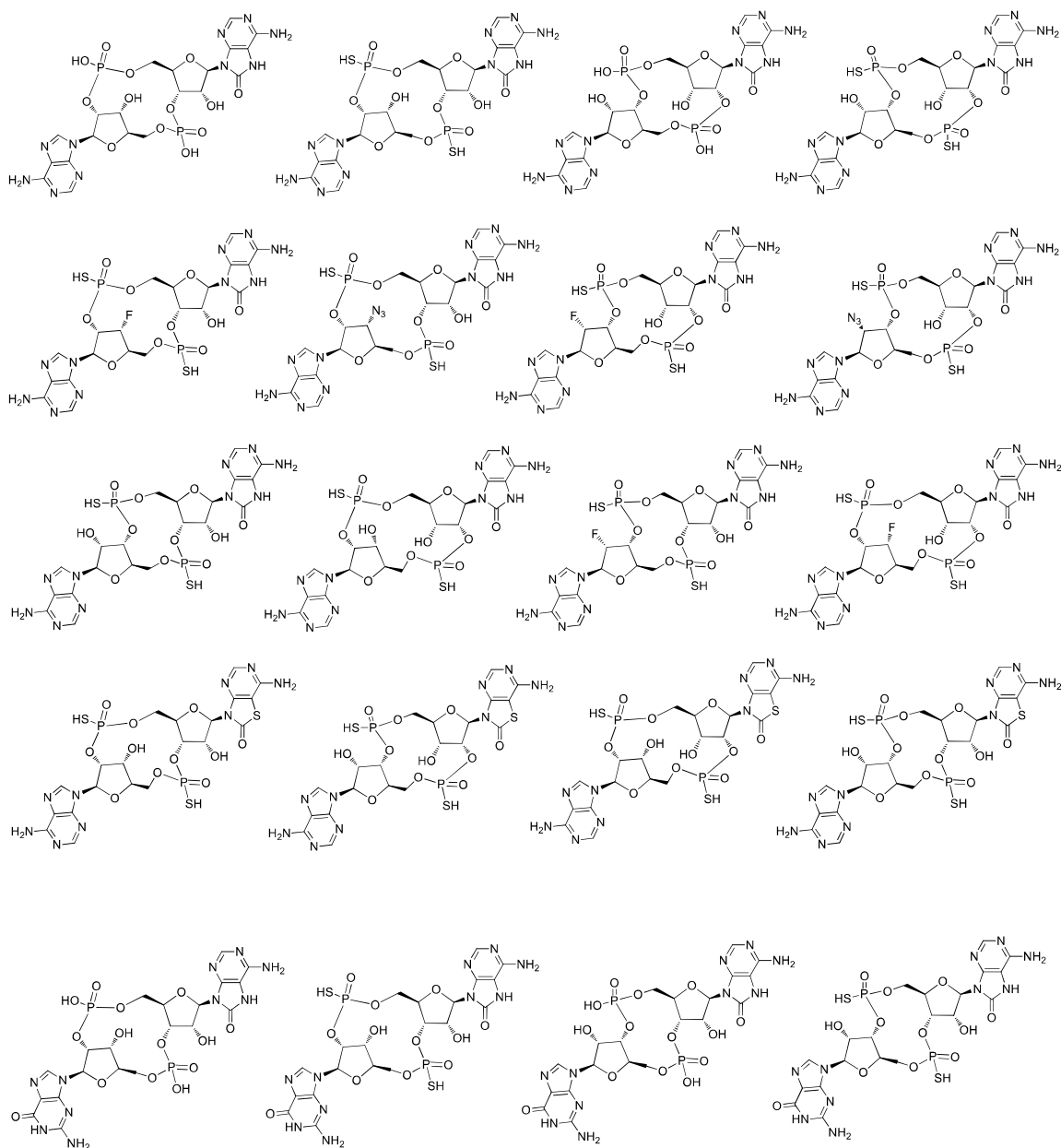


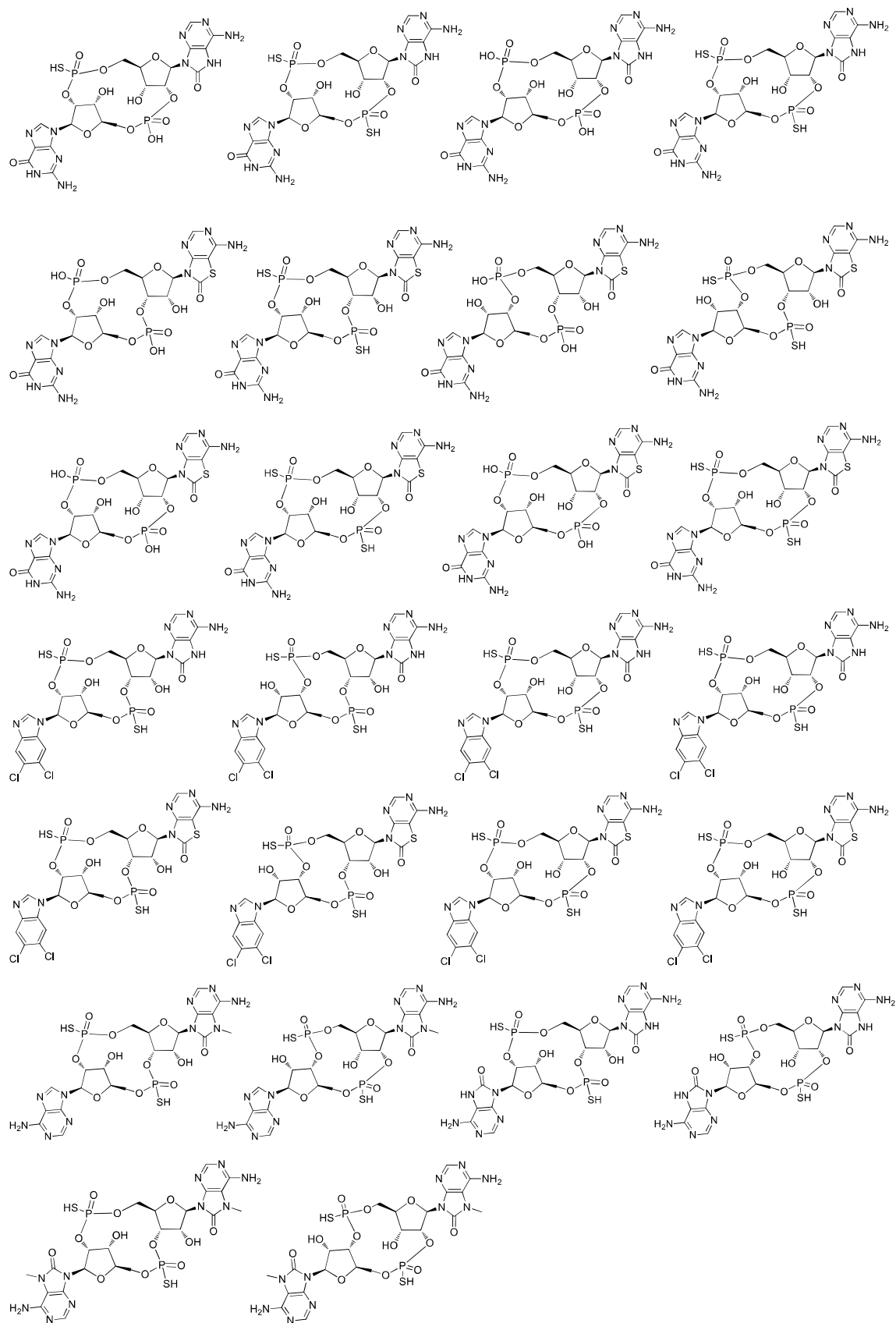
[0274] In some preferred embodiments of Formula VI or VII, the stereo configuration is (Sp, Sp), (Sp, Rp), (Rp, Rp), or (Rp, Sp).

[0275] In some preferred embodiments of Formula VI, R² is -OH; R²¹ is -OH.

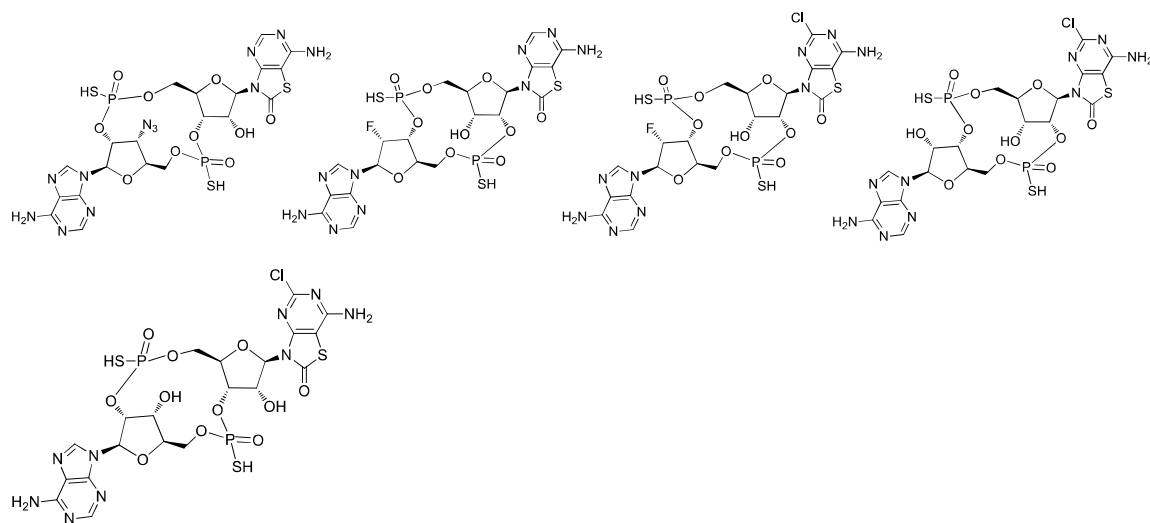
[0276] In some preferred embodiments of Formula VII, R² is -OH or -OCH₃; R²¹ is -OH or F.

[0277] In some embodiments, the compound of Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt is any of the following structures:

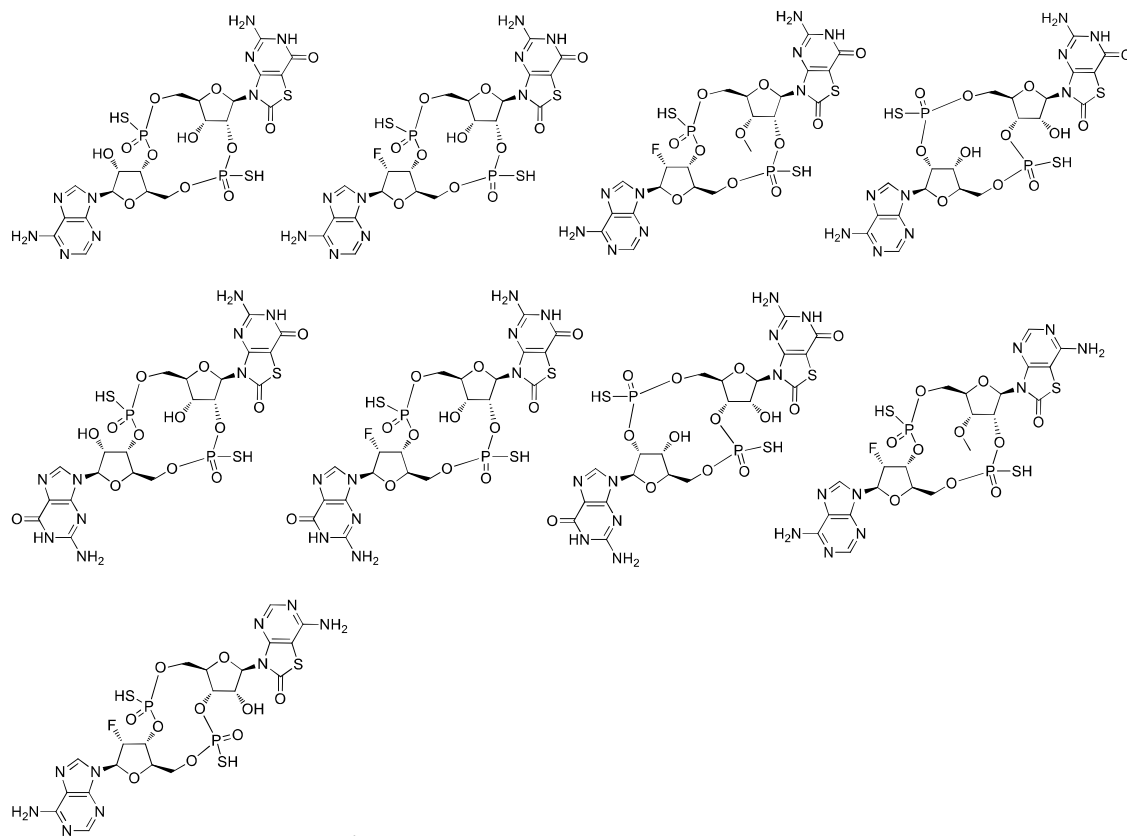




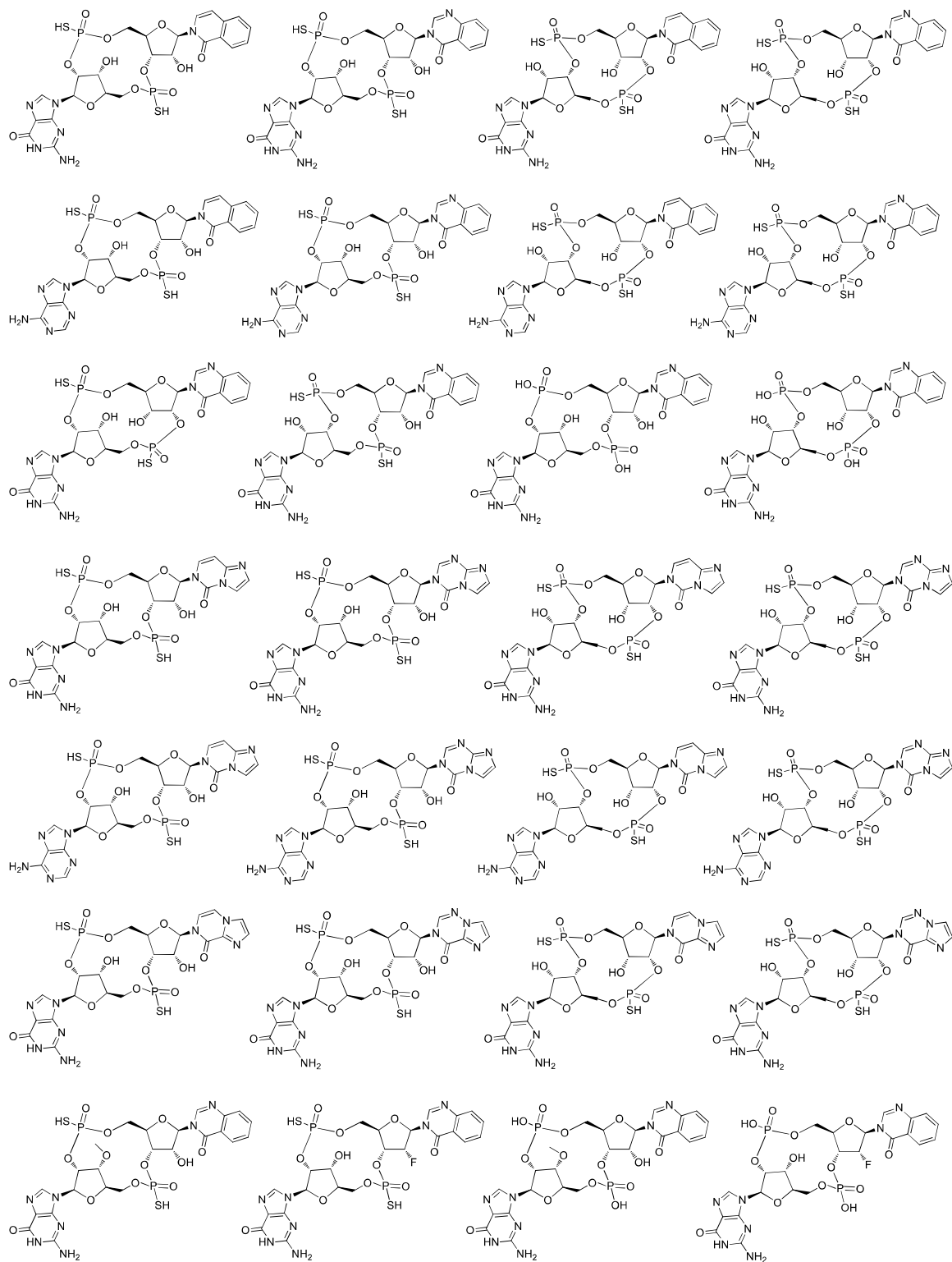
[0278] In some embodiments, the compound of Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt is any of the following structures:

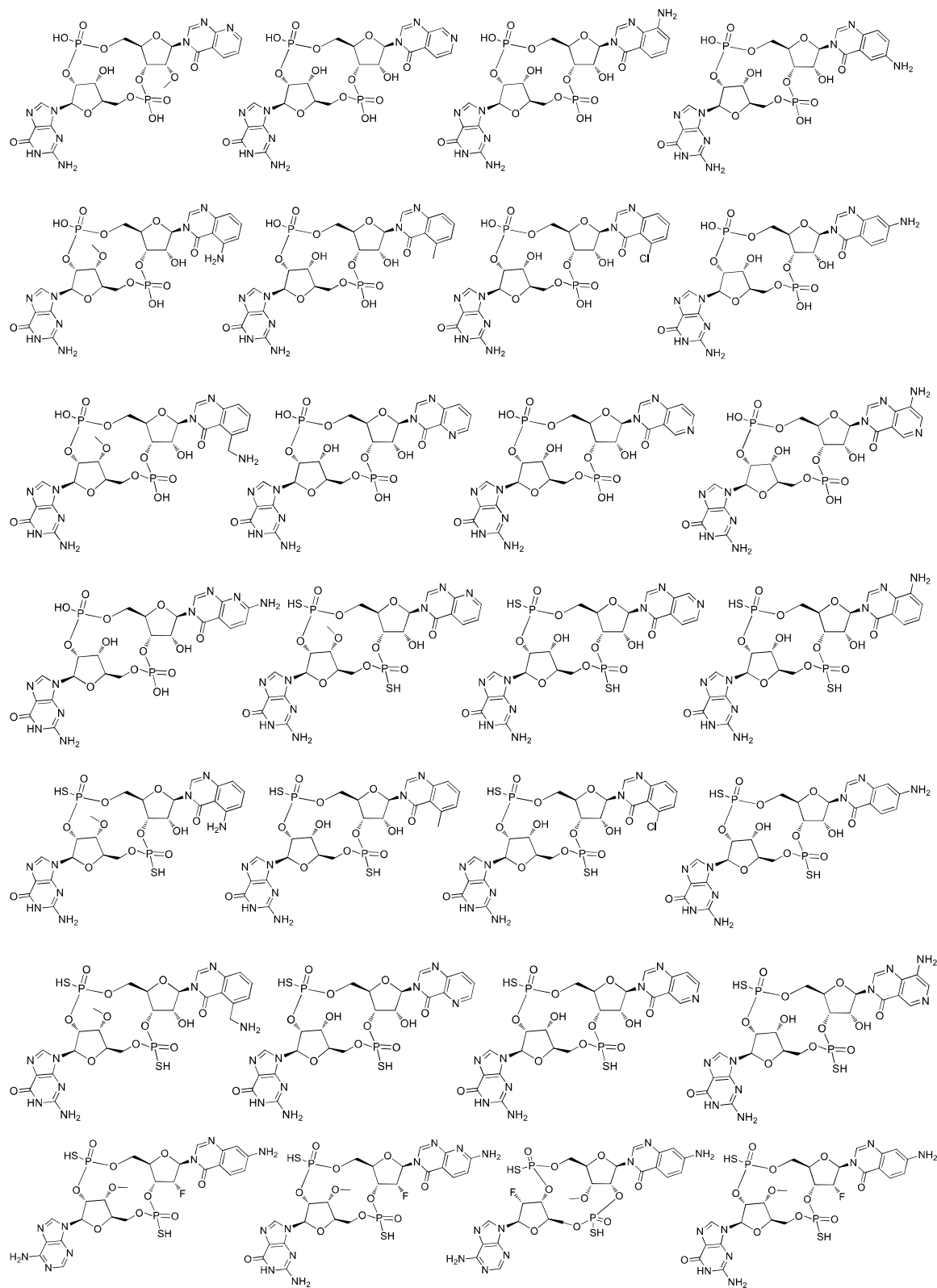


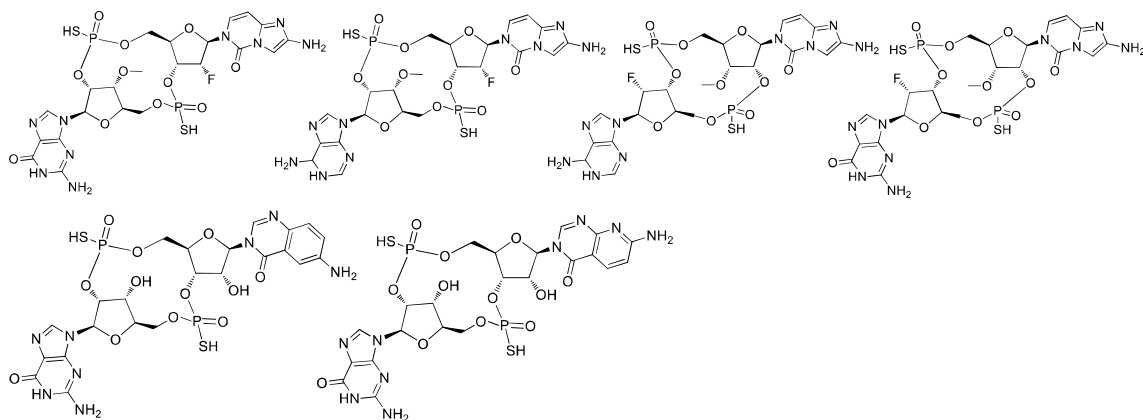
[0279] In some embodiments, the compound of Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt is any of the following structures:



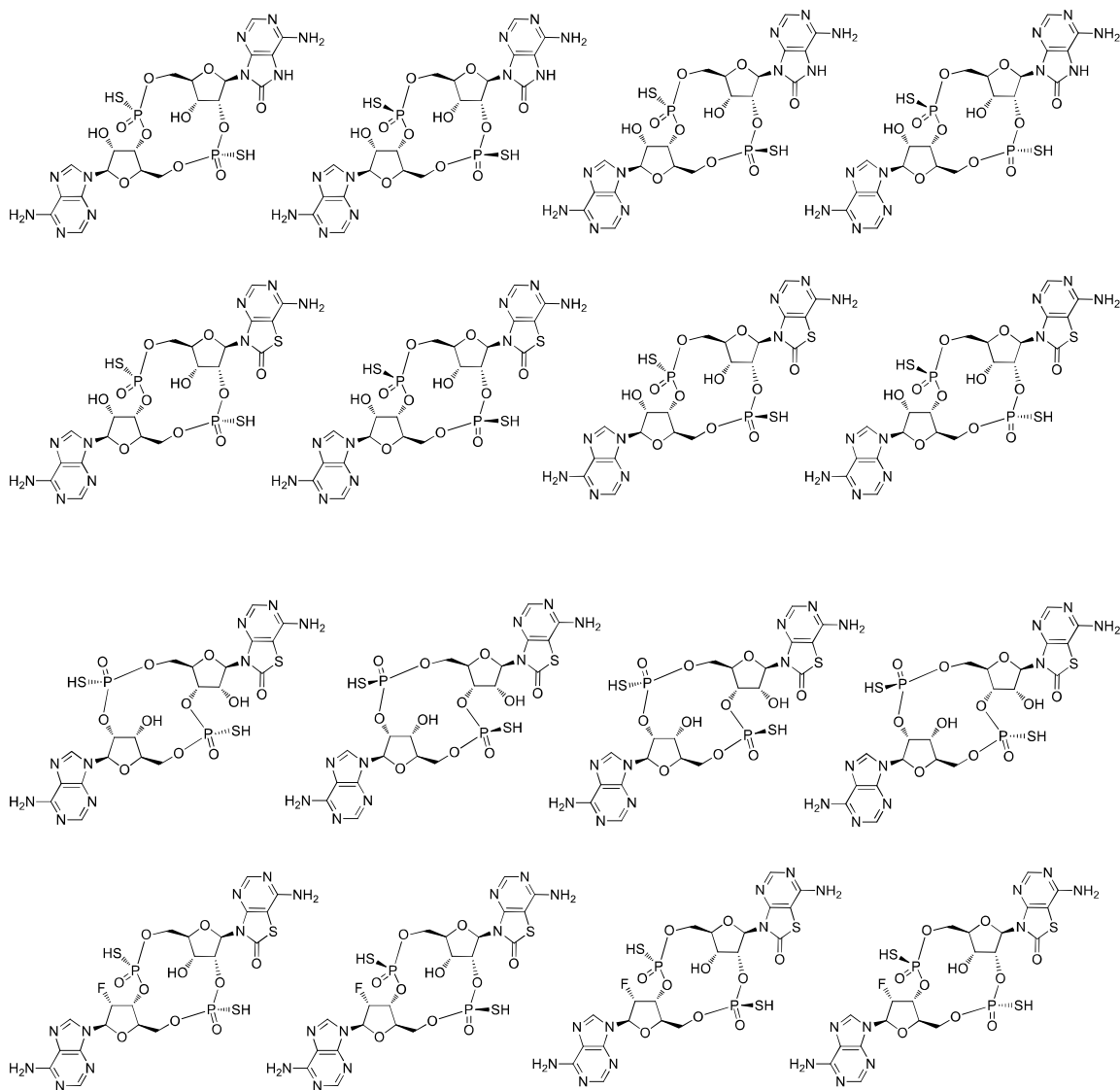
[0280] In some embodiments, the compound of Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt is most preferably any of the following structures:

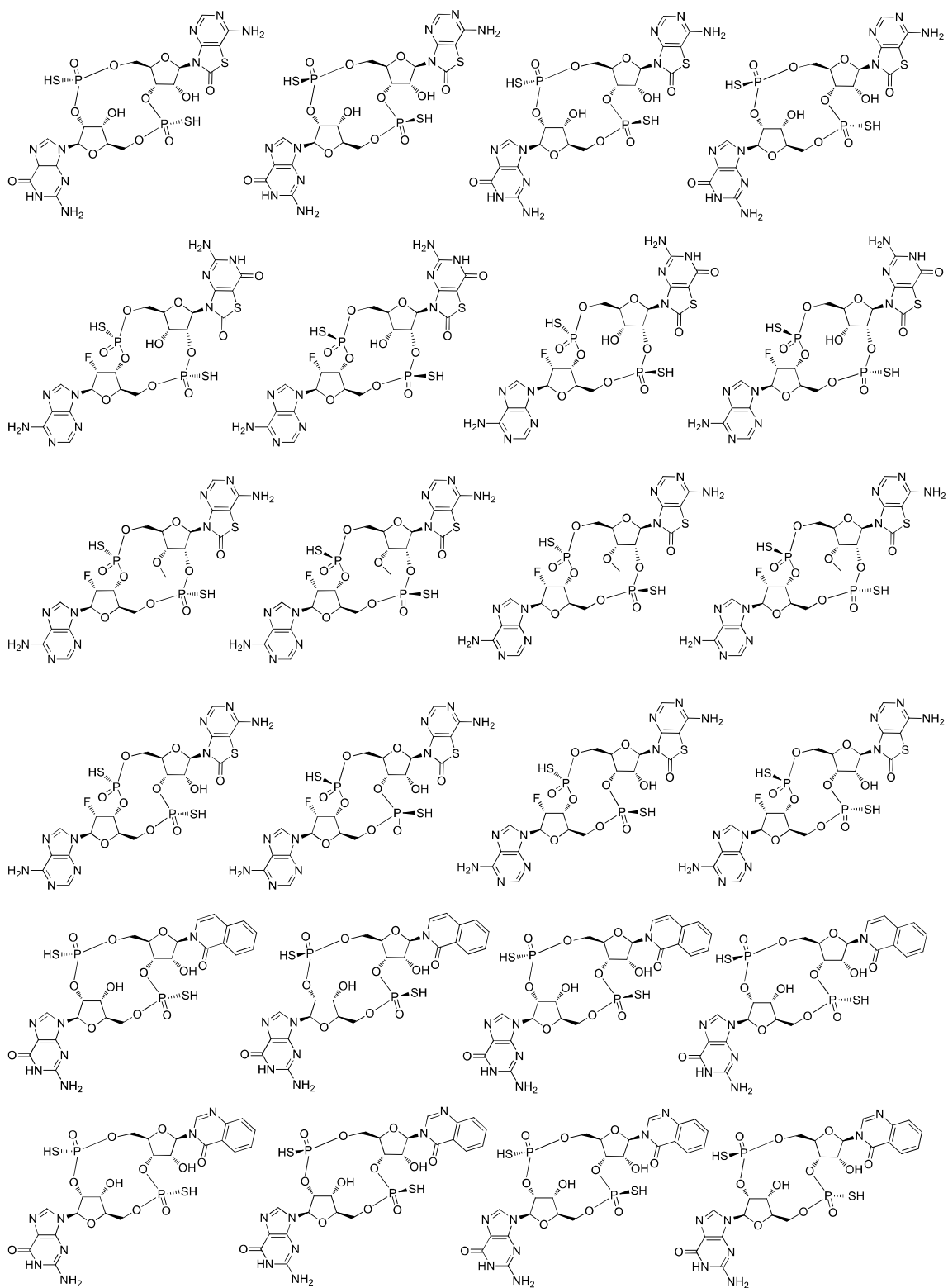


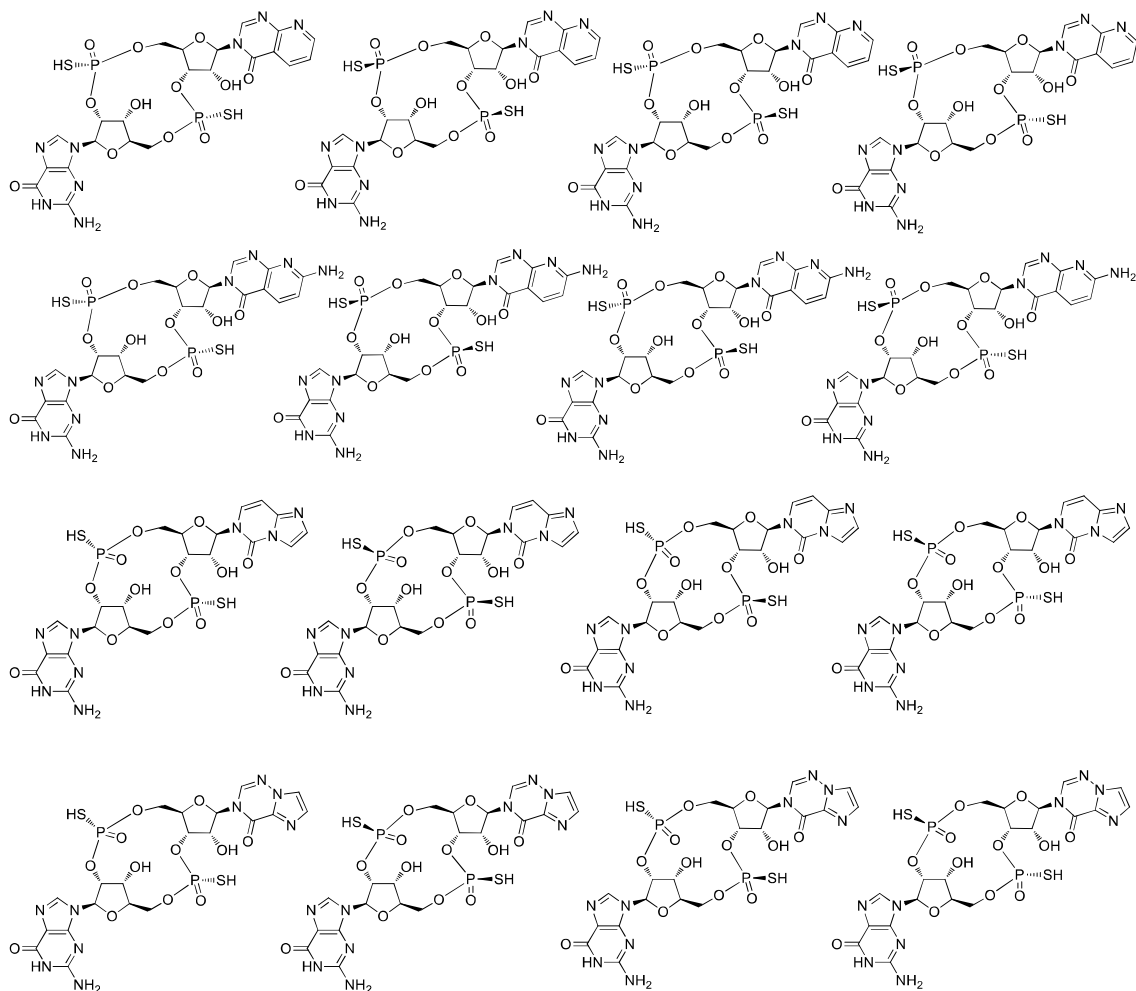




[0281] In some embodiments, the compound of Formula I, the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt is any of the following structures:







[0282] The compound of formula (I), the pharmaceutically acceptable salt thereof can be synthesized by a general chemical method.

[0283] In general, the preparation of the salt can be carried out by reacting the free base or acid with an equivalent chemical equivalent or an excess of an acid (inorganic or organic acid) or a base (inorganic or organic base) in a suitable solvent or solvent composition.

[0284] The present disclosure also provides a pharmaceutical composition comprising a therapeutically effective amount of an active component and a pharmaceutically acceptable excipient; the active component comprises one or more of the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative and pharmaceutically acceptable salt thereof.

[0285] The active component in the pharmaceutical composition can also include other therapeutic agents for viral infections or other infectious diseases (e.g., HIV, HBV, HCV

infection, etc.), autoimmune diseases (e.g., rheumatoid arthritis, lupus erythematosus, psoriasis, etc.) or malignancies.

[0286] In the pharmaceutical composition, the pharmaceutically acceptable excipient can include a pharmaceutically acceptable carrier, diluent, and/or excipient.

[0287] According to the purpose of the treatment, the pharmaceutical composition can be formulated into various types of unit dosage forms, such as tablets, pills, powders, liquids, suspensions, emulsions, granules, capsules, suppositories, and injections (solutions and suspensions) and the like, and preferably liquids, suspensions, emulsions, suppositories and injections (solutions and suspensions), etc.

[0288] In order to shape the pharmaceutical composition in the form of a tablet, any excipient known and widely used in the art can be used. For example, carriers such as lactose, white sugar, sodium chloride, glucose, urea, starch, calcium carbonate, kaolin, crystalline cellulose, and silicic acid; binders such as water, ethanol, propanol, common syrup, dextrose solution, starch solution, gelatin solution, carboxymethyl cellulose, shellac, methyl cellulose and potassium phosphate, polyvinylpyrrolidone, etc.; disintegrating agents, such as dry starch, sodium alginate, agar powder, kelp powder, sodium bicarbonate, calcium carbonate, fatty acid esters of polyethylene dehydrated sorbitol, sodium dodecyl sulfate, monoglyceryl stearate, starch and lactose; disintegration inhibitors such as white sugar, glyceryl tristearate, coconut oil and hydrogenated oil; adsorption promoters such as quaternary ammonium bases and sodium dodecyl sulfate; wetting agents such as glycerin, starch, etc.; adsorbents such as starch, lactose, kaolin, bentonite and colloidal silicic acid; and lubricating agents such as pure talc, stearate, boric acid powder and polyethylene glycol. It is also possible to use a usual coating material to formulate a sugar-coated tablet, a gelatin film tablet, a casing tablet, a film-coated tablet, a two-layer film tablet, and a multilayer tablet.

[0289] In order to shape the pharmaceutical composition in the form of a pill, any excipient known and widely used in the art may be used, for example, carriers such as lactose, starch, coconut oil, hardened vegetable oil, kaolin and talc, etc.; binders such as gum arabic powder, tragacanth powder, gelatin and ethanol, etc.; disintegrating agents such as agar and kelp powder.

[0290] In order to shape the pharmaceutical composition in the form of a suppository, any excipient known and widely used in the art can be used, for example, polyethylene glycol,

coconut oil, higher alcohols, esters of higher alcohols, gelatin and semi-synthetic glycerides, etc.

[0291] For the preparation of a pharmaceutical composition in the form of an injection, the solution or suspension may be sterilized (preferably by adding an appropriate amount of sodium chloride, glucose or glycerin, etc.) to prepare an injection which is isotonic with blood. Any of the commonly used carriers in the art can also be used. For example, water, ethanol, propylene glycol, ethoxylated isostearyl alcohol, polyoxylated isostearyl alcohol, and fatty acid esters of polyethylene dehydrated sorbitol. In addition, usual solubilizers, buffers, analgesics can be added.

[0292] In the present disclosure, the content of the composition in the pharmaceutical composition is not particularly limited and can be selected in a wide range, usually 5-95% by mass, preferably 30-80% by mass.

[0293] In the present disclosure, the administration method of the pharmaceutical composition is not particularly limited. Formulations of various dosage forms can be selected depending on the age, sex and other conditions and symptoms of the patient. For example, tablets, pills, solutions, suspensions, emulsions, granules or capsules are administered orally; injections can be administered alone or in combination with solutions for injection (e.g., glucose solution and amino acid solution); suppositories are given to the rectum.

[0294] The present disclosure also provides a use of the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of interferon gene stimulating factor STING modulators. The interferon gene stimulating factor STING modulator is preferably interferon gene stimulating factor STING agonist. The STING agonist refers to the compound of formula (I), the isomer, prodrug, stable isotope derivative or pharmaceutically acceptable salt thereof, or the pharmaceutical composition capable of activating STING signaling.

[0295] The present disclosure also provides a use of the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a medicament for modulation of the proliferation of T cells or other immune cells.

[0296] The present disclosure also provide a use of the cyclic di-nucleotide analogue (I), the

isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a vaccine adjuvant.

[0297] The present disclosure also provide a use of the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a medicament for the treatment and/or alleviation of a STING-mediated neoplastic disease and non-neoplastic disease. The STING-mediated diseases refer to diseases caused by immunosuppression or hyperactivation due to STING signaling. The types of related diseases include, but are not limited to: viral infections or other infectious diseases, autoimmune diseases, and malignancies.

[0298] The present disclosure preferably provides a use of the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a medicament for the treatment and/or alleviation of malignancies caused by immunosuppression.

[0299] The present disclosure further provides a method of treating viral infections or other infectious diseases, malignancies, autoimmune diseases with the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition, comprising: administering to a mammal a required dose of the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition.

[0300] The mammal is preferably human.

[0301] The present disclosure preferably provides the compound represented by formula (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a medicament for the treatment and/or alleviation of a STING-mediated disease; the STING-mediated disease is those caused by STING mediated immunosuppression, the diseases can include: viral infections or other infectious diseases (e.g., HIV, HBV, HCV infection, etc.), autoimmune diseases (e.g., rheumatoid arthritis, lupus erythematosus, psoriasis, etc.), or malignancies.

[0302] The present disclosure further provides a use of the compound represented by formula (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a medicament for the treatment and/or

alleviation of malignancies.

[0303] The present disclosure further provides a use of the compound represented by formula (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a medicament for the treatment and/or alleviation of viral or other infections.

[0304] The present disclosure further provides a use of the compound represented by formula (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in the preparation of a medicament for the treatment and/or alleviation of autoimmune diseases.

[0305] The present disclosure further provides the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in combination with one or more other kinds of therapeutic agents and/or therapeutic methods for use in the treatment, alleviation and/or prevention of STING-mediated diseases. The STING-mediated diseases are those caused by STING-mediated immunosuppression, and the diseases can include: viral or other infections (e.g., HIV, HBV, HCV infections, etc.), autoimmune diseases (e.g., rheumatoid arthritis, lupus erythematosus, psoriasis, etc.), or cancer.

[0306] The present disclosure preferably provides the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in combination with one or more other kinds of therapeutic agents and/or therapeutic methods for use in the treatment and/or alleviation of cancer.

[0307] The present disclosure preferably provides the cyclic di-nucleotide analogue (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition in combination with one or more other kinds of therapeutic agents and/or therapeutic methods for use in the treatment and/or alleviation of STING-mediated cancer.

[0308] In the present disclosure, the other kinds of therapeutic agents (e.g., other kinds of therapeutic agents for the treatment of cancer) can be made into a therapeutic dosage form with the cyclic di-nucleotide analogue (I) for a single dosage form, or separate therapeutic dosage forms for sequential administration.

[0309] The present disclosure further provides a combination formulation comprising the compound of formula (I), the isomer thereof, prodrug, stable isotope derivative or pharmaceutically acceptable salt, or the pharmaceutical composition and other kinds of therapeutic agents and/or therapeutic methods for the treatment of cancer.

[0310] In the present disclosure, the other kinds of therapeutic agents for the treatment of cancer can include, but are not limited to one or more of: microtubule protein inhibitors, alkylating agents, topozyme I/II inhibitors, platinum compounds, antimetabolites, hormones and hormone analogs, signal transduction pathway inhibitors, angiogenesis inhibitors, targeted therapeutic agents (e.g., specific kinase inhibitors), immunotherapeutic agents, pro-apoptotic agents, and cell cycle signaling pathway inhibitors.

[0311] In the present disclosure, the other kinds of therapeutic methods for the treatment of cancer can include, but are not limited to, one or more of: tumor immunotherapy and radiotherapy.

[0312] In the present disclosure, the other kinds of therapeutic agents for the treatment of cancer are preferably immunotherapeutic agents.

[0313] In the present disclosure, the microtubulin inhibitor may be selected from, but is not limited to, one or more of: the vincristine family (e.g., vinblastine, vincristine, vinorelbine, vindesine sulfate), the taxane family (docetaxel, paclitaxel), and eribulin mesylate.

[0314] In the present disclosure, the alkylating agent may be selected from, but is not limited to: nitrogen mustard, N-oxo-nitrogen mustard hydrochloride, cyclobutoic nitrogen mustard, uracil mustard, cyclophosphamide, ifosfamide, thiotepa, carboquone, trisethyleneiminoquinone, improsulfan tosylate, mannosesufan, treosulfan, busulfan, nimustine hydrochloride, dibromomannitol, melphalan, dacarbazine, ranimustine, carmustine, lomustine, streptozotocin, temozolomide, procarbazine, ethyleneimine derivatives, methanesulfonates, nitrosoureas, triazenes.

[0315] In the present disclosure, the topozyme I/II inhibitors may be selected from, but not limited to, one or more of: doxorubicin, daunorubicin, epirubicin, idarubicin, irinotecan, topotecan, rubitecan, belotecan, etoposide, teniposide, adriamycin, and dexrazoxane, camptothecin.

[0316] In the present disclosure, the platinum compound may be selected from, but not

limited to, one or more of: cisplatin, carboplatin, oxaliplatin, and nedaplatin.

[0317] In the present disclosure, the antimetabolites may be selected from, but not limited to, one or more of: folate antagonists, pyrimidine analogs, purine analogs, adenosine deaminase inhibitors, such as: methotrexate, 5-fluorouracil, floxuridine, cytarabine, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatin, and gemcitabine.

[0318] In the present disclosure, the immunotherapeutic agent may be selected from, but not limited to one or more of: immunomodulators, tumor microenvironment modulators, and anti-angiogenic factors. The immunomodulators may include, but are not limited to: 1) protein antagonists (e.g., immune checkpoint inhibitors) that inhibit T-cell activity: one or more of CTLA4 (e.g., one or more of ipilimumab, tremelimumab, abatacept, belatacept, BMS-986249, BMS-986218, AGEN-1884 and KN-046), PD-1 (e.g., one or more of camrelizumab, toripalimab, sintilimab, cemiplimab, pembrolizumab, nivolumab, tislelizumab, spartalizumab,, dostarlimab, genolimzumab, cetrelimab, HLX-10, BCD-100, AK-105, MEDI-0680, CS-1003, BAT-1306, HX-008, sasanlimab, AGEN-2034, BI-754091, GLS-010, MGA-012, AK-104 and AK-103), PD-L1 (e.g., one or more of durvalumab, avelumab, atezolizumab, envafolimab, cosibelimab, CS1001, SHR-1316, lazertinib, bintrafusp alfa, TQB-2450, CA-170, CX-072, BGB-A333, BMS-936559, GEN-1046, KL-A167 and IO-103), LAG3, and TIM3; 2) protein agonists that stimulate T-cell activity: one or more of GITR, OX40, OX40L, 4-1BB (CD137), CD27 and CD40; 3) one or more of TLR2 agonists, TLR4 agonists, TLR5 agonists, TLR7 agonists, TLR8 agonists and TLR9 agonists; (4) IDO inhibitors, CD73 inhibitors.

[0319] In the present disclosure, the signal transduction pathway inhibitors (STI) may be selected from, but not limited to, one or more of: BCR/ABL kinase inhibitors, epidermal growth factor receptor inhibitors, her-2/neu receptor inhibitors, AKT family kinase inhibitors, PI3K signaling pathway inhibitors, and cell cycle checkpoint inhibitors.

[0320] In the present disclosure, the angiogenesis inhibitors may be selected from, but not limited to, one or more of: VEGF/VEGFR signaling pathway inhibitors, Src family kinase inhibitors, Src signaling pathway inhibitors, and c-Fes kinase inhibitors.

[0321] In the present disclosure, the targeted therapeutic agents may be selected from, but not limited to: one or more of erlotinib, imatinib, apatinib, nilotinib, crizotinib, dasatinib, pazopanib, regorafenib, ruxolitinib, sorafenib, sunitinib, vandetanib, vemurafenib, bosutinib,

gefitinib, afatinib, axitinib, dabrafenib, dacomitinib, nintedanib, levatinib, masitinib, midostaurin, neratinib, ponatinib, radotinib, trametinib, brivanib alaninate, cediranib, cabozantinib malate, ibrutinib, icotinib, lapatinib, cobimetinib, idelalisib, ponatinib, alisertib, dinaciclib, linsitinib, orantinib, rigosertib, tipifarnib, tivozanib, pimasertib, buparlisib, and fedratinib.

[0322] In the present disclosure, the tumor immunotherapy may be selected from, but not limited to, one or more of: antitumor vaccines (e.g., synthetic peptides, DNA vaccines, and recombinant viruses), oncolytic viruses, cytokine therapies (e.g., IL2 and GM-CSF), and chimeric antigen receptor T-cell therapies (CAR-T).

[0323] In the present disclosure, the viral and other infections may include: the infections caused by viruses such as influenza viruses, hepatitis B virus (HBV), hepatitis C virus (HCV), human papillomavirus (HPV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), poliovirus, varicella-zoster virus, coxsackievirus, or human immunodeficiency virus (HIV).

[0324] In the present disclosure, the malignant tumors include metastatic and non-metastatic cancers, also include familial hereditary and sporadic cancers, and may also include solid and non-solid tumors.

[0325] In the present disclosure, specific examples of the solid tumors may include, but not limited to: eye, bone, lung, stomach, pancreas, breast, prostate, brain (including glioblastoma and medulloblastoma), ovary (including those stromal cells, germ cells and mesenchymal cells arising from epithelial cells), bladder, testis, spinal cord, kidney (including adenocarcinoma, nephroblastoma), mouth, lip, throat, oral cavity (including squamous cell carcinoma), nasal cavity, small intestine, colon, rectum, parathyroid gland, gallbladder, bile duct, cervix, heart, subpharyngeal gland, bronchus, liver, ureter, vagina, anus, laryngeal gland, thyroid gland (including thyroid cancer and medullary carcinoma), esophagus, nasopharyngeal gland, pituitary, salivary gland, adrenal gland, intraepithelial neoplasia of head and neck (including Bowen's disease and Paget's disease), sarcoma (including smooth muscle sarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, osteosarcoma), skin (including melanoma, Kaposi's sarcoma, basocellular carcinoma and squamous cell carcinoma) and other related tumors.

[0326] In the present disclosure, the solid tumor is preferably one or more of eye cancer, bone

cancer, lung cancer, stomach cancer, pancreatic cancer, breast cancer, prostate cancer, brain cancer (including but not limited to glioblastoma, adult neural tube cell tumor), ovarian cancer, bladder cancer, cervical cancer, testicular cancer, kidney cancer (including but not limited to adenocarcinoma, nephroblastoma), oral cancer (including squamous cell carcinoma), tongue cancer, laryngeal cancer, nasopharyngeal cancer, head and neck cancer, colon cancer, small intestine cancer, rectal cancer, parathyroid cancer, thyroid cancer, esophageal cancer, gallbladder cancer, bile duct cancer, cervical cancer, liver cancer, lung cancer (including but not limited to small cell lung cancer, non-small cell lung cancer), chorionic epithelioma, osteosarcoma, ewing tumor, soft tissue sarcoma and skin cancer.

[0327] In the present disclosure, specific examples of the non-solid tumors (including hematological tumors) may include, but not limited to: lymphoid leukemia (including acute lymphoblastic leukemia, lymphoma, myeloma, chronic lymphocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, T-cell chronic lymphatic leukemia, B-cell chronic lymphatic leukemia), myeloid-associated leukemia (including acute myeloid leukemia, chronic myeloid leukemia) and AIDs-associated leukemia.

[0328] In the present disclosure, the autoimmune diseases may include, but not limited to: one or more of rheumatoid arthritis, systemic lupus erythematosus, mixed connective tissue disease (MCTD), systemic scleroderma (including: CREST syndrome), dermatomyositis, nodular vasculitis, renal diseases (including: pulmonary hemorrhagic nephritis syndrome, acute glomerulonephritis, primary membranoproliferative glomerulonephritis, etc.), endocrine-related diseases (including: type I diabetes, gonadal insufficiency, pernicious anemia, hyperthyroidism, etc.), liver disease (including: primary biliary cirrhosis, autoimmune cholangitis, autoimmune hepatitis, primary sclerosing cholangitis, etc.) and autoimmune reactions caused by infections (e.g., AIDS, malaria, etc.).

[0329] Unless otherwise stated, the following terms appearing in the specification and claims of the disclosure have the following meanings:

[0330] The term "alkyl" refers to a saturated straight or branched-chain hydrocarbon group comprising 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, more preferably 1 to 8, 1 to 6, 1 to 4, 1 to 3 carbon atoms, representative examples of alkyl groups include, but are not limited to: methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *sec*-butyl, *tert*-butyl, isobutyl, *n*-pentyl, *n*-hexyl,

n-heptyl, octyl, nonyl, decyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1-ethylpropyl, 2-methylbutyl, 3-methylbutyl, 1-ethyl-2-methylpropyl, 1,1,2-trimethylpropyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl, 2-ethylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 4,4-dimethylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 2-ethylpentyl, 3-ethylpentyl, 2,2,4-trimethylpentyl, undecyl, dodecyl, and their various isomers, etc.

[0331] The term "cycloalkyl" refers to a saturated or partially unsaturated (containing 1 or 2 double bonds) monocyclic or polycyclic group containing 3 to 20 carbon atoms. The term "monocyclic cycloalkyl" is preferably a 3 to 10 membered monocyclic alkyl, more preferably a 3 to 8 membered monocyclic alkyl, such as: cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclohexenyl. The term "polycyclic cycloalkyl" includes "bridged cycloalkyl", "fused cycloalkyl" and "spiro cycloalkyl". Monocyclic cycloalkyl or polycyclic cycloalkyl can be linked to the parent molecule by any carbon atom on the ring.

[0332] The term "heterocycloalkyl" refers to a saturated or partially unsaturated (containing 1 or 2 double bonds) non-aromatic cyclic group consisting of carbon atom(s) and heteroatom(s) selected from nitrogen, oxygen or sulfur, which may be monocyclic or polycyclic, in the present disclosure the number of heteroatom(s) in the heterocycloalkyl group is preferably 1, 2, 3 or 4, the nitrogen, carbon or sulfur atom in the heterocycloalkyl group may optionally be oxidized. The nitrogen atom may optionally be further substituted with other groups to form tertiary amines or quaternary ammonium salts. The "monocyclic heterocycloalkyl" is preferably a 3 to 10 membered monocyclic heterocycloalkyl, more preferably a 3 to 8 membered monocyclic heterocycloalkyl. For example: aziridinyl, tetrahydrofuran-2-yl, morpholin-4-yl, thiomorpholin-4-yl, thiomorpholine-S-oxide-4-yl, piperidin-1-yl, *N*-alkylpiperidin-4-yl, pyrrolidin-1-yl, *N*-alkylpyrrolidin-2-yl, piperazin-1-yl, 4-alkylpiperazin-1-yl, etc. "Polycyclic heterocycloalkyl" includes "fused heterocycloalkyl", "spiro heterocycloalkyl" and "bridged heterocycloalkyl". Monocyclic heterocycloalkyl and polycyclic heterocycloalkyl can be linked to the parent molecule by any ring atom on the ring. The above ring atoms refer specifically to the carbon and/or nitrogen atoms that constitute the ring skeleton.

[0333] The term "cycloalkyl alkyl" refers to a cycloalkyl group connected to the parent nucleus structure through an alkyl group. Thus, the term "cycloalkyl alkyl" encompasses the above definitions of alkyl and cycloalkyl.

[0334] The term "heterocycloalkyl alkyl" refers to a heterocycloalkyl group connected to the parent nucleus structure through an alkyl group. Thus, the term "heterocycloalkyl" encompasses the above definitions of alkyl and heterocycloalkyl.

[0335] The term "alkoxy" refers to a cyclic or non-cyclic alkyl group having indicated carbon atoms attached through an oxygen bridge, and includes alkyloxy, cycloalkyloxy and heterocycloalkyloxy. Thus, "alkoxy" includes the above definitions of alkyl, heterocycloalkyl, and cycloalkyl.

[0336] The term "alkenyl" refers to a straight, branched or cyclic non-aromatic hydrocarbon group containing at least 1 carbon-carbon double bond. There may be 1 to 3 carbon-carbon double bonds present, preferably 1 carbon-carbon double bond present. The term "C₂₋₄ alkenyl" refers to an alkenyl group having 2 to 4 carbon atoms, and the term "C₂₋₆ alkenyl" refers to an alkenyl group having 2 to 6 carbon atoms, including vinyl, propylenyl, butenyl, 2-methylbutenyl, and cyclohexenyl. The alkenyl groups can be substituted.

[0337] The term "alkynyl" refers to a straight, branched or cyclic hydrocarbon group containing at least 1 carbon-carbon triple bond. There may be 1 to 3 carbon-carbon triple bonds present, preferably 1 carbon-carbon triple bond present. The term "C₂₋₆ alkynyl" refers to an alkynyl group having 2 to 6 carbon atoms, including ethynyl, propynyl, butynyl and 3-methylbutynyl.

[0338] The term "aryl" refers to any stable 6 to 10 monocyclic or bicyclic aromatic group, such as: phenyl, naphthyl, tetrahydronaphthyl, 2,3-dihydroindenyl, or biphenyl.

[0339] The term "heteroaryl" refers to an aromatic cyclic group formed by replacing at least 1 carbon atom on the ring by a heteroatom selected from nitrogen, oxygen or sulfur, which may be a 5 to 7 membered monocyclic structure or a 7 to 12 membered bicyclic structure, preferably 5 to 6 membered heteroaryl. In the present disclosure, the number of the heteroatom is preferably 1, 2 or 3, and includes: pyridyl, pyrimidinyl, pyridazin-3(2*H*)-one group, furanyl, thienyl, thiazolyl, pyrrolyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, 1,2,5-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,4-triazolyl, 1,2,3-triazolyl, tetrazolyl, indazolyl, isoindazolyl, indolyl,

isindolyl, benzofuranyl, benzothienyl, benzo[d][1,3]dioxolyl, benzothiazolyl, benzoxazolyl, quinolinyl, isoquinolinyl, quinazolinyl, etc.

[0340] The term "arylalkyl" refers to an aryl group connected to the parent nucleus structure through an alkyl group. Thus, the term "arylalkyl" encompasses the above definitions of the alkyl and aryl.

[0341] The term "heteroaryl-alkyl" refers to a heterocycloalkyl group connected to the parent nucleus structure through an alkyl group. Thus, the term "heteroarylalkyl" encompasses the above definitions of the alkyl and heteroaryl.

[0342] The term "halogen" indicates fluorine, chlorine, bromine or iodine.

[0343] The term "halo-alkyl" refers to an alkyl group arbitrarily substituted with halogen. Thus, "halo-alkyl" includes the definitions of the halogen and alkyl above.

[0344] The term "halo-alkoxy" refers to an alkoxy group arbitrarily substituted with halogen. Thus, the term "halo-alkoxy" encompasses the above definitions of the halogen and alkoxy.

[0345] The term "amino" refers to -NH₂ and the term "alkylamino" refers to that at least one hydrogen atom on the amino group is substituted by an alkyl group, including but not limited to: -NHCH₃, -N(CH₃)₂, -NHCH₂CH₃, -N(CH₂CH₃)₂.

[0346] The term "nitro" refers to -NO₂.

[0347] The term "cyano" refers to -CN.

[0348] The term "azido" refers to -N₃.

[0349] The term "room temperature" in the present disclosure refers to 15-30 °C.

[0350] The isotope-substituted derivative includes an isotope-substituted derivative that any hydrogen atom of the compound of formula (I) is replaced by 1 to 5 deuterium atoms, or any carbon atom of the compound of formula (I) is replaced by 1-3 C¹⁴ atom, or any oxygen atom of the compound of formula I is replaced by 1 to 3 O¹⁸ atom.

[0351] The term "prodrug" refers to a compound capable of converting to its original active compound after metabolism *in vivo*. Representatively, a prodrug is an inactive substance or less active than the active parent compound, but may provide convenient handling, administration, or improved metabolic properties.

[0352] The "pharmaceutically acceptable salts" described in the present disclosure are discussed in Berge, *et al.*, "pharmaceutically acceptable salts", *J. Pharm. Sci.*, 66, 1-19 (1977),

and it is apparent to pharmaceutical chemists that the salts are substantially non-toxic and provide the desired pharmacokinetic properties, palatability, absorption, distribution, metabolism or excretion, etc. The compounds described herein may have acidic groups, basic groups, or amphoteric groups, and typical pharmaceutically acceptable salts include those obtained by reaction of compounds of the present disclosure with acids, such as: hydrochloride, hydrobromide, sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogen phosphate, dihydrogen phosphate, metaphosphate, pyrophosphate, nitrate, acetate, propionate, decanoate, octanoate, formate, acrylate, isobutyrate, caproate, heptanoate, oxalate, malonate, succinate, octanedioate, benzoate, methylbenzoate, phthalate, maleate, methanesulfonate, *p*-toluenesulfonate, (*D,L*)-tartaric acid, citric acid, maleic acid, (*D,L*)-malic acid, fumaric acid, succinate, lactate, trifluoromethanesulfonate, naphthalene-1-sulfonate, mandelate, pyruvate, stearate, ascorbate, salicylate. When the compounds of the disclosure contain acidic groups, pharmaceutically acceptable salts thereof may also include: alkali metal salts, such as lithium, sodium or potassium salts; alkaline earth metal salts, such as zinc, calcium or magnesium salts; organic alkali salts, such as salts formed with ammonia, alkylamines (including but not limited to: methylamine, triethylamine), hydroxyalkylamines, amino acids (including but not limited to: lysine, arginine), *N*-methylglucosamine, etc.

[0353] The term "isomer" in the present disclosure means that the compound of formula (I) of the present disclosure may have asymmetric centers and racemates, racemic mixtures and individual diastereoisomers, all of which are included in the present disclosure, including stereoisomers and geometric isomers. In the present disclosure, the individual stereoisomers (enantiomers and diastereoisomers), as well as mixtures thereof, are included within the scope of the present disclosure when the compound of formula (I) or its salt can be present in stereoisomeric form (e.g., it contains one or more asymmetric carbon atoms and/or phosphorus atoms). The present disclosure also includes the individual isomers of the compounds represented by formula (I) or salts, as well as mixtures of isomers with one or more of the chiral centers reversed. The scope of the present disclosure includes mixtures of stereoisomers, and purified enantiomeric or enantiomeric/diastereoisomeric enriched mixtures. The present disclosure includes stereoisomeric mixtures formed by all enantiomers and diastereoisomers in all possible different combinations. The present disclosure includes all combinations and

subsets of stereoisomers of all specific groups as defined above. The compound of formula (I) in the present disclosure contains chiral P-atoms with Rp or Sp conformation, so that compounds with individual stereo-configuration of (Sp, Sp), (Sp, Rp), (Rp, Rp) or (Rp, Sp) and any mixture thereof are included in the scope of the present disclosure.

[0354] The above preferred conditions of the present disclosure may be arbitrarily combined without departing from the general knowledge in the art to obtain the preferred embodiments of the present disclosure.

[0355] The reagents and raw materials used in the present disclosure are commercially available.

Brief description of the drawings

[0356] FIG.1 is the tumor volume change curves of compounds 6-p3 (1 mg/kg, 2 mg/kg, i.t.) and Ref.1 (2 mg/kg, i.t.) in the colon cancer CT26 subcutaneously implanted tumor in mouse.

Detailed description of the embodiment

[0357] The following Embodiments serve to illustrate the present disclosure, but the Embodiments should not be considered as limiting the scope of the disclosure. Some of the experimental methods of the following Embodiments that are not indicated the specific conditions, can in according with the commonly used reaction conditions and procedures, or in accordance with the product specifications.

[0358] All the structures of the compounds in the present disclosure were confirmed by Nuclear Magnetic Resonance (¹H NMR) and/or Mass Spectra (MS).

[0359] ¹H NMR chemical shifts (δ) were recorded in ppm (10⁻⁶). NMR Spectra were recorded on Bruker AVANCE-400 spectrometer. The proper solvents were Chloroform-d (CDCl₃), Methanol-d₄ (CD₃OD), and Dimethyl sulfoxide-d₆ (DMSO-*d*₆), tetramethylsilane as internal standard (TMS).

[0360] The analytical low-resolution mass spectra (LCMS) were recorded on Agilent 1200 HPLC/6120 using an XBridge C18, 3.0×50 mm, 3μm, column temperature: 35°C; or recorded on ThermoUltiMate 3000HPLC/MSQPLUS using an XBridge C18, 3.0×50 mm, 3.5μm, column temperature: 30°C. The gradient elution method 1 of Agilent: 95-5% solvent A₁ and 5-

95% solvent B₁ (0-2.0min), and then 95% solvent B₁ and 5% solvent A₁ (for 1.1min). Percentage as used herein is volume percentage of the volume of a solvent in the total solvent volume. Solvent A₁: 0.01% aqueous solution of trifluoroacetic acid (TFA); Solvent B₁: 0.01% trifluoroacetic acid acetonitrile solution. Percentage is the volume of a solvent in the total solvent volume. The gradient elution method 2 of Thermo: 95-5% solvent A₂ and 5-95% solvent B₂ (0-2min), and then 95% solvent B₂ and 5% solvent A₂ (for 1.8min), Percentage is the volume of a solvent in the total solvent volume. Solvent A₂: 10 mM aqueous solution of ammonium bicarbonate; Solvent B₂: acetonitrile.

[0361] All the compounds in the present disclosure were separated by preparative high-performance liquid chromatography or flash column chromatography.

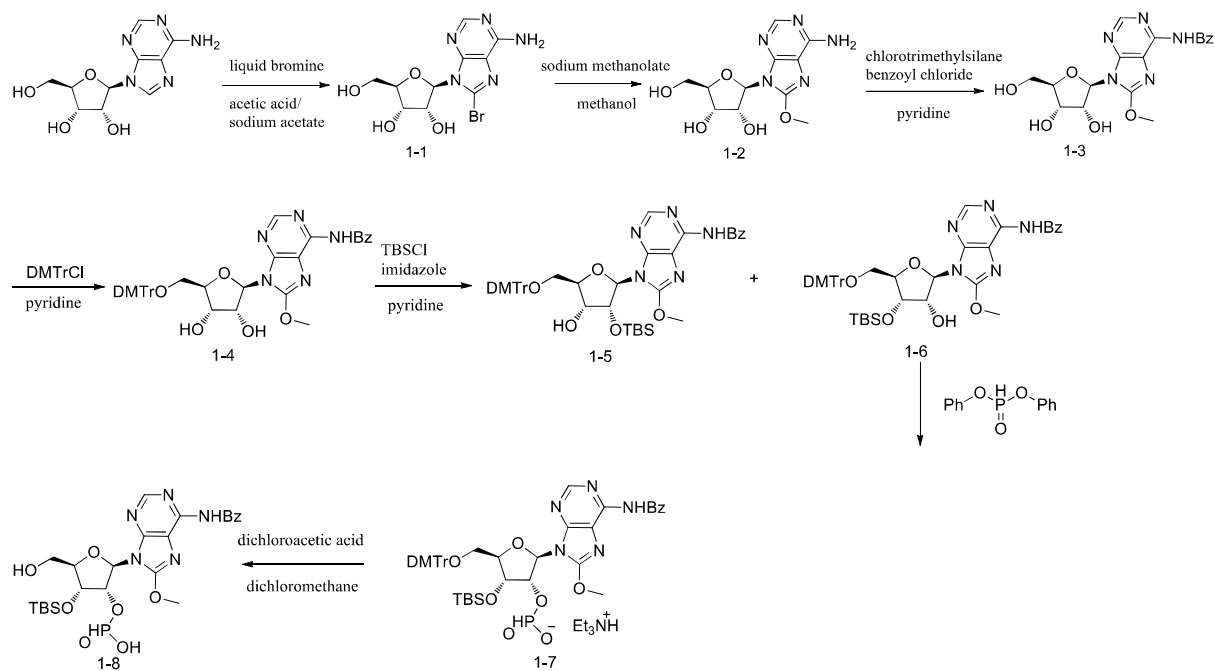
[0362] Preparative high-performance liquid chromatography purification (*prep*-HPLC) was performed on Shimadzu LC-20 HPLC, chromatographic column: waters xbridge Pre C18, 10um, 19mmx250mm. Separation method 1 (acidic condition): mobile phase A: 0.05% aqueous solution of trifluoroacetic acid, mobile phase B: acetonitrile; elution B was 40%, elution time: 20min. Separation method 2 (alkali condition): mobile phase A: 10 mmol/L aqueous solution of ammonium bicarbonate, mobile phase B: acetonitrile; the gradient elution B was from 10% to 80%, elution time: 30min. Separation method 3 (alkali condition): mobile phase A: 10 mmol/L aqueous solution of ammonium bicarbonate, mobile phase B: acetonitrile; the gradient elution B was from 0% to 15%, elution time: 30min. Separation method 4 (alkali condition): mobile phase A: 10 mmol/L aqueous solution of ammonium bicarbonate, mobile phase B: acetonitrile; the gradient elution B was from 0% to 4%, elution time: 10min; the gradient elution B was from 4% to 8%, elution time: 15min. Separation method 5 (alkali condition): mobile phase A: 10 mmol/L aqueous solution of ammonium bicarbonate, mobile phase B: acetonitrile; the gradient elution B was from 0% to 5%, elution time: 10min; the gradient elution B was from 5% to 10%, elution time: 15min. Separation method 6 (alkali condition): mobile phase A: 10 mmol/L aqueous solution of ammonium bicarbonate, mobile phase B: acetonitrile; the gradient elution B was from 10% to 30%, elution time: 5min; the gradient elution B was from 30% to 75%, elution time: 20min. Separation method 7 (alkali condition): mobile phase A: 10 mmol/L aqueous solution of ammonium bicarbonate, mobile phase B: acetonitrile; the gradient elution B was from 0% to 10%, elution time: 7min; the

gradient elution B was from 10% to 40%, elution time: 18min. Detection wavelength: 214nm&254nm; the flow rate: 15.0 mL/min.

[0363] Flash column chromatography (flash system/CheetahTM) was performed on Agela Technologies MP200. Normal-phase chromatography column was Flash column Silica-CS (25g, 40g, 80g, 120g or 330g), Agela Technologies, Tianjing. Ethyl acetate/ petroleum ether or dichloromethane/methanol was chosen as elution system. Reversed-phase chromatography column was C18 column (12g, 20g or 40g), Santai Technologies, Changzhou. Acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L) were chosen as elution system.

[0364] All the compounds in the present disclosure were analyzed by high- performance liquid chromatography. High- performance liquid chromatography (HPLC) was performed on Waters e2695, 2498 UV/VIS Detector, chromatography column: Waters Xselect CHS C18 (4.6*150mm) 5 μ m, mobile phase A: acetonitrile, mobile phase B: acetic acid triethylamine buffer solution which was adjusted pH to 5.0 with acetic acid. Gradient elution of mobile phase B from 95% to 15%, elution time: 30min. Detection wavelength: 214nm&254nm; column temperature: 35°C.

[0365] Embodiment 1: Synthesis of intermediate 1-8



[0366] Step 1: To a suspension of adenosine (50g, 187mmol) in acetic acid/sodium acetate buffer solution (pH=4.0, 0.5M, 1L) was added liquid bromine (60g, 374mmol), keep the system

temperature below 10°C. After addition, the reaction system was stirred at room temperature for 48h. The saturated aqueous solution of sodium bisulfate was added into the reaction solution to remove the excess bromine, and then adjusted pH to neutral with an aqueous solution of sodium hydroxide (1M), the reaction solution was stirred for 2h under ice-water bath. The precipitate was formed and collected by filtration, dried under vacuum to afford intermediate 1-1 (29g). m/z: [M+H]⁺346.0/348.0.

[0367] Step 2: To a suspension of intermediate 1-1 (10g, 28.9mmol) in methanol (100 mL) was added sodium methanolate (9.36g, 173mmol), the reaction system was stirred at reflux for 5h, methanol was concentrated under reduced pressure. The residue was dissolved in a mixed solvent of methanol/ dichloromethane (1/10). The solution was filtered through a Büchner funnel which was covered with a layer of silica gel. The filtrate was concentrated under reduced pressure to afford intermediate 1-2 (3.8g). m/z: [M+H]⁺ 298.2.

[0368] Step 3: To a solution of intermediate 1-2 (10g, 336mmol) in pyridine (40mL) was added chlorotrimethylsilane (16mL, 121mmol) under nitrogen at 0°C. The reaction system was stirred at room temperature for 2h, benzoyl chloride (9.4mL, 80.7mmol) was slowly added to the above reaction system. The resulting solution was stirred at room temperature for overnight, and then ammonium hydroxide solution (25-28%) was added thereto and stirred for 30min. The solvent was concentrate under reduced pressure. The residue was purified by Flash column chromatography (0-10% methanol/ dichloromethane) to afford intermediate 1-3 (7.3g) as a white solid. m/z: [M+H]⁺ 402.2.

[0369] Step 4: To a solution of intermediate 1-3 (7g, 17.4mmol) in anhydrous pyridine (40mL) was added 4,4'-dimethoxytrityl chloride (DMTrCl, 5.9g, 17.4mmol) at 0°C under nitrogen. The reaction system was stirred at room temperature for 3h and then quenched by addition of water (1mL). The solvent was concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-10% methanol/ dichloromethane) to afford intermediate 1-4 (7.4 g) as a yellow solid. m/z: [M+H]⁺ 704.2.

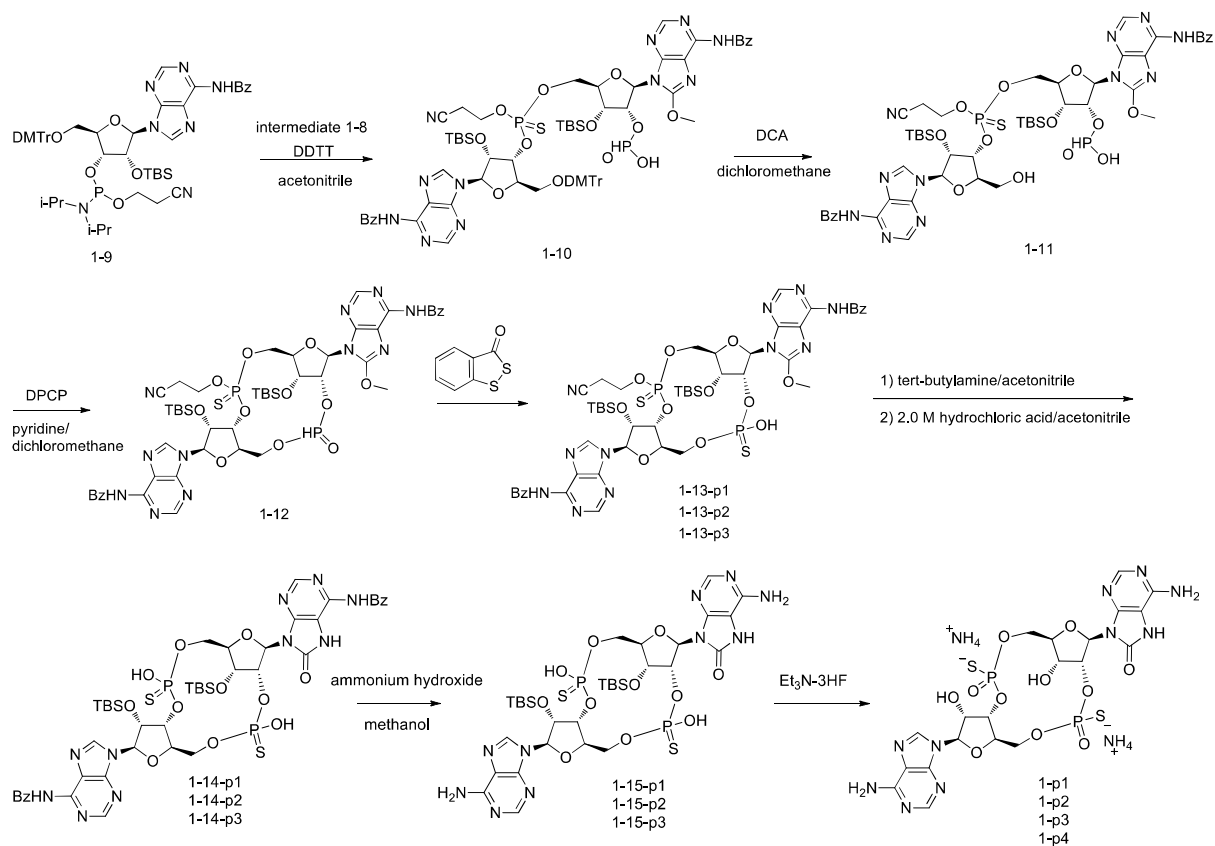
[0370] Step 5: To a solution of intermediate 1-4 (1.2g, 1.70mmol) in pyridine (5mL) was added tert-butyldimethylsilyl chloride (TBSCl, 0.31g, 2.05mmol) and imidazole (0.29g, 4.30mmol) at 0°C under nitrogen. The reaction system was stirred at room temperature for 16h, and then cooled with ice-water, diluted with water (10mL) and ethyl acetate (50mL), the

organic layer washed with brine (50mL×2), the organic layer was separated and dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-60% ethyl acetate/petroleum ether) to afford intermediate 1-5 (less polar, 276mg, white solid) and 1-6 (more polar, 670mg, off-white solid). Intermediate 1-5: m/z: $[M+H]^+$ 818.3; TLC R_f =0.42 (DCM/MeOH =15/1); 1H NMR (400 MHz, DMSO- d_6): δ 11.00 (s, 1H), 8.48 (s, 1H), 8.02 (d, J =7.8 Hz, 2H), 7.62 (t, J =7.4 Hz, 1H), 7.53 (t, J =7.6 Hz, 2H), 7.39 (d, J =7.8 Hz, 2H), 7.29-7.16 (m, 7H), 6.83 (d, J =8.6 Hz, 4H), 5.90 (d, J =5.8 Hz, 1H), 5.13 (d, J =6.0 Hz, 1H), 5.08 (t, J =5.5 Hz, 1H), 4.27 (dd, J =9.6, 5.2 Hz, 1H), 4.07 (d, J =4.0 Hz, 1H), 4.00 (s, 3H), 3.71 (s, 6H), 3.27-2.23 (m, 1H), 3.14-3.10 (m, 1H), 0.74 (s, 9H), 0.10 (d, J =6.6 Hz, 6H). Intermediate 1-6: m/z: $[M+H]^+$ 818.3; TLC R_f =0.23 (DCM/MeOH =10/1); 1H NMR (400 MHz, DMSO- d_6): δ 11.00 (s, 1H), 8.50 (s, 1H), 8.01 (d, J =7.6 Hz, 2H), 7.62 (m, 1H), 7.52 (m, 2H), 7.32 (d, J =7.5 Hz, 2H), 7.26-7.16 (m, 7H), 6.82 (dd, J =8.8, 2.3 Hz, 4H), 5.83 (d, J =5.2 Hz, 1H), 5.40 (d, J =6.1 Hz, 1H), 5.06 (m, 1H), 4.59 (t, J =4.5 Hz, 1H), 4.06 (s, 3H), 3.71 (s, 6H), 3.35-3.33 (m, 1H), 3.31-3.26 (m, 1H), 3.09-3.01 (m, 1H), 0.85 (s, 9H), 0.08 (d, J =7.8 Hz, 6H).

[0371] Step 6: To a solution of intermediate 1-6 (400mg, 0.48mmol) in pyridine (4mL) was added diphenyl phosphite (460mg, 1.92mmol) and then stirred at room temperature for 30min. Triethylamine (0.4mL) and water (0.4 mL) was successively added to the above reaction system and stirred for 30min. Dichloromethane (5 mL) and aqueous solution of sodium bicarbonate (5 mL, 5%) was successively added to the above reaction system. The organic layer washed with water, and then the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (0-10% methanol/dichloromethane) to afford intermediate 1-7 (triethylamine salt, 600mg) as a white solid. m/z: $[M+H]^+$ 882.3.

[0372] Step 7: To a solution of intermediate 1-7 (2.8g, 2.85mmol) in a mixed solvent of dichloromethane (20mL) and water (0.3mL) was added a dichloromethane solution of dichloroacetic acid (DCA) (0.6M, 23.7mL), and then stirred at room temperature for 1h. To the reaction solution was added pyridine (20mL), the resulting mixture was stirred at room temperature for 10min. The solvent was concentrate under reduced pressure to afford intermediate 1-8 (pyridinium salt, curd product). m/z: $[M+H]^+$ 580.1.

[0373] Embodiment 2: Synthesis of compounds 1-p1, 1-p2, 1-p3 and 1-p4



[0374] Step 1: Intermediate 1-8 (2.85mmol) was dissolved in anhydrous acetonitrile (15 mL) and then the solvent was concentrated under reduced pressure, repeated twice and left 10mL acetonitrile at last time, 4A molecular sieve (0.8g) was added thereto. Compound 1-9 (CASNo:104992-55-4, 3.3 g, 3.42 mmol) was dissolved in anhydrous acetonitrile (15mL) and then the solvent was concentrated under reduced pressure, repeated twice and left 5mL acetonitrile at last time. To the solution of 1-8 in acetonitrile was slowly added the acetonitrile solution of compound 1-9 at 0°C, the reaction system was stirred at room temperature for 0.5h, ((Dimethylaminomethylidene)amino)-3H-1,2,4-dithiazoline-3-thione (DDTT, 697mg, 3.42 mmol) was added thereto, and stirred for additional 40min. The molecular sieve was removed by filtration, and the filtrate was concentrated under reduced pressure to afford compound 1-10 (7.5g). m/z: [M+H]⁺ 1499.3.

[0375] Step 2: Compound 1-10 (3.7g, 2.35mmol) was dissolved in dichloromethane (35mL) and water (0.7mL); dichloromethane solution of DCA (0.6 M, 31mL, 18.8mmol) was dropped

thereto at room temperature. The reaction system was stirred at room temperature for 2h. Triethylsilane (20 mL) was dropped thereto, and the reaction solution was stirred at room for additional 1h. Pyridine (10mL) was dropped thereto, the the reaction solution was concentrated under reduced pressure. The residue was purified by *prep*-HPLC to afford compound 1-11 (400 mg) as a white solid. m/z: $[M+H]^+$ 1196.2.

[0376] Step 3&4: To pyridine (5mL) was slowly added diphenyl chlorophosphate (DPCP, 1g, 3.8 mmol) dropwise at -40°C, to the above solution was slowly added anhydrous dichloromethane solution (5mL) of compound 1-11 (230mg, 0.19mmol) dropwise at -40°C, and then stirred at this temperature for 30min, and obtained the reaction solution of compound 1-12. To the solution of compound 1-12 was directly added 3*H*-1,2-benzodithiol-3-one (64mg, 0.38mmol) and stirred for 1h. Water (68mg, 0.38mmol) was added thereto and stirred for additional 1h. The reaction solution was diluted with ethyl acetate and washed with aqueous solution of sodium bicarbonate (2.7%, 30 mL), the organic layer was separated and concentrated to afford compound 1-13. Compound 1-13 was separated by *prep*-HPLC (separation method 2) to afford 3 isomers: 1-13-p1 (60mg) as a yellow solid, 1-13-p2 (30mg) as a white solid, and 1-13-p3 (40mg) as a white solid.

[0377] Step 5: To a solution of compound 1-13-p1 (80mg, 0.066mmol) in acetonitrile (2mL) was added tert-butylamine (2mL). The reaction system was stirred at room temperature for 0.5h. The solvent was concentrate under reduced pressure. The residue was dissolved in methanol (4mL), methanolic hydrochloric acid solution (2M, 4mL) was added thereto, the reaction solution was stirred at 45°C for 1h, and the solvent was concentrate under reduced pressure to afford compound 1-14-p1 (100mg) as a yellow solid. m/z: $[M+H]^+$ 1143.1.

[0378] Step 6: To a solution of compound 1-14-p1 (100mg) in methanol (6 mL) was added ammonium hydroxide solution (6mL). The reaction system was stirred at 45°C for overnight. The solvent was concentrate under reduced pressure. The residual liquid was lyophilized to afford compound 1-15-p1 (100mg, crude compound) as a yellow solid. m/z: $[M+H]^+$ 935.2.

[0379] Step 7: Compound 1-15-p1 (45mg) was subjected to azeotropic dehydration three times with anhydrous pyridine (10mL) and then dissolved in pyridine (2mL), and then triethylamine (0.66mL) and triethylamine trihydrofluoride (387mg) was added thereto under nitrogen. The resulting solution was stirred at 45°C for 3h. The solvent was concentrate under

reduced pressure. The residue was purified by *prep*-HPLC (separation method 3) to afford compound 1-p1 (0.34mg, m/z: [M+H]⁺ 706.8, HPLC-RT: 8.584min) and 1-p2 (0.30mg, m/z: [M+H]⁺ 706.8, HPLC-RT: 8.662 min), as white solids.

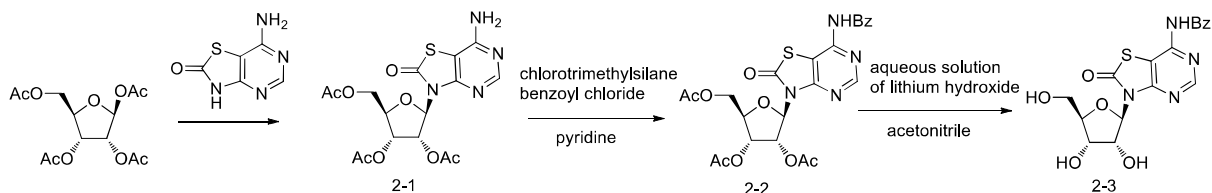
[0380] Synthesis of compound 1-p3:

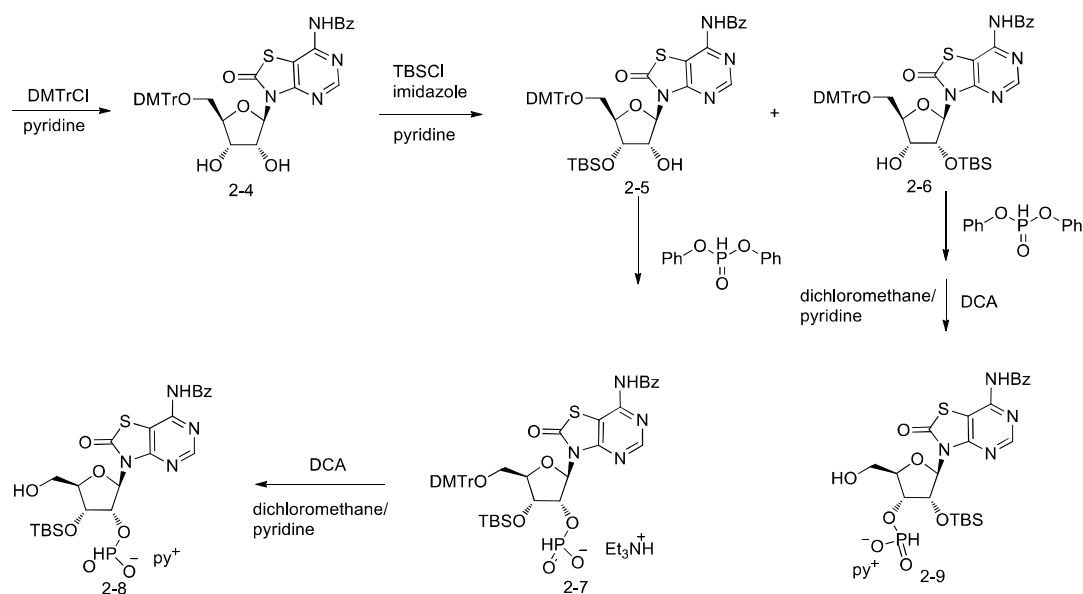
[0381] In a same way, to a solution of compound 1-13-p2 (50mg, 0.041mmol) in acetonitrile (2.0 mL) was added tert-butylamine (2mL), and stirred at room temperature for 0.5h, the solvent was concentrate under reduced pressure. The residue was dissolved in methanol (1mL), methanolic hydrochloric acid solution (2mL, 2M) was added thereto, the reaction solution was stirred at 40°C for 4h, and then the solvent was concentrate under reduced pressure. The residue was dissolved in methanol (1mL), ammonium hydroxide solution (1mL) was added thereto and stirred at 50°C for 16h, and then the reaction solution was purged with nitrogen to remove most of the ammonia. The solvent was concentrate under reduced pressure. The residual liquid was lyophilized. The crude compound was subjected to azeotropic dehydration three times with anhydrous pyridine (10mL) and then dissolved in pyridine (2mL), triethylamine (0.66mL) and triethylamine trihydrofluoride (0.36mL) was added thereto under nitrogen. The resulting solution was stirred at 50°C for 2h. The solvent was concentrate under reduced pressure. The residue was purified by *prep*-HPLC (separation method 3) to afford compound 1-p3 (0.40 mg, m/z: [M+H]⁺ 706.8, HPLC-RT: 9.789 min) as an off-white solid.

[0382] Synthesis of compound 1-p4:

[0383] Compound 1-p4 (2.4mg, m/z: [M+H]⁺ 706.8, HPLC-RT: 9.960 min) was obtained as a white solid in the same manner as compound 1-p3, by using compound 1-13-p3 (40mg, 0.033 mmol) as a starting material. ¹H NMR (400 MHz, DMSO-*d*₆+D₂O): δ 8.37(s, 1H), 8.16(s, 1H), 7.97(s, 1H), 5.93 (d, *J*=7.8 Hz, 1H), 5.74 (d, *J*=8.0, 1H), 5.35 (m, 1H), 5.14 (m, 1H), 4.70 (m, 1H), 4.59 (s, 1H), 4.18 (s, 1H), 4.15 (m, 1H), 3.85 (m, 2H), 3.53 (m, 1H), 3.06 (m, 1H); ³¹P NMR (161 MHz, DMSO-*d*₆+D₂O): δ 58.47, 46.58.

[0384] Embodiment 3: Synthesis of intermediates 2-8 and 2-9





[0385] Step 1: To a solution of 7-aminothiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (refer to *J. Med. Chem.* 1990, 33, 407-415, compound 28) (6.5g, 38.7mmol) and tetraacetylribose (11g, 46.4mmol) in acetonitrile (120mL) was added *N,O*-bis(trimethylsilyl)acetamide (BSA, 13.6g, 116mmol), and then stirred at reflux for 1h. To the above reaction solution was added trimethylsilyl trifluoromethylsulphonate (TMSOTf, 17.2g, 77.4mmol) after cooling to room temperature, and stirred at reflux for additional 48h. The reaction solution was cooled to room temperature, and saturated aqueous solution of sodium bicarbonate was slowly added thereto, the mixture was extracted with ethyl acetate (150mL×3), the combined organic layers were washed with water, the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (ethyl acetate/petroleum ether=3/4) to afford intermediate 2-1 (4.3g) as a light yellow solid. *m/z*: [M+H]⁺ 427.0.

[0386] Step 2: To a solution of intermediate 2-1 (0.5g, 1.17mmol) in pyridine (5mL) was added chlorotrimethylsilane (0.07mL, 0.58mmol) at 0°C under nitrogen, and stirred at for 5min, to the above reaction solution was added benzoyl chloride (0.32mL, 2.81mmol), the reaction system was stirred at room temperature for overnight, and then quenched by addition of water (50mL). The aqueous layer was extracted with ethyl acetate (50mL×2), the combined organic layers were washed with water, and the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column

chromatography (ethyl acetate/ petroleum ether =9/10) to afford intermediate 2-2 (0.45g) as a light yellow solid. m/z: $[M+H]^+$ 634.8.

[0387] Step 3: To a solution of intermediate 2-2 (3g, 4.7mmol) in acetonitrile (150mL) was added aqueous solution of lithium hydroxide (47mL, 1M), the reaction system was stirred at room temperature for 15min and then neutralized pH to 6 with hydrochloric acid (2M). The reaction solution was concentrated to 1/3 of the total volume, the solid was precipitated, filtered, the filter cake washed with water for 3 times, and then dried under vacuum to afford intermediate 2-3 (1g) as a yellow solid. m/z: $[M+H]^+$ 405.0.

[0388] Step 4: To a solution of intermediate 2-3 (7.6g, 18.8mmol) in pyridine (95mL) was added DMTrCl (9.5g, 28.5mmol) under nitrogen. The reaction system was stirred at room temperature for overnight. The solvent was concentrate under reduced pressure. The residue was purified by Flash column chromatography (methanol/dichloromethane=1/25) to afford intermediate 2-4 (8.3g) as an off-white solid. m/z: $[M+H]^+$ 706.8.

[0389] Step 5: To a solution of intermediate 2-4 (8.3g, 11.7mmol) and imidazole (2g, 29.3mmol) in pyridine (60mL) was added TBSCl (2.1g, 14mmol) at 0°C under nitrogen. The reaction system was stirred at room temperature for overnight, and then quenched by addition of water (1mL) and saturated aqueous solution of sodium bicarbonate (100mL), the aqueous layer was extracted with ethyl acetate (200mL×2), the combined organic layers were washed with water, the organic layer was dried over anhydrous sodium sulfate, filtered and concentrated, the residue was purified by Flash column chromatography (methanol/dichloromethane=1/50~1/20) to afford intermediate 2-5 (4g, off-white solid, LCMS-RT (Thermo): 2.813 min) and 2-6 (2g, off-white solid, LCMS-RT (Thermo): 2.763 min. Intermediate 2-5: m/z: $[M+H]^+$ 821.1; 1H NMR (400 MHz, DMSO-*d*₆): δ 11.71 (s, 1H), 8.66 (s, 1H), 8.05-8.03 (m, 2H), 7.68-7.64 (m, 1H), 7.56-7.52 (m, 2H), 7.43-7.32 (m, 2H), 7.27-7.17 (m, 7H), 6.84-6.82 (m, 4H), 6.05-6.04 (m, 1H), 5.29-5.28 (m, 1H), 4.88-4.84 (m, 1H), 4.53-4.50 (m, 1H), 4.05-3.98 (m, 1H), 3.71 (s, 6H), 3.35-3.27 (m, 1H), 3.08-3.04 (m, 1H), 0.82 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H); Intermediate 2-6: m/z: $[M+H]^+$ 821.1; 1H NMR (400 MHz, DMSO-*d*₆): δ 11.73 (s, 1H), 8.62 (s, 1H), 8.06-8.04 (m, 2H), 7.68-7.65 (m, 1H), 7.57-7.53 (m, 2H), 7.41-7.36 (m, 2H), 7.27-7.17 (m, 7H), 6.85-6.82 (m, 4H), 6.09-6.08 (m, 1H), 5.00-4.95 (m, 2H), 4.31-4.27 (m, 1H), 4.05-4.03 (m, 1H), 3.72 (s, 6H), 3.23-3.16 (m, 2H), 0.95 (s, 9H), 0.05

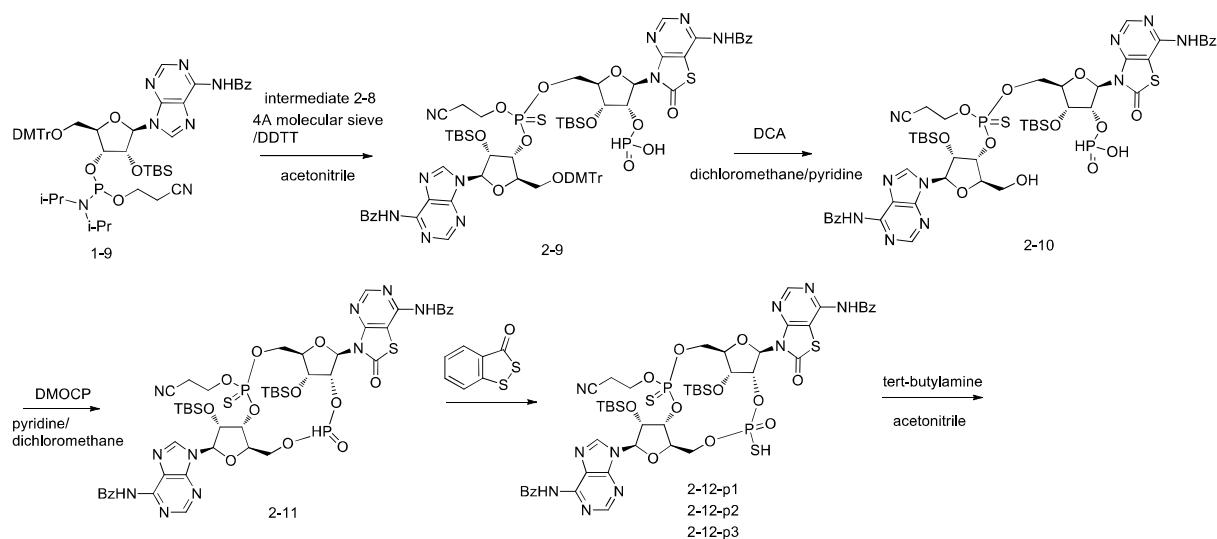
(s, 6H).

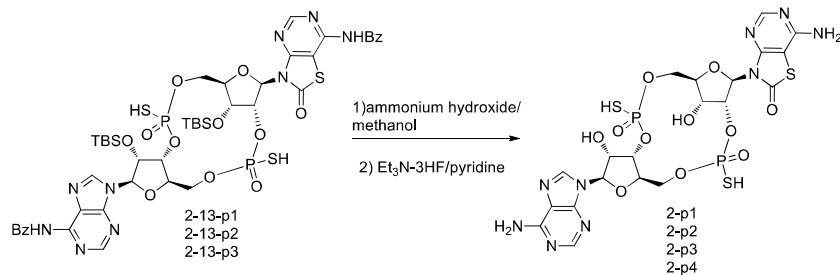
[0390] Step 6: To a solution of intermediate 2-5 (0.5g, 0.61mmol) in pyridine (5mL) was added diphenyl phosphite (0.57g, 1.43mmol) at 0°C under nitrogen, the reaction system was stirred for 1h, and then triethylamine (0.6mL) and water (0.6mL) was added thereto. The resulting mixture was stirred at room temperature for 5min and then diluted with water (50mL), the aqueous layer was extracted with dichloromethane (30mL×2), the combined organic layers were washed with water, and the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (methanol/dichloromethane=1/10) to afford intermediate 2-7 (triethylamine salt, 0.6g) as an off-white solid. m/z: $[M+H]^+$ 884.5.

[0391] Step 7: To a solution of intermediate 2-7 (0.6g, 0.61mmol) in a mixed solvent of dichloromethane (10mL) and water (1mL) was added a dichloromethane solution of DCA (0.6 M, 9.1mL). The reaction mixture was stirred for 0.5h, and then pyridine (20mL) was added thereto, the resulting mixture was concentrate under reduced pressure to afford intermediate 2-8 (pyridinium salt, curd product). m/z: $[M+H]^+$ 582.9.

[0392] Synthesis of intermediate 2-9: intermediate 2-9 (pyridinium salt) was obtained in the same manner as intermediate 2-8, by using intermediate 2-6 as a starting material. m/z: $[M+H]^+$ 583.0.

[0393] Embodiment 4: Synthesis of compounds 2-p1, 2-p2, 2-p3 and 2-p4





[0394] Step 1: Intermediate 2-8 (0.68mmol) and 1-9 (0.81g, 0.82mmol) were subjected to azeotropic dehydration twice with anhydrous acetonitrile (10mL) respectively, and then dissolved in acetonitrile (5mL) respectively for use. To the solution of intermediate 2-8 in acetonitrile, which contained 4A molecular sieve, was slowly added the acetonitrile solution of intermediate 1-9 at 0°C under nitrogen, the resulting mixture was stirred for 1h. To the above reaction system was added DDTT (0.16g, 0.79mmol) and stirred for additional 1h. The molecular sieve was removed by filtration, and the filtrate was concentrated under reduced pressure to afford compound 2-9 (1g). m/z: $[M+H]^+$ 1501.5.

[0395] Step 2: To the solution of compound 2-9 (0.33g, 0.22mmol) in a mixed solvent of dichloromethane (3mL) and water (0.3mL) was added dichloromethane solution of DCA (0.6 M, 2.93mL) under nitrogen. The reaction system was stirred for 0.5h, and then pyridine (1mL) was added thereto and concentrated under reduced pressure, and the residue was purified by Flash column chromatography (acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L) =60%) to afford compound 2-10 (0.25g, pyridinium salt) as a white solid. m/z: $[M+H]^+$ 1199.6.

[0396] Step 3&4: Compound 2-10 (250mg, 0.21mmol) was subjected to azeotropic dehydration three times with pyridine (1mL) and then dissolved in a mixed solvent of pyridine (2mL) and dichloromethane (2mL). 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (DMOPC) (775mg, 4.2mmol) was added thereto, the mixture was stirred at room temperature for 10min to obtain the reaction solution of compound 2-11. To the above reaction solution of compound 2-11 was directly added water (756mg, 42mmol) and 3H-1,2-benzodithiol-3-one (71mg, 0.42mmol), and stirred at room temperature for 20min, aqueous solution of sodium bicarbonate (2.7%, 50mL) was added thereto. The aqueous layer was extracted with ethyl acetate, and the separated organic layer was concentrated. The residue was purified by Flash

column chromatography (acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L) =10-80%) to afford compound 2-12-p1 (30mg, LCMS-RT(Thermo): 2.147min), 2-12-p2 (35 mg, LCMS-RT(Thermo): 2.247min), and 2-12-p3 (50mg, LCMS- RT(Thermo): 2.327min), as white solids.

[0397] Step 5: To a solution of compound 2-12-p1 (30mg, 0.01mmol) in acetonitrile (2mL) was added tert-butylamine (2mL), the reaction system was stirred at room temperature for 0.5h, and then directly concentrated to afford compound 2-13-p1 (30mg, crude product). m/z: $[M+H]^+$ 1159.9.

[0398] Step 6: To a solution of compound 2-13-p1 (30mg, crude) in methanol (2mL) was added ammonium hydroxide solution (2mL), the reaction system was stirred at 45°C for overnight in a sealed tube, and then the reaction solution was concentrated in reduced pressure. The residue was subjected to azeotropic dehydration three times with pyridine (2mL) and then dissolved in pyridine (2mL). To the above reaction system was added triethylamine (0.35g, 3.5mmol) and triethylamine trihydrofluoride (0.28g, 1.75mmol) under nitrogen. The resulting mixture was stirred at 45°C for 6h. The solvent was concentrate under reduced pressure. The residue was adjust pH to 8 with aqueous solution of ammonium bicarbonate (1M), and then purified by *prep*-HPLC (separation method 3) to afford compound 2-p1 (di-ammonium salt, 2mg, m/z: $[M+H]^+$ 723.8, HPLC-RT: 8.116min) and 2-p2 (di-ammonium salt, 2mg, m/z: $[M+H]^+$ 723.8, HPLC-RT: 10.121min), as white solids.

[0399] Synthesis of compound 2-p3:

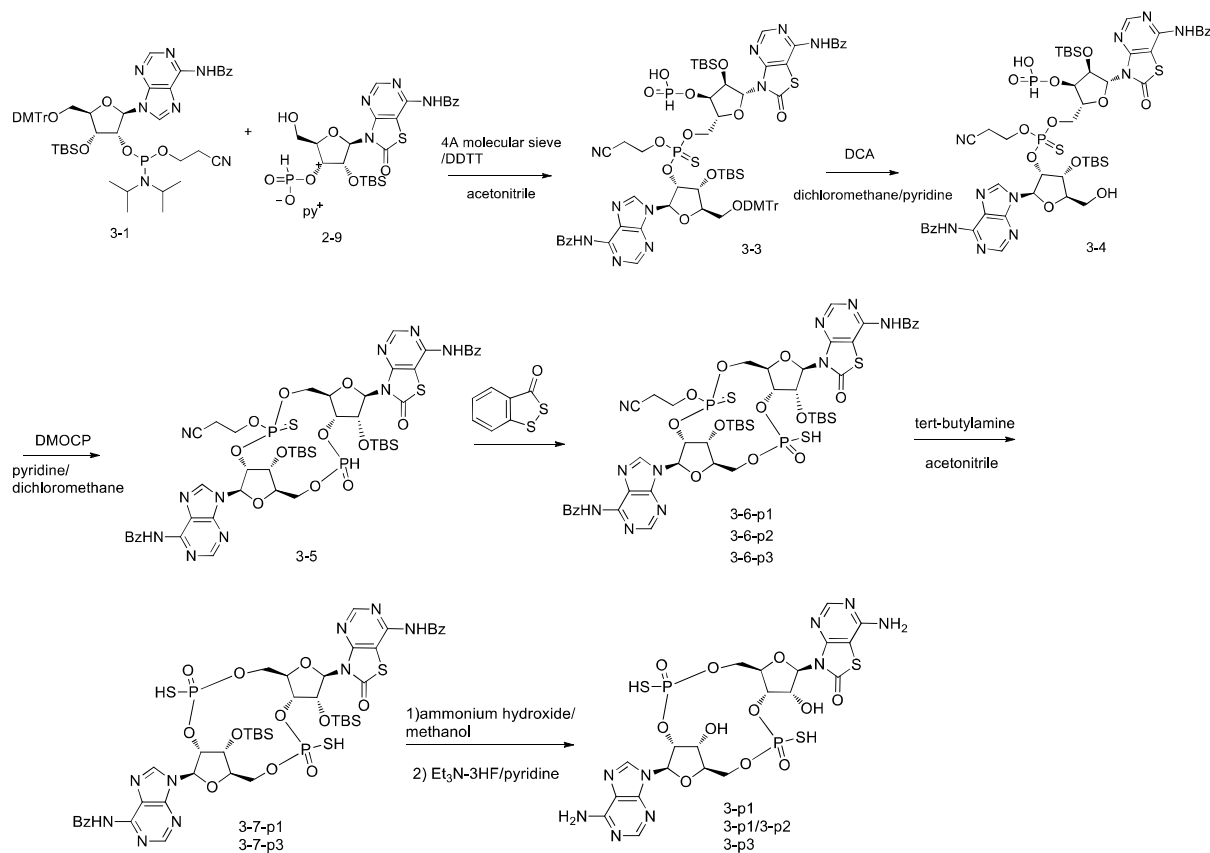
[0400] To a solution of compound 2-12-p2 (30mg, 0.01mmol) in acetonitrile (2mL) was added tert-butylamine (2mL). The mixture was stirred at room temperature for 0.5h, and then concentrated under reduced pressure to afford compound 2-13-p2. To a solution of compound 2-13-p2 in methanol (2mL) was added ammonium hydroxide solution (2mL), the reaction system was stirred in a sealed tube at 45°C for overnight, and then the reaction solution was directly concentrated under reduced pressure. The residue was subjected to azeotropic dehydration three times with pyridine (2mL) and then dissolved in pyridine (2mL). To the above reaction system was added triethylamine (0.35g, 3.5mmol) and triethylamine trihydrofluoride (0.28g, 1.75mmol) under nitrogen. The resulting mixture was stirred at 45°C for 6h. The solvent was concentrate under reduced pressure. The residue was adjust pH to 8

with aqueous solution of ammonium bicarbonate (1M), and then purified by *prep*-HPLC (separation method 3) to afford compound 2-p3 (di-ammonium salt, 0.3mg, m/z: $[M+H]^+$ 723.7, HPLC-RT: 10.121min) as a white solid.

[0401] Synthesis of compound 2-p4:

[0402] Compound 2-p4 (di-ammonium salt, 0.4 mg, m/z: $[M+H]^+$ 723.7, HPLC-RT: 9.632 min) was obtained as a white solid in the same manner as compound 2-p3, by using compound 2-12-p3 (17mg, 0.01mmol) as a starting material.

[0403] Embodiment 5: Synthesis of compounds 3-p1, 3-p1/3-p2 and 3-p3



[0404] Step 1: Compound 3-1 (CAS No.: 129451-95-8) (2.36g, 2.4mmol) and Intermediate 2-9 (1.05g, 2mmol) were subjected to azeotropic dehydration twice with anhydrous acetonitrile (10mL) respectively, and then dissolved in acetonitrile (5mL) respectively for use. To the solution of intermediate 2-9 in acetonitrile, which contained 4A molecular sieve, was slowly added the acetonitrile solution of compound 3-1 at 0°C under nitrogen, the resulting mixture was stirred for 1h, To the above reaction system was added DDTT (0.49g, 2.4mmol), and stirred for additional 1h. The molecular sieve was removed by filtration, and the filtrate was

concentrated under reduced pressure to afford compound 3-3 (1.3g). m/z: $[M+H]^+$ 1501.5.

[0405] Step 2: To the solution of compound 3-3 (1g, 0.66mmol) in a mixed solvent of dichloromethane (3mL) and water (0.3mL) was added dichloromethane solution of DCA (0.6 M, 10mL). The reaction system was stirred for 0.5h. Pyridine (3mL) was added thereto, the mixture was concentrated under reduced pressure. The residue was purified by Flash column chromatography (acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L) =60%) to afford compound 3-4 (390mg, pyridinium salt) as a white solid. m/z: $[M+H]^+$ 1199.6.

[0406] Step 3&4: Compound 3-4 (250mg, 0.21mmol) was subjected to azeotropic dehydration three times with pyridine (1mL) and then dissolved in a mixed solvent of pyridine (2mL) and dichloromethane (2mL). To the above reaction system was added DMOPC (775 mg, 4.2mmol), the mixture was stirred at room temperature for 10min to obtain the reaction solution of compound 3-5. To the reaction solution of compound 3-5 was added water (756mg, 42mmol) and 3H-1,2-benzodithiol-3-one (71mg, 0.42mmol) and stirred at room temperature for 20min, and then aqueous solution of sodium bicarbonate (2.7%, 50mL) was added thereto, the aqueous layer was extracted with ethyl acetate, and the separated organic layer was concentrated. The residue was purified by Flash column chromatography (acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L)=10-80%) to afford compound 3-6-p1 (50mg, LCMS-RT(Thermo): 2.143min), 3-6-p2 (15mg, LCMS-RT(Thermo): 2.230min), and 3-6-p3 (18mg, LCMS- RT(Thermo): 2.320), as white solids.

[0407] Step 5: To a solution of compound 3-6-p1 (20mg, 0.02mmol) in acetonitrile (2mL) was added tert-butylamine (2mL), the reaction system was stirred at room temperature for 0.5h, and then directly concentrated to afford compound 3-7-p1 (25mg, crude product). m/z: $[M+H]^+$ 1159.9.

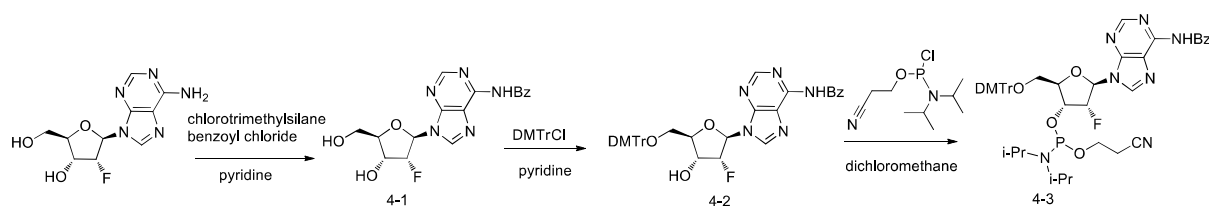
[0408] Step 6: To a solution of compound 3-7-p1 (25mg, crude) in methanol (2mL) was added ammonium hydroxide solution (2mL), the reaction system was stirred in a sealed tube at 45°C for overnight, and then the reaction solution was concentrated in reduced pressure. The residue was subjected to azeotropic dehydration three times with pyridine (2mL) and then dissolved in pyridine (2mL). To the above reaction system was added triethylamine (0.35g, 3.5mmol) and triethylamine trihydrofluoride (0.28g, 1.75mmol) under nitrogen. The resulting mixture was stirred at 50°C for 6h. The solvent was concentrate under reduced pressure. The residue was

adjust pH to 8 with aqueous solution of ammonium bicarbonate (1M), and then purified by *prep*-HPLC (separation method 3) to afford compound 3-p1 (di-ammonium salt, 0.7mg, m/z: $[M+H]^+$ 723.8, HPLC-RT: 9.726min) and a mixture of 3-p1/3-p2 (di-ammonium salt, 1.1mg, m/z: $[M+H]^+$ 723.8, HPLC- RT: 9.726 min and 11.161min).

[0409] Synthesis of compound 3-p3:

[0410] To a solution of compound 3-6-p3 (50mg, 0.04mmol) in acetonitrile (2mL) was added tert-butylamine (2mL). The mixture was stirred at room temperature for 0.5h, and then concentrated under reduced pressure to afford compound 3-7-p3. To a solution of compound 3-7-p3 in methanol (2mL) was added ammonium hydroxide solution (2mL), the reaction system was stirred in a sealed tube at 45°C for overnight, and then the reaction solution was directly concentrated under reduced pressure. The residue was subjected to azeotropic dehydration three times with pyridine (2mL) and then dissolved in pyridine (2mL). To the above reaction system was added triethylamine (0.35g, 3.5mmol) and triethylamine trihydrofluoride (0.28g, 1.75mmol) under nitrogen. The resulting mixture was stirred at 45°C for 6h. The solvent was concentrate under reduced pressure. The residue was adjust pH to 8 with aqueous solution of ammonium bicarbonate (1M), and then purified by *prep*-HPLC (separation method 3) to afford compound 3-p3 (di-ammonium salt, 2.2mg, m/z: $[M+H]^+$ 723.7, HPLC-RT: 11.76min) as a white solid.

[0411] Embodiment 6: Synthesis of intermediate 4-3



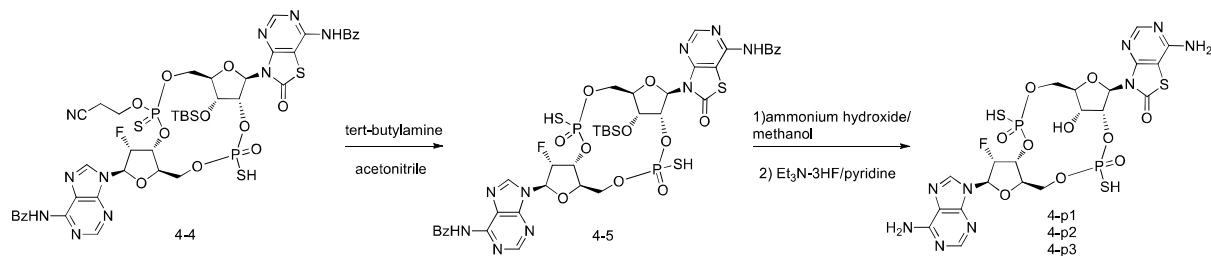
[0412] Step 1: To a solution of 2'-fluoro-2'-deoxyadenosine (CAS No.: 64183-27-3) (9.7g, 36.0mmol) in pyridine (110mL) was added chlorotrimethylsilane (23.5g, 216mmol) at 0°C under nitrogen. The mixture was stirred at room temperature for 2h, and then benzoyl chloride (7.6g, 54 mmol) was added thereto. The resulting mixture was stirred at room temperature for overnight. To the reaction solution was added water (40mL) and stirred for 1h, and then ammonium hydroxide solution (40mL) was added thereto and stirred for additional 2h.

Additional water (40mL) was added thereto, the mixture was extracted with ethyl acetate (500mL×2), and the combined organic layers were dried over anhydrous sodium sulfate, and then concentrated to 1/5 of the total volume, filtered, the filter cake was dried under vacuum to afford intermediate 4-1 (10g) as a white solid. m/z: $[M+H]^+$ 374.0.

[0413] Step 2: To a solution of intermediate 4-1 (1.2g, 3.2mmol) in pyridine (15mL) was added DMTrCl (1.6g, 4.8mmol) under nitrogen. The reaction system was stirred at room temperature for overnight. To the reaction solution was added water (50mL), the mixture was extracted with ethyl acetate (40mL×3), and the combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by Flash column chromatography (2% methanol/dichloromethane) to afford intermediate 4-2 (1.9g) as a light yellow solid. m/z: $[M+H]^+$ 676.0.

[0414] Step 3: To a solution of intermediate 4-2 (1.35g, 2mmol) in dichloromethane (10mL) was added *N,N*-diisopropylethylamine (1.1mL, 6mmol) and 2-cyanoethyl *N,N*-diisopropylchloro- phosphoramidite (947mg, 4mmol) at 0°C under nitrogen. The resulting mixture was stirred at room temperature for 2h. To the reaction solution was added water (50mL) and saturated aqueous solution of sodium bicarbonate (20mL). The resulting mixture was extracted with ethyl acetate (50mL×3), and the combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by Flash column chromatography (3% methanol/ dichloromethane) to afford intermediate 4-3 (1.2g) as a yellow solid. m/z: $[M+H]^+$ 876.1.

[0415] Embodiment 7: Synthesis of compounds 4-p1, 4-p2 and 4-p3

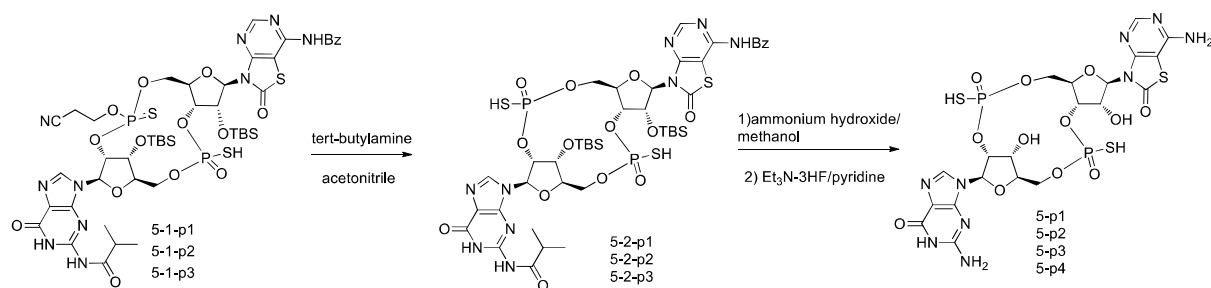


[0416] Step 1: To a solution of intermediate 4-4 (4-4 was obtained as a mixture of stereoisomers in the same manner as Embodiment 4 steps 1~4, by using intermediates 4-3 and 2-8 as starting materials) (50mg, 0.05mmol) in acetonitrile (2mL) was added *tert*-butylamine

(2mL). The resulting mixture was stirred at room temperature for 0.5h, and then concentrated under reduced pressure. The residue was purified by Flash column chromatography (acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L) =10-80%) to afford compound 4-5 (15mg) as a white solid. m/z: $[M+H]^+$ 1047.8.

[0417] Step 2: To the solution of compound 4-5 (30mg, 0.03mmol) in methanol (2mL) was added ammonium hydroxide solution (2mL), the reaction solution was stirred in a sealed tube at 45°C for overnight, and then the solvent was concentrated in reduced pressure. The residue was subjected to azeotropic dehydration three times with anhydrous pyridine (2mL) and then dissolved in pyridine (1mL), and then triethylamine (0.91g, 9mmol) and triethylamine trihydrofluoride (0.58g, 3.6mmol) was added thereto under nitrogen. The resulting mixture was stirred at 50°C for 6h. The solvent was concentrate under reduced pressure. The residue was adjusted pH to 8 with ammonium hydroxide solution, and then directly purified by *prep*-HPLC (separation method 3) to afford compound 4-p1 (di-ammonium salt, 0.76mg, m/z: $[M+H]^+$ 725.8, HPLC-RT: 9.04min), 4-p2 (di-ammonium salt, 0.82mg, m/z: $[M+H]^+$ 725.8, HPLC-RT: 10.45min), and 4-p3 (di-ammonium salt, 0.97mg, m/z: $[M+H]^+$ 725.8, HPLC-RT: 10.35min), as white solids.

[0418] Embodiment 8: Synthesis of compounds 5-p1, 5-p2, 5-p3 and 5-p4



[0419] Step 1: To a solution of compound 5-1-p1 (5-1-p1, 5-2-p2 and 5-3-p3 were obtained in the same manner as Embodiment 4 steps 1~4, by using intermediates 3-2 and 3'-TBDMS-IBU-RG phosphoramidite (CASNo: 1445905-51-0) as starting materials, LCMS- RT(Thermo) of 5-1-p1, 5-2-p2 and 5-3-p3 were 2.11min, 2.14min and 2.31min, respectively) (30mg, 0.03mmol) in acetonitrile (2mL) was added *tert*-butylamine (2mL). The resulting mixture was stirred at room temperature for 0.5h, and then concentrated to afford compound 5-2-p1 (30mg,

crude product). m/z : $[M+H]^+$ 1141.6.

[0420] Step 2: To the solution of compound 5-2-p1 (30mg, crude) in methanol (2mL) was added ammonium hydroxide solution (2mL), the reaction solution was stirred in a sealed tube at 45°C for overnight, and then the solvent was concentrated in reduced pressure. The residue was subjected to azeotropic dehydration three times with anhydrous pyridine (2mL) and then dissolved in pyridine (1mL), and then triethylamine (0.35g, 3.5mmol) and triethylamine trihydrofluoride (0.28g, 1.75mmol) was added thereto under nitrogen. The resulting mixture was stirred at 50°C for 6h. The solvent was concentrate under reduced pressure. The residue was adjusted pH to 8 with ammonium hydroxide solution, and then directly purified by *prep*-HPLC (separation method 3) to afford compound 5-p1 (di-ammonium salt, 0.89mg, m/z : $[M+H]^+$ 739.8, HPLC-RT: 6.495min) as a white solid.

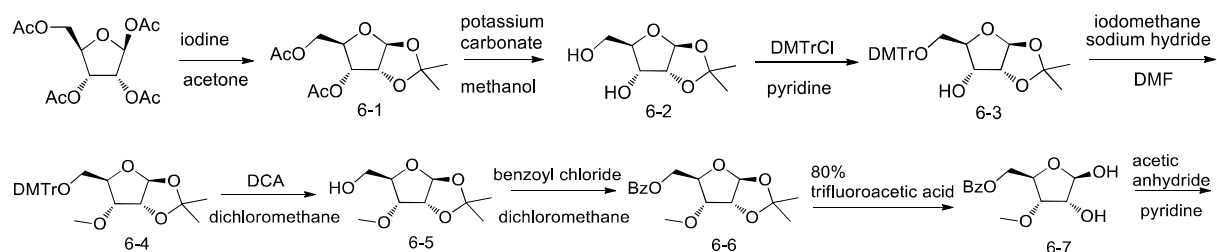
[0421] Synthesis of compounds 5-p2 and 5-p3

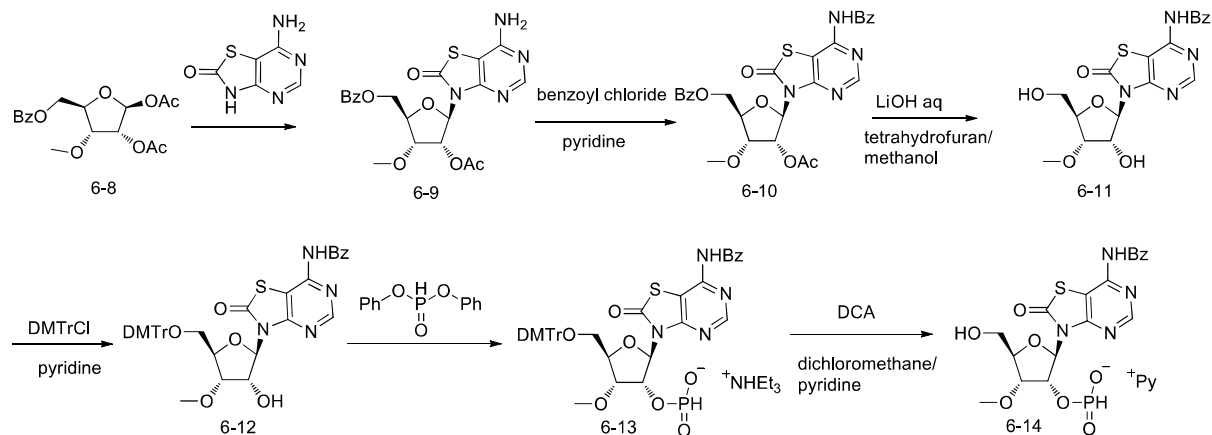
[0422] Compounds 5-p2 (di-ammonium salt, 13mg, m/z : $[M+H]^+$ 739.8, HPLC-RT: 10.666min, 1H NMR (400 MHz, DMSO- d_6 +D $_2$ O): δ 8.22 (s, 1H), 8.10 (s, 1H), 5.99 (d, $J=7.7$ Hz, 1H), 5.85 (d, $J=8.5$ Hz, 1H), 5.34-5.28 (m, 1H), 5.21-5.25 (m, 1H), 5.12-5.16 (m, 1H), 4.43-4.31 (m, 1H), 4.16-4.12 (m, 1H), 4.08-4.06 (m, 1H), 4.01-3.97 (m, 2H), 3.66 (d, $J=11.8$ Hz, 1H), 3.59-3.55 (m, 1H); ^{31}P NMR (162 MHz, DMSO- d_6): δ 59.36, 57.52) and 5-p3 (di-ammonium salt, 0.7mg, m/z : $[M+H]^+$ 739.9, HPLC-RT: 10.663min) were obtained as white solids in the same manner as compound 5-p1, by using compound 5-1-p2 (76.5mg, crdue) as a starting material.

[0423] Synthesis of compound 5-p4

[0424] Compounds 5-p4 (di-ammonium salt, 1.41mg, m/z : $[M+H]^+$ 739.9, HPLC-RT: 11.973min) was obtained as a white solid in the same manner as compound 5-p1, by using compound 5-1-p3 (100 mg, crude) as a starting material.

[0425] Embodiment 9: Synthesis of intermediate 6-14





[0426] Step 1: Tetraacetylribose (150g, 472mmol) was dissolved in acetone (1L), and iodine (11.9g, 47.2mmol) was added thereto at 0°C. After addition, the reaction system was stirred at room temperature for 12h. The saturated aqueous solution of sodium bisulfate was added into the reaction solution to remove the excess iodine, and then extracted with ethyl acetate (500mL×3), and the combined organic layers were washed with brine, the separated organic layer was dried over anhydrous sodium sulfate, the filtrate was concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-60% ethyl acetate/petroleum ether) to afford intermediate 6-1 (100g) as a yellow oil. m/z: [M+H]⁺ 275.0.

[0427] Step 2: To the suspension of intermediate 6-1 (100g, 365mmol) in methanol (100mL) was added potassium carbonate (150g, 1.09mmol). The reaction system was stirred at room temperature for 12h, and then filtered, the filtrate was concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-10% methanol/dichloromethane) to afford intermediate 6-2 (60.3g) as a white solid. m/z: [M+H]⁺ 191.0.

[0428] Step 3: To a solution of intermediate 6-2 (60g, 316mmol) in pyridine (300mL) was added DMTrCl (128g, 379mmol) in small portions at 0°C under nitrogen. The reaction system was stirred at room temperature for 12h and then quenched by addition of water (100mL). The solvent was concentrate under reduced pressure. The residue was purified by Flash column chromatography (0-50% petroleum ether/ ethyl acetate) to afford intermediate 6-3 (150.3g) as a white solid.

[0429] Step 4: To a solution of intermediate 6-3 (150g, 305mmol) in DMF (500mL) was added sodium hydride (18.3g, 458mmol, 60%) under nitrogen at 0°C. The reaction system was

stirred at 0°C for 0.5h. To the reaction system was added iodomethane (56.3g, 396mmol) and stirred at room temperature for 3h. The mixture was diluted with water (100mL) and ethyl acetate (300mL × 3). The organic layer washed with brine, and then separated and dried over anhydrous sodium sulfate, the filtrate was concentrated under reduced pressure to afford intermediate 6-4 (150 g) as a white solid. m/z: [M+H]⁺507.1.

[0430] Step 5: To a solution of intermediate 6-4 (150g, 296mmol) in dichloromethane (500mL) was added DCA (344g, 2.67mol) at 0°C. The reaction system was stirred at 0°C for 3h, and then quenched by addition of saturated aqueous solution of sodium carbonate, the aqueous layer was extracted with dichloromethane (300mL×3), the combined organic layers were washed with brine (150mL×2), the separated organic layer was dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-10% methanol/dichloromethane) to afford intermediate 6-5 (50.2g) as a white solid. m/z: [M+H]⁺ 205.0.

[0431] Step 6: To a solution of intermediate 6-5 (50g, 245mmol) in dichloromethane (500mL) was added benzoyl chloride (41.2g, 294mmol) at 0°C. The reaction system was stirred at room temperature for 12h, and then the reaction was quenched by addition of water, the aqueous layer was extracted with dichloromethane (150mL×3), the combined organic layers were washed with brine (150mL×2), the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-70% petroleum ether/ethyl acetate) to afford intermediate 6-6 (65.3g) as a white solid. m/z: [M+H]⁺ 309.0.

[0432] Step 7: Intermediate 6-6 (65g, 211mmol) was dissolved in an aqueous solution of trifluoroacetic acid (150mL, 80%), the reaction system was stirred at room temperature for 5h. Most of the solvent was removed under reduced pressure, and then the reaction solution washed with saturated aqueous solution of sodium bicarbonate, the aqueous layer was extracted with dichloromethane (100mL×3), the organic layer washed with brine (150 mL×2), the separated organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford intermediate 6-7 (50.2g) as an off-white solid.

[0433] Step 8: To a solution of intermediate 6-7 (50g, 187mmol) in pyridine (150mL) was slowly added acetic anhydride (114g, 1.11mol), the reaction system was stirred at room

temperature for 12h. Most of the solvent was removed under reduced pressure, and then the reaction solution washed with saturated aqueous solution of sodium bicarbonate, the aqueous layer was extracted with ethyl acetate (100mL×3), the combined organic layers were washed with brine (100mL×2), the separated organic layer was dried over anhydrous sodium sulfate, filtered, the filtrate was concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-70% petroleum ether/ ethyl acetate) to afford intermediate 6-8 (60.2g) as an off-white solid.

[0434] Step 9: To a solution of 7-aminothiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (6.5g, 38.7mmol) and intermediate 6-8 (16.3g, 46.4mmol) in acetonitrile (120mL) was added BSA (13.6g, 116mmol), the reaction system was stirred at reflux for 1h. To the reaction solution was added TMSOTf (17.2g, 77.4mmol) after the reaction solution was cooled to room temperature and stirred at reflux for additional 72h, and then to the reaction solution was slowly added saturated aqueous solution of sodium bicarbonate after the reaction solution was cooled to room temperature, and then the aqueous layer was extracted with ethyl acetate (150mL×3), the combined organic layers were washed with water, the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (ethyl acetate/ petroleum ether=3/4) to afford intermediate 6-9 (5g) as a light yellow solid. m/z: [M+H]⁺ 461.0.

[0435] Step 10: To a solution of intermediate 6-9 (5g, 10.9mmol) in pyridine (5mL) was added benzoyl chloride (5.3g, 38mmol) at 0°C. The reaction system was stirred at room temperature for overnight, and then quenched by addition of water (50mL), the aqueous layer was extracted with dichloromethane (150mL×3), the combined organic layers were washed with water, the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (petroleum ether/ethyl acetate=1/1) to afford intermediate 6-10 (6.8g) as a white solid. m/z: [M+H]⁺ 669.1.

[0436] Step 11: To a solution of intermediate 6-10 (6.8g, 10.1mmol) in a mixed solvent of tetrahydrofuran and methanol (60/20mL) was added aqueous solution of lithium hydroxide (36 mL, 1M) at 0°C, the reaction system was stirred at 0°C for 2h and then neutralized pH to 6 with acetic acid. The reaction solution was concentrated to 1/3 of the total volume, the solid was precipitated, filtered, the filter cake washed with water for 3 times, and then dried under

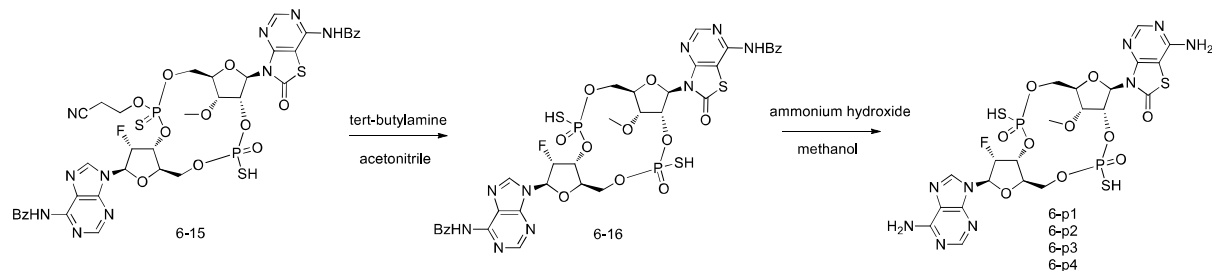
vacuum to afford intermediate 6-11 (3.8g) as a yellow solid. m/z : $[M+H]^+$ 419.0.

[0437] Step 12: To a solution of intermediate 6-11 (3.8g, 9.09mmol) in pyridine (60mL) was added DMTrCl (3.6g, 10.9mmol) under nitrogen. The reaction system was stirred at room temperature for overnight. The solvent was concentrate under reduced pressure. The residue was purified by Flash column chromatography (petroleum ether/ethyl acetate=1/1) to afford intermediate 6-12 (6g) as a light yellow solid.

[0438] Step 13: To a solution of intermediate 6-12 (5g, 6.94mmol) in pyridine (5mL) was added diphenyl phosphite (4.9g, 20.8mmol), the reaction solution was stirred at room temperature for 1h. Triethylamine (2mL) and water (1mL) was successively added thereto. The reaction solution was stirred at room temperature for 5min, and then diluted with water (50mL) and extracted with dichloromethane (60mL×3), the combined organic layers were washed with aqueous solution of sodium bicarbonate (5%), the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (methanol/dichloromethane=1/10) to afford intermediate 6-13 (5.1g, triethylamine salt) as an off-white solid. m/z : $[M+H]^+$ 886.3.

[0439] Step 14: To a solution of intermediate 6-13 (3g, 3.39mmol) in dichloromethane (20mL) was added a dichloromethane solution of DCA (0.6M, 50mL) at 0°C. The reaction mixture was stirred at room temperature for 1h and then triethylsilane (1mL) and pyridine (3mL) were added thereto, the resulting mixture was stirred at room temperature for additional 10min, the solvent was concentrated under reduced pressure to afford intermediate 6-14 (2.5g, pyridinium salt).

[0440] Embodiment 10: Synthesis of compounds 6-p1, 6-p2, 6-p3 and 6-p4

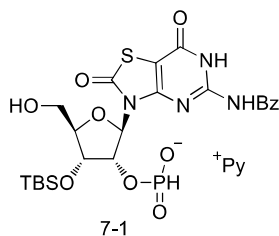


[0441] Step 1: To a solution of compound 6-15 (6-15 was obtained as a mixture of stereoisomers in the same manner as Embodiment 4 steps 1~4, by using intermediates 6-14 and 4-3 as starting materials) (50mg, 0.05mmol) in acetonitrile (2mL) was added tert-butylamine

(2mL). The resulting mixture was stirred at room temperature for 0.5h, and then concentrated under reduced pressure to afford compound 6-16 (40mg). m/z : $[M+H]^+$ 948.0.

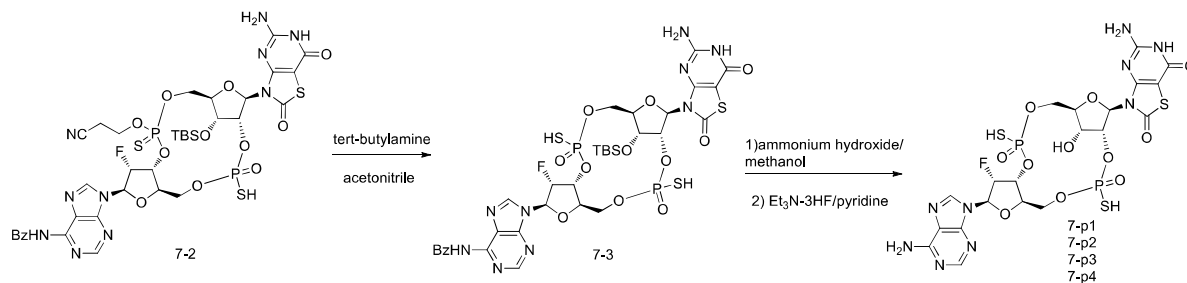
[0442] Step 2: To a solution of compound 6-16 (40mg, crude) in methanol (1mL) was added ammonium hydroxide solution (1mL), the mixture was stirred in a sealed tube at 50°C for 5h, and then cooled to room temperature and quenched by addition of acetic acid, the mixture was lyophilized. The residue was purified by *prep*-HPLC (separation method 4) to afford compound 6-p1 (di-ammonium salt, 3.6mg, m/z : $[M+H]^+$ 740.0, HPLC-RT: 10.814min), 6-p2 (di-ammonium salt, 3.2mg, m/z : $[M+H]^+$ 740.0, HPLC-RT: 11.380min), 6-p3 (di-ammonium salt, 3.9mg, m/z : $[M+H]^+$ 740.0, HPLC-RT: 10.370min; 1H NMR (400 MHz, D_2O): δ 8.36 (s, 2H), 8.12(s, 1H), 6.61 (s, 1H), 6.11 (s, 1H), 5.79 (s, 1H), 5.39 (d, $J=51.6$ Hz, 1H), 4.93-5.02 (m, 1H), 4.36-4.48 (m, 3H), 3.84-4.03(m, 4H), 3.51 (s, 3 H); ^{31}P NMR (162 MHz, D_2O): δ 56.49, 51.13; ^{19}F NMR (162 MHz, D_2O): δ -202.92.) and 6-p4 (di-ammonium salt, 2.3mg, m/z : $[M+H]^+$ 740.0, HPLC-RT: 11.650min), as white solids.

[0443] **Embodiment 11:** Synthesis of intermediate 7-1



[0444] Intermediate 7-1 was obtained as a yellow solid in the same manner as Embodiment 3 intermediate 2-8, by using tetraacetylribose and 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (refer to *J. Med. Chem.* 1990, 33, 407-415, compound 4) as starting materials. m/z : $[M+H]^+$ 599.1.

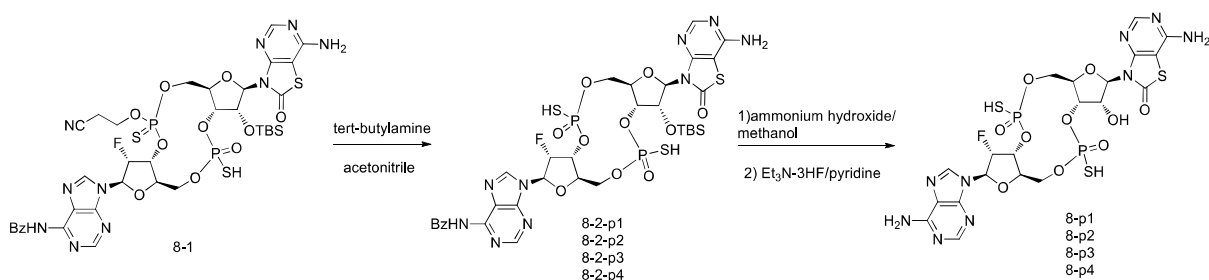
[0445] **Embodiment 12:** Synthesis of compounds 7-p1, 7-p2, 7-p3 and 7-p4



[0446] Step 1: To a solution of compound 7-2 (7-2 was obtained as a mixture of stereoisomers in the same manner as Embodiment 4 steps 1~4, by using intermediates 7-1 and 4-3 as starting materials) (330mg, 0.3mmol) in acetonitrile (2mL) was added tert-butylamine (2mL). The resulting mixture was stirred at room temperature for 1h, and then concentrated under reduced pressure. The residue was purified by Flash column chromatography (acetonitrile/aqueous solution of ammonium bicarbonate (10mmol/L)=0~60%) to afford compound 7-3 (100mg) as a white solid. m/z: $[M+H]^+$ 1063.8.

[0447] Step 2: To the solution of compound 7-3 (100 mg, 94 μ mol) in methanol (2mL) was added ammonium hydroxide solution (2mL), the reaction solution was stirred in a sealed tube at 55°C for 5h, and then the solvent was concentrated in reduced pressure. The residue was subjected to azeotropic dehydration three times with anhydrous pyridine (2mL) and then dissolved in pyridine (1mL) and then triethylamine (1mL) and triethylamine trihydrofluoride (0.5mL) was added thereto under nitrogen. The resulting mixture was stirred at 50°C for 3h. The solvent was concentrate under reduced pressure. The residue was neutralized with ammonium hydroxide solution, and then directly purified by *prep*-HPLC (separation method 5) to afford compound 7-p1 (di-ammonium salt, 1.29mg, m/z: $[M+H]^+$ 741.3, HPLC-RT: 9.058min), 7-p2 (di-ammonium salt, 4.41mg, m/z: $[M+H]^+$ 741.3, HPLC-RT: 9.590min), 7-p3 (di-ammonium salt, 2.3mg, m/z: $[M+H]^+$ 741.3, HPLC-RT: 10.438min) and 7-p4 (di-ammonium salt, 24mg, m/z: $[M+H]^+$ 741.7, HPLC-RT: 10.929min), as white solids.

[0448] Embodiment 13: Synthesis of compounds 8-p1, 8-p2, 8-p3 and 8-p4



[0449] Step 1: To a solution of compound 8-1 (8-1 was obtained as a mixture of stereoisomers in the same manner as Embodiment 4 steps 1~4, by using intermediates 2-9 and 4-3 as starting materials) (618mg, 0.61mmol) in acetonitrile (3mL) was added tert-butylamine (3mL). The

resulting mixture was stirred at room temperature for 1h, and then concentrated under reduced pressure. The residue was purified by Flash column chromatography (acetonitrile/aqueous solution of ammonium bicarbonate (10mmol/L) =10~80%) to afford compounds 8-2-p1 (67 mg), 8-2-p2 (40mg), 8-2-p3 (40mg), and 8-2-p4 (87mg), as white solids. m/z: $[M+H]^+$ 1047.7.

[0450] Step 2: To a solution of compound 8-2-p1 (50mg, 52 μ mol) in methanol (1mL) was added ammonium hydroxide solution (1mL), the reaction solution was stirred in a sealed tube at 50°C for 4h, and then the solvent was concentrated in reduced pressure. The residue was subjected to azeotropic dehydration three times with anhydrous pyridine (1mL) and then dissolved in pyridine (1mL) and then triethylamine (1mL) and triethylamine trihydrofluoride (0.5mL) was added thereto under nitrogen. The resulting mixture was stirred at 55°C for 4h. The solvent was concentrate under reduced pressure. The residue was neutralized with ammonium hydroxide solution, and then directly purified by *prep*-HPLC (separation method 5) to afford compound 8-p1 (di-ammonium salt, 7mg, m/z: $[M+H]^+$ 725.5, HPLC-RT: 11.702min) as a white solid.

[0451] Synthesis of compound 8-p2:

[0452] Compound 8-p2 (di-ammonium salt, 1.98mg, m/z: $[M+H]^+$ 725.6, HPLC-RT: 10.602 min) was obtained as a white solid in the same manner as compound 8-p1, by using compound 8-2-p2 (40mg, 0.04mmol) as a starting material.

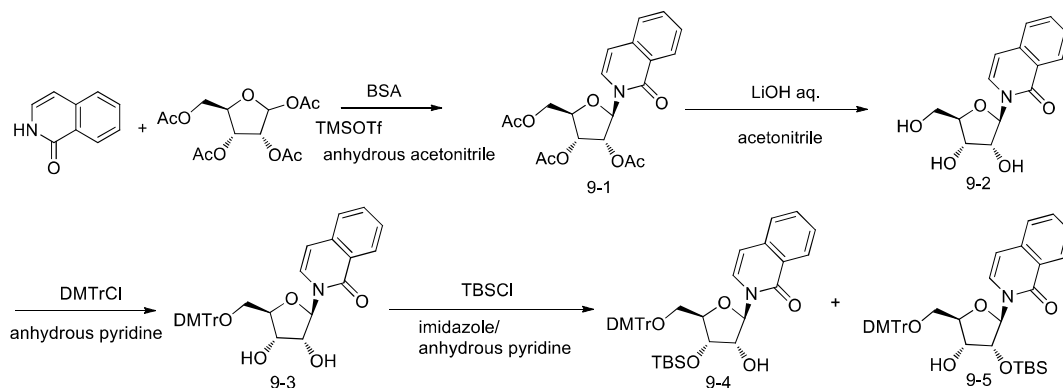
[0453] Synthesis of compound 8-p3:

[0454] Compound 8-p3 (di-ammonium salt, 1.1mg, m/z: $[M+H]^+$ 726.1, HPLC-RT: 10.556min) was obtained as a white solid in the same manner as compound 8-p1, by using compound 8-2-p3 (40mg, 0.04mmol) as a starting material.

[0455] Synthesis of compound 8-p4:

[0456] Compound 8-p4 (di-ammonium salt, 7.5mg, m/z: $[M+H]^+$ 725.6, HPLC-RT: 12.102min) was obtained as a white solid in the same manner as compound 8-p1, by using compound 8-2-p4 (80mg, 0.08mmol) as a starting material.

[0457] **Embodiment 14:** Synthesis of intermediates 9-4 and 9-5



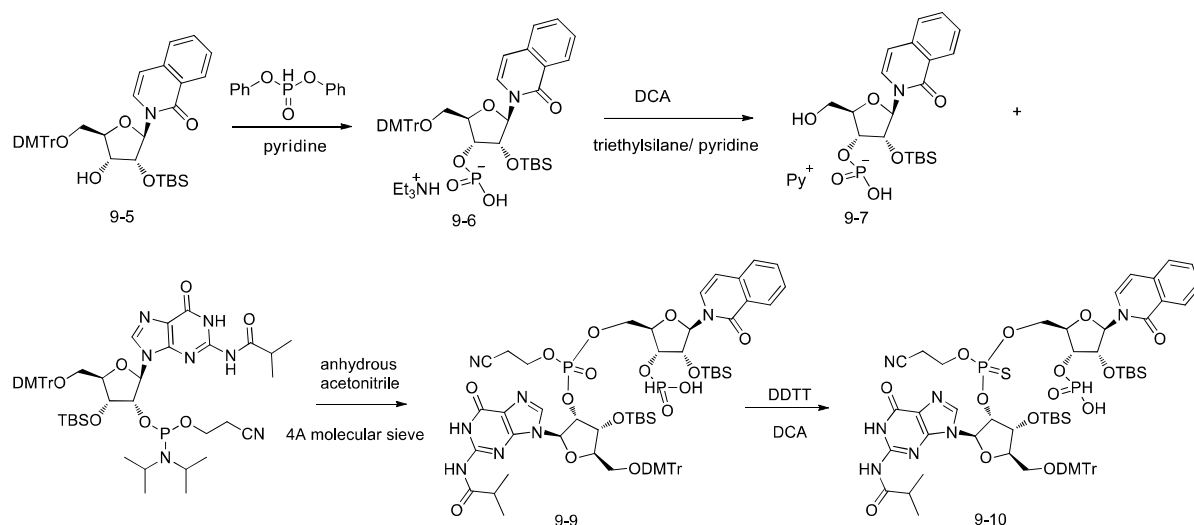
[0458] Step 1: The solution of isocarbofuran (25g, 172mmol), tetraacetylribose (137g, 431mmol) and BSA (105g, 517mmol) in anhydrous acetonitrile was stirred at reflux for 1h, and then TMSOTf (62mL, 344mmol) was added thereto after the reaction solution was cooled to room temperature, the mixture was stirred at reflux for additional 5h, saturated aqueous solution of sodium bicarbonate was slowly added thereto to adjusted pH to about 7 after the reaction solution was cooled to room temperature, the aqueous layer was extracted with ethyl acetate (150mL×3), the combined organic layers were washed with water, the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (petroleum ether/ethyl acetate=2/1) to afford intermediate 9-1 (41.1g) as an orange oil. m/z : $[M+H]^+$ 404.0.

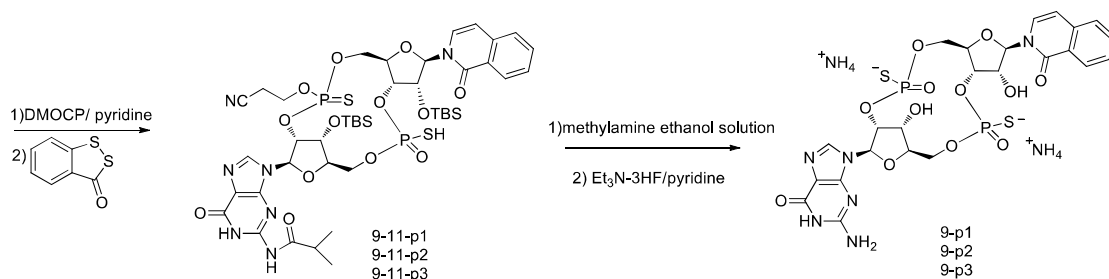
[0459] Step 2: To a solution of intermediate 9-1 (41.1g, 102mmol) in acetonitrile (300mL) was added aqueous solution of lithium hydroxide (510mL, 1M), and then the reaction system was stirred at room temperature for 1.5h. An amount of white solid was precipitated, filtered, the filter cake washed with water for 3 times, and then dried under vacuum to afford intermediate 9-2 (18.5g) as a white solid. m/z : $[M+H]^+$ 278.0.

[0460] Step 3: Intermediate 9-2 (17.5g, 63.1mmol) was subjected to azeotropic dehydration three times with anhydrous pyridine and then dissolved in pyridine (100mL) under nitrogen. To the above reaction solution was added pyridine solution of DMTrCl (22.5g, 66.3mmol, 50mL) at 0°C. The resulting mixture was stirred at this temperature for 3h. The reaction was quenched by addition of water (50mL), the aqueous layer was extracted with ethyl acetate (100mL×2), and the combined organic layers were concentrated under reduced pressure. The residue was purified by Flash column chromatography (petroleum ether/ethyl acetate=1/1) to afford intermediate 9-3 (32.6 g) as an off-white solid. m/z : $[M+Na]^+$ 602.0.

[0461] Step 4: Intermediate 9-3 (12g, 20.7mmol) and imidazole (4.9g, 72.5mmol) were subjected to azeotropic dehydration three times with anhydrous pyridine and then dissolved in pyridine (50mL) under nitrogen, TBSCl (3.4g, 22.8mmol) was added thereto at 0°C. The reaction system was stirred at room temperature for overnight, and water (50mL) was added thereto, the aqueous layer was extracted with ethyl acetate (100mL×2), the combined organic layers were washed with water, dried over anhydrous sodium sulfate, filtered and concentrated, the residue was purified by Flash column chromatography (petroleum ether/ethyl acetate=10/1~6/1) to afford intermediates 9-4 (4.3g, off-white solid, less polar) 9-5 (3.5g, off-white solid, more polar). 9-4: m/z: [M+Na]⁺716.0; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.28 (d, *J*=8.4 Hz, 1H), 7.71 (t, *J*=8.0 Hz, 2H), 7.65 (d, *J*=8.0 Hz, 1H), 7.53-7.45 (m, 3H), 7.36-7.32 (m, 6H), 7.26 (t, *J*=8.0 Hz, 1H), 6.93 (d, *J*=8.4 Hz, 4H), 6.46 (d, *J*=8.0 Hz, 1H), 6.29 (d, *J*=4.0 Hz, 1H), 5.14 (d, *J*=6.0 Hz, 1H), 4.28-4.26 (m, 1H), 4.20-4.16 (m, 1H), 4.14-4.20 (m, 1H), 4.05-4.00 (m, 1H), 3.74 (s, 6H), 3.37-3.35 (m, 1H), 0.82 (m, 9H), 0.03 (s, 3H), 0.01 (s, 3H); 9-5: m/z: [M+Na]⁺716.0; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.27 (d, *J*=8.0 Hz, 1H), 7.75-7.70 (m, 2H), 7.66-7.64 (m, 1H), 7.52 (t, *J*=8.0 Hz, 1H), 7.45-7.43 (m, 2H), 7.35-7.25 (m, 7H), 6.92 (d, *J*=8.4 Hz, 4H), 6.50 (d, *J*=7.6 Hz, 1H), 6.27 (d, *J*=3.6 Hz, 1H), 5.39 (d, *J*=6.0 Hz, 1H), 4.28-4.26 (m, 1H), 4.05-4.00 (m, 2H), 3.74 (s, 6H), 3.46-3.44 (m, 1H), 3.22-3.18 (m, 1H), 0.78 (m, 9H), 0.03 (s, 3H), 0.01 (s, 3H).

[0462] Embodiment 15: Synthesis of compounds 9-p1, 9-p2 and 9-p3





[0463] Step 1: To a solution of intermediate 9-5 (3.1g, 4.47mmol) in pyridine (20mL) was added diphenyl phosphite (3.1g, 13.4mmol) at 0°C under nitrogen, the reaction system was stirred for 0.5h, and then triethylamine (3mL) and water (3mL) was added thereto. The resulting mixture was stirred at room temperature for 5min and then diluted with water (50mL), extracted with propan-2-ol/ chloroform (30mL×2), the combined organic layers were washed with water, the separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (dichloromethane/methanol/ triethylamine=100/5/1) to afford compound 9-6 (4.5g, triethylamine salt) as an off-white solid. m/z: [M-H]⁻756.0.

[0464] Step 2: To a solution of compound 9-6 (3g, 3.49mmol) in a mixed solvent of dichloromethane (28mL) and water (1mL) was added dichloromethane solution of DCA (0.6 M, 46.5mL). The mixture was stirred at room temperature for 30min, to the the reaction solution was added triethylsilane (28 mL) and stirred for additional 1h. When the color of the reaction solution changes from brown to colorless, pyridine (28mL) was added thereto, the solvent was concentrated under reduced pressure to afford compound 9-7 (3g, pyridinium salt, curd product). m/z: [M+H]⁺ 445.0.

[0465] Step 3&4: Compound 9-7 (3g, crude) was dissolved in anhydrous acetonitrile (15mL) and then concentrated under reduced pressure, repeated three times. The residue was dissolved in acetonitrile (50mL), and 4A molecular sieve (1g) was added thereto. 3'-TBDMS-ibu-rG Phosphoramidite (CAS No.: 1445905-51-0, 3.4g, 3.49mmol) was dissolved in anhydrous acetonitrile (15 mL) and concentrated under reduced pressure, repeated three times. The residue was dissolved in acetonitrile (20mL), and 4A molecular sieve (2g) was added thereto. To a solution of 9-7 in acetonitrile was slowly added the acetonitrile solution of 3'-TBDMS-ibu-rG Phosphoramidite at 0°C, the resulting mixture was stirred at room temperature for 0.5h,

and then DDTT (697 mg, 3.42 mmol) was added thereto and stirred at room temperature for additional 3h. The molecular sieve was removed by filtration. To the mixture was added water (1mL) and then slowly added DCA (3.6g, 27.9mmol) dropwise, the resulting mixture was stirred at room temperature for 2h, triethylsilane (28mL) was added thereto and stirred for additional 1h, pyridine (28mL) was added thereto, and the reaction solution was concentrated under reduced pressure. The residue was purified by Flash column chromatography (acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L)=50%) to afford compound 9-10 (680mg) as a white solid. m/z : $[M+H]^+$ 1053.9.

[0466] Step 5: To a solution of compound 9-10 (200mg, 0.19mmol) in anhydrous pyridine (8mL) was added DMOPC (0.7g, 3.8mmol) for one charge, the mixture was stirred at room temperature for 0.5h, to the above reaction solution was added 3*H*-1,2-benzodithiol-3-one (38mg, 0.23mmol), and then stirred at room temperature for 0.5h, the reaction was quenched by addition of aqueous solution of sodium bicarbonate (2.7%). The mixture was extracted with ethyl acetate (50mL×2), and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by *prep*-HPLC (separation method 5) to afford compound 9-11-p1 (13.5mg, LCMS-RT (Thermo): 2.023min), 9-11-p2 (30.2mg, LCMS-RT (Thermo): 2.157min), and 9-11-p3 (38.0mg, LCMS-RT (Thermo): 2.300min), as white solids. m/z : $[M+H]^+$ 1067.9.

[0467] Step 6: Compound 9-11-p1 (10mg, 0.094mmol) was dissolved in methylamine ethanol solution (1mL, 30%) and stirred at room temperature for 1h, and then concentrated under reduced pressure, the residue was dissolved in pyridine (0.5mL), triethylamine (0.5mL) and triethylamine trihydrofluoride (0.25mL) was added thereto, the reaction system was stirred at 50°C for 5h and then concentrated under reduced pressure. The residue was diluted with methanol, and then ammonium hydroxide solution was slowly dropped to adjusted pH to 8~9. The resulting mixture was purified by *prep*-HPLC (separation method 5) to afford compound 9-p1 (4.07mg, HPLC-RT: 11.311min; m/z : $[M+H]^+$ 716.8; 1H NMR (400 MHz, DMSO- d_6): δ 8.26 (d, $J=6.0$ Hz, 1H), 8.19 (s, 1H), 7.73 (t, $J=7.6$ Hz, 1H), 7.68 (d, $J=8.0$ Hz, 1H), 7.53 (t, $J=8.0$ Hz, 1H), 7.42 (d, $J=7.6$ Hz, 1H), 6.69 (d, $J=7.6$ Hz, 1H), 6.51-6.47 (m, 2H), 6.22 (d, $J=4.0$ Hz, 1H), 5.90-5.82 (m, 2H), 5.52-5.51 (m, 1H), 5.08-5.06 (m, 1H), 4.95-4.90 (m, 1H), 4.88-4.86 (m, 1H), 4.65-4.61 (m, 1H), 4.57-4.55 (m, 1H), 4.40-4.36 (m, 2H), 4.25-4.19 (m,

2H), 4.10-3.99 (m, 2H), 3.94-3.87 (m, 2H); ^{31}P NMR (162 MHz, $\text{DMSO}-d_6$): δ 56.49, 54.10.)
a as white solid.

[0468] Synthesis of compounds 9-p2:

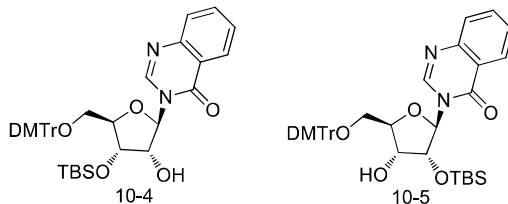
[0469] Compound 9-p2 (4.45mg, HPLC-RT: 11.389min; m/z : $[\text{M}+\text{H}]^+$ 706.8; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.26 (d, $J=8.0$ Hz, 1H), 8.02 (s, 1H), 7.74 (t, $J=7.2$ Hz, 1H), 7.67 (d, $J=7.6$ Hz, 1H), 7.53 (t, $J=7.6$ Hz, 1H), 7.37 (d, $J=7.6$ Hz, 1H), 6.91-6.34 (m, 4H), 5.87-5.85 (m, 1H), 5.37-5.19 (m, 2H), 4.38-4.37 (m, 1H), 4.21-4.19 (m, 1H), 4.11-4.05 (m, 2H), 3.74-3.70 (m, 1H), 3.01-2.99 (m, 8H); ^{31}P NMR (162 MHz, $\text{DMSO}-d_6$): δ 59.18, 56.65, 54.32, 47.74.) was obtained as a white solid in the same manner as compound 9-p1, by using compound 9-11-p2 as a starting material and purified by *prep*-HPLC (separation method 5).

[0470] Synthesis of compounds 9-p3:

[0471] Compound 9-p3 (7.0 mg, HPLC-RT: 10.912min; m/z : $[\text{M}+\text{H}]^+$ 716.8; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.26 (d, $J=8.0$ Hz, 1H), 7.97 (s, 1H), 7.73 (t, $J=7.6$ Hz, 1H), 7.65 (d, $J=7.6$ Hz, 1H), 7.53 (t, $J=7.6$ Hz, 1H), 7.44 (d, $J=7.6$ Hz, 1H), 6.62 (d, $J=8.0$ Hz, 1H), 6.56-6.52 (m, 1H), 6.33 (d, $J=6.8$ Hz, 1H), 5.86 (d, $J=8.4$ Hz, 1H), 5.27-5.23 (m, 1H), 5.06-5.04 (m, 2H), 4.46-4.40 (m, 2H), 4.22-4.18 (m, 1H), 4.12-4.08 (m, 2H), 3.96-3.92 (m, 2H), 3.78-3.75 (m, 1H), 3.01-2.95 (m, 4H); ^{31}P NMR (162 MHz, $\text{DMSO}-d_6$): δ 57.77, 50.27.) was obtained as a white solid in the same manner as compound 9-p1, by using compound 9-11-p3 as a starting material, and purified by *prep*-HPLC (separation method 5).

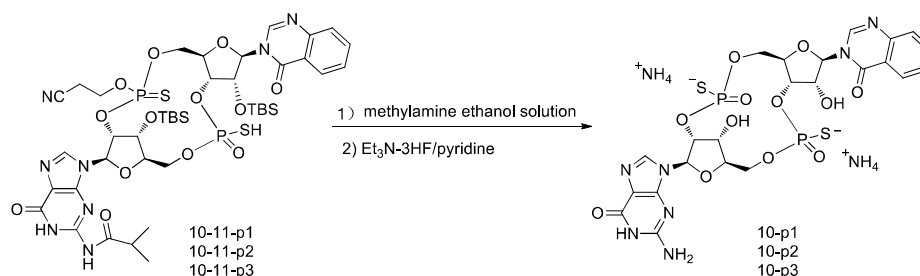
[0472] **Embodiment 16:** Synthesis of compounds 10-p1, 10-p2 and 10-p3

[0473] Synthesis of intermediates 10-4 and 10-5:



[0474] Intermediates 10-4 (2.0g, more polar) and 10-5 (3.4g, less polar) were obtained as white solids in the same manner as compounds 9-4 and 9-5, by using quinazolin-4(3H)-one as a starting material. m/z : $[\text{M}+\text{H}]^+$ 695.0; 10-4: ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.50 (s, 1H), 8.16 (d, $J=8.0$ Hz, 2H), 7.86-7.82 (m, 1H), 7.67 (d, $J=8.0$ Hz, 2H), 7.58-7.54 (m, 1H), 7.40 (d, $J=8.0$ Hz, 2H), 7.31-7.22 (m, 7H), 6.89-6.87 (m, 4H), 6.09 (d, $J=4.0$ Hz, 1H), 5.10 (d, $J=8.0$

Hz, 1H), 4.38-4.36 (m, 1H), 4.13-4.09 (m, 2H), 3.72 (s, 6H), 0.81 (s, 9H), 0.01-0.00 (s, 6H); 10-5: ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.53 (s, 1H), 8.15 (d, *J*=8.0 Hz, 1H), 7.86-7.82 (m, 1H), 7.67 (d, *J*=8.0 Hz, 1H), 7.58-7.54 (m, 1H), 7.41-7.39 (m, 2H), 7.30-7.19 (m, 7H), 6.85 (d, *J*=8.0 Hz, 4H), 6.06-6.05 (m, 1H), 5.36 (d, *J*=8.0 Hz, 1H), 4.31-4.26 (m, 2H), 4.02-4.00 (m, 1H), 3.71 (s, 6H), 0.79 (s, 9H), 0.02-0.00 (s, 6H).



[0475] Synthesis of compounds 10-11-p1, 10-11-p2 and 10-11-p3: compounds 10-11-p1 (7.0mg, LCMS-RT (Thermo): 2.020min), 10-11-p2 (26mg, LCMS-RT (Thermo): 2.170min) and 10-11-p3 (19mg, LCMS-RT (Thermo): 2.350min) were obtained as white solids in the same manner as Embodiment 15 steps 1~5, by using compound 10-5 as a starting material, and purified by *prep*-HPLC (separation method 6). *m/z*: [M+H]⁺ 1068.9.

[0476] Synthesis of compound 10-p1:

[0477] To compound 10-11-p1 (7.0mg, 0.006mmol) was added methylamine ethanol solution (1 mL, 30%), the reaction system was stirred at room temperature for 1h and then concentrated under reduced pressure, the residue was dissolved in anhydrous pyridine (0.5mL), triethylamine (0.5mL) and triethylamine trihydrofluoride (0.25mL) was added thereto under nitrogen, the reaction solution was stirred at 50°C for 1h. The solvent was concentrated under reduced pressure, the residue was diluted with methanol, and then ammonium hydroxide solution was slowly dropped to adjusted pH to 8~9. The mixture was purified by *prep*-HPLC (separation method 4) to afford compound 10-p1 (0.73mg, HPLC-RT: 10.671min, *m/z*: [M+H]⁺717.8) as a white solid.

[0478] Synthesis of compound 10-p2:

[0479] Compound 10-p2 (7.10mg, HPLC-RT: 10.826min; *m/z*: [M+H]⁺717.8; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.41 (s, 1H), 8.25-8.20 (m, 1H), 8.03 (s, 1H), 7.89-7.85 (m, 1H), 7.72-6.69 (m, 1H), 7.61-7.57 (m, 1H), 7.61-7.57 (m, 1H), 6.52-6.48 (m, 2H), 6.07-6.00 (m, 1H), 5.91-

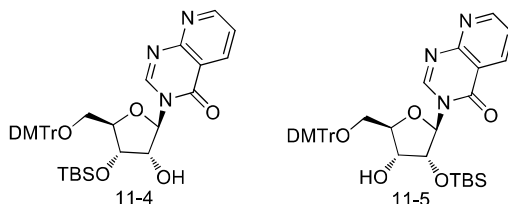
5.79 (m, 1H), 0.56 (s, 1H), 5.34-5.30 (m, 1H), 5.20-5.19 (m, 1H), 5.09-5.01 (m, 1H), 4.70-4.64 (m, 1H), 4.36-4.33 (m, 1H), 4.27-4.18 (m, 1H), 4.14-4.05 (m, 2H), 3.95-3.70 (m, 3H), 1.24 (s, 2H).) was obtained as a white solid in the same manner as compound 10-p1, by using compound 10-11-p2 as a starting material, and purified by *prep*-HPLC (separation method 4).

[0480] Synthesis of compound 10-p3:

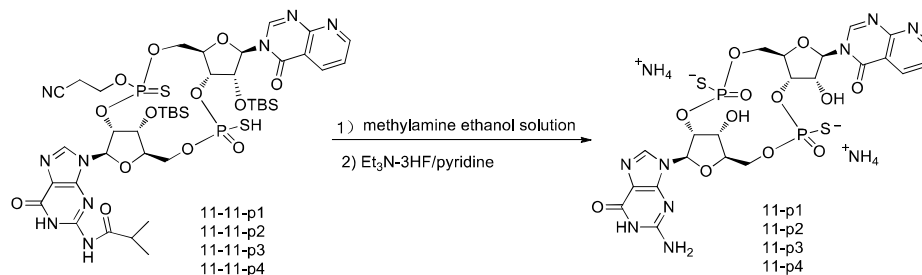
[0481] Compound 10-p3 (5.46mg, HPLC-RT:10.925min; m/z: [M+H]⁺717.8; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.44 (s, 1H), 8.22-8.20 (m, 1H), 7.99 (s, 1H), 7.89-7.85 (m, 1H), 7.68 (d, *J*=8.0 Hz, 1H), 7.61-7.57 (m, 1H), 6.56 (s, 2H), 6.09 (d, *J*=4.0 Hz, 2H), 5.83 (d, *J*=8.0 Hz, 1H), 5.34-5.32 (m, 1H), 5.26 (s, 1H), 5.08 (m, 1H), 4.71-4.68 (m, 1H), 4.46-4.45 (m, 1H), 4.25 (m, 1H), 4.14-3.95 (m, 5H), 3.79-3.76 (m, 1H), 1.24 (s, 2H).) was obtained as a white solid in the same manner as compound 10-p1, by using compound 10-11-p3 as a starting material, and purified by *prep*-HPLC (separation method 4).

[0482] **Embodiment 17:** Synthesis of compounds 11-p1, 11-p2, 11-p3 and 11-p4

[0483] Synthesis of intermediates 11-4 and 11-5:



[0484] Synthesis of compounds 11-4 and 11-5: compounds 11-4 (650mg, more polar) and 11-5 (610mg, less polar) were obtained as white solids in the same manner as compounds 9-4 and 9-5, by using pyrido[2,3-*d*]pyrimidin-4-one as a starting material. m/z: [M+H]⁺ 696.0; 11-4: ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.97 (d, *J*=4.0 Hz, 1H), 8.74 (s, 1H), 8.56 (d, *J*=8.0 Hz, 1H), 7.60-7.57 (m, 1H), 7.42 (d, *J*=8.0 Hz, 2H), 7.31-7.20 (m, 1H), 6.88 (d, *J*=12.0 Hz, 4H), 6.02 (s, 1H), 5.40 (d, *J*=4.0 Hz, 1H), 4.30-4.29 (m, 2H), 4.01-3.98 (m, 1H), 3.71 (s, 6H), 3.40 - 3.37 (m, 1H), 3.21-3.18 (m, 1H), 0.74 (s, 9H), 0.00 (s, 6H); 11-5: ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.94 (d, *J*=4.0 Hz, 1H), 8.67 (s, 1H), 8.55 (d, *J*=8.0 Hz, 1H), 7.56-7.53 (m, 1H), 7.41 (d, *J*=8.0 Hz, 2H), 7.29-7.25 (m, 1H), 6.86 (d, *J*=8.0 Hz, 4H), 6.00 (s, 1H), 5.13 (d, *J*=4.0 Hz, 1H), 4.13-4.08 (m, 2H), 4.00-3.98 (m, 1H), 3.39 (s, 6H), 3.36-3.32 (m, 1H), 3.27-3.25 (m, 1H), 0.79 (s, 9H), 0.00 (s, 6H).



[0485] Synthesis of compounds 11-11-p1, 11-11-p2, 11-11-p3 and 11-11-p4: compounds 11-11-p1 (27mg), 11-11-p2 (15mg), 11-11-p3 (19mg) and 11-11-p4 (36mg) were obtained as white solids in the same manner as Embodiment 15 steps 1~5, by using compound 11-5 as a starting material, and purified by *prep*-HPLC (separation method 6).

[0486] Synthesis of compound 11-p1:

[0487] To compound 11-11-p1 (20mg, 0.019mmol) was added methylamine ethanol solution (2 mL, 30%), the reaction system was stirred at room temperature for 10h and then concentrated under reduced pressure, the residue was dissolved in anhydrous pyridine (0.5mL), and triethylamine (0.5mL) and triethylamine trihydrofluoride (0.25mL) were added thereto under nitrogen, the reaction solution was stirred at 50°C for 1h. The solvent was concentrated under reduced pressure. The residue was diluted with methanol, and then ammonium hydroxide solution was slowly added to dropped pH to 8~9. The mixture was purified by *prep*-HPLC (separation method 7) to afford compound 11-p1 (2.45mg, HPLC-RT: 8.963min, m/z: [M+H]⁺718.8) as a white solid.

[0488] Synthesis of compound 11-p2:

[0489] Compound 11-p2 (3.07mg, HPLC-RT: 8.527min, m/z: [M+H]⁺718.7) was obtained as a white solid in the same manner as compound 11-p1, by using compound 11-11-p2 as a starting material, and purified by *prep*-HPLC (separation method 5).

[0490] Synthesis of compound 11-p3:

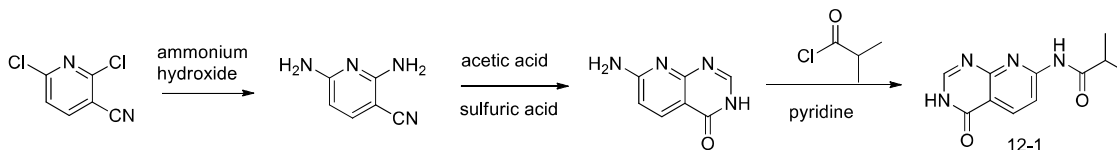
[0491] Compound 11-p3 (1.28mg, HPLC-RT: 9.103min, m/z: [M+H]⁺719.1) was obtained as a white solid in the same manner as compound 11-p1, by using compound 11-11-p3 as a starting material, and purified by *prep*-HPLC (separation method 5).

[0492] Synthesis of compound 11-p4:

[0493] Compound 11-p4 (3.03mg, HPLC-RT: 9.403min, m/z: [M+H]⁺718.6) was obtained as

a white solid in the same manner as compound 11-p1, by using compound 11-11-p4 as a starting material, and purified by *prep*-HPLC (separation method 5).

[0494] Embodiment 18: Synthesis of intermediate 12-1



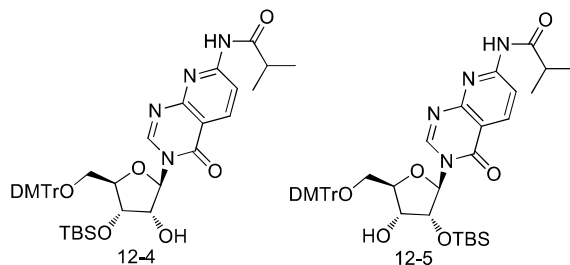
[0495] Step 1: 2,6-dichloronicotinonitrile (25g, 145mmol) and ammonium hydroxide solution (250mL) was charged into a sealed tube. The reaction system was stirred at 120°C for 48h and then cooled to 10°C, the solid was filtered and washed with cold water, the filter cake was dried under vacuum to afford 2,6-diaminonicotinonitrile (12.5g) as a light yellow solid. m/z: $[M+H]^+$ 135.0.

[0496] Step 2: To a solution of 2,6-diaminonicotinonitrile (12.5g, 93.2mmol) in acetic acid (120mL) was slowly added concentrated sulfuric acid (3mL), the reaction system was stirred at reflux for 9h, and then cooled to room temperature and concentrated under reduced pressure. The residue was triturated with ammonium hydroxide solution, the solid was filtered, the filter cake washed with cold water, and dried under vacuum to afford 7-aminopyrido[2,3-*d*]pyrimidin-4(3*H*)-one (15.1g) as a light yellow solid. m/z: $[M+H]^+$ 163.0.

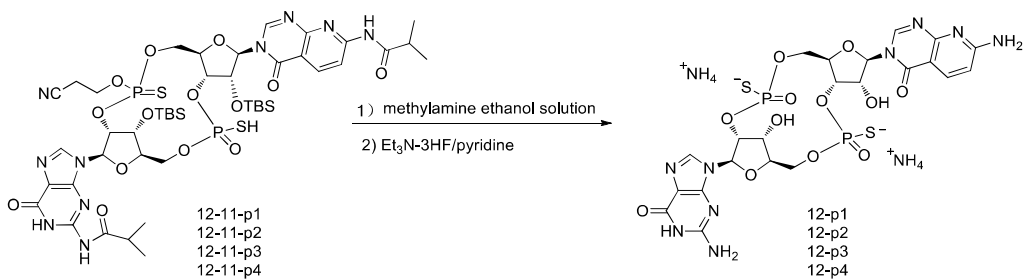
[0497] Step 3: 7-aminopyrido[2,3-*d*]pyrimidin-4(3*H*)-one (15.1g, 93.1mmol) was dissolved in pyridine (1.5L), isobutyryl chloride (29.7g, 279mmol) was slowly added thereto, the reaction system was stirred at room temperature for 18h and then water (1.5L) was added thereto, the reaction solution was extracted with chloroform /propan-2-ol (1.5L), the organic layer was concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-6.2% methanol/ dichloromethane) to afford compound 12-1 (11.9g) as a white solid. m/z: $[M+H]^+$ 233.1.

[0498] Embodiment 19: Synthesis of compounds 12-p1, 12-p2, 12-p3 and 12-p4

[0499] Synthesis of intermediates 12-4 and 12-5:



[0500] Intermediates 12-4 (1.73g, more polar) and 12-5 (1.1g, less polar) were obtained as white solids in the same manner as compounds 9-4 and 9-5, by using compound 12-1 as a starting material. m/z : $[M+H]^+781.0$; 12-4: 1H NMR (400 MHz, $DMSO-d_6$): δ 11.15 (s, 1H), 8.70 (s, 1H), 8.52 (d, $J=8.8$ Hz, 1H), 8.33 (d, $J=8.8$ Hz, 1H), 7.43 (d, $J=7.6$ Hz, 2H), 7.32 (d, $J=7.6$ Hz, 2H), 7.29 (d, $J=8.4$ Hz, 4H), 6.89 (d, $J=8.4$ Hz, 4H), 6.03 (d, $J=2.8$ Hz, 1H), 5.40 (d, $J=6.0$ Hz, 1H), 4.30 (t, $J=3.2$ Hz, 1H), 4.05-4.02 (m, 2H), 3.73 (s, 6H), 3.40-3.36 (m, 1H), 3.25-3.22 (m, 1H), 2.83-2.79 (m, 1H), 1.12 (s, 3H), 1.11 (s, 3H), 0.77 (s, 9H), 0.03 (s, 3H), -0.04 (s, 3H); 12-5: 1H NMR (400 MHz, $DMSO-d_6$): δ 11.13 (s, 1H), 8.67 (s, 1H), 8.54 (d, $J=8.8$ Hz, 1H), 8.31 (d, $J=8.8$ Hz, 1H), 7.45 (d, $J=7.6$ Hz, 2H), 7.33 (d, $J=7.6$ Hz, 2H), 7.31 (d, $J=8.4$ Hz, 4H), 6.90 (d, $J=8.4$ Hz, 4H), 6.04 (d, $J=2.8$ Hz, 1H), 5.16 (d, $J=6.0$ Hz, 1H), 4.40 (t, $J=3.2$ Hz, 1H), 4.17-4.12 (m, 2H), 3.74 (s, 6H), 3.43-3.40 (m, 1H), 3.30-3.27 (m, 1H), 2.82-2.79 (m, 1H), 1.13 (s, 3H), 1.11 (s, 3H), 0.85 (s, 9H), 0.05 (s, 6H).



[0501] Synthesis of compounds 12-11-p1, 12-11-p2, 12-11-p3 and 12-11-p4: compounds 12-11-p1 (25mg), 12-11-p2 (7mg), 12-11-p3 (40mg) and 12-11-p4 (32mg) were obtained as white solids in the same manner as Embodiment 15 steps 1~5, by using compound 12-5 as a starting material, and purified by *prep*-HPLC (separation method 6).

[0502] Synthesis of compound 12-p1:

[0503] To compound 12-11-p1 (25mg, 19 μ mol) was added methylamine ethanol solution (2mL, 30%), the reaction system was stirred at room temperature for 3h and then concentrated under reduced pressure, the residue was dissolved in anhydrous pyridine (0.5mL),

triethylamine (0.5mL) and triethylamine trihydrofluoride (0.25mL) was added thereto under nitrogen, the reaction solution was stirred at 50°C for 1h. The solvent was concentrated under reduced pressure. The residue was diluted with methanol, and then ammonium hydroxide solution was slowly dropped to adjusted pH to 8~9. The mixture was purified by *prep*-HPLC (separation method 4) to afford compound 12-p1 (0.9mg, HPLC-RT: 8.891min, m/z: [M+H]⁺733.7) as a white solid.

[0504] Synthesis of compound 12-p2:

[0505] Compound 12-p2 (0.5mg, HPLC-RT: 8.571min, m/z: [M+H]⁺733.7) was obtained as a white solid in the same manner as compound 12-p1, by using compound 12-11-p2 as a starting material, and purified by *prep*-HPLC (separation method 4).

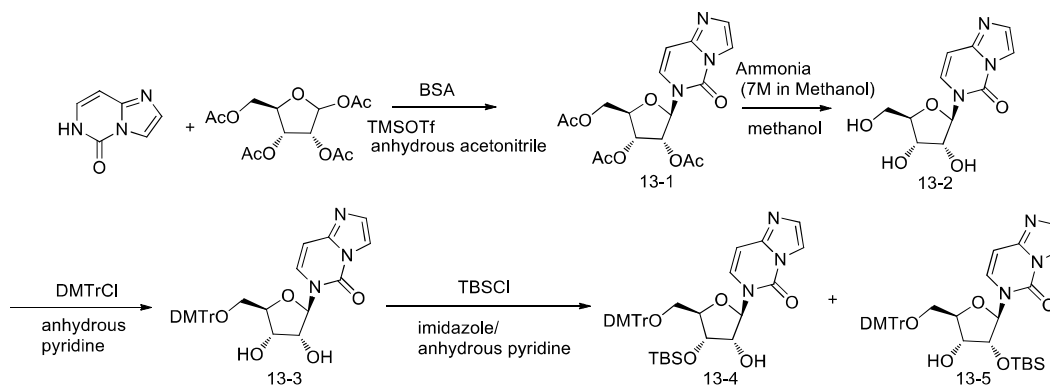
[0506] Synthesis of compound 12-p3:

[0507] Compound 12-p3 (1.5mg, HPLC-RT: 10.020min, m/z: [M+H]⁺733.8) was obtained as a white solid in the same manner as compound 12-p1, by using compound 12-11-p3 as a starting material, and purified by *prep*-HPLC (separation method 4).

[0508] Synthesis of compound 12-p4:

[0509] Compound 12-p4 (3.07mg, HPLC-RT: 9.468min, m/z: [M+H]⁺733.8) was obtained as a white solid in the same manner as compound 12-p1, by using compound 12-11-p4 as a starting material, and purified by *prep*-HPLC (separation method 4)

[0510] Embodiment 20: Synthesis of intermediates 13-4 and 13-5



[0511] Synthesis of imidazo[1,2-*c*]pyrimidin-5(6*H*)-one: The suspension of cytosine (18g, 0.16mol), aqueous solution of chloroacetaldehyde (63.6g, 0.32mol, 40%) and sodium acetate (32.8g, 0.40mol) in water (180mL) was stirred at 80°C for 3h, and then the reaction system was cooled to 10°C, the solid was filtered and washed with cold water, the filter cake was dried

under vacuum to afford imidazo[1,2-*c*]pyrimidin-5(6*H*)-one (17.8g) as a brown solid. m/z: [M+H]⁺ 136.0.

[0512] Step 1: To a suspension of imidazo[1,2-*c*]pyrimidin-5(6*H*)-one (17.8g, 0.13mmol), tetraacetylribose (46.1g, 0.14mmol) and BSA (39.7g, 0.19mmol) in acetonitrile (200mL) was added TMSOTf (43.3g, 0.19mol) dropwise at 0°C, the reaction system was stirred at 60°C for 3h and then concentrated under reduced pressure to afford compound 13-1 (100g, crude product) as a brown oil. m/z: [M+H]⁺ 394.0.

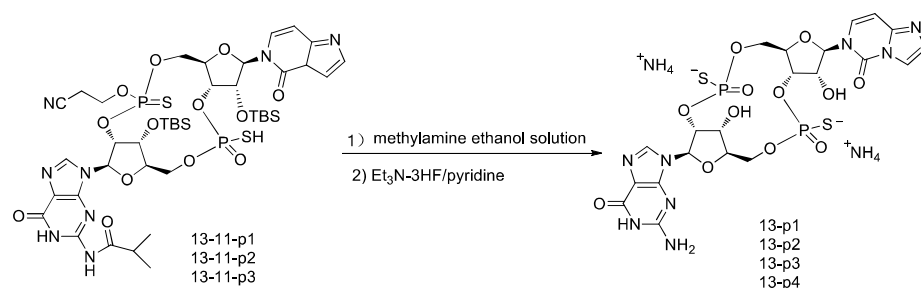
[0513] Step 2: Compound 13-1 (45g, crude) was dissolved in methanol (100mL), ammonia in methanol (81.7mL, 7M) was slowly added thereto, the reaction system was stirred at room temperature for overnight, the solid was filtered and washed with methanol, the filter cake was dried under vacuum to afford compound 13-2 (8g) as a brown solid. m/z: [M+H]⁺ 268.0.

[0514] Step 3: Compound 13-2 (12.5g, 5.76mmol) was subjected to azeotropic dehydration three times with anhydrous pyridine (20mL) and then dissolved in pyridine (100mL). To the above reaction solution was added DMTrCl (19.52g, 5.76mmol) in small portions at 0~5°C under nitrogen. The reaction system was stirred at room temperature for overnight, the solvent was concentrated under reduced pressure, and the residue was diluted with chloroform (100mL). The organic layer washed with saturated aqueous solution of sodium bicarbonate (30mL×2), the organic layer was separated and dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Flash column chromatography (methanol/dichloromethane=0-3%) to afford compound 13-3 (16.65g) as a brown solid. m/z:[M+Na]⁺ 570.0.

[0515] Step 4: Compound 13-3 (5.38g, 9.44mmol) was subjected to azeotropic dehydration three times with anhydrous pyridine (20mL) and then dissolved in pyridine (60mL). To the mixture was added TBSCl (2.13g, 14.2mmol) and imidazole (2.25g, 33.1mmol) at 0°C under nitrogen. The reaction system was stirred at room temperature for overnight, the solvent was concentrated under reduced pressure, and the residue was diluted with chloroform (100mL). The organic layer washed with saturated aqueous solution of sodium bicarbonate (30mL×2), the separated organic layer was dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by Flash column chromatography (0-3% methanol/ dichloromethane) to afford compounds 13-4 (1.32g, less

polar) and 13-5 (3.68g, more polar), as white foam solids. m/z : $[M+H]^+$ 684.0; 13-4: 1H NMR (400 MHz, DMSO- d_6): δ 7.88 (d, $J=5.6$ Hz, 1H), 7.82 (s, 1H), 7.76 (s, 1H), 7.40-7.26 (m, 9H), 6.92-6.90 (m, 4H), 6.68 (d, $J=6.0$ Hz, 1H), 5.90 (d, $J=9.2$ Hz, 1H), 5.33 (d, $J=4.8$ Hz, 1H), 4.50 (t, $J=4.4$ Hz, 1H), 4.15-4.14 (m, 2H), 3.74 (s, 6H), 3.28-3.26 (m, 2H), 0.77 (s, 9H), 0.02 (s, 3H), -0.12 (s, 3H). 13-5: 1H NMR (400 MHz, DMSO- d_6): δ 7.92 (d, $J=6.0$ Hz, 1H), 7.78 (s, 2H), 7.36-7.21 (m, 9H), 6.88-6.86 (m, 4H), 6.68 (d, $J=8.8$ Hz, 1H), 5.81 (d, $J=4.8$ Hz, 1H), 5.63 (d, $J=6.0$ Hz, 1H), 4.40-4.35 (m, 2H), 4.27 (t, $J=4.8$ Hz, 1H), 4.06-4.04 (m, 1H), 3.74 (s, 6H), 3.20-3.16 (m, 1H), 0.80 (s, 9H), 0.06 (s, 3H), -0.01 (s, 3H).

[0516] Embodiment 21: Synthesis of compounds 13-p1, 13-p2, 13-p3 and 13-p4



[0517] Synthesis of compounds 13-11-p1, 13-11-p2, 13-11-p3 and 13-11-p4: compounds 13-11-p1 (55mg, LCMS-RT (Thermo):1.874, 1.944min), 13-11-p2 (25mg, LCMS-RT (Thermo):2.030min), and 13-11-p3 (11mg, LCMS-RT (Thermo):2.170min) were obtained as white solids in the same manner as Embodiment 15 steps 1~5, by using compound 13-5 as a starting material, and purified by Flash column chromatography (acetonitrile/ aqueous solution of ammonium bicarbonate (10mmol/L) =40%). m/z : $[M+H]^+$ 1057.9.

[0518] Synthesis of compounds 13-p1 and 13-p2:

[0519] Compound 13-11-p1 (43mg, 0.04mmol) was dissolved in methylamine ethanol solution (3 mL, 30%) and stirred at room temperature for 3h, and then concentrated under reduced pressure, the residue was dissolved in anhydrous pyridine (0.5mL), triethylamine (0.5mL) and triethylamine trihydrofluoride (0.25mL) was added thereto under nitrogen, the reaction solution was stirred at 50°C for 3h and then concentrated under reduced pressure. The residue was diluted with methanol and ammonium hydroxide solution was slowly dropped to adjusted pH to 8~9. The mixture was purified by *prep*-HPLC (separation method 5) to afford compounds 13-p1 (2.28mg, HPLC-RT: 6.048min, m/z : $[M+H]^+$ 706.8) and 13-p2 (0.68mg,

HPLC-RT: 7.781min, m/z: $[M+H]^+$ 706.8), as white solids.

[0520] Synthesis of compound 13-p3:

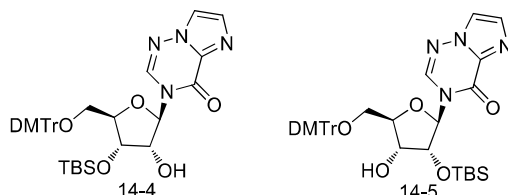
[0521] Compound 13-p3 (3.82mg, HPLC-RT: 6.445min, m/z: $[M+H]^+$ 706.8) was obtained as a white solid in the same manner as compounds 13-p1/13-p2, by using compound 13-11-p2 as a starting material, and purified by *prep*-HPLC (separation method 4).

[0522] Synthesis of compound 13-p4:

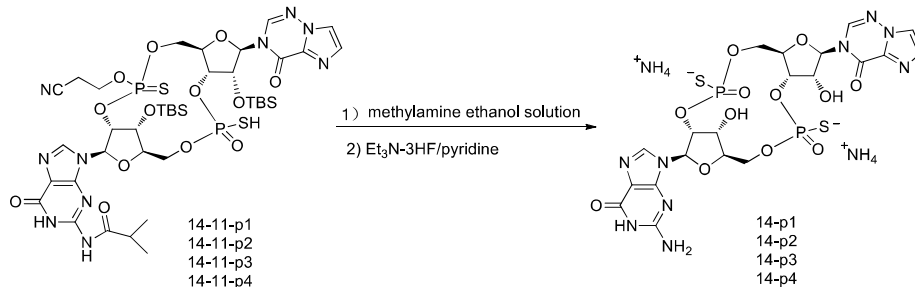
[0523] Compound 13-p4 (4.03mg, HPLC-RT: 10.395min, m/z: $[M+H]^+$ 706.8) was obtained as a white solid in the same manner as compounds 13-p1/13-p2, by using compound 13-11-p3 as a starting material, and purified by *prep*-HPLC (separation method 5).

[0524] **Embodiment 22:** Synthesis of compounds 14-p1, 14-p2, 14-p3 and 14-p4

[0525] Synthesis of intermediates 14-4 and 14-5:



[0526] Intermediates 14-4 (1.3g, more polar) and 14-5 (0.56g, less polar) were obtained as white solids in the same manner as compounds 13-4 and 13-5, by using imidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one (CAS No.: 865444-76-4) as a starting material. m/z: $[M+H]^+$ 685.0.



[0527] Synthesis of compounds 14-11-p1, 14-11-p2, 14-11-p3 and 14-11-p4: compounds 14-11-p1 (5mg, LCMS-RT (Thermo):1.850min), 14-11-p2 (17mg, LCMS-RT (Thermo): 1.880min), 14-11-p3 (22mg, LCMS-RT (Thermo): 2.020min) and 14-11-p4 (18mg, LCMS-RT (Thermo): 2.140min) were obtained as white solids in the same manner as Embodiment 15 steps 1~5, by using compound 14-5 as a starting material, and purified by *prep*-HPLC (separation method 7). m/z: $[M+H]^+$ 1059.0.

[0528] Synthesis of compound 14-p1:

[0529] Compound 14-11-p1 (5.0mg, 0.05mmol) was dissolved in methylamine ethanol solution (3.0mL, 30%), the mixture was stirred at room temperature for 3h and then concentrated under reduced pressure, the residue was dissolved in anhydrous pyridine (0.5mL), triethylamine (0.5mL) and triethylamine trihydrofluoride (0.3mL) was added thereto under nitrogen, the reaction solution was stirred at 50°C for 2h. The solvent was concentrated under reduced pressure. The residue was diluted with methanol and then ammonium hydroxide solution was slowly dropped to adjusted pH to 8~9. The mixture was purified by *prep*-HPLC (separation method 5) to afford compound 14-p1 (3.65mg, HPLC-RT: 1.914min, m/z: [M+H]⁺707.5) as a white solid.

[0530] Synthesis of compound 14-p2:

[0531] Compound 14-p2 (17.5mg, HPLC-RT: 3.123min, m/z: [M+H]⁺707.5) was obtained as a white solid in the same manner as compound 14-p1, by using compound 14-11-p2 as a starting material, and purified by *prep*-HPLC (separation method 4).

[0532] Synthesis of compound 14-p3:

[0533] Compound 14-p3 (1.04mg, HPLC-RT: 3.193min, m/z: [M+H]⁺707.5) was obtained as a white solid in the same manner as compound 14-p1, by using compound 14-11-p3 as a starting material, and purified by *prep*-HPLC (separation method 4).

[0534] Synthesis of compound 14-p4:

[0535] Compound 14-p4 (1.04mg, HPLC-RT: 36.036min, m/z: [M+H]⁺707.5) was obtained as a white solid in the same manner as compound 14-p1, by using compound 14-11-p4 as a starting material, and purified by *prep*-HPLC (separation method 4).

[0536] Embodiments of bioassays

[0537] Embodiment 1: Type 1 IFN activation assay

[0538] Plated THP-1 dual cells (Invivogen) as 100000 cells/well in a 96 well-plate, and then phorbol 12-myristate 13-acetate (PMA) was added and the final concentration of PMA was 30ng/mL. After 24h incubation, cells were washed with fresh medium for twice, and 3-folds dilution compounds were added to appropriate wells. The compounds were diluted by PB buffer (50mM HEPES, 100mM KCl, 3mM MgCl₂, 0.1mM DTT, 85mM sucrose, 1mM ATP,

0.1mM GTP, 0.2% bovine serum albumin and 5µg/ml digitonin) and the top dose was 10µM , minimum dose was 0.0015µM. The cells were incubated for 30min, and then washed with fresh medium for twice again. Add fresh medium, and the cells were incubated for additional 24h. After incubation, 10ul supernant and 50µL QUANTI-Luc (Invivogen) was add to a 96 well-plate, fluorescence was read with TECAN. The express level of IFN was proportional to the fluorescence intensity. Drew the curve with Graphpad Prism and analyse EC₅₀ of the compounds.

Compound No.	EC ₅₀ (µM)	Compound No.	EC ₅₀ (µM)
1-p1	>10	8-p3	99.53
1-p2	>10	8-p4	38.80
1-p3	>10	9-p1	>100
1-p4	25.615	9-p2	>100
2-p1	45.755	9-p3	4.865
2-p2	>100	10-p1	70.69
2-p3	97.411	10-p2	118.8
2-p4	2.077	10-p3	0.494
4-p1	6.834	11-p1	60.81
4-p2	0.625	11-p2	9.805
4-p3	0.417	11-p3	17.50
5-p1	>10	11-p4	0.421
5-p2	51.981	12-p1	>100
5-p3	58.238	12-p2	19.01
5-p4	3.223	12-p3	8.628
6-p1	2.273	12-p4	0.565
6-p2	1.063	13-p1	188.1
6-p3	0.429	13-p2	351.6
6-p4	0.311	13-p3	176.0
7-p1	3.464	13-p4	4.991

7-p2	40.62	14-p1	>100
7-p3	0.275	14-p2	>100
7-p4	2.307	14-p3	40.35
8-p1	>100	14-p4	21.52
8-p2	>100	Ref.1	2.942

[0539] Embodiment 2: STING IFN β secretion assay

[0540] Plated 40uL THP-1 cells (ATCC) as 16000 cells/well in a 96 well-plate (Corning, 3596). 3-folds dilution compounds were added to appropriate wells. The compounds were diluted with buffer (RPMI1640+2mM L-glutamine + 1x non-essential amino acids + 1mM sodium pyruvate + 0.5% Fetal bovine serum), and the top dose of the compound was 100 μ M, minimum dose was 1.23 μ M. After 5h incubation, 2 μ L supernant was added to 384-well plate (Greiner, Cat: 784075) and the secretion of IFN- β was detected with AllphaLISA IFN- β kit(PerkinElmer, Cat:AL577C). 1x buffer was prepared to dilute the receptor and dornor, and then 4 μ L 20 μ g/mL Anti-pIFN β AlphaLISA receptor was added to every well. After 30mins incubation at room temperature, 4 μ L 2nM biotinylated Anti-pIFN β antibody was added to a 384 well-plate, incubated overnight at 4°C. 10 μ L 40 μ g/mL Streptavidin (SA) dornor which was diluted with 1xbuffer was added, and then incubated at room temperature for 30min. Fluorescence was read with TECAN. The expressed level of IFN- β was proportional to the fluorescence intensity. Drew the curve with Graphpad Prism and analysed EC₅₀ of the compounds.

Compound No.	EC ₅₀ (μ M)
2-p4	47.64
4-p2	36.22
4-p3	94.39
6-p2	58.09
6-p3	9.84
6-p4	9.40

[0541] Embodiment 3: Efficacy study in a CT-26 colon tumor xenograft model in mice

[0542] Cell Culture: The CT26 colon tumor cells (ATCC) were maintained in vitro as a

monolayer culture in RPMI1640 medium supplemented with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely passaged twice per week by trypsin-EDTA treatment. The cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

[0543] Animals: BALB/c nude mice, 6-8weeks, 19-22g, animal supplier: Beijing Vital River Laboratory Animal Technology Co., Ltd.

[0544] 4 groups were set up as follows:

Group	No.	Treatment	Dose	Dosing Route	Actual Schedule
1	8	Vehicle	--	i.t.	Q3D x 3 doses
2	8	Ref.1	2 mg/kg	i.t.	Q3D x 3 doses
3	8	Compound 6-p3	1 mg/kg	i.t.	Q3D x 3 doses
4	8	Compound 6-p3	2 mg/kg	i.t.	Q3D x 3 doses

[0545] Note: i.t.: intra-tumor injection, Q3D: once every 3 days

[0546] Experiment method: Each mouse was inoculated subcutaneously with CT-26 tumor cells (1×10^5) in 0.1mL over right flank region. The growth of tumor was observed regularly, when the tumor volume reached 150mm³, mice were randomized based on tumor volume and body weight, and treated with schedule. The weight and tumor size of mice were measured 2-3 times a week during the whole experiment.

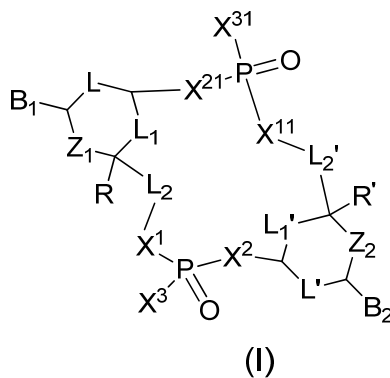
[0547] Tumor size formula: Tumor volumes (mm³)=0.5×(long diameters of the tumor×short diameters of the tumor²).

[0548] The tumor growth curves of different 4 groups are shown in Figures 1. The result indicated that compared with the positive control Ref.1, the compound of the disclosure can show better efficacy on the subcutaneous CT-26 colon tumor model in mice.

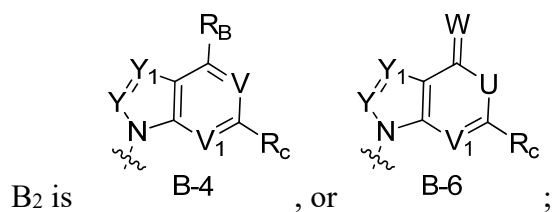
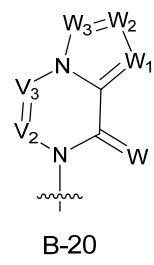
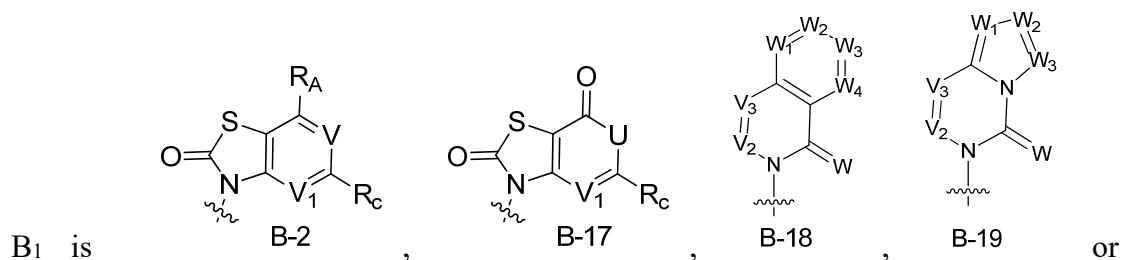
[0549] Note: Ref.1 used in embodiments of bioassays 1 and 3 was MLRR-CDA (ammonia salt), CASNo: 1638750-96-5, the synthesis method could refer to PCT patent application WO2014/189805A1 compound 22.

Claims:

1. A cyclic di-nucleotide analogue (I), a stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof;



wherein each of Z_1 , Z_2 is independently O;



L , L_1 , L' and L'_1 are as defined in the following combinations:

- 1) L is a connecting bond, L_1 is CR^1R^2 , L' is $CR^{11}R^{21}$, L'_1 is a connecting bond, or
- 2) L is CR^1R^2 , L_1 is a connecting bond, L' is a connecting bond, L'_1 is $CR^{11}R^{21}$;

L_2 is CH_2 ; L'_2 is CH_2 ;

X^1 is O; X^{11} is O;

X^2 is O; and X^{21} is O;

X^3 and X^{31} are each independently OH, SH or BH_3^- ;

R and R' are each independently hydrogen;

R^1 and R^2 are each independently hydrogen, halogen, cyano, hydroxyl, thiol, amino, azido, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkyl, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, halo-C₁₋₆ alkylthio, C₁₋₆ alkylamino, OC(O)R^a or OR^a; the C₂₋₆ alkenyl, C₂₋₆ alkynyl, or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido, and cyano;

R^{11} and R^{21} are each independently hydrogen, halogen, cyano, hydroxyl, thiol, amino, azido, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkyl, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, halo-C₁₋₆ alkylthio, C₁₋₆ alkylamino, OC(O)R^a, or OR^a; the C₂₋₆ alkenyl, C₂₋₆ alkynyl, or C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by 1 to 3 substituents selected from halogen, hydroxyl, amino, azido, and cyano;

Y and Y₁ are independently CR_E or N;

U is CHR_{E'} or NR_{D'};

V, V₁, V₂ and V₃ are each independently CR_{E''} or N;

W is O or S;

W₁, W₂, W₃ and W₄ are each independently N or CR_{F'};

each of R_A, R_B, R_C, R_E, R_{E'}, R_{E''} and R_{F'} are independently H, halogen, -CN, -NO₂, -N₃, R^c, -SR^c, -OR^c, -OC(O)R^c, -OC(O)OR^c, -OC(O)NR^bR^c, -C(O)OR^c, -C(O)R^c, -C(O)NR^bR^c, -NR^bR^c, -NR^bC(O)R^c, -N(R^b)C(O)OR^c, -N(R^a)C(O)NR^bR^c, -NR^bS(O)₂R^c, -NR^bC(=NH)R^c, -NR^bC(=NR^c)NH₂, -S(O)₁₋₂R^c, -S(O)₂NR^bR^c or -NR^aS(O)₂NR^bR^c;

R_{D'} is independently H or R^c;

each of R^a and R^b is independently H, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ alkyl, halo-C₁₋₆ alkyl;

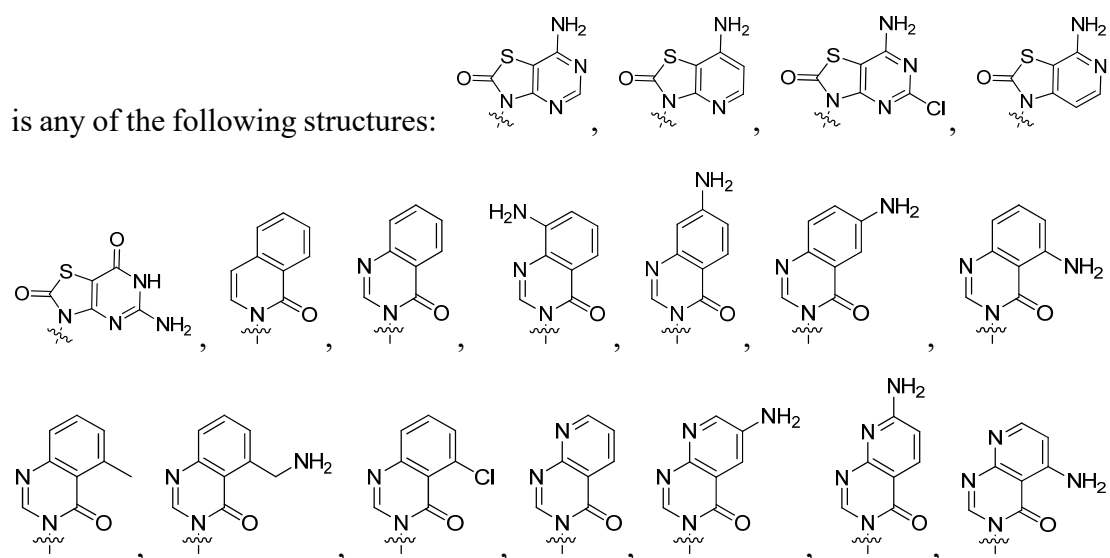
each R^c is independently H, substituted or unsubstituted C₁₋₁₀ alkyl, substituted or unsubstituted C₂₋₈ alkenyl, substituted or unsubstituted C₂₋₈ alkynyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 3-10 membered heterocycloalkyl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₆₋₁₀ aryl-C₁₋₆ alkyl, substituted or unsubstituted C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 3-10 membered

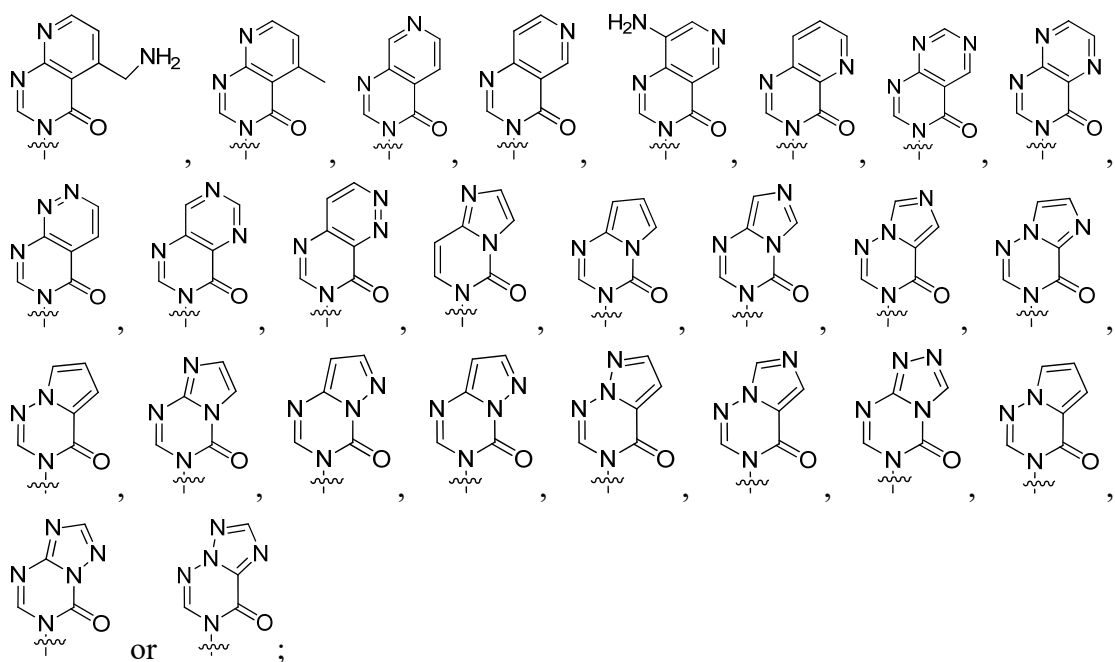
heterocycloalkyl-C₁₋₆ alkyl, substituted or unsubstituted 5-10 membered heteroaryl-C₁₋₆ alkyl; the C₁₋₁₀ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₁₀ cycloalkyl, C₆₋₁₀ aryl, 3-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₃₋₁₀ cycloalkyl-C₁₋₆ alkyl, 3-10 membered heterocycloalkyl-C₁₋₆ alkyl, or 5-10 membered heteroaryl-C₁₋₆ alkyl is unsubstituted or selectively substituted at any position by one or more R^d;

each R^d is independently halogen, halo-C₁₋₆ alkyl, halo-C₁₋₆ alkoxy, C₁₋₆ alkyl, -CN, -N₃, -SR^e, -OR^e, -C(O)R^e, -NR^eR^{e'}, substituted or unsubstituted C₆₋₁₀ aryl, substituted or unsubstituted 5-10 membered heteroaryl, substituted or unsubstituted C₃₋₁₀ cycloalkyl, or substituted or unsubstituted 3-10 membered heterocycloalkyl; the C₆₋₁₀ aryl, 5-10 membered heteroaryl, C₃₋₁₀ cycloalkyl or 3-10 membered heterocycloalkyl is unsubstituted or selectively substituted at any position by one or more substituents selected from halogen, hydroxyl, cyano, amino, C₁₋₄ alkyl, halo-C₁₋₄ alkyl, C₁₋₄ alkoxy, C₁₋₄ alkylamino and halo-C₁₋₄ alkoxy;

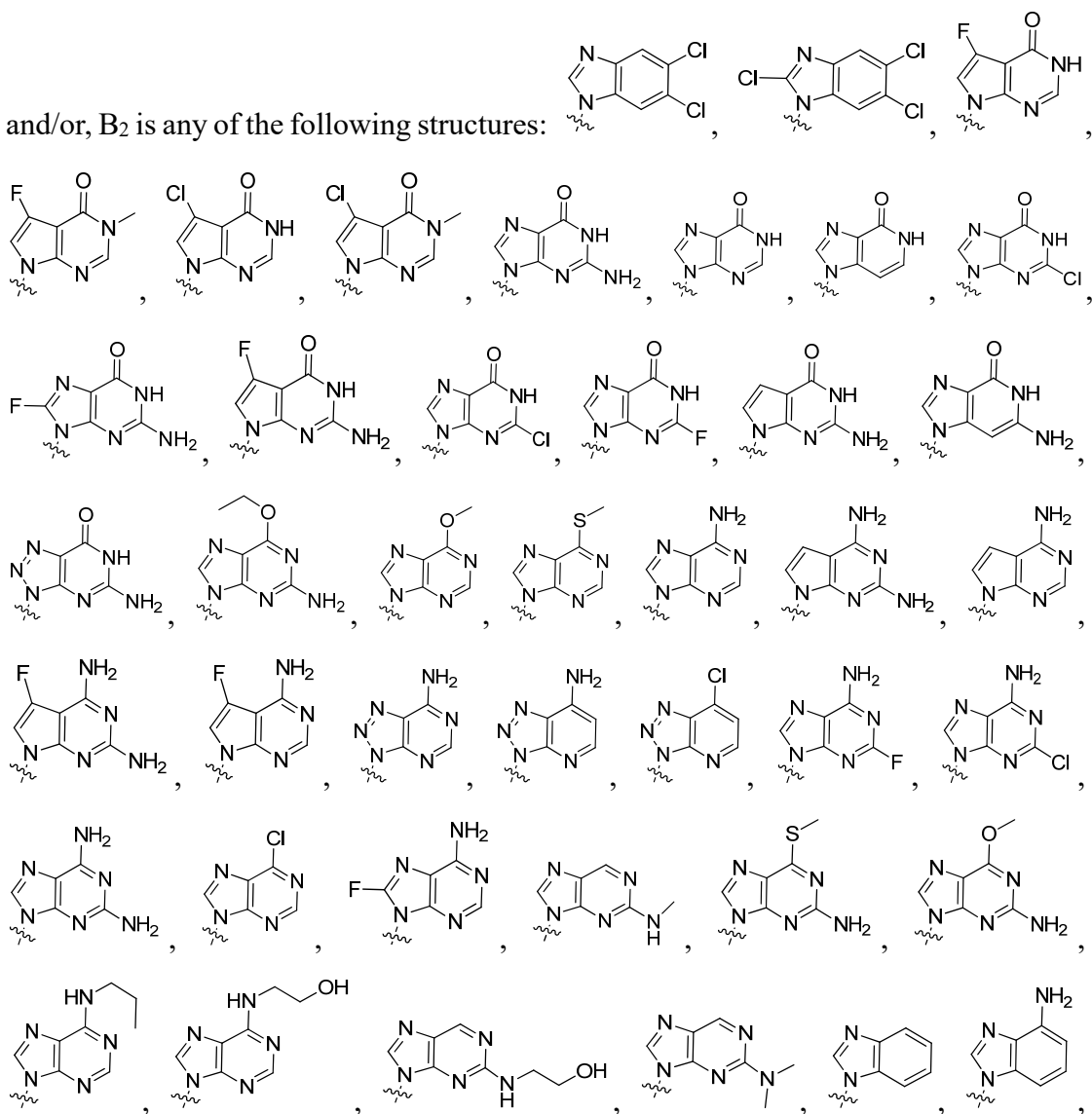
each of R^e and R^{e'} is independently C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ alkyl, halo-C₁₋₆ alkyl.

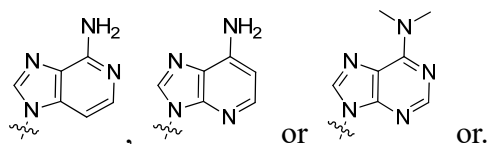
2. The cyclic di-nucleotide analogue of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to claim 1, wherein B₁





and/or, B₂ is any of the following structures:





3. The cyclic di-nucleotide analogue of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to claim 1,

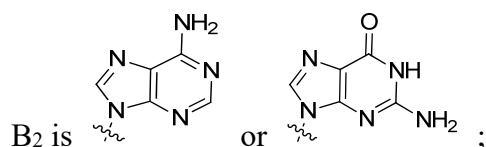
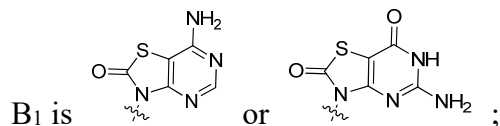
R^1 and R^2 are each independently hydrogen, halogen, hydroxyl, or OR^a ;

and/or, R^{11} and R^{21} are each independently hydrogen, halogen, hydroxyl, or OR^a ;

and/or, each R^a is independently C_{1-4} alkyl or halo- C_{1-4} alkyl.

4. The cyclic di-nucleotide analogue of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to claim 1, wherein Z_1 is O;

Z_2 is O;



L and L_1 are each independently a connecting bond or CR^1R^2 ;

L' and L_1' are each independently a connecting bond or $CR^{11}R^{21}$; and, L , L_1 , L' and L_1' are as defined in the following combinations:

1) L is a connecting bond, L_1 is CR^1R^2 , L' is $CR^{11}R^{21}$, L_1' is a connecting bond, or

2) L is CR^1R^2 , L_1 is a connecting bond, L' is a connecting bond, L_1' is $CR^{11}R^{21}$;

L_2 is CH_2 ;

L_2' is CH_2 ;

X^1 is O;

X^{11} is O;

X^2 is O;

X^{21} is O;

X^3 and X^{31} are each independently OH or SH;

R and R' are each independently hydrogen;

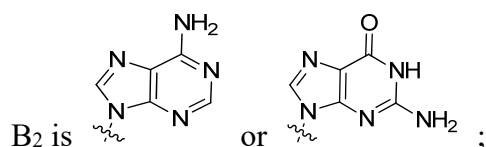
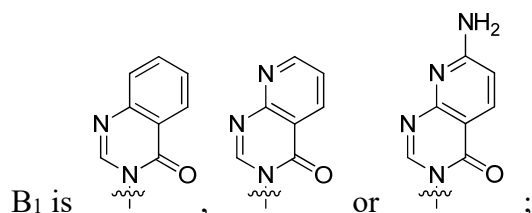
R^1 and R^2 are each independently hydrogen, halogen, hydroxyl or OR^a ;

R^{11} and R^{21} are each independently hydrogen, halogen, hydroxyl or OR^a ;

each R^a is independently C_{1-4} alkyl or halo- C_{1-4} alkyl.

5. The cyclic di-nucleotide analogue for formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to claim 1, wherein Z_1 is O;

Z_2 is O;



L and L_1 are each independently a connecting bond or CR^1R^2 ;

L' and L_1' are each independently a connecting bond or $CR^{11}R^{21}$; and, L, L_1 , L' and L_1' are as defined in the following combinations:

1) L is a connecting bond, L_1 is CR^1R^2 , L' is $CR^{11}R^{21}$, L_1' is a connecting bond, or

2) L is CR^1R^2 , L_1 is a connecting bond, L' is a connecting bond, L_1' is $CR^{11}R^{21}$;

L_2 is CH_2 ;

L_2' is CH_2 ;

X^1 is O;

X^{11} is O;

X^2 is O;

X^{21} is O;

X^3 and X^{31} are each independently OH or SH;

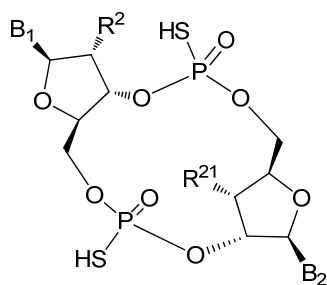
R and R' are each independently hydrogen;

R¹ and R² are each independently hydrogen, halogen, hydroxyl or OR^a;

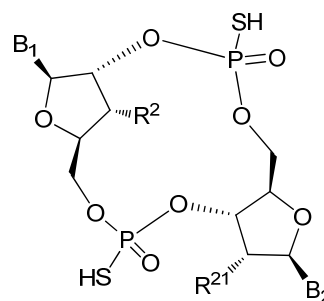
R¹¹ and R²¹ are each independently hydrogen, halogen, hydroxyl or OR^a;

each R^a is independently C₁₋₄ alkyl or halo-C₁₋₄ alkyl.

6. The cyclic di-nucleotide analogue of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to any one of claims 1 to 5, wherein, which is shown as the compound of formula (VI) or (VII), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof:



(VI)

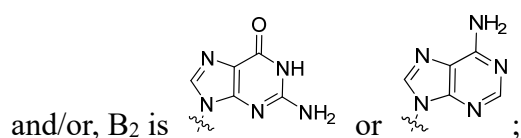
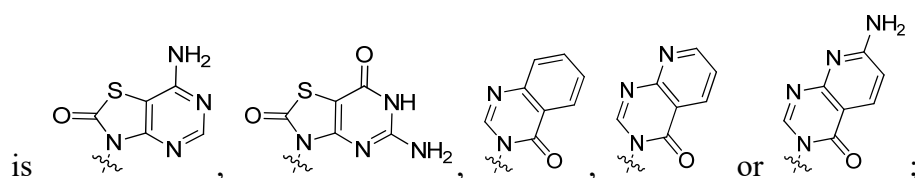


(VII)

wherein, B₁, B₂, R² and R²¹ are as defined in any one of claims 1 to 5.

7. The cyclic di-nucleotide analogue of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to claim 6, wherein the stereo-configuration of P is (Sp, Sp), (Sp, Rp), (Rp, Rp) or (Rp, Sp).

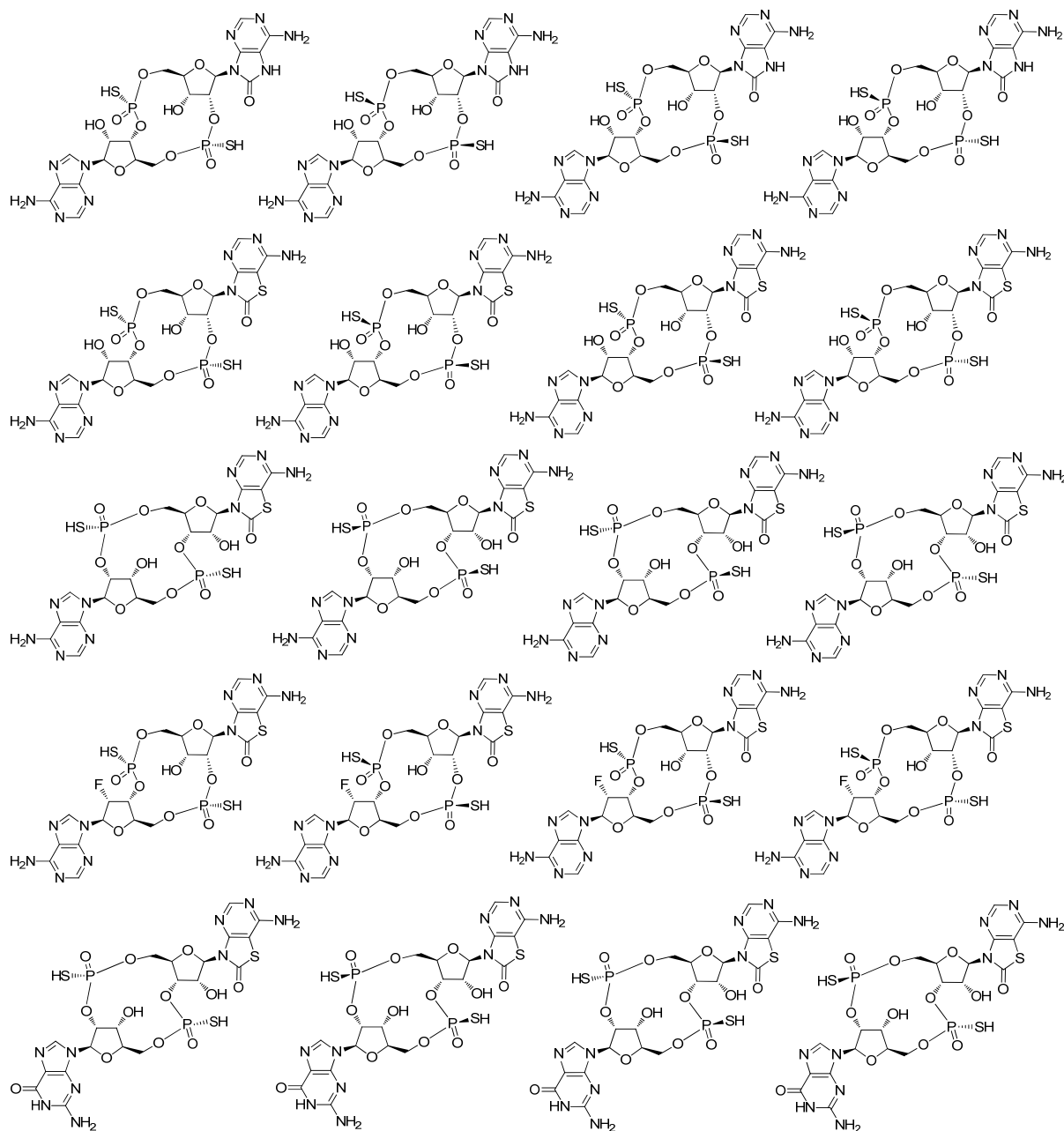
8. The cyclic di-nucleotide analogue of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to claim 6, wherein B₁

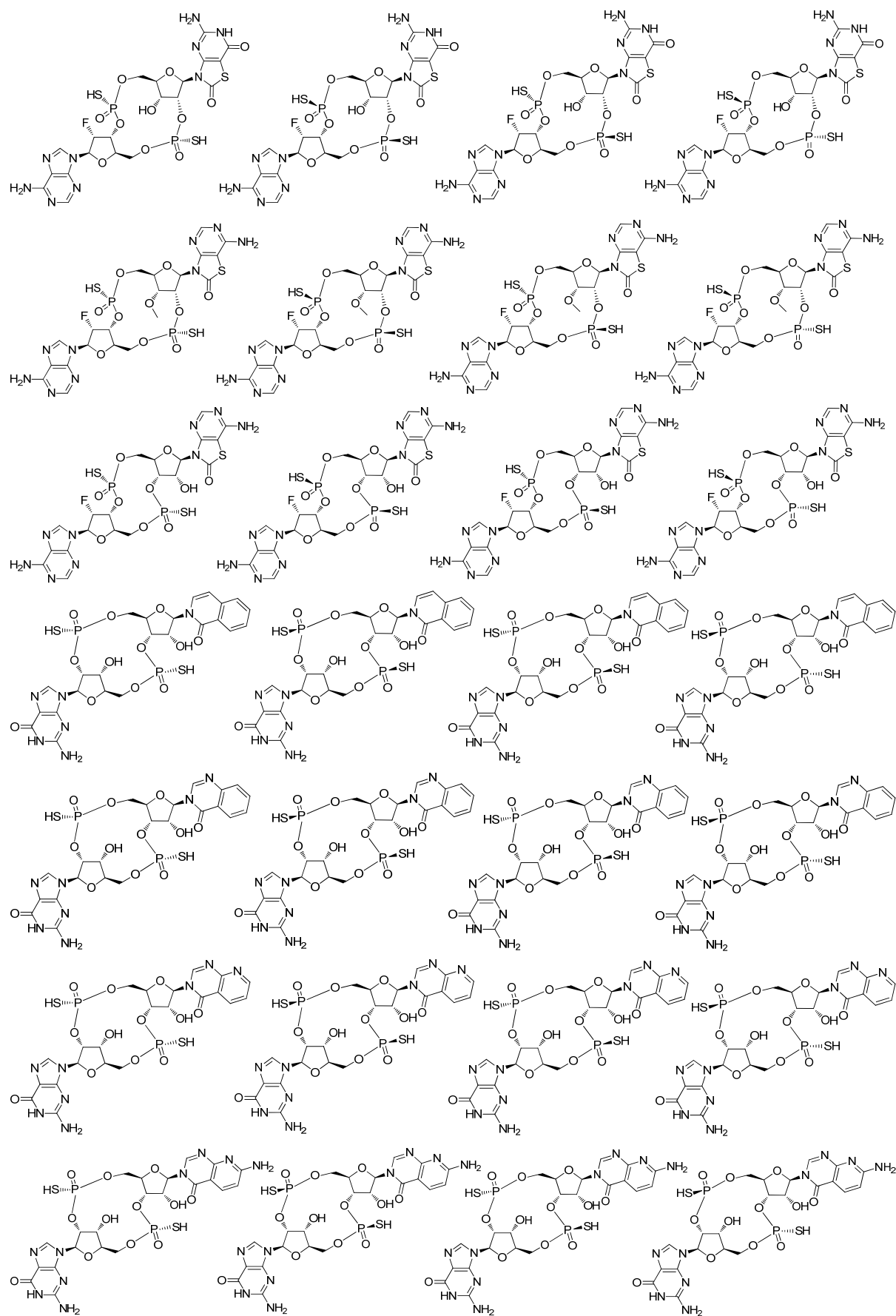


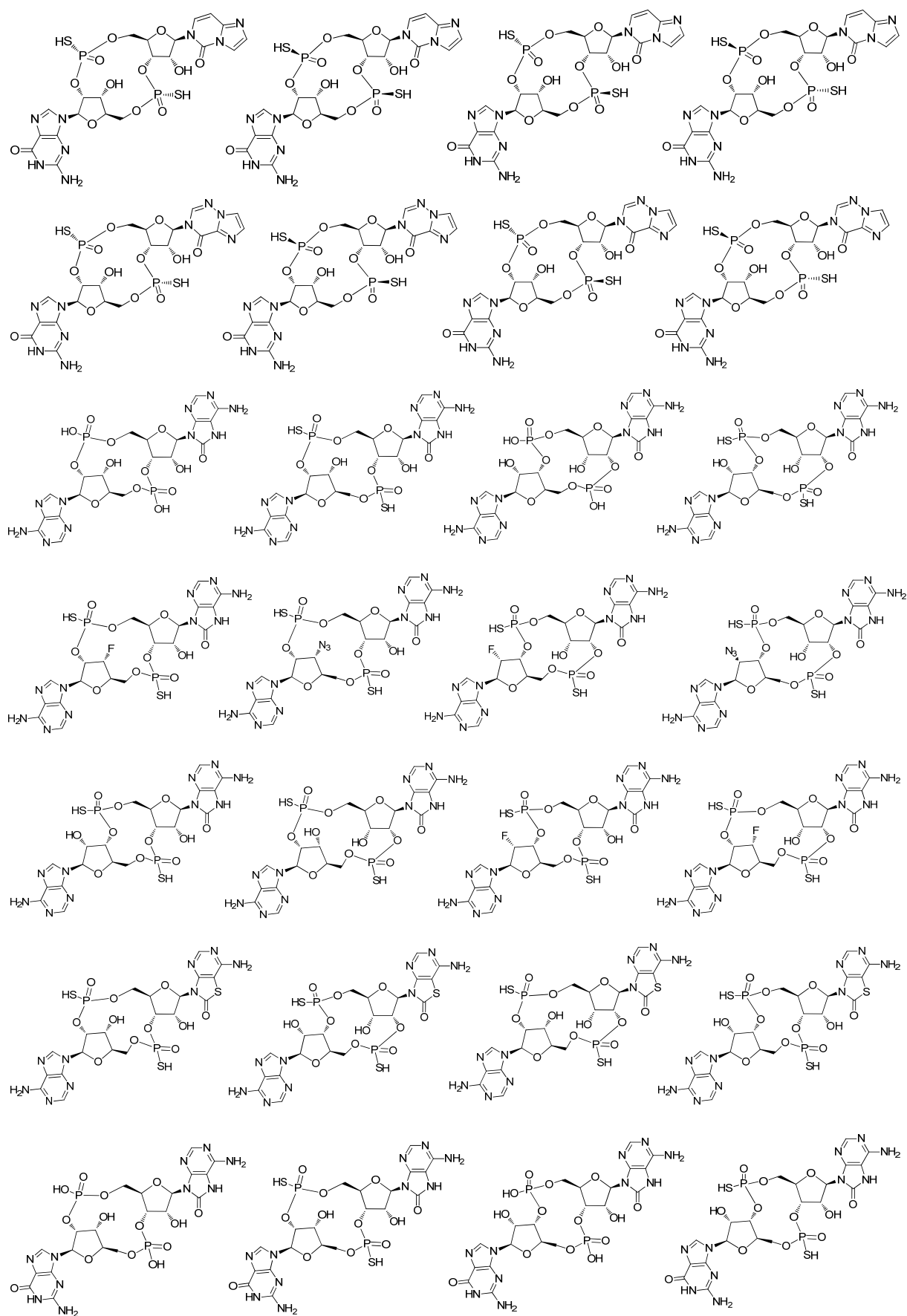
in Formula VI, R² is -OH; R²¹ is -OH;

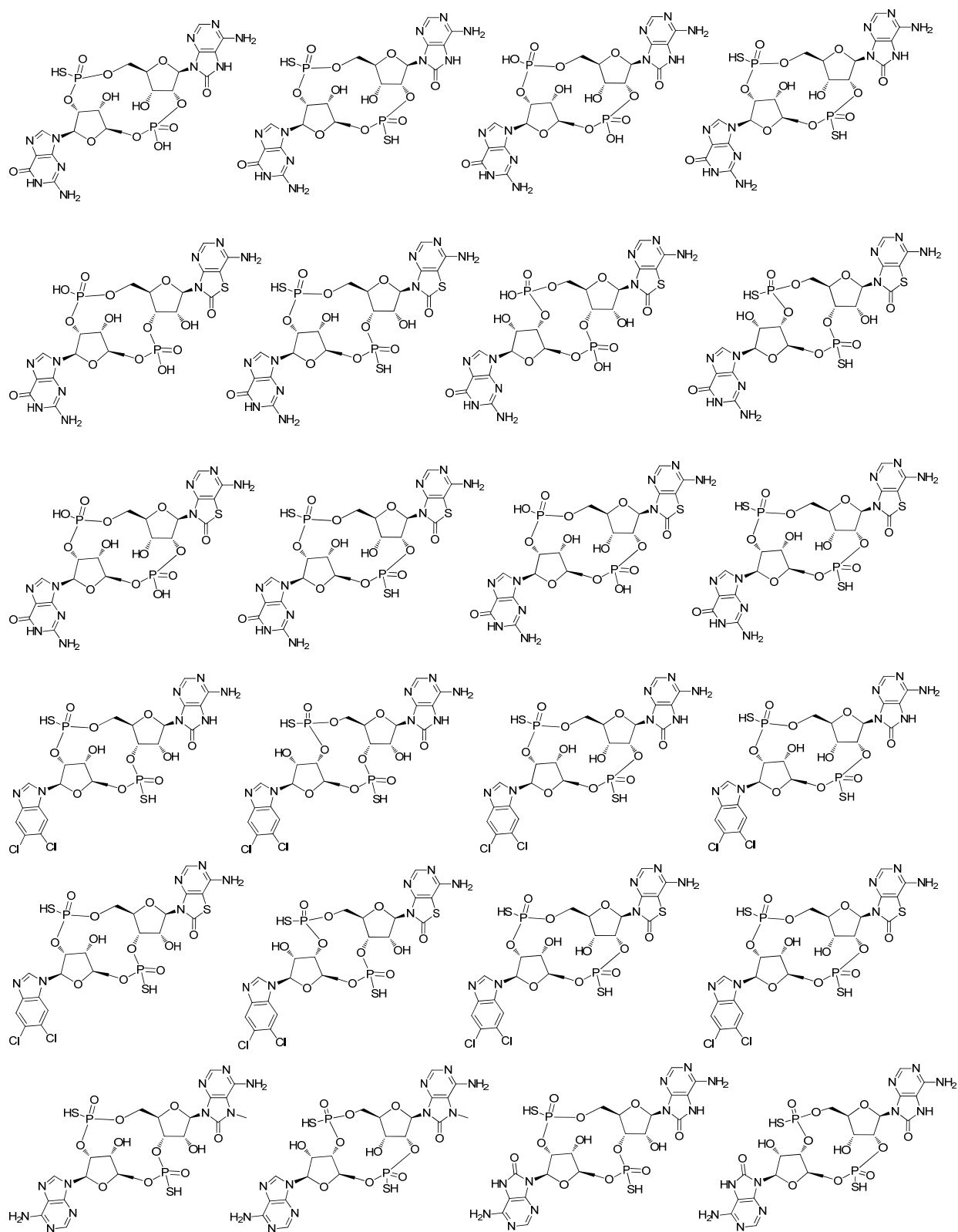
in Formula VII, R^2 is -OH or -OCH₃; R^{21} is -OH or F.

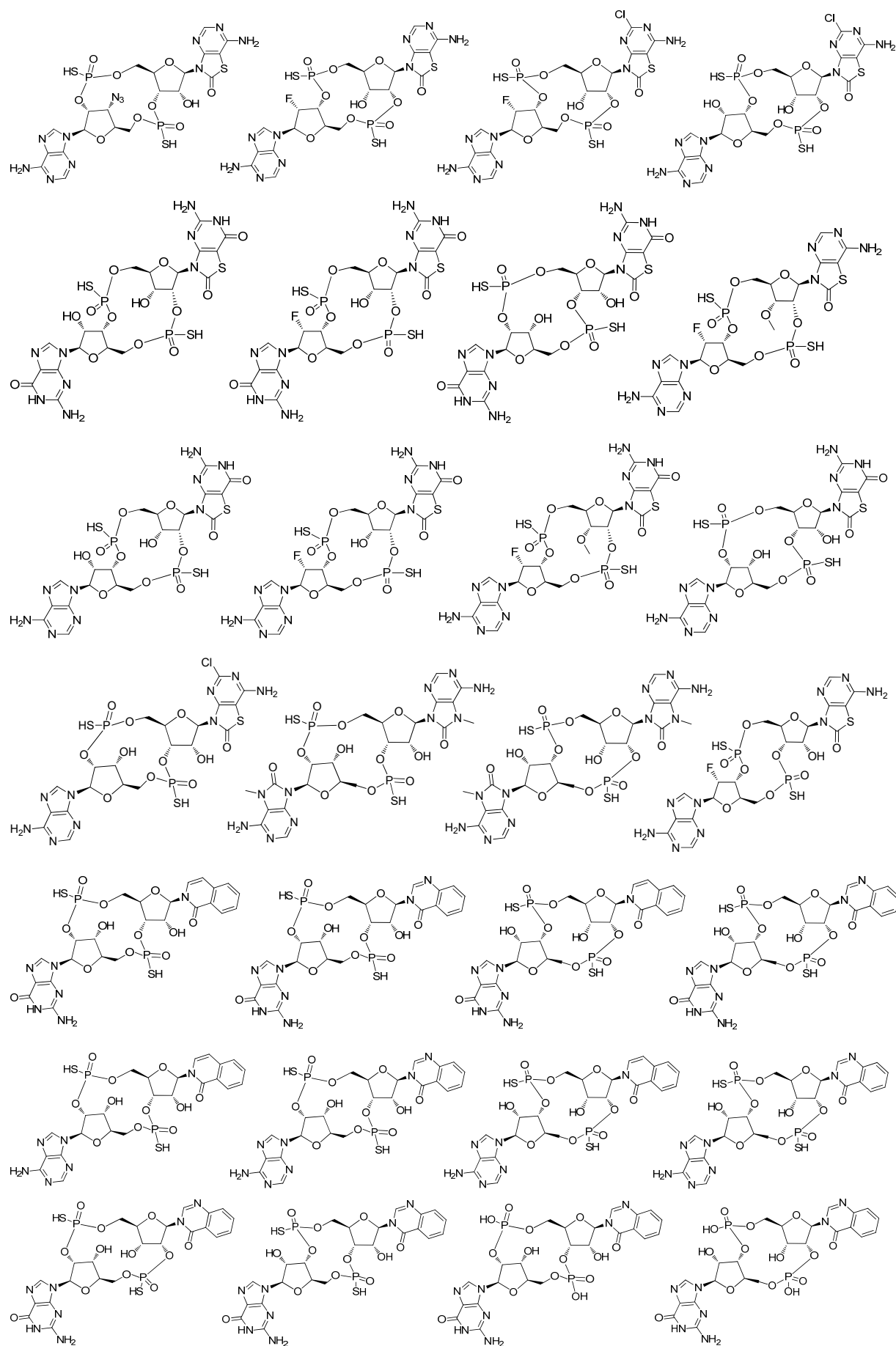
9. A cyclic di-nucleotide analogue of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof, which is any of the following structures:

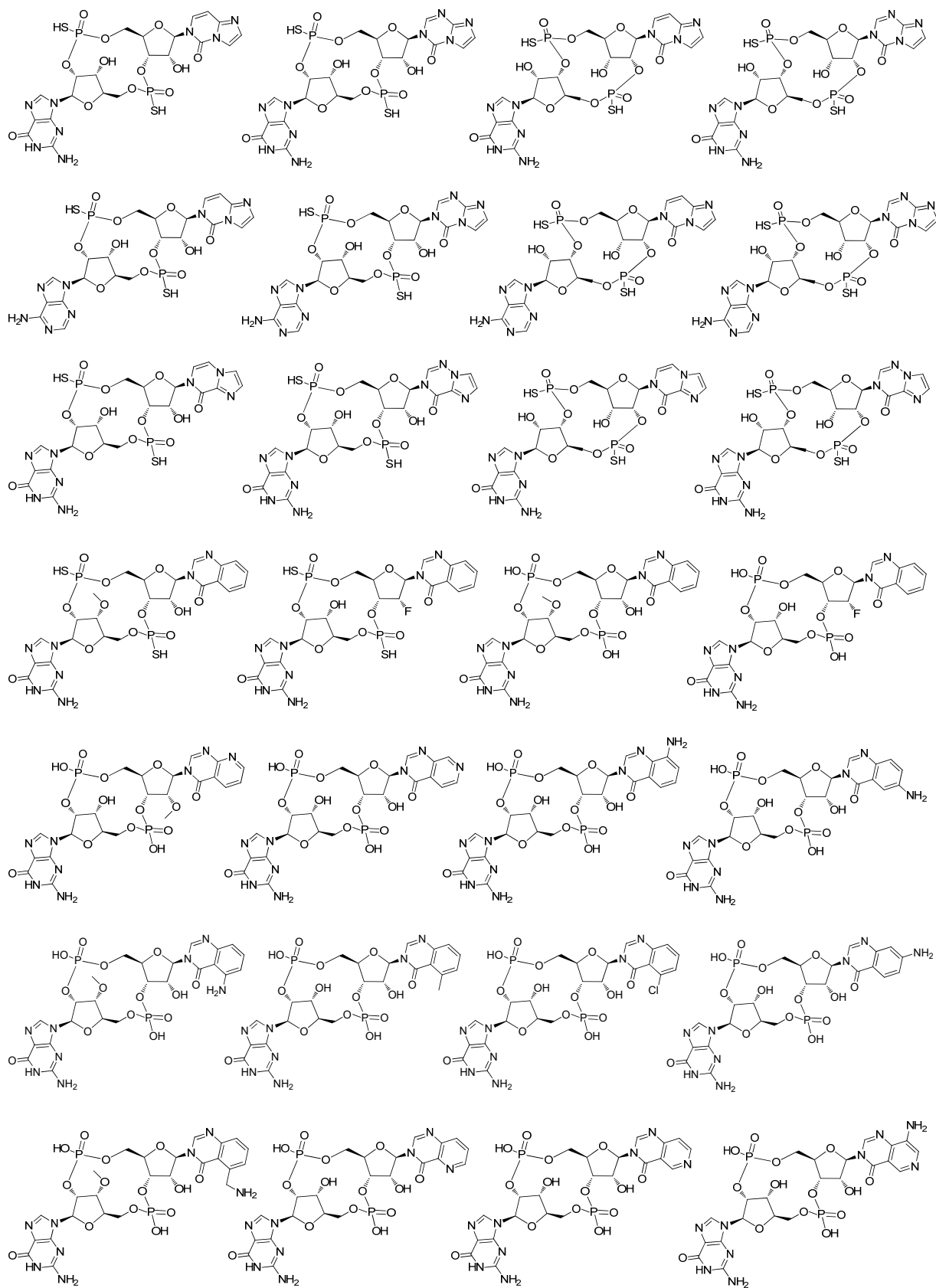


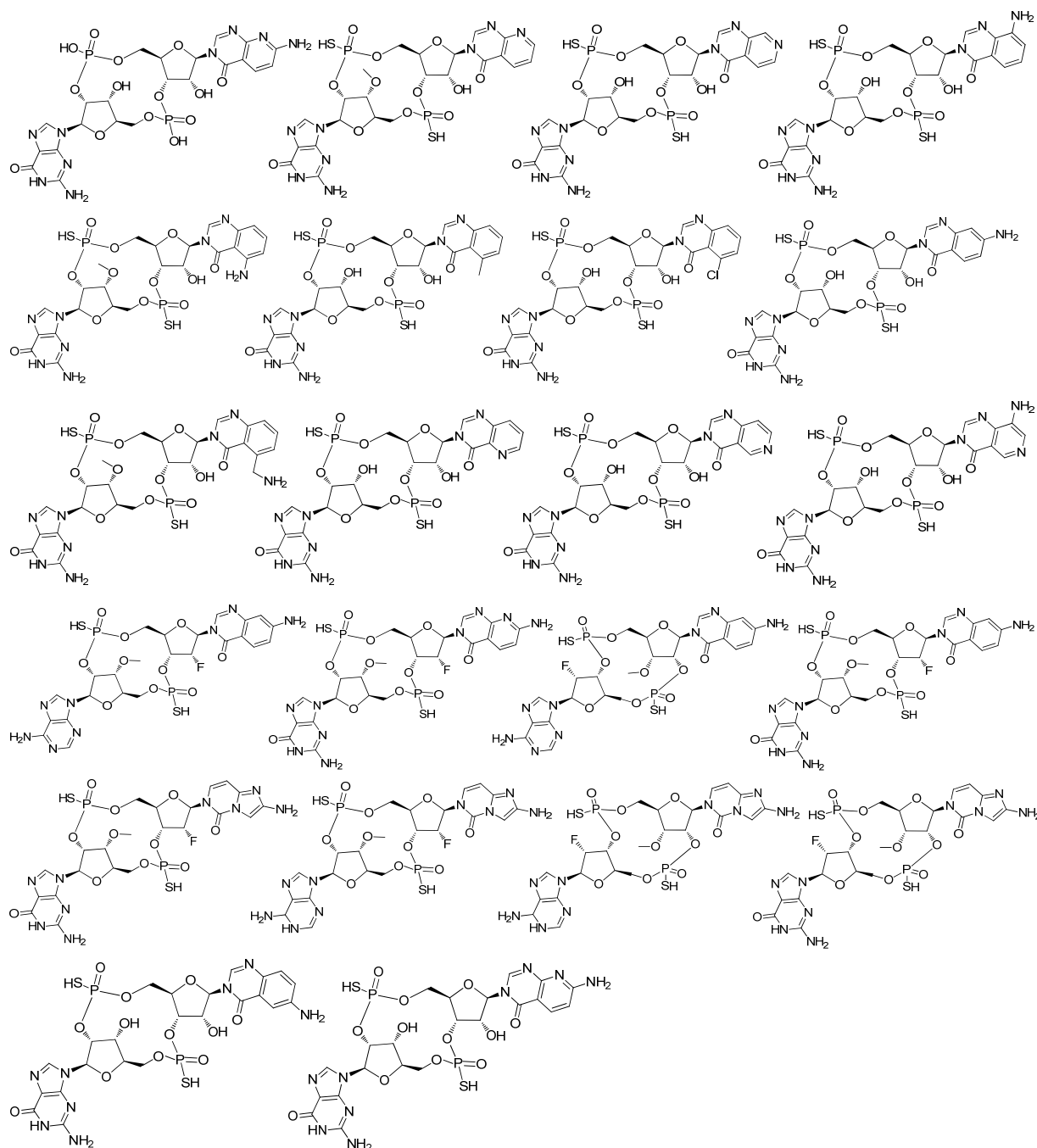












10. A pharmaceutical composition comprising a therapeutically effective amount of the compound of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to at least one of claims 1 to 9, and a pharmaceutically acceptable excipient.

11. The compound of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to any of claims 1 to 9 or the

pharmaceutical composition according to claim 10, comprised in a STING modulator.

12. The compound of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to claim 11 or the pharmaceutical composition according to claim 11, wherein the STING modulator is a STING agonist.

13. A use of the compound of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to at least one of claims 1 to 9 or the pharmaceutical composition according to claim 10, in the preparation of a medicament for the treatment, alleviation and/or prevention of a STING-mediated disease, wherein the STING-mediated disease is viral infection or other infectious diseases, autoimmune diseases, or malignancies.

14. A use of the compound of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to at least one of claims 1 to 9 or the pharmaceutical composition according to claim 10 in the preparation of medicament for the treatment and/or alleviation of malignancies.

15. The compound of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to at least one of claims 1 to 9 or the pharmaceutical composition according to claim 10 comprised in a vaccine adjuvant.

16. Use of a combination formulation, wherein the combination formulation comprises a compound of formula (I), the stereoisomer, stable isotope derivative or pharmaceutically acceptable salt thereof according to at least one of claims 1 to 9 or the pharmaceutical composition according to claim 10 is in combination with other kinds of therapeutic agents, in the manufacture of a medicament for the treatment of cancer.