Supporting information for

GPT-Driven Generation and Biological Activity Evaluation of Novel mRNA Trinucleotide Cap1 Analogs with Modified Ribose†

Honglei Zhang[a,‡], Jingxuan Ma [a,‡], Teng Ma[a], Yuqing Ma[a], Lijie Jin[a], Lijie Liu[a], Yangjian Liu[a], Kai Dong[a], Man Zhang[a], Dawei Huang[a], Fei Yu[a], Gengshen Song[a,*]

[a] Beijing Youcare Kechuang Pharmaceutical Technology Co., Ltd., Beijing 100176, China

‡ These authors contributed equally to this work

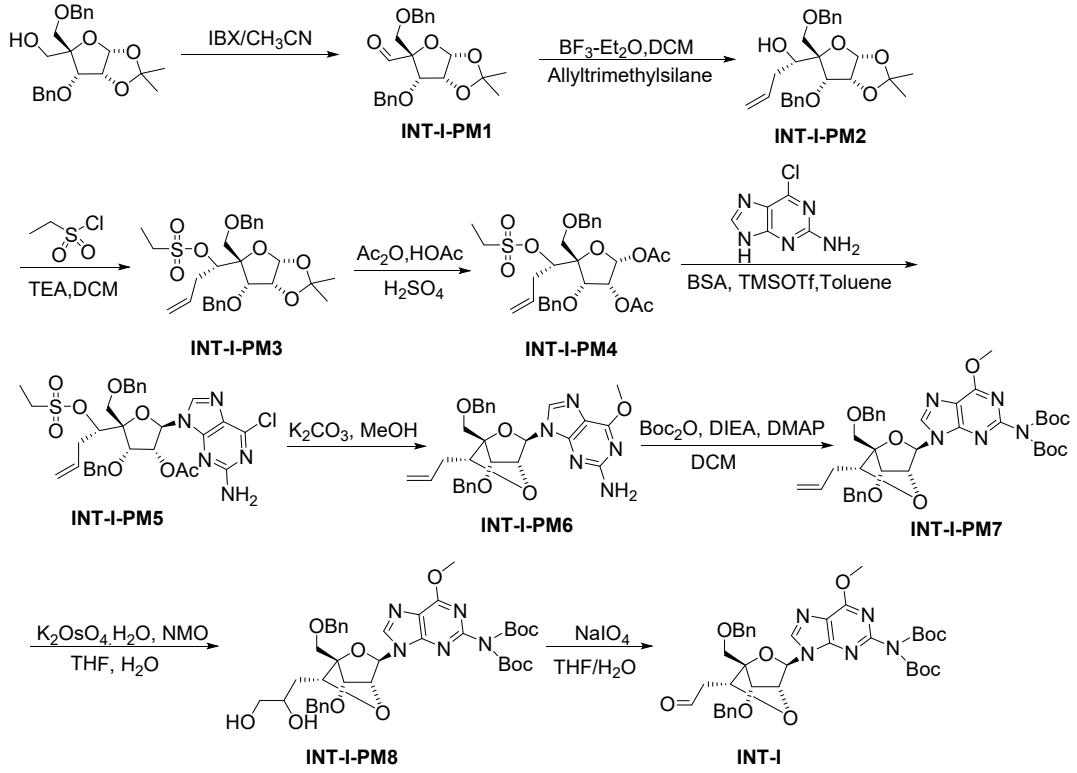
* Correspondence to: G.S. songgengshen@youcareyk.com

Abbreviation

IBX: 2-iodoacylbenzoic acid; $BF_3{\cdot}Et_2O$: boron trifluoride diethyl etherate; Allyltrimethylsilane: allyltrimethylsilane; TEA: triethylamine; Ac_2O: acetic anhydride; HOAc: acetic acid; conc H_2SO_4: concentrated sulfuric acid; BSA: *N,O*-bis(trimethylsilyl)acetamide; TMSOTf: Trifluoromethanesulfonate trimethylsilane; Toluene: Toluene; MeOH: Methanol; $(Boc)_2O$: Di-tert-butyl dicarbonate; DIEA: *N,N*-Diisopropylethylamine; DMAP: 4-Dimethylaminopyridine; DMSO: Dimethyl sulfoxide; HATU: 2-(7-Azabenzotriazol)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; THF: Tetrahydrofuran; TBSCl: *tert*-Butyldimethylchlorosilane; Imidazole: Imidazole; DMF: *N,N*-Dimethylformamide; TBAF: *tetra-n*-butylammonium fluoride; TBSOTf: *tert*-Butyldiphenylsilane triflate; NMO: *N*-Methylmorpholine *N*-oxide; *m*-CPBA: *meta*-Chloroperbenzoic acid; DIAD: Diisopropyl azodicarboxylate; NCS: *N*-Chlorosuccinimide; $PO(MeO)_3$: Triethyl phosphate; PySSPy: 2,2'-Dipyridyldisulfide; imidazole: Imidazole; PPh_3: Triphenylphosphine; TEAP: Triethylamine phosphate; TEAB: Triethylammonium bicarbonate; MTBE: Methyl *tert*-butyl ether; DCM: Dichloromethane; EA: Ethyl acetate; DAST: Diethylamine sulfonotriflate; AcSH: Thioacetic acid.

Chemical Synthesis

1. Synthesis of intermediate INT-I



Step 1: Synthesis of INT-I-PM1

1,2-O-(1-methylethylidene)-4-C-[(phenylmethoxy)methyl]-3-O-(phenylmethyl)-L-Lyxofuranose (100.0 g, 0.25 mol) was dissolved in acetonitrile (500 mL), and then IBX (104.9 g, 0.37 mol) was added to the solution and stirred at 70 ºC for 2 h. After cooling to room temperature, the mixture was filtrated and acetonitrile was removed by rotary evaporation under vacuum to obtain INT-I-PM1 (102.1 g, > 100%), which was used directly in the next step.

Step 2: Synthesis of INT-I-PM2

INT-I-PM1 (102.1 g, 0.25 mol) was dissolved in DCM (400 mL), and $BF_3 \cdot Et_2O$ (50.82 g, 0.35 mol) was slowly added to the solution at -40 ºC under N_2 and stirred for 5 minutes. Then allyltriethylsilane (52.9 mL, 0.33 mol) was slowly added to the solution and the mixture was stirred for 2 h. Upon completion, the reaction was quenched with saturated sodium bicarbonate aqueous solution (1000 mL) and extracted with DCM (500 mL × 2). The combined organic layers were dried by anhydrous sodium sulfate and evaporated under reduced pressure to obtain INT-I-PM2 (103.0 g, 0.23 mol).

Step 3: Synthesis of INT-I-PM3

INT-I-PM2 (103.0 g, 0.23 mol) and TEA (70.9 g, 0.70 mol) was dissolved in DCM (500 mL), and ethyl chlorosulfonate (60.4 g, 0.47 mol) was dropwise added to the solution at 0 ºC. The mixture was stirred at room temperature for 2 h. Upon completion, the reaction was quenched with saturated sodium bicarbonate aqueous solution (1000 mL) and extracted with DCM (500 mL × 2). The combined organic layers were dried by anhydrous sodium sulfate and evaporated under reduced pressure. INT-I-PM3 (106.0 g, 0.20 mol, 86.5%) was purified by silica gel chromatography.

Step 4: Synthesis of INT-I-PM4

INT-I-PM3 (106.0 g, 0.20 mol) was dissolved in HOAc (200 mL), and then Ac_2O (125.6 g, 1.23 mol) and H_2SO_4 (2.4 mL) was added to the solution. The mixture was stirred at room temperature for 1 h. Upon completion, the reaction was quenched with EA (800 mL) and water (800 mL), and then extracted with EA (800 mL). The combined organic phase was washed with water (800 mL × 3), saturated sodium bicarbonate solution (800 mL) and saturated brine (800 mL), dried by anhydrous sodium sulfate, and evaporated under reduced pressure to obtain INT-I-PM4 (86.5 g, 0.15 mol, 75.0%).

Step 5: Synthesis of INT-I-PM5

6-Chloroguanine (30.50 g, 0.18 mol) was added to toluene (250 mL), and then BSA (61.00 g, 0.30 mol) was added to the solution, the reaction mixture was stirred at 80 ℃ to dissolve. A solution of INT-I-PM4 (86.5 g, 0.15 mol) in toluene (150 mL) was added to the above mixture at room temperature and stirring for 5 min, and then TMSOTf (40.00 g, 0.18 mol) was added to the solution. The reaction mixture was stirred at 110 ºC for 3 h. Upon completion, the reaction was quenched with EA (300 mL) and saturated sodium bicarbonate aqueous solution (500 mL), filtered and extracted with EA (500 mL). The combined organic layers were dried by anhydrous sodium sulfate and evaporated under reduced pressure. INT-I-PM5 (103.02 g, 0.15 mol, 100%) was purified by silica gel chromatography. $C_{32}H_{36}ClN_5O_8S$, MS(ES): (m/z) $[M + H]^+$ = 686.2.

Step 6: Synthesis of INT-I-PM6

INT-I-PM5 (103.00 g, 0.15 mol) was dissolved in methanol (500 mL), and potassium carbonate (103.73 g, 0.75 mol) was added to the solution and the reaction mixture was stirred at room temperature for 23 h. Upon completion, the mixture was filtrated and methanol was removed by rotary evaporation under vacuum. The residue was extracted with EA (800 mL) and water (500 mL), and then the aqueous phase was extracted with EA (300 mL). The combined organic layers were dried by anhydrous sodium sulfate and evaporated under reduced pressure. INT-I-PM6 (44.03 g, 0.083 mol, 55.4%) was purified by silica gel chromatography. $C_{29}H_{31}N_5O_5$, MS(ES): (m/z) $[M + H]^+$ = 530.2.

Step 7: Synthesis of INT-I-PM7

INT-I-PM6 (44.00 g, 0.083 mol) was dissolved in DCM (500 mL), and DIEA (32.15 g, 0.24 mol), DMAP (1.01 g, 0.01 mol) and $(Boc)_2O$ (54.45 g, 0.24 mol) were added to the solution. The reaction mixture was stirred at 30 ºC overnight and evaporated under reduced pressure. INT-I-PM7 was purified by silica gel chromatography (49.81 g, 68.25 mmol, 82.2%). $C_{39}H_{47}N_5O_9$, MS(ES): (m/z) $[M + H]^+$ = 730.2.

Step 8: Synthesis of INT-I-PM8

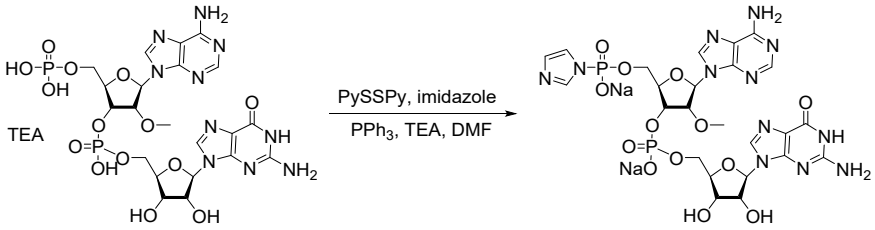
INT-I-PM7 (49.81 g, 68.25 mmol) was dissolved in THF (400 mL) and H_2O (100 mL), and NMO (12.00 g, 102.37 mmol) and potassium osmate dihydrate (1.26 g, 3.41 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 5 h and quenched with saturated sodium sulfite, extracted with EA (500 mL × 2). The combined organic layers were washed with saturated brine (200 mL), dried by anhydrous sodium sulfate and evaporated under reduced pressure to abtain INT-I-PM8 (52.00 g, 68.08 mmol, 99.8%). $C_{39}H_{49}N_5O_{11}$, MS(ES): (m/z) $[M + H]^+$ = 764.3.

Step 8: Synthesis of INT-I

INT-I-PM8 (52.00 g, 68.08 mmol) was dissolved in THF (500 mL) and H_2O (125 mL), and

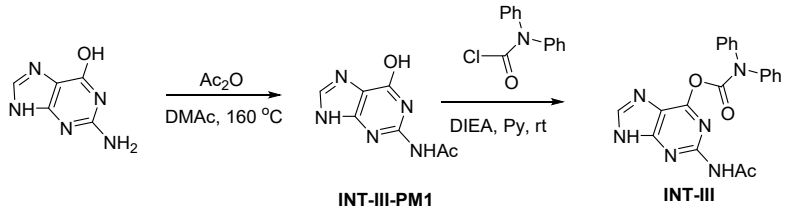
sodium periodic (21.84 g, 102.11 mmol) was added to the solution. The reaction mixtrue was stirred at 30 ºC overnight and quenched with saturated sodium sulfite, extracted with EA (500 mL × 3). The combined organic layers were washed with saturated brine, dried by anhydrous sodium sulfate and evaporated under reduced pressure to obtain INT-I (45.5 g, 62.2 mmol, 91.3%). $C_{38}H_{45}N_5O_{10}$, MS(ES): (m/z) $[M + H]^+$ = 732.3.

2. Synthesis of intermediate INT-II



pA(2'-OMe)mpG•TEA (300.1 mg, 0.37 mmol), Imidazole (347.2 mg, 5.10 mmol), PySSPy (1123.6 mg, 5.10 mmol) and TEA (516.1 mg, 5.10 mmol) were dissolved in 2.0 mL ultra-dried DMF, PPh_3 (1337.7 mg, 5.10 mmol) was added to the solution under nitrogen. The resulting mixture was stirred at 25 ºC for 4 h and slowly added to a pre-cooled acetone solution of NaI (598.1 mg, 3.99 mmol) and crystallize at 25 ºC for 30 mins. The white solid INT-II (240.6 mg, 0.30 mmol, 80.9 %) was obtained after centrifugation. $C_{24}H_{30}N_{12}O_{13}P_2$, MS(ES): (m/z) $[M - H]^-$ = 755.2.

3. Synthesis of intermediate INT-III



Step 1: Synthesis of INT-III-PM1

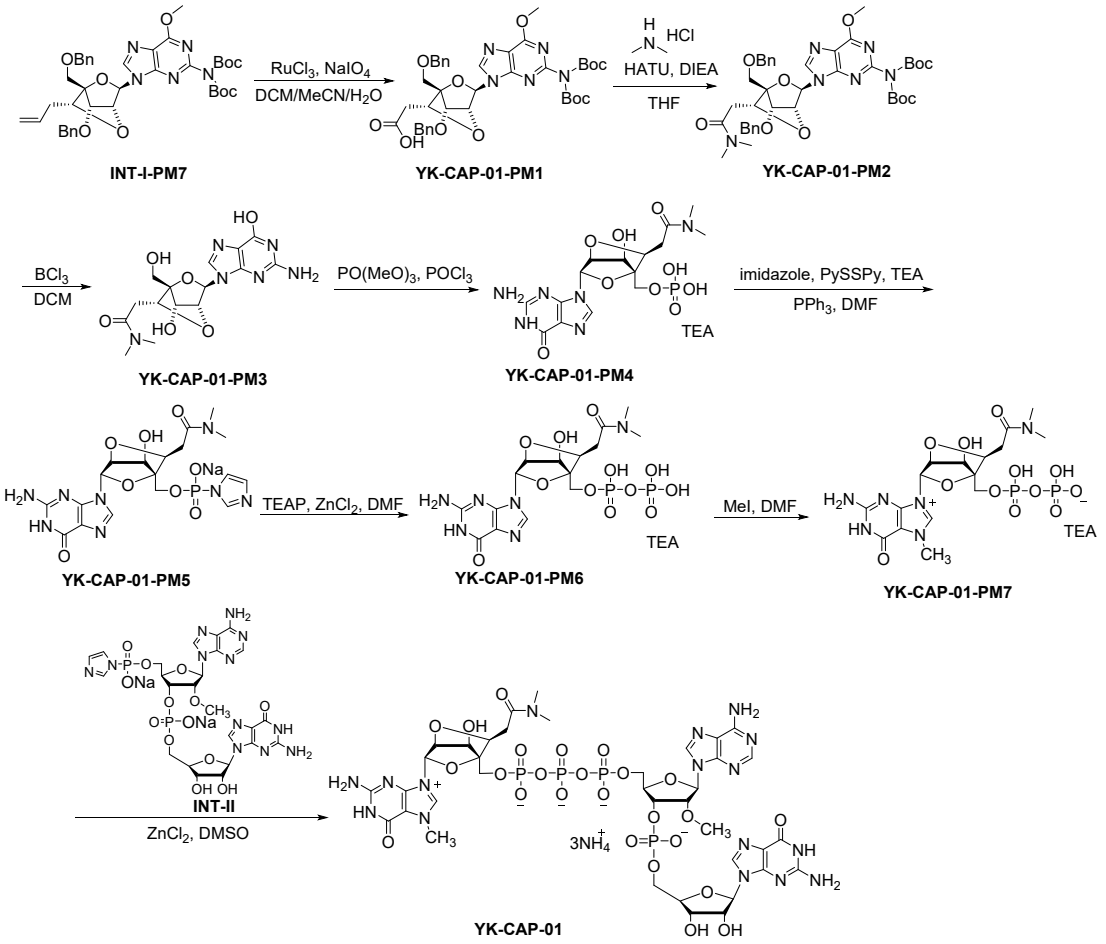
2-amino-9H-purine-6-ol (50.0 g, 0.33 mol) was dissolved in 500 mL N, N-Dimethylacetamide. Subsequently, Ac_2O (100 mL, 1.06 mol) was added to the solution. The mixture was then stirred at 160 ºC until the solution became clear, signifying the completion of the reaction. After the reaction system had naturally cooled to room temperature, the precipitated products were filtered out. The resulting filter cake was washed with ethanol to yield INT-III-PM1 (60.0 g, 0.31 mol, 94.1%). $C_7H_7N_5O_2$, MS(ES): (m/z) $[M + H]^+$ = 194.1.

Step 2: Synthesis of INT-III

INT-III-PM1 (60.0 g, 0.31 mol) was initially dissolved in 200 mL pyridine. Subsequently, DIEA (120.2 g, 0.93 mol) was introduced to the solution. A solution containing diphenylcarbamyl chloride (86.2 g, 0.37 mol) in 100 mL pyridine was then gradually added to the reaction mixture at 0 ºC. The reaction mixture was allowed to warm to room temperature and stirred for an additional 3 h, as monitored by liquid chromatography-mass spectrometry (LCMS) to confirm the absence of starting materials. The reaction was quenched with 100 mL water, concentrated under reduced pressure. The resulting residue was dissolved in a 1:1 mixture of ethanol and water (800 mL), heated to reflux for 2 h, and then cooled to room temperature. The precipitated solids were filtered out, washed with

ethanol, and dried to yield INT-III (59.7 g, 0.15 mol, 49.6%). $C_{20}H_{16}N_6O_3$, MS(ES): (m/z) $[M + H]^+$ = 389.1.

4. Synthesis of YK-CAP-01



Step 1: Synthesis of YK-CAP-01-PM1

INT-I-PM7(22.00 g, 30.14 mmol) was dissolved in a mixed solution of DCM (220 mL), acetonitrile (220 mL) and H_2O (330 mL), and then $NaIO_4$ (52.06 g, 241.12 mmol) and $RuCl_3$ (250 mg, 1.21 mmol) was added to the solution. The reaction was stirred at 30 ºC for 48 h and then concentrated by rotary evaporation under vacuum to remove solvents. The residue was diluted with EA (400 mL), washed with H_2O (120 mL × 3). The organic phase was dried over Na_2SO_4 and concentrated by rotary evaporation under vacuum. YK-CAP-01-PM1 (19.87 g, 26.58 mmol, 88.2%) was purified by silica gel column chromatography. $C_{38}H_{45}N_5O_{11}$, MS(ES): (m/z) $[M + H]^+$ = 748.3.

Step 2: Synthesis of YK-CAP-01-PM2

YK-CAP-01-PM1 (19.87 g, 26.58 mmol) was dissolved in THF (200 mL), and then dimethylamine hydrochloride (2.38 g, 29.23 mmol), HATU (12.13 g, 31.90 mmol) and DIEA (10.31 g, 79.74 mmol) were added to the solution. The resulting mixture was stirred at room temperature overnight and then concentrated by rotary evaporation under vacuum to remove solvent. The residue was diluted with EA (400 mL), washed with H_2O (120 mL × 3). The organic phase was dried over Na_2SO_4 and concentrated by concentrated by rotary evaporation under vacuum. The YK-CAP-01-PM2 (13.10 g, 16.91 mmol, 63.6%) was purified by silica gel column chromatography. $C_{40}H_{50}N_6O_{10}$, MS(ES): (m/z) $[M + H]^+$ = 775.3.

Step 3: Synthesis of YK-CAP-01-PM3

YK-CAP-01-PM2 (13.0 g, 16.78 mmol) was dissolved in DCM (195 mL), and then 1 M BCl_3 solution in DCM (134 mL, 134 mmol) was slowly added to the solution at -40 ºC under N_2. The resulting mixture was stirred at 0 ºC for 6 h. The reaction was quenched with MeOH (200 mL) and concentrated by rotary evaporation under vacuum, and kept at room temperature for 24 h. The crude product was diluted with DCM (500 mL) and filtered to obtain crude brown products, which were purified by preparative HPLC to obtain YK-CAP-01-PM3 (956.04 mg, 2.51 mmol, 15.0%). $C_{15}H_{20}N_6O_6$, MS(ES): (m/z) $[M + H]^+$ = 381.32.

^1H NMR (400 MHz, CD_3OD) δ 7.97 (s, 1H), 5.84 (s, 1H), 4.70 - 4.63 (m, 1H), 4.45 - 4.38 (m, 2H), 3.91 - 3.75 (m, 2H), 3.15 (s, 3H), 2.99 - 2.70 (m, 5H).

Step 4: Synthesis of YK-CAP-01-PM4

YK-CAP-01-PM3 (956.04 mg, 2.51 mmol) was dissolved in $PO(MeO)_3$ (10 mL), $POCl_3$ (1.69 g, 10.37 mmol) was slowly added to the solution at 0 ºC under N_2. The resulting mixture was stirred at 0 ºC for 4 h. The reaction mixture was quenched with 10 mL H_2O, stirred at 0 ºC for 1 h, washed by DCM (10 mL × 3). The upper aqueous phase was separated and concentrate under reduced pressure. The residue was diluted with H_2O (200 mL), and YK-CAP-01-PM4 (TEA salt, 921.3 mg, 1.64 mmol, 65.3%) was purified by ion-exchange chromatography on DEAE Sephadex. $C_{15}H_{21}N_6O_9P$, MS(ES): (m/z) $[M - H]^-$ = 459.11.

Step 5: Synthesis of YK-CAP-01-PM5

YK-CAP-01-PM4 (921.3 mg, 1.64 mmol), Imidazole (1.67 g, 24.6 mmol), PySSPy (1.19 g, 24.6 mmol), TEA (2.48 g, 24.6 mmol), and PPh_3 (6.45 g, 24.6 mmol) were dissolved in extra dried DMF (10 mL). The reaction mixture was stirred at room temperature for 4 h under N_2, and then poured into a solution of NaI (4.43 g, 29.61 mmol) in acetone (30 mL), stirred at room temperature for 30 minutes, then centrifuged to obtain the crude product as a precipitate, which was washed with acetone, centrifuged, and lyophilized to obtain YK-CAP-01-PM5 (Na salt, 510.3 mg, 0.96 mmol, 58.4%). $C_{18}H_{23}N_8O_8P$, MS(ES): (m/z) $[M - H]^-$ = 509.12.

Step 6: Synthesis of YK-CAP-01-PM6

YK-CAP-01-PM5 (510.3 mg, 0.96 mmol) and TEAP (668 mg, 3.36 mmol) were dissolved in extra dried DMF (10 mL), and $ZnCl_2$ (313 mg, 2.30 mmol) was added to the solution. The resulting mixture was stirred at room temperature for 21 h under N_2, and then MTBE (30 mL) was added to the reaction system, which was then ultrasonically stirred. The supernatant was decanted, and the bottom material was collected and concentrated under reduced pressure. YK-CAP-01-PM6 (TEA salt, 853.8 mg, 1.33 mmol, > 100%) was purified by ion-exchange chromatography on DEAE Sephadex. $C_{15}H_{22}N_6O_{12}P_2$, MS(ES): (m/z) $[M - H]^-$ = 539.08.

Step 7: Synthesis of YK-CAP-01-PM7

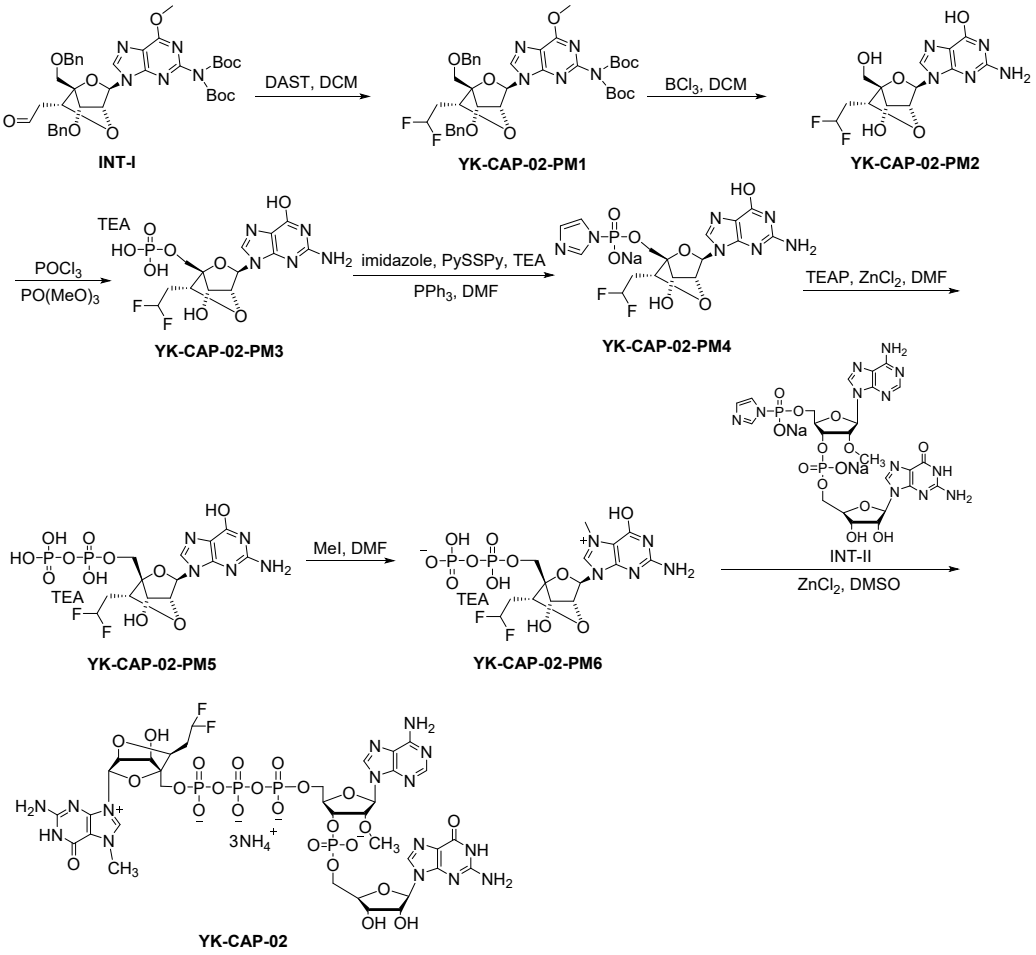
YK-CAP-01-PM6 (853.8 mg, 1.33 mmol) and CH_3I (2.26 g, 15.96 mmol) were dissolved in dry DMF (10 mL) and stirred at 37 ºC for 23 h. H_2O (20 mL) was added to the reaction system, washed with EA (60 mL), and the aqueous layer was separated, collected and concentrated under reduced pressure. YK-CAP-01-PM7 (TEA salt, 170.0 mg, 0.26 mmol, 19.2%) was purified by ion-exchange chromatography on DEAE Sephadex and reversed-phase high-performance liquid chromatography. $C_{16}H_{24}N_6O_{12}P_2$, MS(ES): (m/z) $[M - H]^-$ = 553.09.

Step 9: Synthesis of YK-CAP-01

YK-CAP-01-PM7 (170.0 mg, 0.26 mmol) and INT-II (332 mg, 0.44 mmol) were dissolved in extra dried DMSO (2 mL), and $ZnCl_2$ (842 mg, 6.17 mmol) was added to the solution. The

resulting mixture was stirred at 37 ºC for 3 days under N_2, and then EDTA solution (0.25 M) was added to the reaction system, which pH was adjusted to 6 ~ 7 with 1.5 M TEAB. YK-CAP-01 (20.3 mg, 15.7 μmol, 6.0%) was purified by ion-exchange chromatography on DEAE Sephadex and preparative HPLC. $C_{37}H_{50}N_{16}O_{25}P_4$, MS(ES): (m/z) $[M - H]^-$ = 1241.19. ^{1}H NMR (400 MHz, D_2O) δ 8.80 (s, 1H), 8.28 (s, 1H), 7.99 (s, 1H), 7.84 (s, 1H), 5.91 (d, *J* = 5.2 Hz, 1H), 5.73 - 5.70 (m, 2H), 4.79 - 4.83 (m, 2H), 4.58 (t, *J* = 5.2 Hz, 2H), 4.50 (s, 1H), 4.37 - 4.42 (m, 3H), 4.31 (t, *J* = 4.8 Hz, 1H), 4.24 - 4.28 (m, 4H), 4.09 - 4.13 (m, 2H), 3.93 (s, 3H), 3.37 (s, 3H) , 3.01 (s, 3H), 2.84 (s, 3H), 2.82 - 2.75 (m, 1H), 2.65 - 2.70 (m, 1H); ^{31}P NMR (162 MHz, D_2O) δ -1.02, -10.96, -11.47, -22.56 (4P).

5. Synthesis of YK-CAP-02



Step 1: Synthesis of YK-CAP-02-PM1

INT-I (12.65 g, 17.29 mmol) was dissolved DCM (70 mL), and a solution of DAST (8.36 g，51.86 mmol) in DCM was added to the solution at -40 °C under N_2. The reaction was stirred at 0 ºC for 4 h. The mixture was diluted by saturated sodium bicarbonate aqueous solution and extracted by DCM (200 mL × 3). The combined organic layers were dried by anhydrous sodium sulfate and filtered. The filtrate was concentrated under vacuum and YK-CAP-02-PM1 (7.92 g, 10.51 mmol, 60.89%) was purified by silica gel chromatography. $C_{38}H_{45}F_2N_5O_9$, MS(ES): (m/z) $[M + H]^+$ = 754.3.

Step 2: Synthesis of YK-CAP-02-PM2

Following the procedure described for YK-CAP-01-PM3, YK-CAP-02-PM2 (926.34 mg, 2.58 mmol, 48.68%) was made from YK-CAP-02-PM1 (4.00 g, 5.31 mmol). $C_{13}H_{15}F_2N_5O_5$, MS(ES):

(m/z) $[M + H]^+$ = 360.1.

^{1}H NMR (400 MHz, MeOD) δ 7.94 (s, 1H), 6.34 - 5.94 (m, 1H), 5.86 (s, 1H), 4.62 (s, 1H), 4.44 (dd, *J* = 24.4, 8.8 Hz, 3H), 3.99 - 3.84 (m, 2H), 2.48 - 2.08 (m, 2H)

Step 3: Synthesis of YK-CAP-02-PM3

Following the procedure described for YK-CAP-01-PM4, YK-CAP-02-PM3 (1.48 g, 2.74 mmol, > 100%) was made from YK-CAP-02-PM2 (926.34 mg, 2.58 mmol). $C_{13}H_{16}F_2N_5O_8P$, MS(ES): (m/z) $[M - H]^-$ = 438.07.

Step 4: Synthesis of YK-CAP-02-PM4

Following the procedure described for YK-CAP-01-PM5, YK-CAP-02-PM4 (771.8 mg, 1.51 mmol, 57.7%) was made from YK-CAP-02-PM3 (1.48 g, 2.74 mmol). $C_{16}H_{18}F_2N_7O_7P$, MS(ES): (m/z) $[M - H]^-$ = 488.08.

Step 5: Synthesis of YK-CAP-02-PM5

Following the procedure described for YK-CAP-01-PM6, YK-CAP-02-PM5 (1.22 g, 1.97 mmol, > 100%) was made from YK-CAP-02-PM4 (771.8 mg, 1.51 mmol). $C_{13}H_{17}F_2N_5O_{11}P_2$, MS(ES): (m/z) $[M - H]^-$ = 518.04.

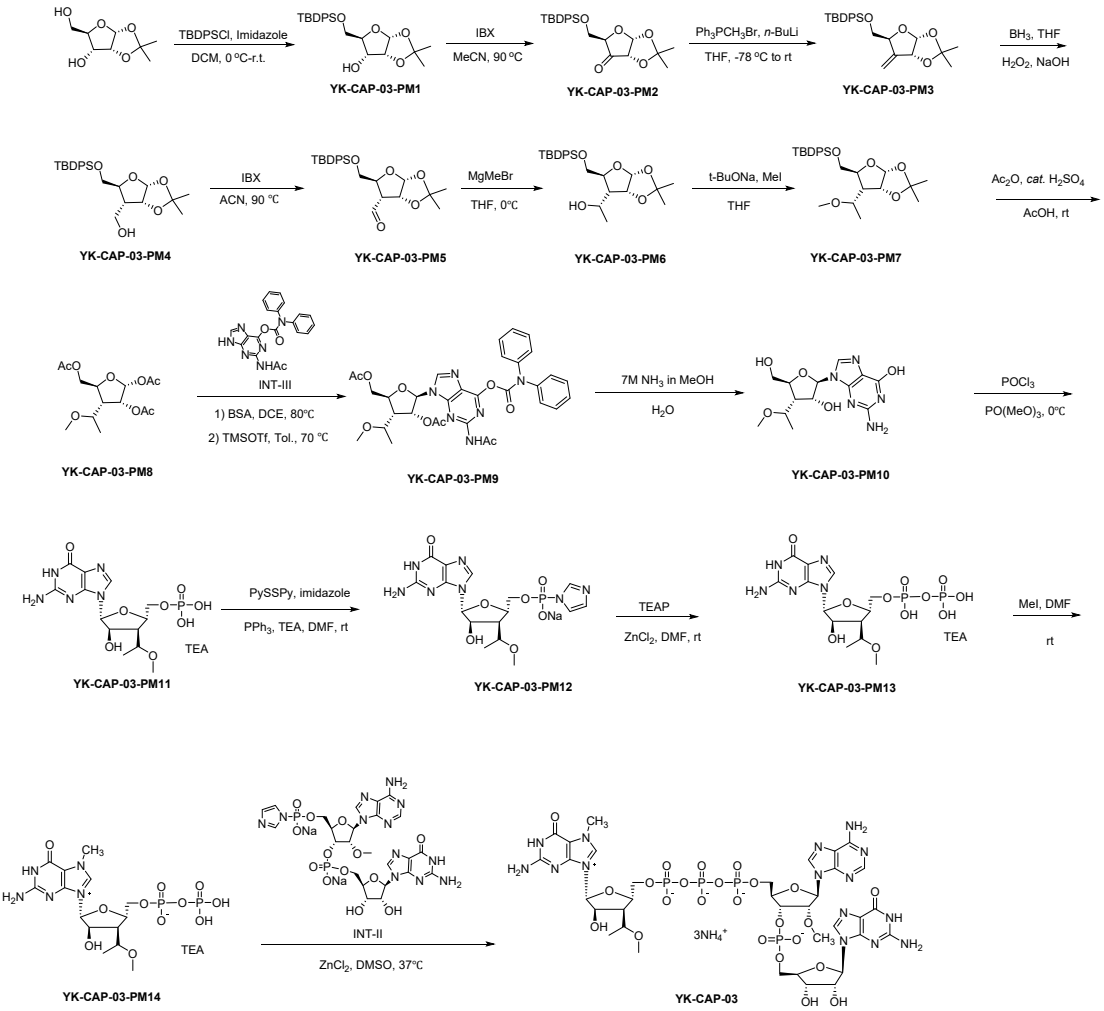
Step 6: Synthesis of YK-CAP-02-PM6

Following the procedure described for YK-CAP-01-PM7, YK-CAP-02-PM6 (172.6 mg, 0.27 mmol, 13.6%) was made from YK-CAP-02-PM5 (1.22 g, 1.97 mmol). $C_{14}H_{19}F_2N_5O_{11}P_2$, MS(ES): (m/z) $[M - H]^-$ = 532.05.

Step 7: Synthesis of YK-CAP-02

Following the procedure described for YK-CAP-01, YK-CAP-02 (22.3 mg, 17.5 µmol, 6.4%) was made from YK-CAP-02-PM6 (172.6 mg, 0.27 mmol). $C_{35}H_{42}F_2N_{15}O_{24}P_4$, MS(ES): (m/z) $[M - H]^-$ = 1220.15. ^{1}H NMR (400 MHz, D_2O) δ 8.88 (s, 1H), 8.43 (s, 1H), 8.14 (s, 1H), 7.91 (s, 1H), 6.28 - 5.95 (m, 2H), 5.98 (d, *J* = 6.0 Hz, 2H), 4.91 - 4.84 (m, 1H), 4.70 - 4.66 (m, 2H), 4.54 (s, 1H), 4.50 - 4.44 (m, 3H), 4.43 - 4.40 (m, 1H), 4.39 - 4.34 (m, 1H), 4.32 - 4.24 (m, 4H), 4.23 - 4.15 (m, 1H), 4.14 - 4.09 (m, 2H), 3.96 (s, 3H), 3.39 (s, 3H), 3.17 - 2.95 (m, 1H); ^{31}P NMR (162 MHz, D_2O) δ -0.90, -11.03, -11.59, -22.83 (4P).

6. Synthesis of YK-CAP-03



Step 1: Synthesis of YK-CAP-03-PM1

1,2-O-isopropylidene-α-D-ribofuranose (20.0 g, 0.11 mol) was dissolved in 200 mL DCM, then imidazole (11.6 g, 0.17 mol) and TBDPSCl (33.0 g, 0.12 mol) were added to the solution. The reaction was stirred at room temperature for 15 h. The reaction solution was poured into saturated $NaHCO_3$ solution, extracted with DCM, washed with saturated NaCl solution, dried with Na_2SO_4, filtered, concentrated by rotary evaporation under vacuum. The residue was purified by silica gel chromatography (0-17% ethyl acetate/hexane) to obtain YK-CAP-03-PM1 (33.5 g, 78.16 mmol, 71.1%).

Step 2: Synthesis of YK-CAP-03-PM2

YK-CAP-03-PM1 (33.5 g, 78.16 mmol) was dissolved in 300 mL ACN, then IBX (28.5 g, 101.8 mmol) was added to the solution. The resulting mixture was stirred at 90 ºC for 5 h. The reaction solution was filtered, concentrated by rotary evaporation under vacuum to obtain YK-CAP-03-PM2 (32.7 g, 76.66 mmol, 98.1%).

Step 3: Synthesis of YK-CAP-03-PM3

Ph_3PCH_3Br (60.3 g, 138.3 mmol) was dissolved in 400 mL THF, then 2.5M n-BuLi in THF solution (76 mL, 190 mmol) was slowly added at -78 ºC. The reaction system was stirred at 0 ºC for 2 h. The THF (100 mL) solution of YK-CAP-03-PM2 (48.0 g, 112.52 mmol) was slowly added at -78 ºC. The reaction system was stirred at room temperature overnight. The reaction system was quenched by 200 mL saturated NH_4Cl solution, extracted with EA (300 mL × 3),

the organic phase was combined, washed with saturated NaCl solution, dried with Na_2SO_4, filtered, and concentrated by rotary evaporation under reduced pressure. The residue was purified by silica gel chromatography (0-20% ethyl acetate/hexane) to obtain YK-CAP-03-PM3 (39.0 g, 91.85 mmol, 81.6%).

Step 4: Synthesis of YK-CAP-03-PM4

1 M BH_3 in THF solution (325 mL, 0.325 mol) was added to a three-necked flask, and the THF (180 mL) solution of YK-CAP-03-PM3 (60.00 g, 0.14 mol) was slowly added to the solution at 0 ºC. The resulting mixture was stirred at room temperature for 2 h. THF/H_2O (1:1, 120 mL), 2 N NaOH (261 mL), and 30% H_2O_2 solution (271 mL) were sequentially and slowly added to the reaction system at 0 ºC. The resulting mixture was stirred at room temperature overnight, then extracted with H_2O. The organic phase was washed sequentially with saturated $Na_2S_2O_3$ solution and saturated NaCl solution, dried with Na_2SO_4, filtered, concentrated by rotary evaporation. The residue was purified by silica gel chromatography (0-25% ethyl acetate/hexane) to obtain a colorless oily compound YK-CAP-03-PM4 (48.80 g, 0.11 mol, 78.8%).

Step 5: Synthesis of YK-CAP-03-PM5

YK-CAP-03-PM4 (34.20 g, 77.27 mmol) was dissolved in ACN, IBX (28.10 g, 100.5 mmol) was added to the solution. The resulting mixture was stirred at 90 ºC for 5 h. The reaction mixture was filtered after cooling to room temperature, the filtrate was concentrated by rotary evaporation to obtain crude product YK-CAP-03-PM5 (34.50 g) as pale-yellow oil, which was used in next step without further purification.

Step 6: Synthesis of YK-CAP-03-PM6

YK-CAP-03-PM5 (32.90 g, 74.67 mmol) was dissolved in THF, then 1 M CH_3BrMg in THF solution (97.1 mL, 97.1 mmol) was slowly added to the solution at 0 ºC under N_2. The resulting mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with saturated NH_4Cl solution at 0 ºC, extracted with EA. The organic phase was dried with Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (0-41% ethyl acetate/hexane) to obtain light yellow oil YK-CAP-03-PM6 (16.80 g, 36.79 mmol, 49.3%).

Step 7: Synthesis of YK-CAP-03-PM7

YK-CAP-03-PM6 (16.80 g, 36.79 mmol) was dissolved in THF, the THF solution of *t*-BuONa (11.20 g, 116.7 mmol) was slowly added to the solution at 0 ºC under N_2. The resulting mixture was stirred at room temperature for 1.5 h. CH_3I (27.60 g, 194.5 mmol) was slowly added to the above system. The resulting mixture was stirred at room temperature for 3 h, and quenched with saturated NH_4Cl solution, extracted with EA. The organic phase was washed with saturated NaCl solution 3-5 times, dried with Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (0-12% ethyl acetate/hexane) to obtain light yellow oil YK-CAP-03-PM7 (13.16 g, 27.96 mmol, 76.0%).

Step 8: Synthesis of YK-CAP-03-PM8

YK-CAP-03-PM7 (13.16 g, 27.96 mmol) was dissolved in 130 mL HOAc, Ac_2O (17.13 g, 167.8 mmol) and H_2SO_4 (0.52 mL) was sequentially added to the solution. The resulting mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with EA, washed with H_2O once, saturated $NaHCO_3$ solution three times. The organic phase was dried, concentrated under by rotary evaporation reduced pressure to obtain yellow oil YK-CAP-03-PM8 (11.38 g), which was used directly in the next step.

Step 9: Synthesis of YK-CAP-03-PM9

INT-III (6.30 g, 16.22 mmol) was dissolved in 100 mL DCE, then BSA (6.60 g, 32.4 mmol) was added to the solution. The resulting mixture was stirred at 80 ºC for 1.5 h. The reaction solvent was removed by rotary evaporation under reduced pressure. A solution of YK-CAP-03-PM8 (6.00 g, 18.85 mmol) in 100 mL toluene and TMSOTf (3.6 g, 16.2 mmol) were sequentially added to the above system. The resulting mixture was stirred at 70 ºC for 3.5 h. The reaction mixture was diluted with EA and washed once with saturated $NaHCO_3$ solution. The insoluble material was filtered off, and the filtrate was separated. The organic phase was dried and then concentrated by rotary evaporation under reduced pressure. The crude product was purified by silica gel chromatography (0-52% ethyl acetate/ dichloromethane) to obtain a yellow solid YK-CAP-03-PM9 (3.20 g, 4.95 mmol, 26.3%). $C_{32}H_{34}N_6O_9$, MS(ES): (m/z) $[M + H]^+$ = 647.2.

Step 10: Synthesis of YK-CAP-03-PM10

YK-CAP-03-PM9 (3.20 g, 4.95 mmol) was dissolved in 7M NH_3 in MeOH solution and H_2O (5:1, 24 mL), then stirred at 50 ºC for 6 h. The reaction mixture was concentrated by rotary evaporation under reduced pressure, stirred with EA, filtered. The filter cake was dried to obtain a white solid YK-CAP-03-PM10 (1.38 g, 4.24 mmol, 85.7%). $C_{13}H_{19}N_5O_5$, MS(ES): (m/z) $[M + H]^+$ = 326.2.

^{1}H NMR (400 MHz, MeOD) δ 8.07 (s, 1H), 5.82 (d, *J* = 2.6 Hz, 1H), 4.53 (dd, *J* = 6.1, 2.7 Hz, 1H), 4.36 (ddd, *J* = 8.3, 3.9, 2.6 Hz, 1H), 3.95 (dd, *J* = 12.1, 2.4 Hz, 1H), 3.78 -3.67 (m, 2H), 3.34 (s, 3H), 2.11 (q, *J* = 7.4 Hz, 1H), 1.23 (d, *J* = 6.1 Hz, 3H).

Step 11: Synthesis of YK-CAP-03-PM11

Following the procedure described for YK-CAP-01-PM4, YK-CAP-03-PM11 (TEA salt, 644 mg, 1.27 mmol, 34.4%) was made from YK-CAP-03-PM10 (1.20 g, 3.69 mmol). $C_{13}H_{20}N_5O_8P$, MS(ES): (m/z) $[M - H]^-$ = 404.1.

Step 12: Synthesis of YK-CAP-03-PM12

Following the procedure described for YK-CAP-01-PM5, YK-CAP-03-PM12 (Na salt, 549 mg, 1.15 mmol, 90.6%) was made from YK-CAP-03-PM11 (644 mg, 1.27 mmol). $C_{16}H_{22}N_7O_7P$, MS(ES): (m/z) $[M - H]^-$ = 454.2.

Step 13: Synthesis of YK-CAP-03-PM13

Following the procedure described for YK-CAP-01-PM6, YK-CAP-03-PM13 (TEA salt, 531 mg, 0.91 mmol, 78.7%) was made from YK-CAP-03-PM12 (549 mg, 1.15 mmol). $C_{13}H_{21}N_5O_{11}P_2$, MS (ES): (m/z) $[M - H]^-$ = 484.0.

Step 14: Synthesis of YK-CAP-03-PM14

Following the procedure described for YK-CAP-01-PM7, YK-CAP-03-PM14 (TEA salt, 163 mg, 0.27 mmol, 29.8%) was made from YK-CAP-03-PM13 (531 mg, 0.91 mmol). $C_{14}H_{23}N_5O_{11}P_2$, MS(ES): (m/z) $[M - H]^-$ = 498.1.

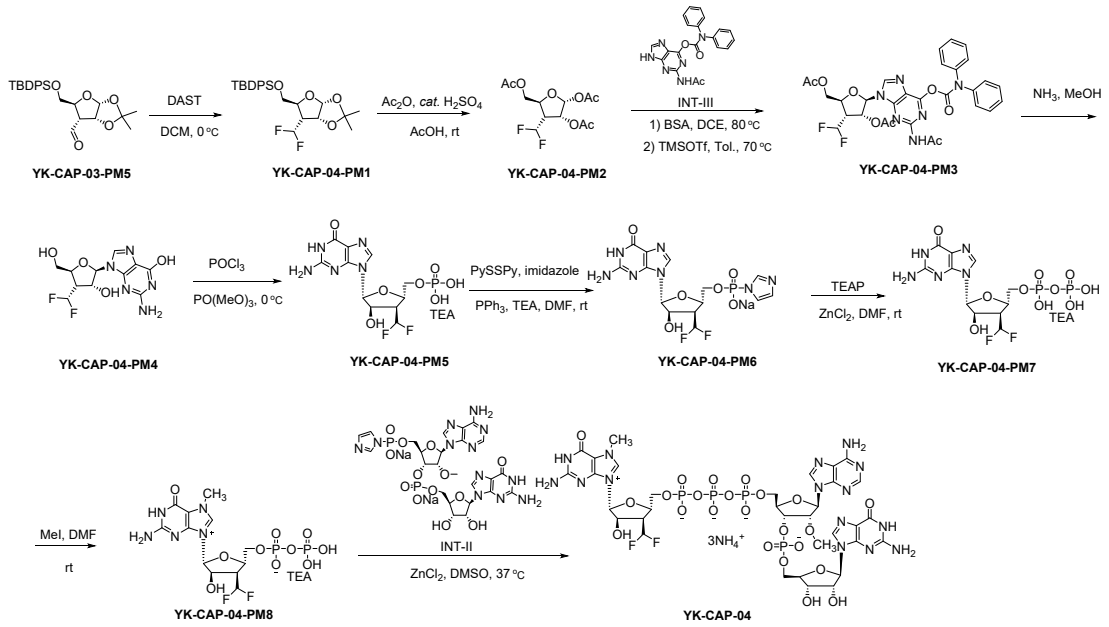
Step 15: Synthesis of YK-CAP-03

Following the procedure described for YK-CAP-01, YK-CAP-03 (35 mg, 28.25 µmol, 10.5%) was made from YK-CAP-03-PM11 (163 mg, 0.27 mmol). $C_{35}H_{49}N_{15}O_{24}P_4$, MS(ES): (m/z) $[M - H]^-$ = 1186.1.

^{1}H NMR (400 MHz, D2O) δ 9.09 (s, 1H), 8.43 (s, 1H), 8.17 (s, 1H), 7.89 (s, 1H), 6.03 (d, *J* = 4.4 Hz, 1H), 5.71 (d, *J* = 5.6 Hz, 1H), 5.68 - 5.64 (m, 1H), 4.90 - 4.83 (m, 1H), 4.67 (t, *J* = 5.4 Hz, 2H), 4.52 (d, *J* = 5.2 Hz, 1H), 4.48 - 4.42 (m, 2H), 4.41 - 4.38 (m, 1H), 4.34 (t, *J* = 4.8 Hz, 1H), 4.31 - 4.23 (m, 2H), 4.21 - 4.15 (m, 1H), 4.14 - 4.11 (m, 2H), 4.10 - 4.06 (m, 1H), 3.93 (s, 3H),

3.66 - 3.55 (m, 1H), 3.42 (s, 3H), 3.25 (s, 3H), 2.33 - 2.24 (m, 1H), 1.12 (d, *J* = 6.0 Hz, 3H). ^{31}P NMR (162 MHz, D_2O) δ -0.87, -11.44, -11.55, -22.56 (4P).

7. Synthesis of YK-CAP-04



Step 1: Synthesis of YK-CAP-04-PM1

YK-CAP-03-PM5 (14.4 g, 32.68 mmol) was dissolved in DCM, then DAST (16.7 g, 103.5 mmol) was slowly added at 0 ºC. The resulting mixture was stirred at 0 ºC for 4 h. The reaction was slowly quenched by saturated $NaHCO_3$ solution and extracted with DCM. The organic phase was separated, washed with saturated $NaHCO_3$ solution twice, saturated NaCl solution 3 times, dried over Na_2SO_4, filtered, and concentrated by rotary evaporation under reduced pressure. The residue was purified by silica gel column chromatography (0-30% ethyl acetate/hexane) to obtain YK-CAP-04-PM1 (10.6 g, 22.91 mmol, 70.1%).

Step 2: Synthesis of YK-CAP-04-PM2

YK-CAP-04-PM1 (5.6 g, 12.11 mmol) was dissolved in HOAc, then Ac_2O (24.7 g, 242.1 mmol) and H_2SO_4 (280 µl) were sequentially added to the solution. The resulting mixture was stirred at room temperature for 16 h. The reaction mixture was added 200 mL H_2O, extracted with EA. The organic phase was washed with saturated $NaHCO_3$ solution three times, dried, concentrated by rotary evaporation under reduced pressure to obtain the crude product YK-CAP-04-PM2 (6.0 g) as yellow oil, which was used directly in the next step.

Step 3: Synthesis of YK-CAP-04-PM3

The intermediate INT-III (5.2 g, 13.4 mmol) was dissolved in DCE, then BSA (7.4 g, 36.3 mmol) was added to the solution. The resulting mixture was stirred at 80 ºC for 2 h. The reaction solvent was removed by rotary evaporation under reduced pressure. A toluene solution of YK-CAP-04-PM2 (6.0 g, 12.11 mmol) and TMSOTf (3.0 g, 13.4 mmol) were sequentially added to the above residue. The resulting mixture was stirred at 70 ºC for 2 h. The reaction mixture was diluted with EA and washed once with saturated $NaHCO_3$ solution. The insoluble material was filtered off, and the filtrate was separated. The organic phase was dried and then concentrated by rotary evaporation under reduced pressure. The residue was purified by silica gel chromatography (0-30% ethyl acetate/dichloromethane) to obtain YK-CAP-04-PM3 (3.5 g, 5.48 mmol). $C_{30}H_{28}F_2N_6O_8$, MS(ES): (m/z) $[M + H]^+$ = 639.1.

Step 4: Synthesis of YK-CAP-04-PM4

YK-CAP-04-PM3 (3.50 g, 5.48 mmol) was dissolved in 4M NH_3 in MeOH solution and H_2O (5:1), then stirred at room temperature for 16 h. The reaction mixture was concentrated by rotary evaporation under reduced pressure, stirred with EA, filtered. The filter cake was dried to obtain YK-CAP-04-PM4 (1.3 g, 4.10 mmol, 74.7%). $C_{11}H_{13}F_2N_5O_4$, MS(ES): (m/z) $[M + H]^+$ = 318.1.

^{1}H NMR (400MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.00 (s, 1H), 6.40 (s, 2H), 5.71 - 5.56 (m, 2H), 4.86 - 4.83 (m, 1H), 4.36 - 4.32 (m, 1H), 4.19 - 4.15 (m, 1H), 4.62 (s, 1H), 3.75 - 3.73 (m, 1H), 3.62 - 3.49 (m, 1H), 3.27 - 3.22 (m, 1H).

Step 5: Synthesis of YK-CAP-04-PM5

Following the procedure described for YK-CAP-01-PM4, YK-CAP-04-PM5 (TEA salt, 1.1 g, 2.21 mmol, 53.8%) was made from YK-CAP-04-PM4 (1.3 g, 4.10 mmol). $C_{11}H_{14}F_2N_5O_7P$, MS(ES): (m/z) $[M - H]^-$ = 396.1.

Step 6: Synthesis of YK-CAP-04-PM6

Following the procedure described for YK-CAP-01-PM5, YK-CAP-04-PM6 (Na salt, 1.0 g, 2.13 mmol, 96.4%) was made from YK-CAP-04-PM5 (1.1 g, 2.21 mmol). $C_{14}H_{16}F_2N_7O_6P$, MS(ES): (m/z) $[M - H]^-$ = 446.1.

Step 7: Synthesis of YK-CAP-04-PM7

Following the procedure described for YK-CAP-01-PM6, YK-CAP-04-PM7 (TEA salt, 900 mg, 1.56 mmol, 73.1%) was made from YK-CAP-04-PM6 (1.0 g, 2.13 mmol). $C_{11}H_{15}F_2N_5O_{10}P_2$, MS(ES): (m/z) $[M - H]^-$ = 476.1.

Step 8: Synthesis of YK-CAP-04-PM8

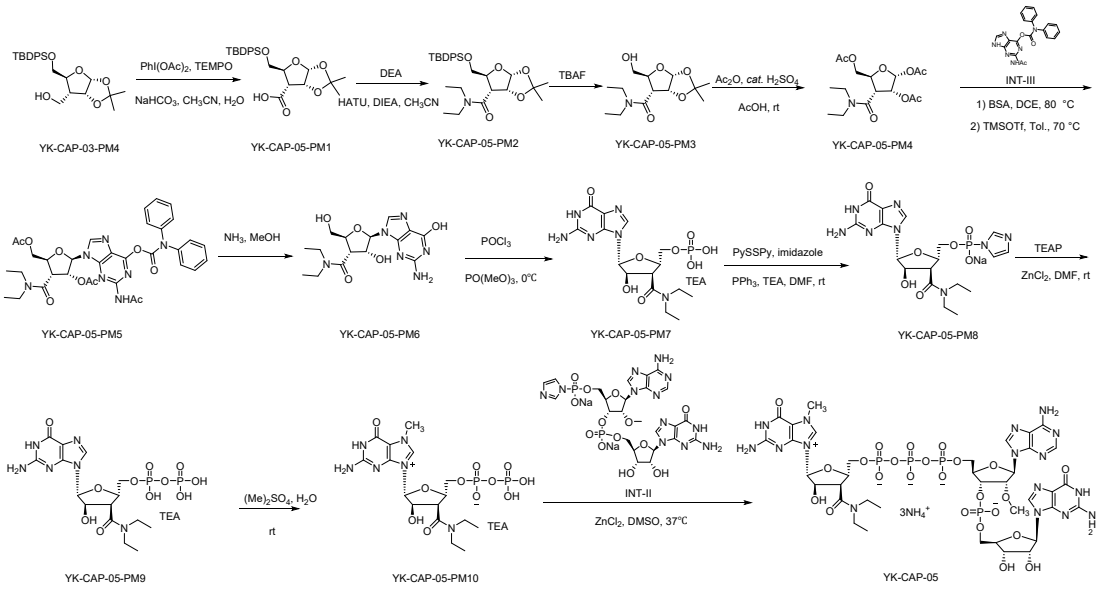
Following the procedure described for YK-CAP-01-PM7, YK-CAP-04-PM8 (TEA salt, 500 mg, 0.84 mmol, 54.1%) was made from YK-CAP-04-PM7 (900 mg, 1.56 mmol). $C_{12}H_{17}F_2N_5O_{10}P_2$, MS(ES): (m/z) $[M - H]^-$ = 490.2.

Step 9: Synthesis of YK-CAP-04

Following the procedure described for YK-CAP-01, YK-CAP-04 (25 mg, 22.03 μmol, 13.0%) was made from YK-CAP-04-PM8 (100 mg, 0.17 mmol). $C_{33}H_{43}F_2N_{15}O_{23}P_4$, MS(ES): (m/z) $[M - H]^-$ = 1178.1.

^{1}H NMR (400 MHz, D_2O) δ 9.01 (s, 1H), 8.33 (s, 1H), 8.06 (s, 1H), 7.87 (s, 1H), 6.30 - 5.99 (m, 1H), 5.96 (d, *J* = 5.3 Hz, 1H), 5.75 - 5.71 (m, 2H), 4.90 - 4.82 (m, 1H), 4.81 - 4.76 (m, 1H), 4.68 - 4.61 (m, 2H), 4.45 - 4.31 (m, 4H), 4.30 - 4.21 (m, 2H), 4.20 - 4.10 (m, 3H), 4.07 - 4.00 (m, 1H), 3.93 (s, 3H), 3.38 (s, 3H), 2.97 - 2.85 (m, 1H). ^{31}P NMR (162 MHz, D_2O) δ -0.88, -11.50, -11.64, -22.76 (4P).

8. Synthesis of YK-CAP-05



Step 1: Synthesis of YK-CAP-05-PM1

YK-CAP-03-PM4 (35.0 g, 79.07 mmol) was dissolved in ACN and H_2O (1:1, 280 mL), $PhI(OAc)_2$ (53.5 g, 166.03 mmol), $NaHCO_3$ (9.96 g, 118.56 mmol), and TEMPO (1.85 g, 11.85 mmol) were sequentially added under an ice bath. The resulting mixture was stirred at room temperature for 2 h. The reaction was quenched by saturated $Na_2S_2O_3$ solution, extracted with EA. The organic phase was separated, dried with Na_2SO_4, filtered, and concentrated by rotary evaporation under reduced pressure to obtain crude brown oil YK-CAP-05-PM1 (68.0 g), which was directly used for the next step.

Step 2: Synthesis of YK-CAP-05-PM2

YK-CAP-05-PM1 (34.0 g, 39.54 mmol) was dissolved in ACN, DIEA (14.0 g, 108.33 mmol) and HATU (17.86 g, 46.97 mmol) were added to the solution at 0 ºC and stirred for 20 minutes, then DEA (6.6 g, 90.24 mmol) was added and stirred at room temperature for 4 h. The reaction mixture was concentrated by rotary evaporation under reduced pressure and purified by silica gel column chromatography (0-20% ethyl acetate/hexane) to obtain YK-CAP-05-PM2 (17.5 g, 34.20 mmol, 86.5%).

Step 3: Synthesis of YK-CAP-05-PM3

YK-CAP-05-PM2 (17.5 g, 34.20 mmol) was dissolved in THF, TBAF (13.4 g, 51.25 mmol) was added to the solution and stirred at room temperature for 2 h. The reaction mixture was concentrated by rotary evaporation under reduced pressure and purified by silica gel column chromatography (0-90% ethyl acetate/hexane) to obtain YK-CAP-05-PM3 (8.9 g, 32.56 mmol, 95.2%).

Step 4: Synthesis of YK-CAP-05-PM4

YK-CAP-05-PM3 (4.0 g, 14.63 mmol) was dissolved in HOAc, H_2SO_4 (300 µl) was added to the solution and stirred at room temperature for 30 minutes, Ac_2O (30.0 g, 293.86 mmol) was added and stirred at room temperature for 18 h. The reaction mixture was added 200 mL H_2O, extracted with EA. The organic phase was washed with saturated $NaHCO_3$ solution three times, dried, concentrated by rotary evaporation under reduced pressure to obtain the crude product YK-CAP-05-PM4 (3.19 g) as yellow oil, which was used directly in the next step.

Step 5: Synthesis of YK-CAP-05-PM5

The intermediate INT-III (3.79 g, 9.76 mmol) was dissolved in DCE, then BSA (5.42 g, 26.64

mmol) was added to the solution. The resulting mixture was stirred at 80 ºC for 2 h. The reaction solvent was removed by rotary evaporation under reduced pressure. A toluene solution of YK-CAP-05-PM4 (3.19 g) and TMSOTf (2.96 g, 13.32 mmol) were sequentially added to the above residue. The resulting mixture was stirred at 70 ºC for 2 h. The reaction mixture was diluted with EA and washed once with saturated $NaHCO_3$ solution. The insoluble material was filtered off, and the filtrate was separated. The organic phase was dried and then concentrated by rotary evaporation under reduced pressure. The residue was purified by silica gel column chromatography (0-60% ethyl acetate/dichloromethane) to obtain YK-CAP-05-PM5 (2.20 g, 3.20 mmol).

Step 6: Synthesis of YK-CAP-05-PM6

YK-CAP-05-PM5 (2.20 g, 3.20 mmol) was dissolved in 4 M NH_3 in MeOH solution and H_2O (5:1), then stirred at room temperature for 16 h. The reaction mixture was concentrated by rotary evaporation under reduced pressure, stirred with EA, filtered. The filter cake was dried to obtain YK-CAP-05-PM6 (890 mg, 2.43 mmol, 75.9%). $C_{15}H_{22}N_6O_5$, MS(ES): (m/z) $[M + H]^+$ = 367.1.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.31 (s, 1H), 6.38 (s, 2H), 6.16 (d, J = 4.3 Hz, 1H), 5.38 - 5.41 (m, 1H), 4.84 - 4.91 (m, 1H), 4.28 - 4.31 (m, 1H), 3.98 - 4.02 (m, 1H), 3.45 - 3.49 (m, 2H), 3.33 - 3.21 (q, J = 4.1 Hz, 4H), 2.65 - 2.62 (m, 1H), 1.17 - 1.15 (t, J = 4.1 Hz, 6H).

Step 7: Synthesis of YK-CAP-05-PM7

Following the procedure described for YK-CAP-01-PM4, YK-CAP-05-PM7 (TEA salt, 900 mg, 1.64 mmol, 68.5%) was made from YK-CAP-05-PM6 (880 mg, 2.42 mmol). $C_{15}H_{23}N_6O_8P$, MS(ES): (m/z) $[M - H]^-$ = 445.1.

Step 8: Synthesis of YK-CAP-05-PM8

Following the procedure described for YK-CAP-01-PM5, YK-CAP-05-PM8 (Na salt, 730 mg, 1.41 mmol, 85.7%) was made from YK-CAP-05-PM7(900 mg, 1.64 mmol). $C_{18}H_{25}N_8O_7P$, MS(ES): (m/z) $[M - H]^-$ = 495.1.

Step 9: Synthesis of YK-CAP-05-PM9

Following the procedure described for YK-CAP-01-PM6, YK-CAP-05-PM9 (TEA salt, 750 mg, 1.20 mmol, 86.1%) was made from YK-CAP-05-PM8 (720 mg, 1.39 mmol). $C_{15}H_{24}N_6O_{11}P_2$, MS(ES): (m/z) $[M - H]^-$ = 525.1.

Step 10: Synthesis of YK-CAP-05-PM10

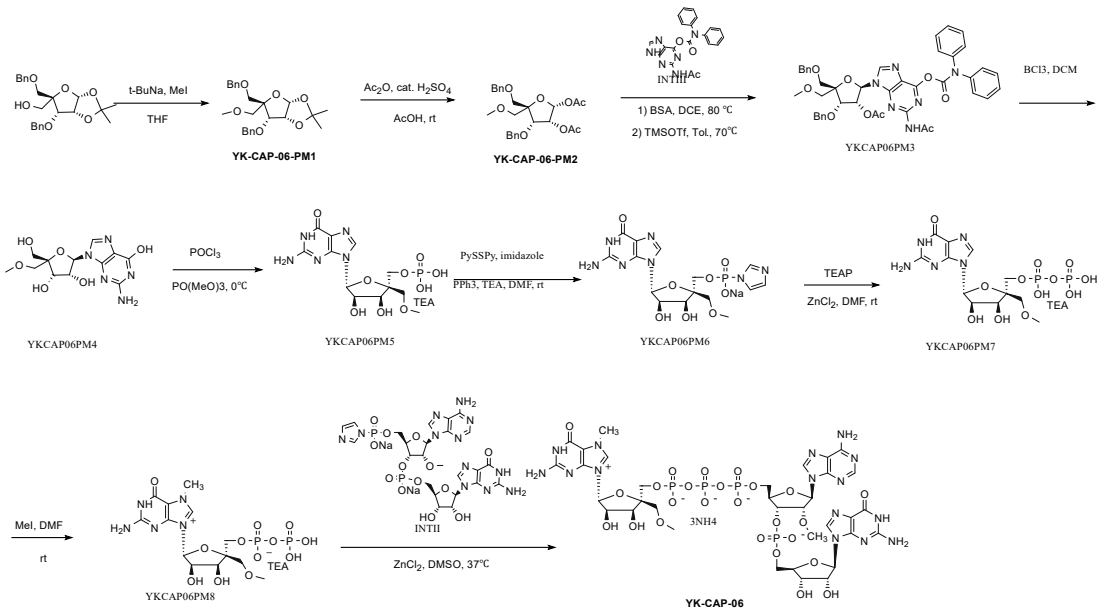
Following the procedure described for YK-CAP-01-PM6, YK-CAP-05-PM10 (TEA salt, 350 mg, 0.55 mmol, 85.6%) was made from YK-CAP-05-PM9 (400 mg, 0.64 mmol). $C_{16}H_{26}N_6O_{11}P_2$, MS (ES): (m/z) $[M - H]^-$ = 539.1.

Step 11: Synthesis of YK-CAP-05

Following the procedure described for YK-CAP-01, YK-CAP-05 (50 mg, 39.07 μmol, 25.1%) was made from YK-CAP-05-PM10 (100 mg, 0.16 mmol). $C_{37}H_{52}N_{16}O_{24}P_4$, MS(ES): (m/z) $[M - H]^-$ = 1127.2.

^{1}H NMR (400 MHz, D_2O) δ 9.11 (s, 1H), 8.82 (s, 1H), 8.60 (s, 1H), 8.36 (s, 1H), 6.09 (d, *J* = 4.8 Hz, 1H), 5.82 (d, *J* = 3.6 Hz, 2H), 4.90 - 4.84 (m, 2H), 4.82 - 4.78 (m, 1H), 4.62 (t, *J* = 4.4 Hz, 1H), 4.52 - 4.36 (m, 4H), 4.34 - 4.09 (m, 5H), 4.08 - 4.00 (m, 1H), 3.95 (s, 3H), 3.72 (t, *J* = 6.4 Hz, 1H), 3.43 (s, 3H), 3.39 - 3.21 (m, 4H), 1.05 (dt, *J* = 21.2, 7.2 Hz, 6H). ^{31}P NMR (162 MHz, D_2O) δ -0.92, 11.48, -11.73, -22.57 (4P).

9. Synthesis of YK-CAP-06



Step 1: Synthesis of YK-CAP-06-PM1

1,2-O-(1-methylethylidene)-4-C-[(phenylmethoxy)methyl]-3-O-(phenylmethyl)-L-Lyxofuranose (10.00 g, 24.97 mmol) was dissolved in THF, a THF solution of t-BuONa (7.61 g, 79.20 mmol) was slowly added to the solution at 0 ºC under N_2. The resulting mixture was stirred at room temperature for 1.5 h. CH_3I (18.73 g, 132.0 mmol) was slowly added to the above system. The resulting mixture was stirred at room temperature for 3 h, and quenched with saturated NH_4Cl solution, extracted with EA. The organic phase was washed with saturated NaCl solution 3-5 times, dried with Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-12% ethyl acetate/hexane) to obtain light yellow oil YK-CAP-06-PM1 (8.62 g, 20.80 mmol, 83.3%).

Step 2: Synthesis of YK-CAP-06-PM2

YK-CAP-06-PM1 (8.62 g, 20.80 mmol) was dissolved in HOAc, then Ac_2O (21.23 g, 207.95 mmol) and H_2SO_4 (380 µl) were sequentially added to the solution. The resulting mixture was stirred at room temperature for 16 h. The reaction mixture was added 300 mL H_2O, extracted with EA. The organic phase was washed with saturated $NaHCO_3$ solution three times, dried, concentrated under reduced. The residue was purified by silica gel column chromatography (0-40% ethyl acetate/hexane) to obtain light yellow oil YK-CAP-06-PM2 (8.13 g, 17.73 mmol, 85.3%).

Step 3: Synthesis of YK-CAP-06-PM3

The intermediate INT-III (7.57 g, 19.50 mmol) was dissolved in 100 mL DCE, BSA (9.01 g, 44.32 mmol) was added to the solution. The resulting mixture was stirred at 80 ºC for 2 h. The reaction solvent was removed by rotary evaporation under reduced pressure. A toluene solution of YK-CAP-06-PM2 (8.13 g, 17.73 mmol) and TMSOTf (1.31 g, 26.59 mmol) were sequentially added to the above residue. The resulting mixture was stirred at 70 ºC for 2 h. The reaction mixture was diluted with EA and washed once with saturated $NaHCO_3$ solution. The insoluble material was filtered off, and the filtrate was separated. The organic phase was dried and then concentrated by rotary evaporation under reduced pressure. The residue was purified by silica gel column chromatography (0-60% ethyl acetate/ dichloromethane) to

obtain YK-CAP-06-PM3 (6.24 g, 7.93 mmol, 44.7%). $C_{43}H_{42}N_6O_9$, MS(ES): (m/z) $[M + H]^+$ = 787.3.

Step 4: Synthesis of YK-CAP-06-PM4

YK-CAP-06-PM3 (6.24 g, 7.93 mmol) was dissolved in 200 mL DCM, a DCM solution of 1 M BCl_3 (79.3 mL, 79.30 mmol) was slowly added to the solution at -40 °C under N_2. The resulting mixture was stirred at 0 ºC for 3 h. The reaction was monitored by TLC, quenched with MeOH at -40 ºC, concentrated under reduced pressure, and left at room temperature for 24 h. The crude product was dropwised to DCM to precipitate a solid, filtered to obtain a crude brown product which was purified by preparative HPLC to obtain YK-CAP-06-PM4 (1.19 g, 3.64 mmol, 45.8%). $C_{12}H_{17}N_6O_5$, MS (ES): (m/z) $[M + H]^+$ = 328.1.

^{1}H NMR (400 MHz, MeOD) δ 8.15 (s, 1H), 4.51 (s, 1H), 4.41 (s, 1H), 4.21 - 4.17 (m, 1H), 3.81 (s, 2H), 3.44 - 3.31 (m, 2H), 2.97 (s, 3H).

Step 5: Synthesis of YK-CAP-06-PM5

Following the procedure described for YK-CAP-01-PM4, YK-CAP-06-PM5 (TEA salt, 1.23 g, 2.42 mmol, 66.5%) was made from YK-CAP-06-PM4 (1.19 g, 3.64 mmol). $C_{12}H_{18}N_5O_9P$, MS(ES): (m/z) $[M - H]^-$ = 406.1.

Step 6: Synthesis of YK-CAP-06-PM6

Following the procedure described for YK-CAP-01-PM5, YK-CAP-06-PM6 (Na salt, 993 mg, 2.07 mmol, 85.6%) was made from YK-CAP-06-PM5 (1.23 g, 2.42 mmol). $C_{15}H_{20}N_7O_8P$, MS(ES): (m/z) $[M - H]^-$ = 456.1.

Step 7: Synthesis of YK-CAP-06-PM7

Following the procedure described for YK-CAP-01-PM6, YK-CAP-06-PM7 (TEA salt, 794 mg, 1.35 mmol, 65.1%) was made from YK-CAP-06-PM6 (993 mg, 2.07 mmol). $C_{12}H_{19}N_5O_{12}P_2$, MS(ES): (m/z) $[M - H]^-$ = 486.1.

Step 8: Synthesis of YK-CAP-06-PM8

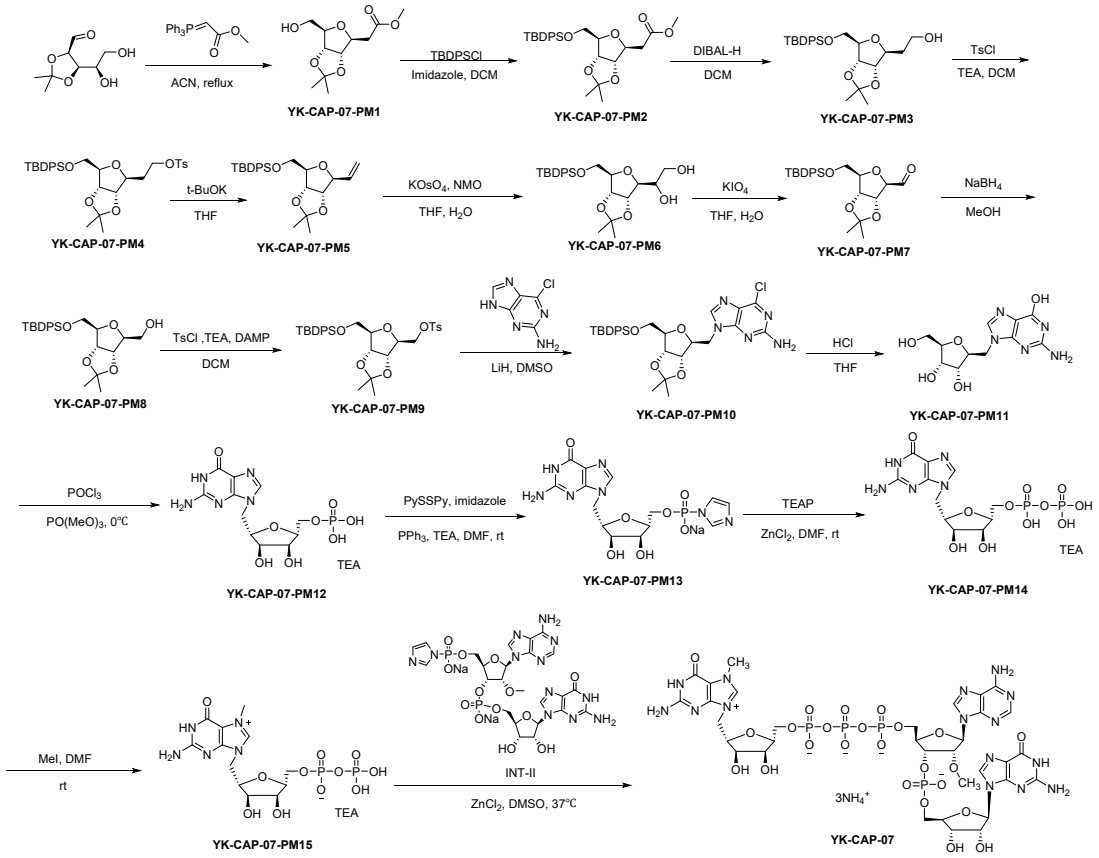
YK-CAP-06-PM7 (794 mg, 1.35 mmol) and CH_3I (2.29 g, 16.20 mmol) were dissolved in 10 mL dry DMF and stirred at 37 ºC for 23 h. 20 mL H_2O was added to the reaction system, washed with 60 mL EA, the aqueous layer was separated, collected and concentrated under reduced pressure. The residue was dissolved with 100 mL H_2O, purified by a gel column (H_2O and 1.5 M TEAB = 10:1), the target product peak was collected, concentrated, lyophilized and then purified through high-efficiency preparative HPLC (mobile phase system of 50 mM TEAB and MeOH) to desalt, obtaining YK-CAP-06-PM8 (TEA salt, 520 mg, 0.86 mmol, 64.0%). $C_{13}H_{21}N_5O_{12}P_2$, MS(ES): (m/z) $[M - H]^-$ = 500.1.

Step 9: Synthesis of YK-CAP-06

YK-CAP-06-PM8 (150 mg, 0.25 mmol) and INT-II (319 mg, 0.42 mmol) were dissolved in 2 mL dry DMSO, $ZnCl_2$ (809 mg, 5.93 mmol) was added to the solution. The resulting mixture was stirred at 37 ºC for 3 days under N_2. 0.25 M EDTA solution was added to the reaction system, which pH was adjusted to 6-7 with 1.5 M TEAB, and purified by gel column (H_2O and 1.5 M TEAB = 10:1). The target product peak was collected, concentrated and lyophilized, further purified by high-performance preparative liquid chromatography to obtain the final product YK-CAP-06 (26 mg, 20.95 μmol, 8.4%). $C_{34}H_{47}N_{15}O_{25}P_4$, MS(ES): (m/z) $[M - H]^-$ = 1188.1. ^{1}H NMR (400 MHz, D_2O) δ 9.01 (s, 1H), 8.36 (s, 1H), 8.09 (s, 1H), 7.88 (s, 1H), 5.95 (d, *J* = 5.2 Hz, 1H), 5.86 (d, *J* = 5.2 Hz, 1H), 5.73 (d, *J* = 5.6 Hz, 1H), 4.87 - 4.81 (m, 1H), 4.68 - 4.64 (m, 2H), 4.53 (d, *J* = 5.6 Hz, 1H), 4.44 - 4.39 (m, 2H), 4.33 (t, *J* = 5.2 Hz, 2H), 4.28 - 4.21 (m, 2H), 4.19 - 4.15 (m, 2H), 4.14 - 4.09 (m, 3H), 3.95 (s, 3H), 3.69 (d, *J* = 10.8 Hz, 1H), 3.59 (d, *J* = 10.8 Hz, 1H),

3.38 (s, 3H), 3.35 (s, 3H). ^{31}P NMR (162 MHz, D_2O) δ -0.92, -11.44, -11.55, -22.74 (4P).

6. Synthesis of YK-CAP-07



Step 1: Synthesis of YK-CAP-07-PM1

2,3-O-Isopropylidene-D-ribofuranoside (10.00 g, 52.58 mmol) and methyl (triphenylphosphoranylidene)acetate (21.6 g, 64.60 mmol) were dissolved in 200 mL ACN. The resulting mixture was stirred at 90 ºC for 10 h and diluted with 300 mL EA and washed by saturated NaCl solution. The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation to obtain a yellow oil compound YK-CAP-07-PM1 (26.7 g) which was used in the next step without further purification.

Step 2: Synthesis of YK-CAP-07-PM2

YK-CAP-07-PM1 (12.8 g, 25.21 mmol), TBDPSCl (17.2 g, 62.42 mmol) and imidazole (5.31 g, 78.03 mmol) were dissolved in 200 mL DCM and the reaction was stirred at room temperature overnight. The resulting mixture was diluted with 100 mL DCM and washed by saturated NaCl solution (3 × 200 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation. The residue was purified by silica gel chromatography (0-17% ethyl acetate/hexane) to obtain YK-CAP-07-PM2 (20.0 g, 41.27 mmol).

Step 3: Synthesis of YK-CAP-07-PM3

YK-CAP-07-PM2 (20.0 g, 41.27 mmol) was dissolved in 150 mL DCM, and a toluene solution of diisobutylaluminum hydride (1.5M, 63.2 mL, 94.8 mmol) was slowly added to the solution at -78 ºC. The reaction system was allowed to warm to room temperature and stirred overnight. 65 mL MeOH was slowly added under an ice bath, followed by the formation of white flocculent solid, then sodium sulfate decahydrate was added and stirred for 20 minutes. The solid was filtered off and the filtrate was concentrated by vacuum evaporation,

the residue was purified by silica gel chromatography (0-30% ethyl acetate/hexane) to obtain YK-CAP-07-PM3 (15.00 g, 32.85 mmol, 79.6%).

Step 4: Synthesis of YK-CAP-07-PM4

YK-CAP-07-PM3 (15.00 g, 32.85 mmol), TsCl (7.5 g, 39.40 mmol) and TEA (5.0 g, 49.30 mmol) were dissolved in 60 mL DCM and the reaction was stirred at room temperature overnight. The resulting mixture was diluted with 50 mL DCM and washed by saturated NaCl solution (2 × 100 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation. The residue was purified by silica gel chromatography (0-20% ethyl acetate/hexane) to obtain YK-CAP-07-PM4 (17.00 g, 27.83 mmol, 84.7%).

Step 5: Synthesis of YK-CAP-07-PM5

YK-CAP-07-PM4 (17.00 g, 27.83 mmol) was dissolved in 150 mL THF, and a THF solution of t-BuOK (1 M, 61.0 mL, 61.0 mmol) was slowly added at -40 ºC. The reaction was stirred at room temperature for 3 h and diluted with EA (200 mL) and washed with saturated NaCl solution (2 × 150 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation. The residue was purified by silica gel chromatography (0-11% ethyl acetate/hexane) to obtain YK-CAP-07-PM5 (3.29 g, 7.50 mmol, 27.0%).

Step 6: Synthesis of YK-CAP-07-PM6

YK-CAP-07-PM5 (3.29 g, 7.50 mmol) was dissolved in a mixed solution of 25 mL THF and 5 mL H_2O, $K_2OsO_4 \cdot 2H_2O$ (140 mg, 0.38 mmol) and NMO (1.05 g, 9.00 mmol) were slowly added to the above reaction system. The reaction was stirred at 40 ºC for 6 h and diluted with 100 mL EA, washed with saturated Na_2SO_3 solution (2 × 80 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation to obtain YK-CAP-07-PM6 (3.48 g, 7.36 mmol, 98.2%).

Step 7: Synthesis of YK-CAP-07-PM7

YK-CAP-07-PM6 (3.48 g, 7.36 mmol) was dissolved in a mixed solution of 25 mL THF and 5 mL H_2O, KIO_4 (2.54 g, 11.04 mmol) was added to the above reaction system. The reaction was stirred at 40 ºC for 6 h and diluted with 100 mL EA, washed with saturated Na_2SO_3 solution (2 × 50 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation to obtain YK-CAP-07-PM7 (3.14 g, 7.13 mmol, 96.9%).

Step 8: Synthesis of YK-CAP-07-PM8

YK-CAP-07-PM7 (5.19 g, 11.78 mmol) was dissolved in 100 mL MeOH, $NaBH_4$ (0.54 g, 13.18 mmol) was added to the solution under an ice bath. The reaction mixture was stirred at room temperature for 3 h and diluted with 150 mL H_2O, extracted with EA (2 × 150 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation. The residue was purified by silica gel chromatography (0-13% ethyl acetate/hexane) to obtain YK-CAP-07-PM8 (3.30 g, 7.46 mmol, 63.3%).

Step 9: Synthesis of YK-CAP-07-PM9

YK-CAP-07-PM8 (3.30 g, 7.46 mmol), TsCl (1.71 g, 8.95 mmol) and TEA (1.13 g, 11.19 mmol) were dissolved in 13 mL DCM and the reaction was stirred at room temperature overnight. The resulting mixture was diluted with 20 mL DCM and washed by saturated NaCl solution (2 × 50 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation. The residue was purified by silica gel chromatography (0-20% ethyl acetate/hexane) to obtain YK-CAP-07-PM9 (3.42 g, 5.73 mmol, 76.8%).

Step 10: Synthesis of YK-CAP-07-PM10

2-amino-6-chloropurine (1.17 g, 6.90 mmol) and LiH (55 mg, 6.88 mmol) were dissolved in 30 mL DMSO and stirred at 90 ºC for 1 h, then a DMSO solution (15 mL) of YK-CAP-07-PM9 (3.42 g, 5.73 mmol) was added to the above reaction system and continued stirring for 5 h. The reaction was diluted with 100 mL H_2O, extracted with DCM (2 × 150 mL). The organic phase was dried with Na_2SO_4 and concentrated by vacuum evaporation. The residue was purified by silica gel chromatography (0-25% ethyl acetate/dichloromethane) to obtain YK-CAP-07-PM10 (1.90 g, 3.20 mmol, 55.9%). $C_{30}H_{36}ClN_5O_4Si$, MS(ES): (m/z) $[M + H]^+$ = 595.2.

Step 11: Synthesis of YK-CAP-07-PM11

YK-CAP-07-PM10 (1.90 g, 3.20 mmol) was dissolved in 40 mL THF, 1 M HCl (80 mL) was added to the solution. The resulting mixture was stirred at 90 ºC for 7 h. The reaction mixture was concentrated by vacuum evaporation. The residue was purified by preparative HPLC to obtain YK-CAP-07-PM11 (587 mg, 1.97 mmol, 61.7%) as a white solid. $C_{11}H_{15}N_5O_5$, MS(ES): (m/z) $[M + H]^+$ = 298.2.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H), 7.68 (s, 1H), 6.46 (s, 2H), 5.00 - 4.53 (m, 2H), 4.17 - 4.09 (m, 1H), 4.00 - 3.87 (m, 2H), 3.77 - 3.61 (m, 3H), 3.46 - 3.32 (m, 3H).

Step 12: Synthesis of YK-CAP-07-PM12

Following the procedure described for YK-CAP-01-PM4, YK-CAP-07-PM12 (TEA salt, 350 mg, 0.73 mmol, 72.5%) was made from YK-CAP-07-PM11 (300 mg, 1.01 mmol). $C_{11}H_{16}N_5O_8P$, MS(ES): (m/z) $[M - H]^-$ = 376.1.

Step 13: Synthesis of YK-CAP-07-PM13

Following the procedure described for YK-CAP-01-PM5, YK-CAP-07-PM13 (Na salt, 320 mg, 0.71 mmol, 97.4%) was made from YK-CAP-07-PM12 (350 mg, 0.73 mmol). $C_{14}H_{18}N_7O_7P$, MS(ES): (m/z) $[M - H]^-$ = 426.1.

Step 14: Synthesis of YK-CAP-07-PM14

Following the procedure described for YK-CAP-01-PM6, YK-CAP-07-PM14 (TEA salt, 250 mg, 0.45 mmol, 62.9%) was made from YK-CAP-07-PM13 (320 mg, 0.71 mmol). $C_{11}H_{17}N_5O_{11}P_2$, MS (ES): (m/z) $[M - H]^-$ = 456.0.

Step 15: Synthesis of YK-CAP-07-PM15

Following the procedure described for YK-CAP-01-PM7, YK-CAP-07-PM15 (TEA salt, 90 mg, 0.16 mmol, 34.9%) was made from YK-CAP-07-PM14 (250 mg, 0.45 mmol). $C_{12}H_{20}N_5O_{11}P_2$, MS(ES): (m/z) $[M - H]^-$ = 470.1.

Step 16: Synthesis of YK-CAP-07

Following the procedure described for YK-CAP-01, YK-CAP-07 (29 mg, 23.95 μmol, 15.0%) was made from YK-CAP-101-PM6 (90 mg, 0.16 mmol). $C_{33}H_{45}N_{15}O_{24}P_4$, MS(ES): (m/z) $[M - H]^-$ = 1158.2. ^{1}H NMR (400 MHz, D_2O) δ 8.81 (s, 1H), 8.25 (s, 1H), 8.01 (s, 1H), 7.85 (s, 1H), 5.98 - 5.96 (m, 1H), 5.75 - 5.72 (m, 1H), 4.86 - 4.80 (m, 1H), 4.68 - 4.65 (m, 2H), 4.48 - 4.43 (m, 1H), 4.43 - 4.39 (m, 1H), 4.35 - 4.28 (m, 2H), 4.28 - 4.25 (m, 1H), 4.24 - 4.21 (m, 1H), 4.19 - 4.16 (m, 1H), 4.15 - 4.09 (m, 3H), 4.08 - 4.01 (m, 4H), 4.01 - 3.95 (m, 1H), 3.93 (s, 3H), 3.38 (s, 3H).

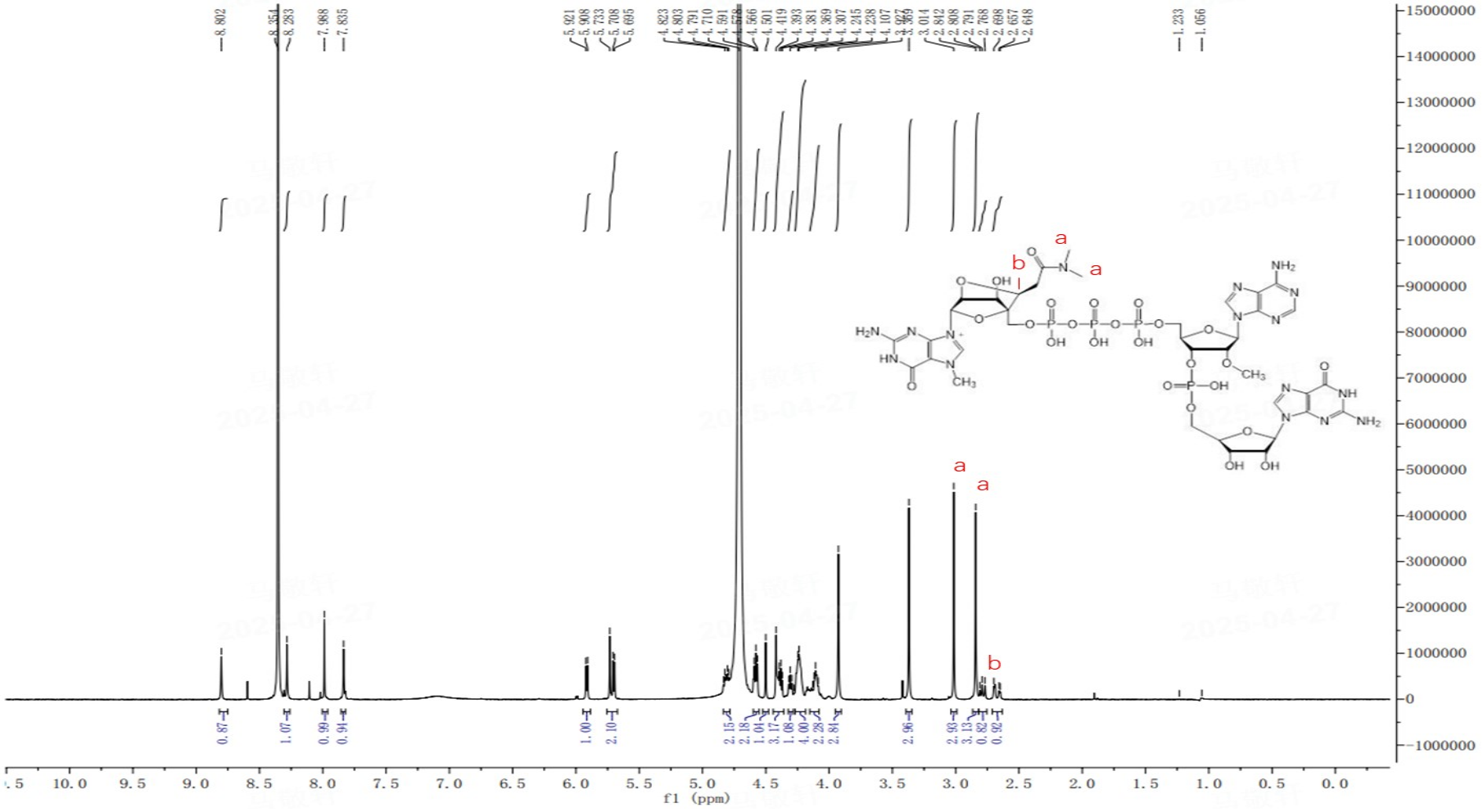


Figure S1. ^{1}H NMR spectrum of YK-CAP-01.

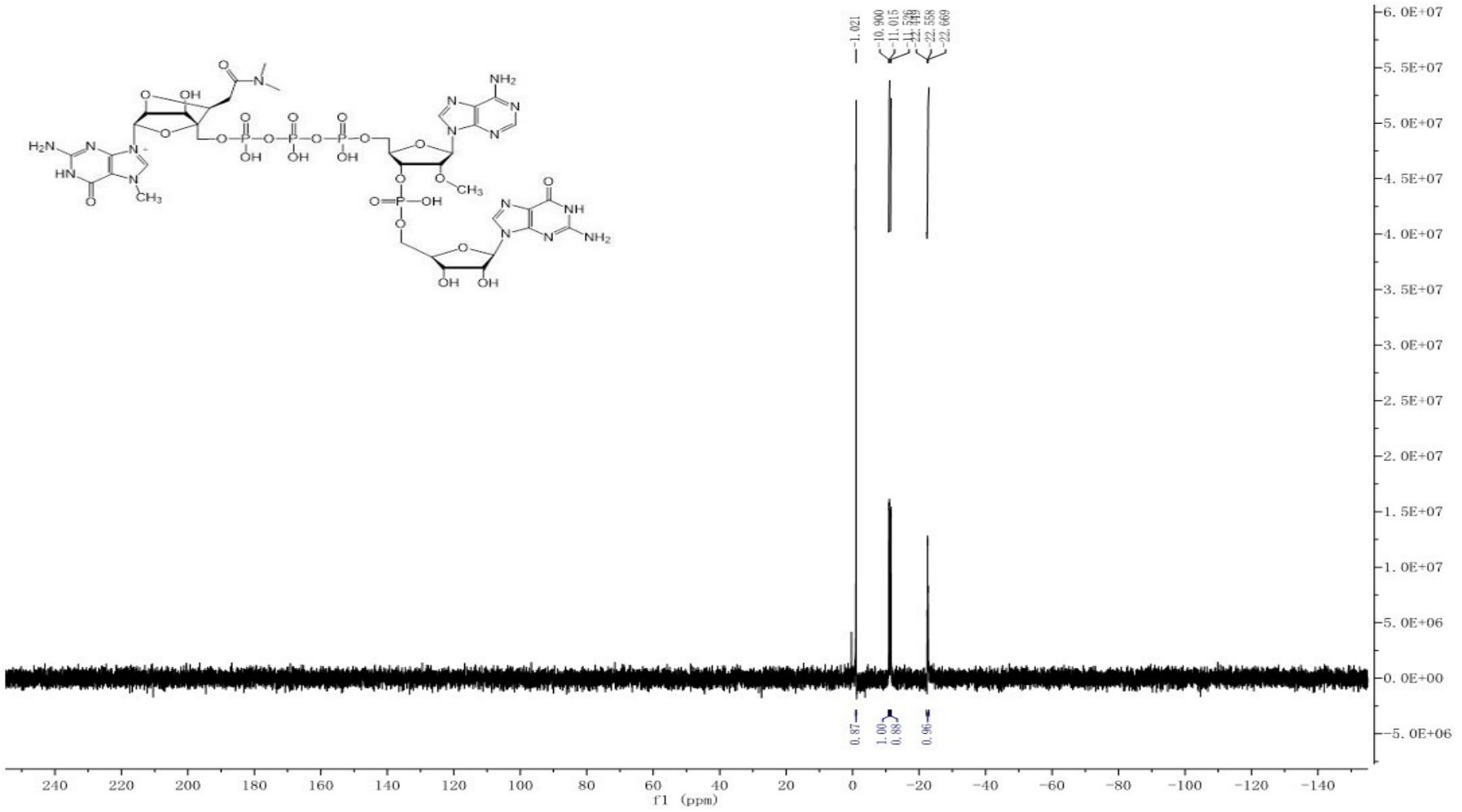


Figure S2. ^{31}P NMR spectrum of YK-CAP-01.

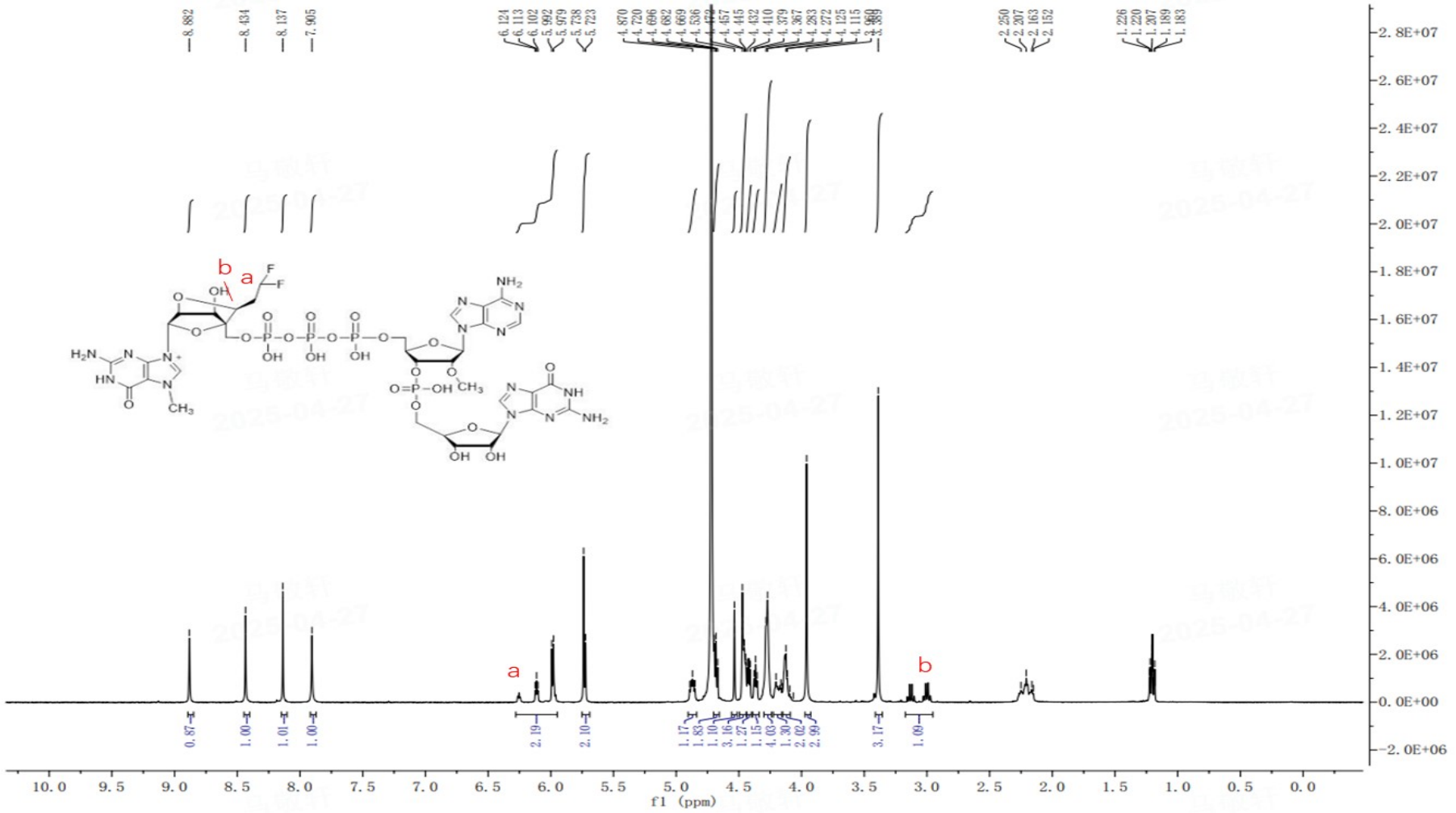


Figure S3. ^{1}H NMR spectrum of YK-CAP-02.

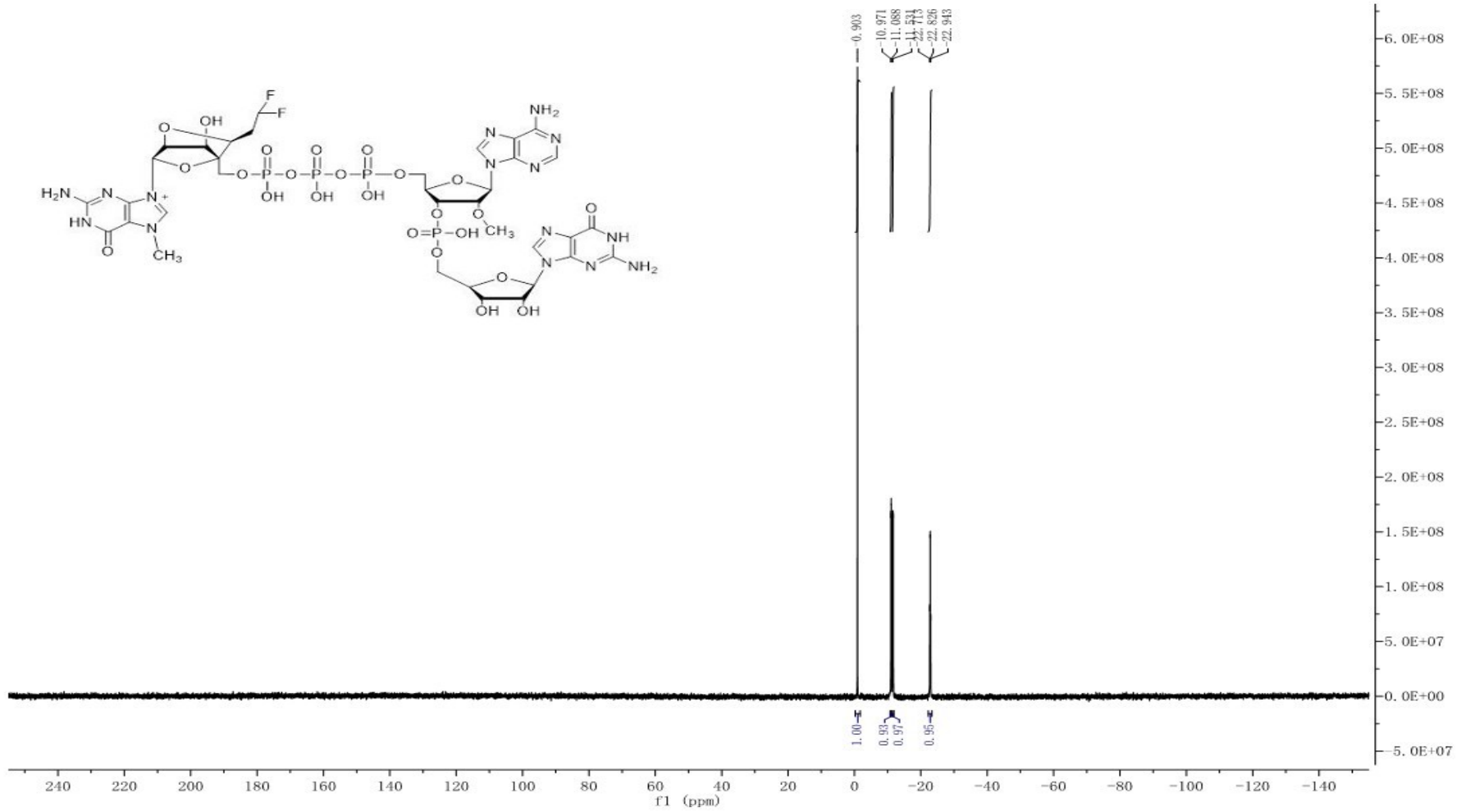


Figure S4. ^{31}P NMR spectrum of YK-CAP-02.

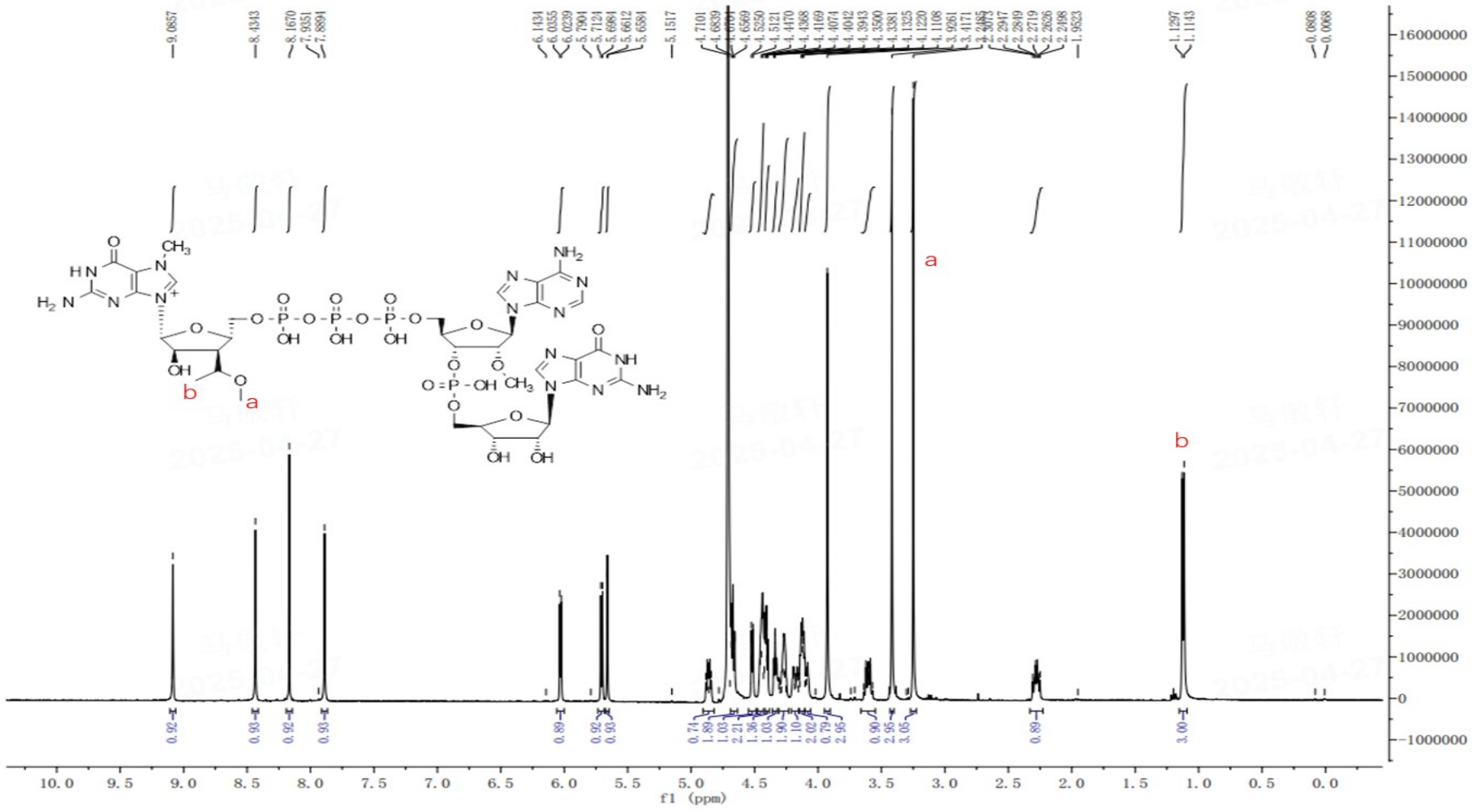


Figure S5. ^{1}H NMR spectrum of YK-CAP-03.

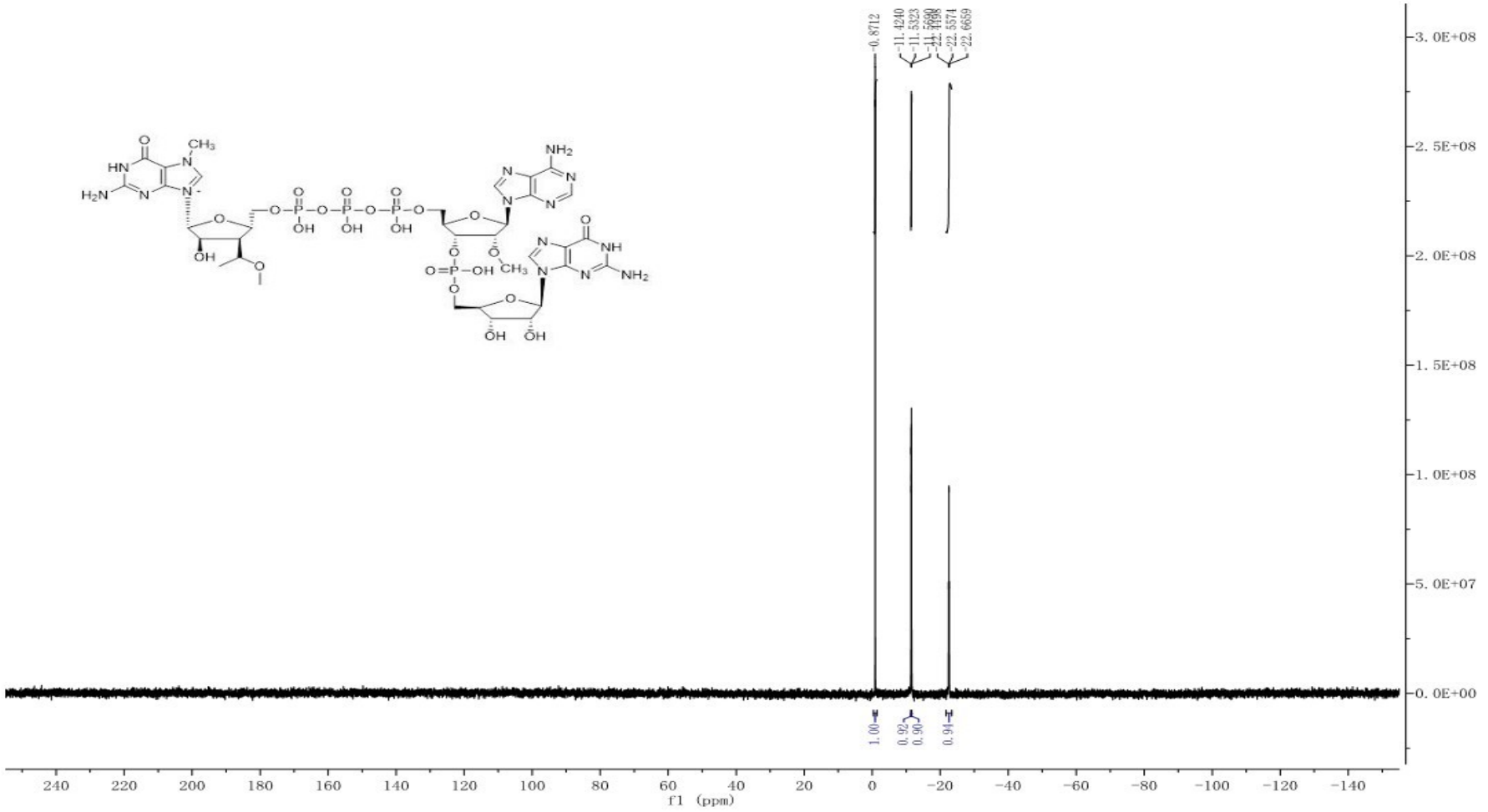


Figure S6. ^{31}P NMR spectrum of YK-CAP-03.

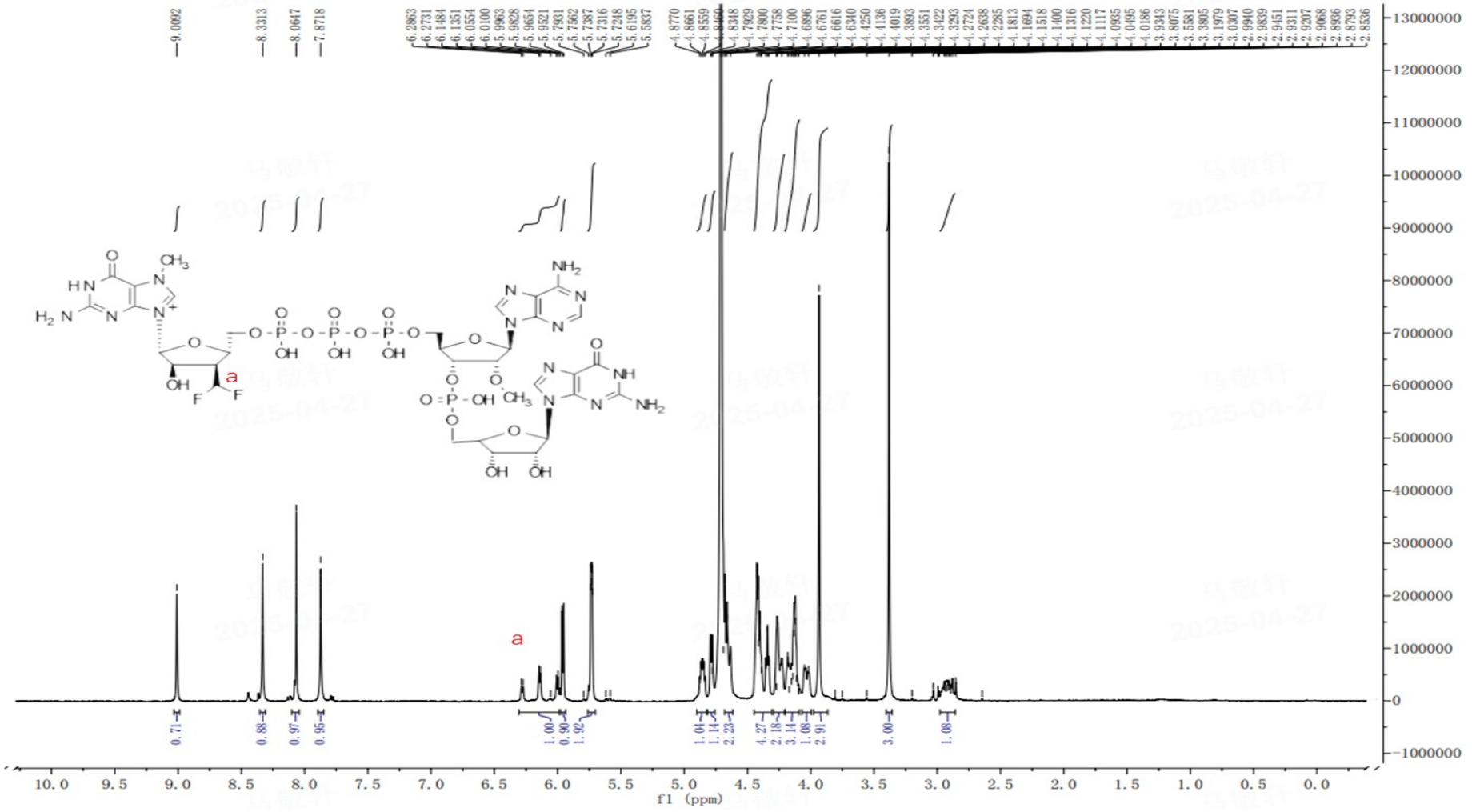


Figure S7. ^{1}H NMR spectrum of YK-CAP-04.

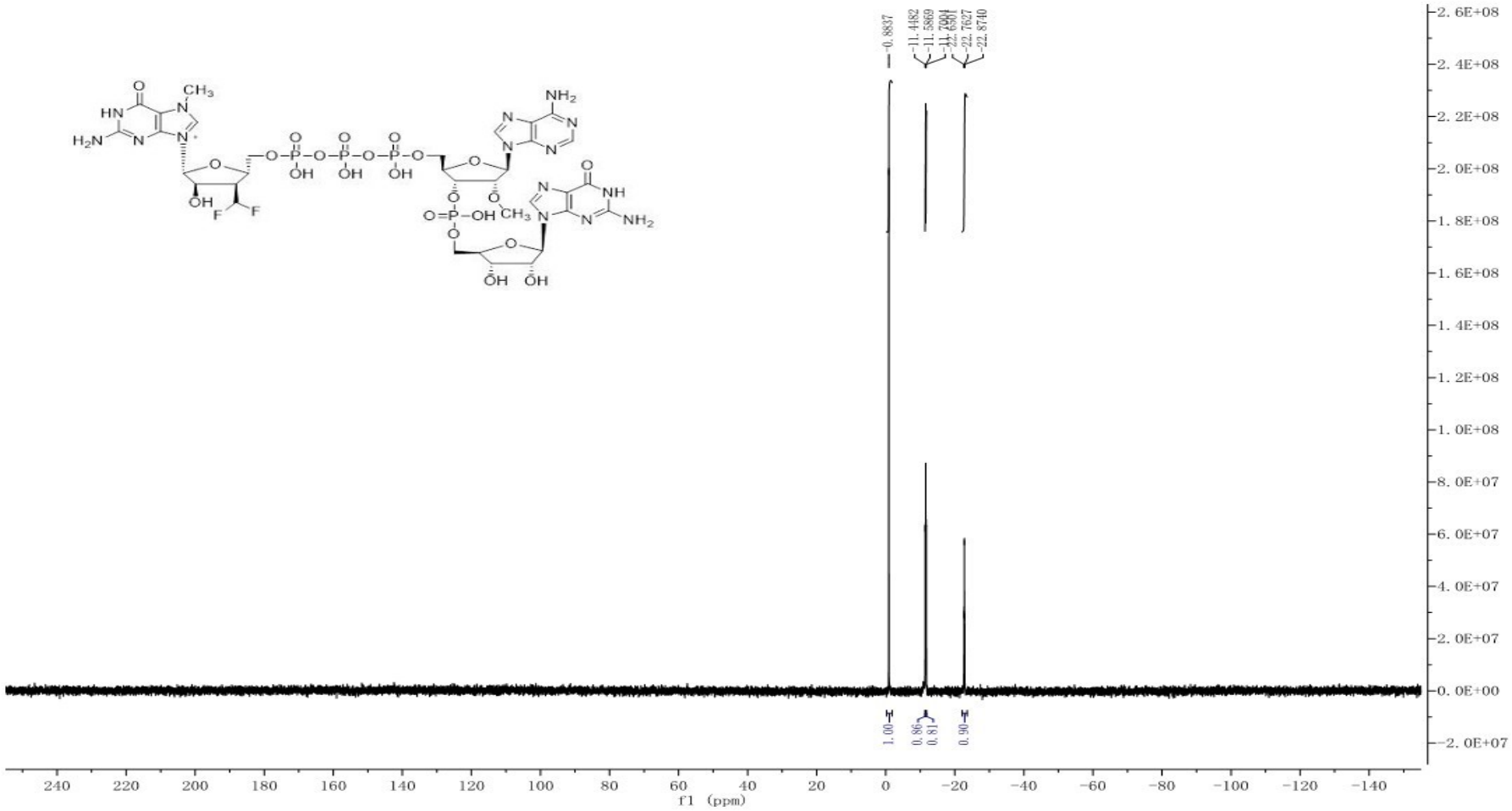


Figure S8. ^{31}P NMR spectrum of YK-CAP-04.

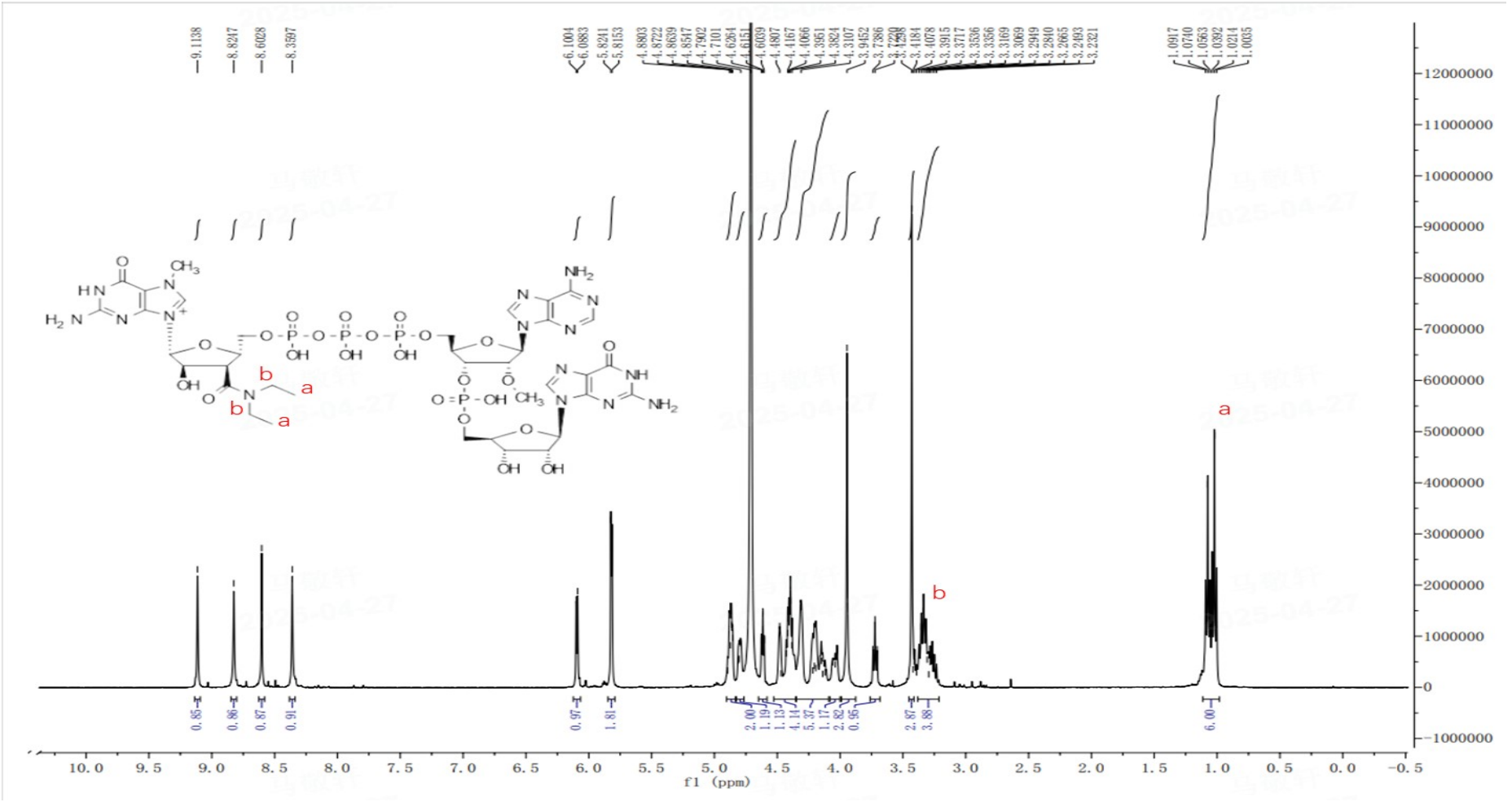


Figure S9. ^{1}H NMR spectrum of YK-CAP-05.

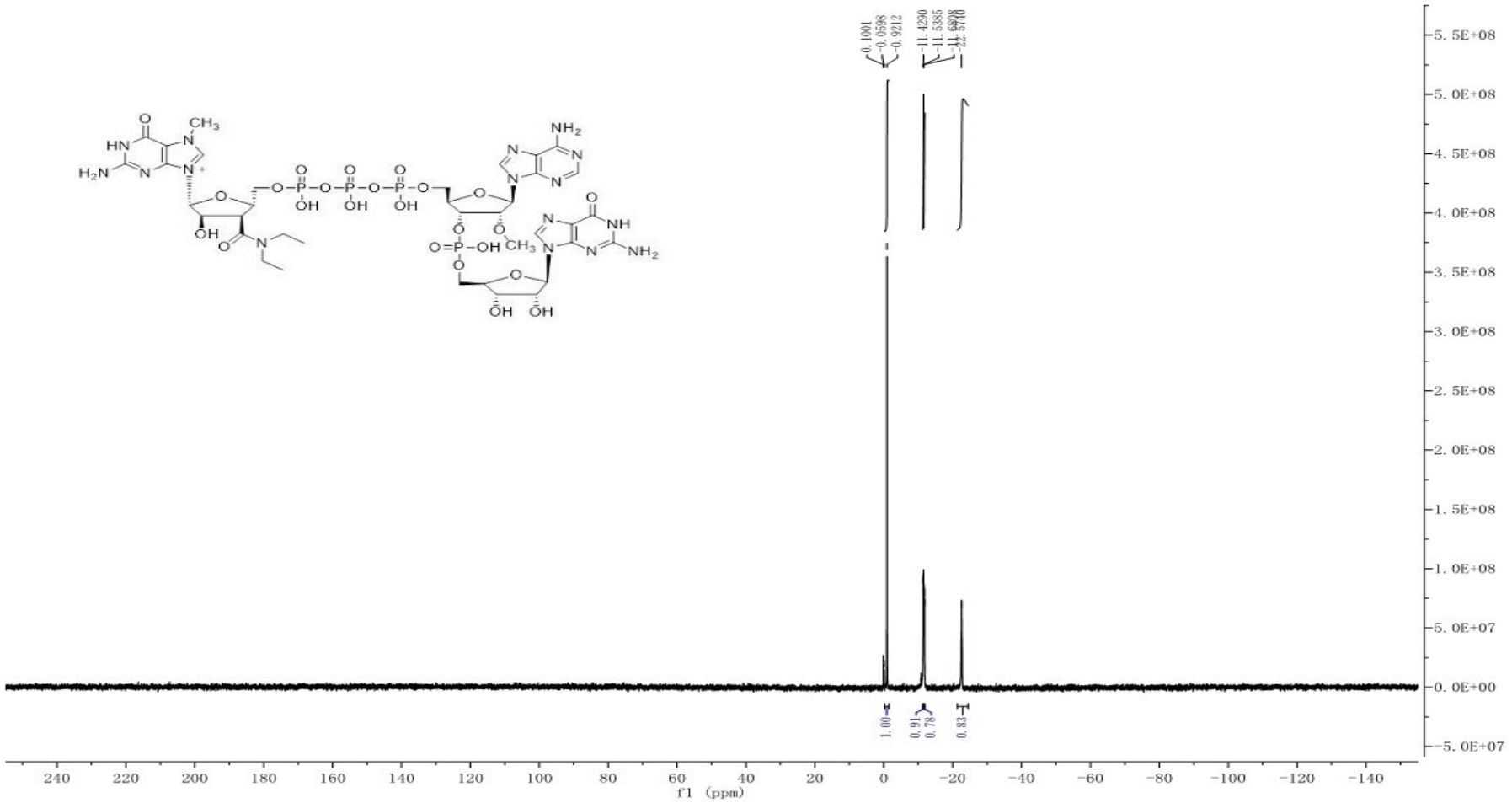


Figure S10. ^{31}P NMR spectrum of YK-CAP-05.

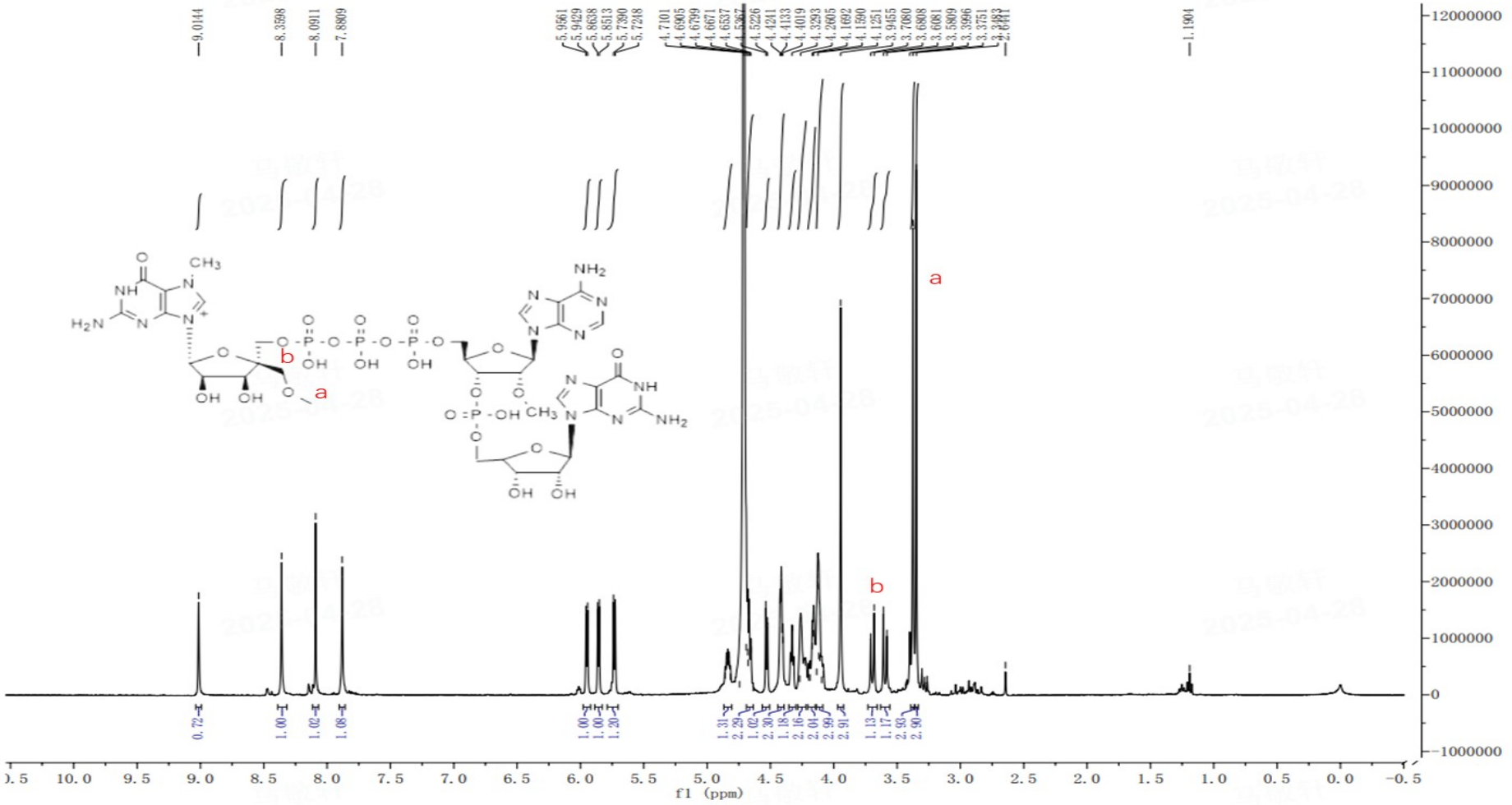


Figure S11. ^{1}H NMR spectrum of YK-CAP-06.

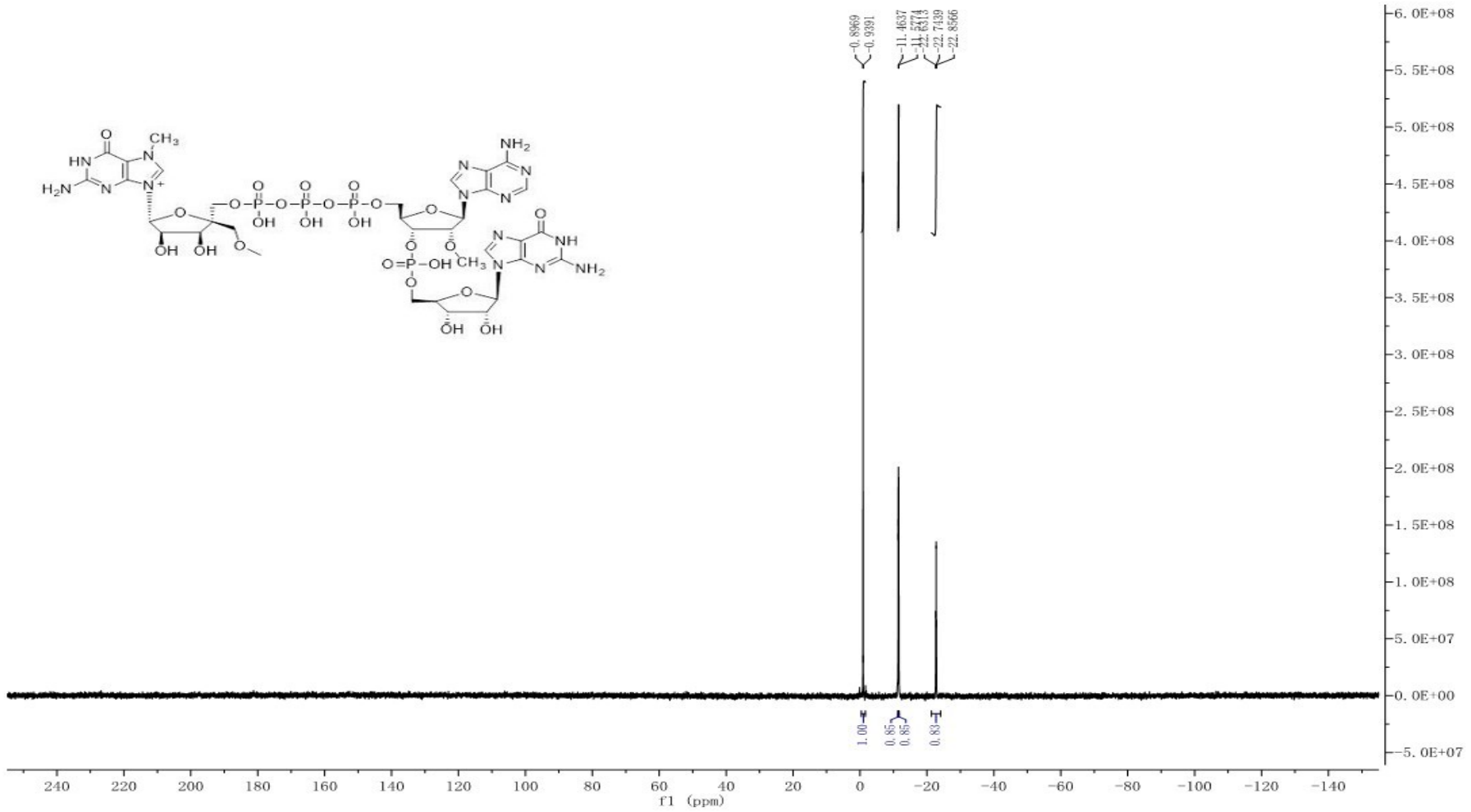


Figure S12. **^{31}P NMR spectrum of YK-CAP-06.**

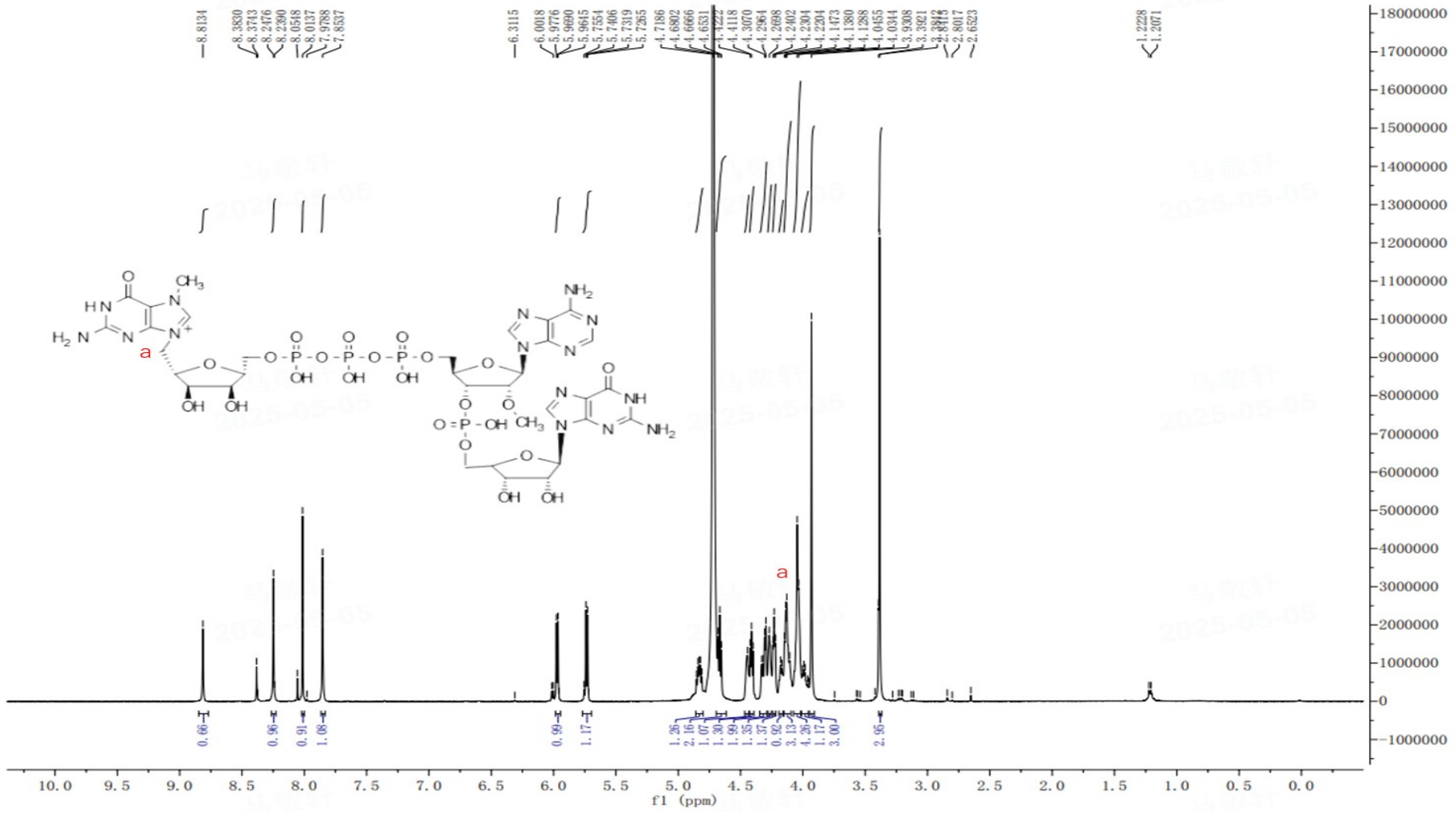


Figure S13. **^{1}H NMR spectrum of YK-CAP-07.**

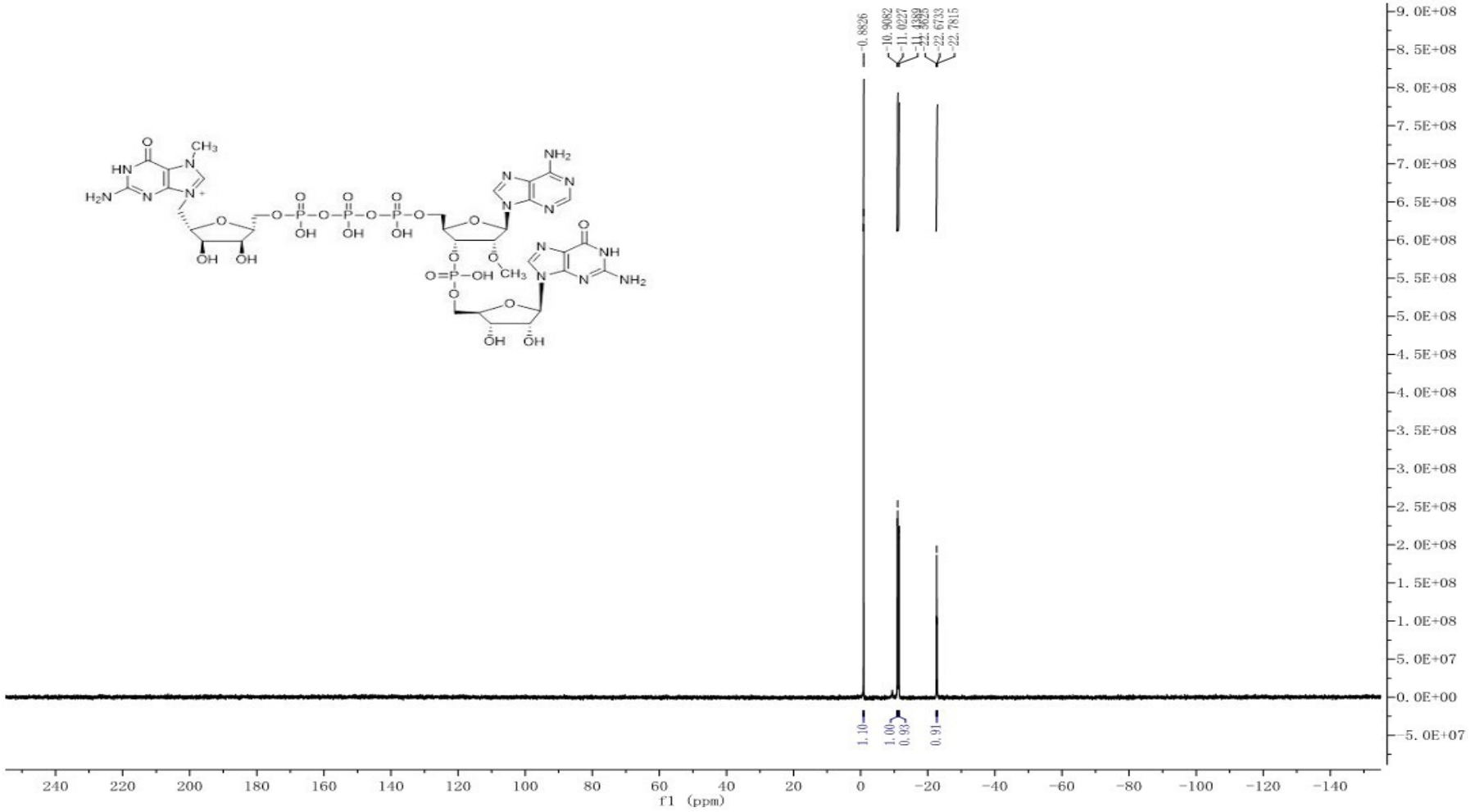


Figure S14. ^{31}P NMR spectrum of YK-CAP-07.

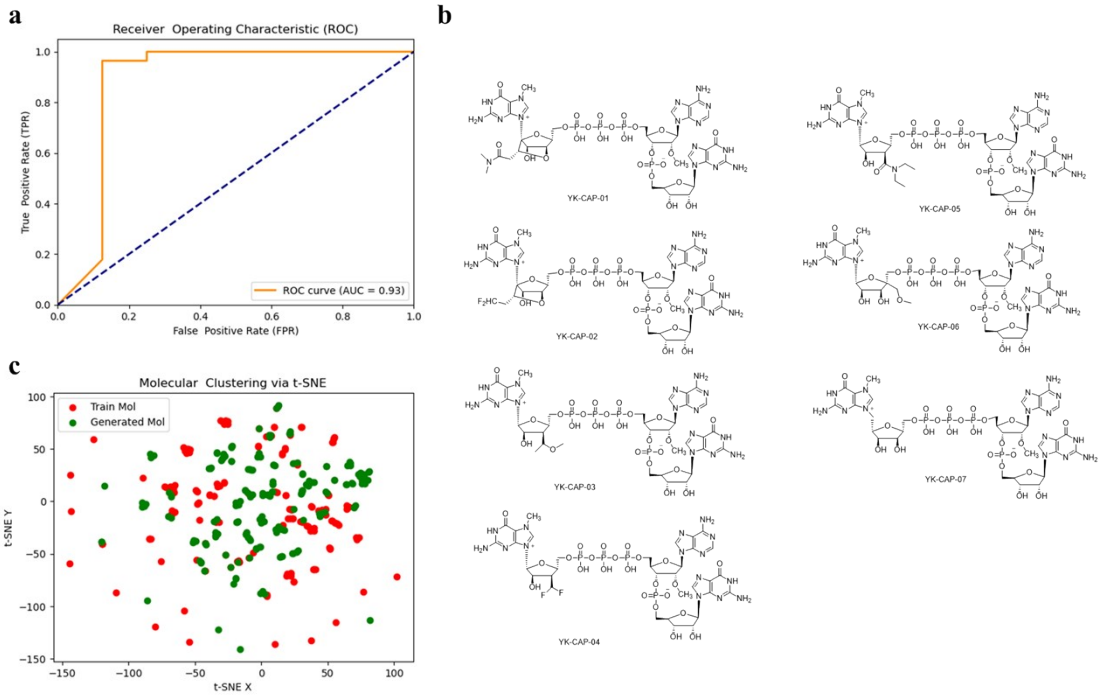


Figure S15. Model results. (a) Receiver Operating Characteristic (ROC) curve. (b) Examples of generated molecules. (c) Visual analysis of generated molecules and molecules in the training set.

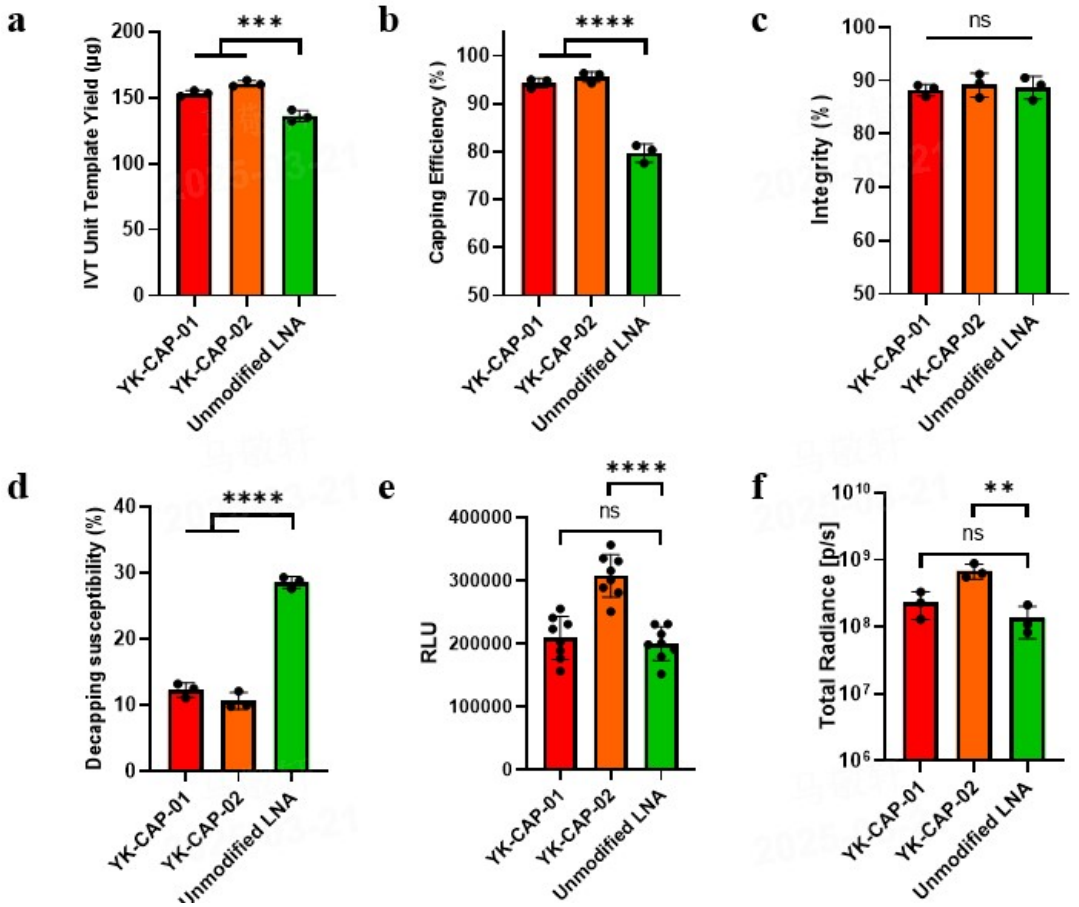


Figure S16. Comparison of modified and unmodified LNA. (a) IVT reactions were incubated at 37 ºC for 2 h containing 2 µg template, 2 µL 100 mM ATP, GTP, CTP, and 1-Methyl-Pseudouridine-5'-Triphosphate, 2 µL 100 mM various cap analogues in 20 µL reaction volume. (b) RNA capping efficiency was calculated as a fraction of capped RNA versus total RNA. (c) Integrity was determined by a capillary electrophoresis system after initial purification. (d) Fraction of decapped RNA after 45 min of incubation with hDcp2. (e) Relative luciferase expression of HEK-293T cells after incubation with LNPs-Fluc mRNA for 24 h. (f) Quantified total luminescence of the muscle sites of female BALB/c mice injected via intramuscular route at 6 h. Statistical significance was calculated using one-way ANOVA (Analysis of Variance) with Dunnett's multiple comparisons test, and data are shown as mean ± SD. (ns: no significant difference, **P < 0.01, ***P < 0.001, ****P < 0.0001).

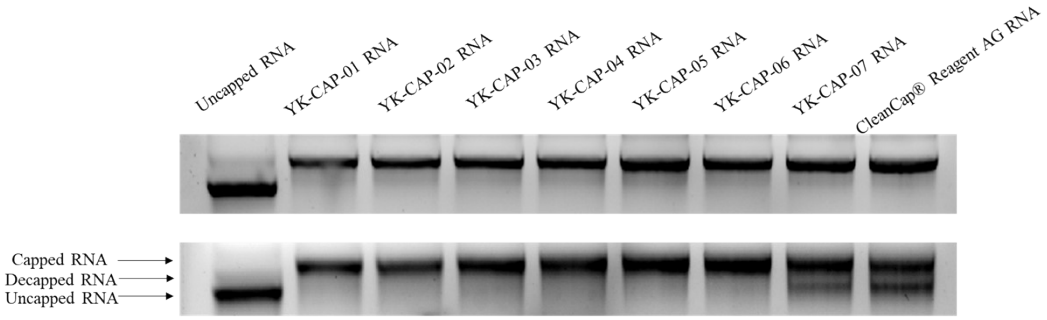


Figure S17 5'capped mRNAs with YK-CAPs prevents RNA from decapping by hDCP2. Short capped RNAs were subjected to treatment with hDCP2 over a 45 min time-course. Reactions with uncapped RNA served as controls. Figure presents representative result of one biological

repetition.

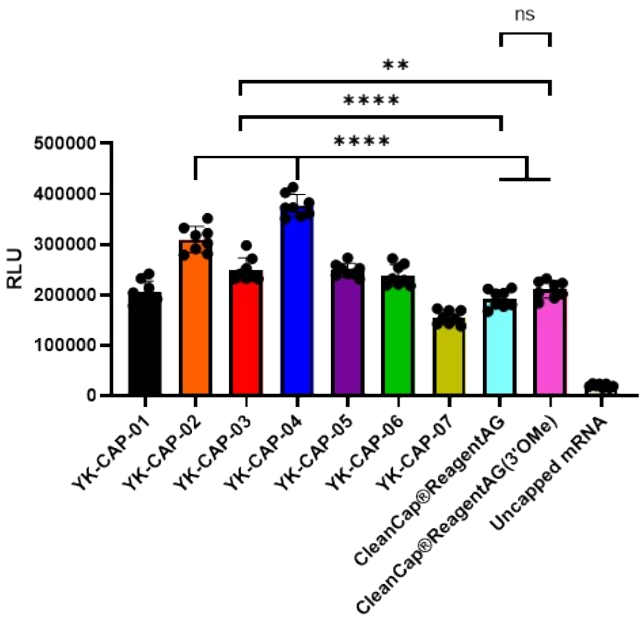


Figure S18. *In vitro* translational efficiency of LNP-mRNA-capped with cap analogues. Relative luciferase expression of HEK-293T cells after incubation with LNPs-Fluc mRNA for 24 h. Statistical significance was calculated using one-way ANOVA (Analysis of Variance) with Dunnett's multiple comparisons test, ns: no significant difference, **P < 0.01, ****P < 0.0001. Data represents the mean ± SD.

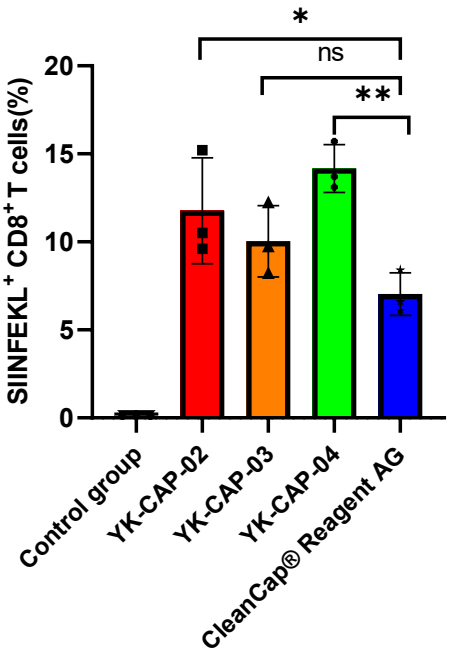


Figure S19. *In vivo* immunotherapeutic efficacy of 5'capped mRNAs with YK-CAPs. Flow

cytometry analysis of SIINFEKL^{+} % in CD8^{+} T cells in spleens was measured at day 12 after three injections of LNPs encapsulated with 5'capped OVA mRNAs with YK-CAP-02~04 and CleanCap® Reagent AG at day 0, 3 and 7. Control group refers to DPBS-treatment. Statistical significance was calculated using one-way ANOVA with Dunnett's multiple comparisons test and data are shown as mean ± SEM. (ns: no significant difference, *P < 0.05, **P < 0.01).

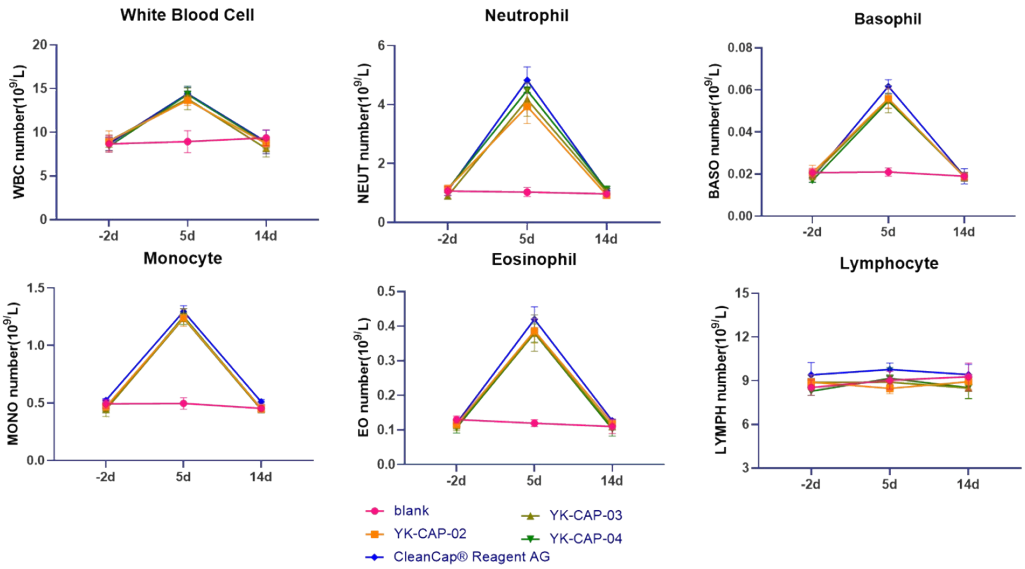


Figure S20. Hematological changes of 5'capped mRNAs with YK-CAPs. The counts of various white blood cells (white blood cells, neutrophils, basophils, monocytes, eosinophils and lymphocytes) in Sprague-Dawley rats , were measured at day -2, 5 and 14 after one vaccination with LNPs encapsulated with 5'capped OVA mRNAs with YK-CAP-02~04 and CleanCap® Reagent AG at day 0 (n = 3). Blank refers to DPBS-treatment.

Table S1 The information of hyper-parameters

Hyper-parameters	Value
Learning Rate	0.001
Batch size	32
Epoch	500
Optimizer	Adam
Loss Fuction (generative model)	CrossEntropyLoss
Loss Fuction (discriminative mode)	BCELoss