Supplementary Materials Part I for

Scalable convergent synthesis of therapeutic oligonucleotides

Xuan Zhou, Wuming Yan, William F. Kiesman, Yannick A. Fillon, Hong Jiang, Firoz D. Antia, Li Xiao, Jing Yang, Armin Delavari, Robert S. Gronke, Xianglin Shi*

Correspondence to: xianglin.shi@biogen.com

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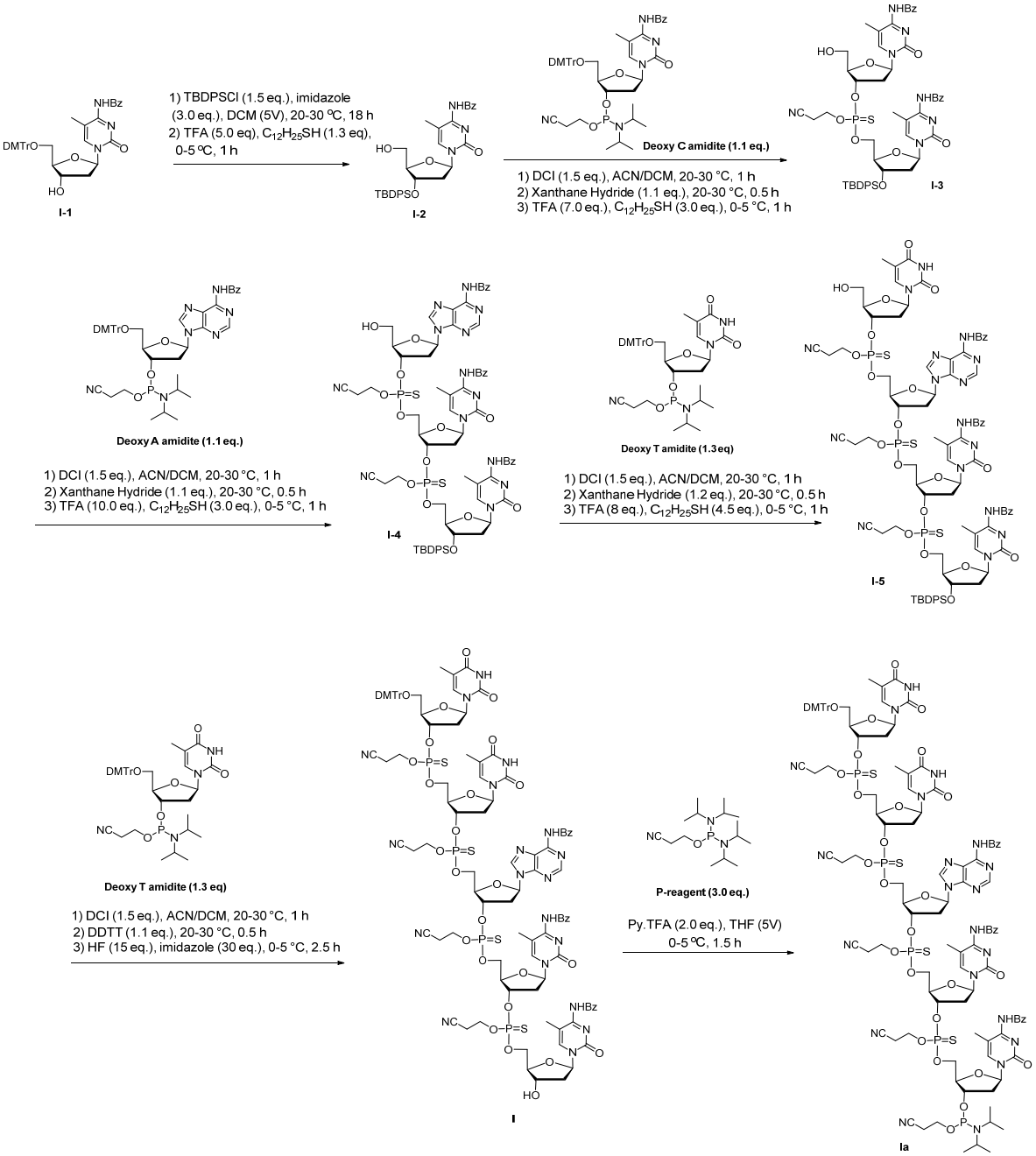
List of Schemes

Abbreviations

Ac	acetyl
ACN	acetonitrile anhydrous
AEX	Anion-exchange chromatography
ASOs	antisense oligonucleotides
Bz	benzoyl
CE	2-cyanoethyl
DCA	dichloroacetic acid
DCC	*N,N′*-dicyclohexylcarbodiimide
DCI	4,5-dicyanoimidazole
DCM	dichloromethane
DDTT	3-[(Dimethylaminomethylene)amino]-3H-1,2,4-dithiazole-5-thione
DIPE	Diisopropyl ether
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DMTr	4,4′-dimethoxytrityl
DMTrCl	4,4′-dimethoxytrityl chloride
EA/EtOAc	ethyl acetate
EDTA	Ethylenediamine tetra acetic acid
Eq.	equivalents
ETT	5-ethylthio-1*H*-tetrazole
h	hours
HIC	Hydrophobic Interaction Chromatography
HF	hydrogen fluoride
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
Im	imidazole
i-Pr	isopropyl
IPA	2-Propanol
Kg	kilogram
LPOS	Liquid phase oligonucleotide synthesis
MOE	Methoxyethyl
Me	methyl
MeOH	methanol
min	Minutes
MS	mass spectrometry
3Å MS	3Å molecular sieves
MTBE	Methyl tert-butyl ether
NH_4OH	Ammonium hydroxide
NMI	*N*-methylimidazole
NMI•TFA	*N*-methylimidazole trifluoroacetate
NMR	nuclear magnetic resonance
Py	pyridine
Py•TFA	pyridine trifluoroacetic acid
t-Bu	*tert*-butyl
TBDPS	*tert*-butyldiphenylsilyl
TBDPSCl	*tert*-butyl(chloro)diphenylsilane
TBuAA	Tributyl Ammonium Acetate
TCA	trichloroacetic acid
TEA	triethylamine

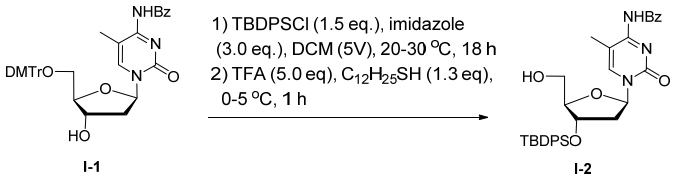
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
XH	Xanthane hydride

1. Synthesis of fragment I and its amidite Ia



Scheme 1. *Synthesis route of fragment **I***

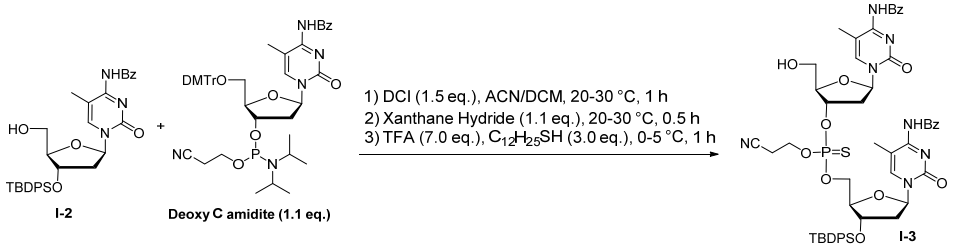
5'-OH-Deoxy C-3'-TBDPS, I-2



I-1 I-2

A mixture of **I-1** (150.2 g, 232.0 mmol, 1.00 *eq*), DCM (750 mL), imidazole (47.4 g, 696 mmol, 3.0 *eq*), TBDPSCl (95.0 g, 342.7 mmol, 1.49 *eq*) was stirred at 25 °C for 18 h (HLPC indicated compound **I-1** was consumed > 99.9%). After addition of IPA (14.0 g, 17.9 mL, 1.00 eq) the mixture was stirred at 25 °C for 0.5 h. The mixture was cooled to 0 °C and 1-dodecanethiol (60.8 g, 301.6 mmol, 1.30 *eq*) was added. TFA (132 g, 1160 mmol, 5.00 *eq*) was then added dropwise in 0.5 h and the mixture was stirred at 0 °C for 1 h (HPLC indicated the reaction conversion > 99.9%). The reaction mixture was added into Na_2CO_3 solution (74.2 g Na_2CO_3 dissolved in 750 mL DI water) in 30 min with vigorous stirring. The organic layer was separated, washed with brine (2 x 450 mL), dried over $MgSO_4$ (100 g), filtered, and concentrated in vacuo. The crude product was dissolved in DCM (375 mL) and added to a mixture of heptane/TBME (4.3 L, 18:1, v/v) with stirring. The precipitated product was filtered and washed with heptane (2 x 150 mL), dried under vacuum at 20-30 °C for 40 h to afford **I-2** as a white solid (128.2 g, 94.7% yield). HRMS calcd for $C_{33}H_{38}N_3O_5Si^+$ $[M+H]^+$: 584.2581, found: 584.2656.

5'-OH-Deoxy CC-3'-TBDPS, I-3



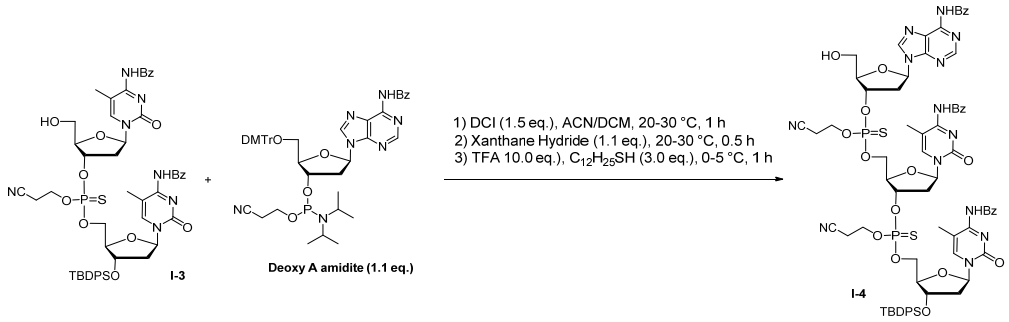
I-2 Deoxy C amidite (1.1 eq.) I-3

A mixture of **I-2** (100 g, 171.4 mmol, 1.00 eq), ACN/DCM (1000 mL, 1:1, v/v), deoxy C amidite (159.8 g, 188.5 mmol, 1.10 eq) and 3 Å MS (100.0 g) was stirred at 20-30 °C for 1 h. DCI (30.3 g, 257.1 mmol, 1.50 eq) was added and the reaction mixture was stirred at 20-30 °C for 1.0 h (HPLC indicated the conversion of **I-2** was > 99.9%). To the mixture was added xanthane hydride (28.3 g, 188.5 mmol, 1.10 eq) and the mixture was stirred at 20~30 °C for 0.5 h. The reaction mixture was cooled to 0 ± 5 °C and dodecane-1-thiol (105.7 g, 522.8 mmol, 3.05 eq) and TFA (137.8 g, 1.21 mol, 7.05 eq) were added slowly. The mixture was stirred at 0 ± 5 °C for 2 h (HPLC indicated the reaction conversion > 99.9%) and NMI (99.3 g, 1.21 mol, 7.0 eq) and EtOAc (3.0 L) were added and the mixture was stirred vigorously for 30 min at 20-30 °C.

The reaction mixture was filtered to remove 3 Å MS, washed with brine (5%wt, 1.0 L), aqueous $NaHCO_3$ solution (5%wt, 1.0 L), brine (2 x 1.0 L) and diluted with DCM (3.0 L). The solution was filtered through the silica gel pad (1500 g) and the pad was washed with a mixture of DCM/EtOAc (3.0 L, 1:1, v/v). The filtrate was concentrated, and the residue was dissolved in ACN (1000 mL) and then brine (5% wt, 0.5 L) was added. The mixture was extracted with heptane/TBME (2 x 2.5 L, 4:1, v/v). The bottom layers were collected and extracted with TBME

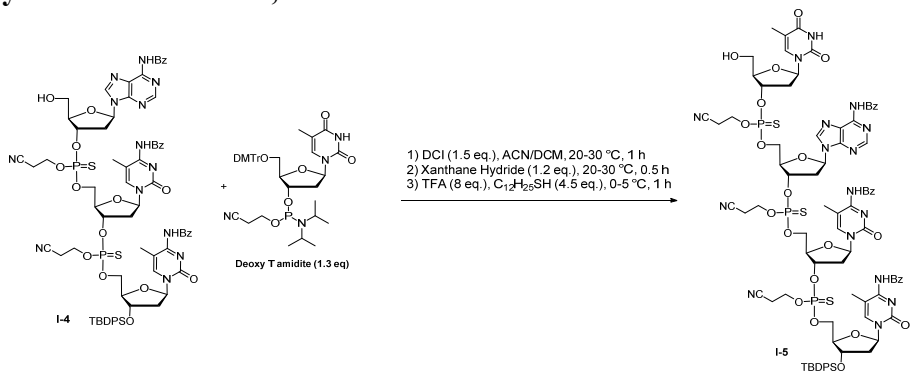
(720 mL) after addition of brine (1000 mL). The organic layer was separated and washed with brine (2 x 1.0 L) and then concentrated. The residue was dissolved in DCM azeotropically distilled and the **I-3** solution in DCM (600 mL) was used for the next step without further purification. HRMS calcd for $C_{53}H_{59}N_7O_{11}PSSi^+$ $[M+H]^+$: 1060.3500, found: 1060.3591.

5'-OH-Deoxy ACC-3'-TBDPS, I-4



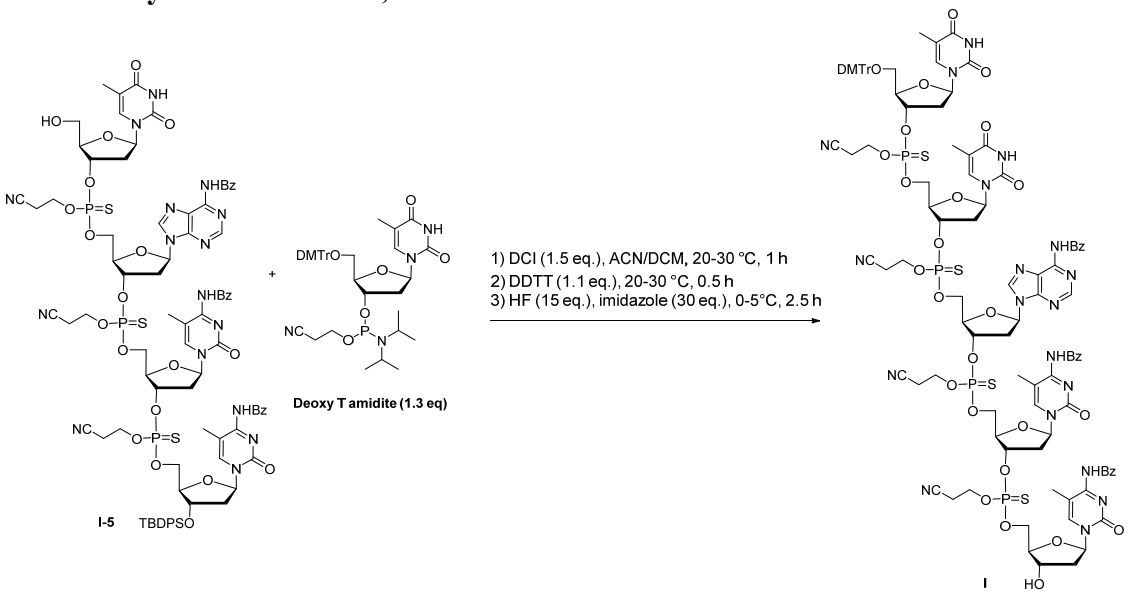
The DCM solution of **I-3** (181.6 g, 171.4 mmol, 1.00 eq) from the previous step was added to a mixture of deoxy A amidite (161.6 g, 188.5 mmol, 1.10 eq), 3 Å MS (120.0 g), and ACN (600 mL) and the mixture was stirred at 20-30 °C for 1 h. DCI (30.3 g, 257.1 mmol, 1.50 eq) was added and the mixture was stirred for 1.0 h at 20-30 °C (HPLC indicated the reaction conversion > 99.9%). Xanthane hydride (28.3 g, 188.5 mmol, 1.10 eq) was added, and the mixture was stirred at 20-30 °C for 0.5 h. The reaction mixture was cooled to 0 ± 5 °C and dodecane-1-thiol (105.0 g, 519.4 mmol, 3.03 eq) and TFA (194.7 g, 1.71 mol, 9.97 eq) were added slowly, and stirred at 0 ± 5 °C for 2 h (HPLC indicated the reaction conversion > 99.9%). NMI (155.1 g, 1.89 mol, 11.0 eq) and EtOAc/MTBE (3.0 L, 2:1, v/v) were added and the mixture was stirred vigorously for 30 min at 20-30 °C. The reaction mixture was filtered to remove 3 Å MS, washed with brine (5%wt, 2.0 L), aqueous $NaHCO_3$ solution (5%wt, 2 x 2.0 L), and brine (2 x 2.0 L), dried over $MgSO_4$ (100 gram), filtered, and concentrated in vacuo. The crude product was dissolved in DCM (1300 mL) and slowly added into MTBE (9.0 L). The precipitated product was filtered and washed with TBME (2 x 400 mL) and dried under vacuum at 20-30 ºC for 40 h to yield **I-4** as a yellowish solid (219.6 g, 82.9% yield). HRMS calcd for $C_{73}H_{78}N_{13}O_{16}P_2S_2Si^+$ $[M+H]^+$: 1546.4375, found: 1546.4368.

5'-OH-Deoxy-TACC-3'-TBDPS, I-5



A mixture of **I-4** (212.0 g, 137.2 mmol, 1.00 eq), ACN/DCM (1280 mL, 1:1, v/v), deoxy T amidite (132.7 g, 178.3 mmol, 1.30 eq), 3 Å MS (128.0 g) was stirred at 20-30 °C for 1 h and then DCI (24.2 g, 205.8 mmol, 1.50 eq) was added. The reaction mixture was stirred for 1.0 h at 20-30 °C (HPLC indicated the reaction conversion > 99.9%). IPA (0.103 eq.) was added and stirred for 0.5 h at 20~30 °C to quench the excess amidite. Xanthane hydride (24.7 g, 164.6 mmol, 1.20 eq) was and the mixture was stirred at 20-30 °C for 0.5 h. The reaction mixture was cooled to 0 ± 5 °C and dodecane-1-thiol (124.8 g, 617.4 mmol, 4.5 eq) and TFA (125.0 g, 1.10 mol, 8.0 eq) were added slowly and the reaction mixture was stirred at 0 ± 5 °C for 1 hour (HPLC indicated the reaction conversion > 99.9%). NMI (101.3 g, 1.23 mol, 9.0 eq) and EtOAc (6.4 L) were added and the mixture was stirred vigorously for 30 min at 20~30 °C. The reaction mixture was filtered to remove 3 Å MS, washed with brine (5%wt, 2.1 L), aqueous $NaHCO_3$ solution (5%wt, 2 x 4.2 L), brine (2 x 4.2 L), dried over $MgSO_4$ (100 gram), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in DCM (1500 mL) and slowly added to MTBE (8.5 L) to precipitate the product, which was filtered, washed with TBME (2 x 400 mL), dried under vacuum at 20-30 ºC for 40 h to afford **I-5** as a light-yellow solid (242.0 g, 92.0% yield). HRMS calcd for $C_{86}H_{94}N_{16}O_{22}P_3S_3Si^+$ $[M+H]^+$: 1919.4873, found: 1919.4801.

5'-DMTr-Deoxy TTACC-3'-OH, I

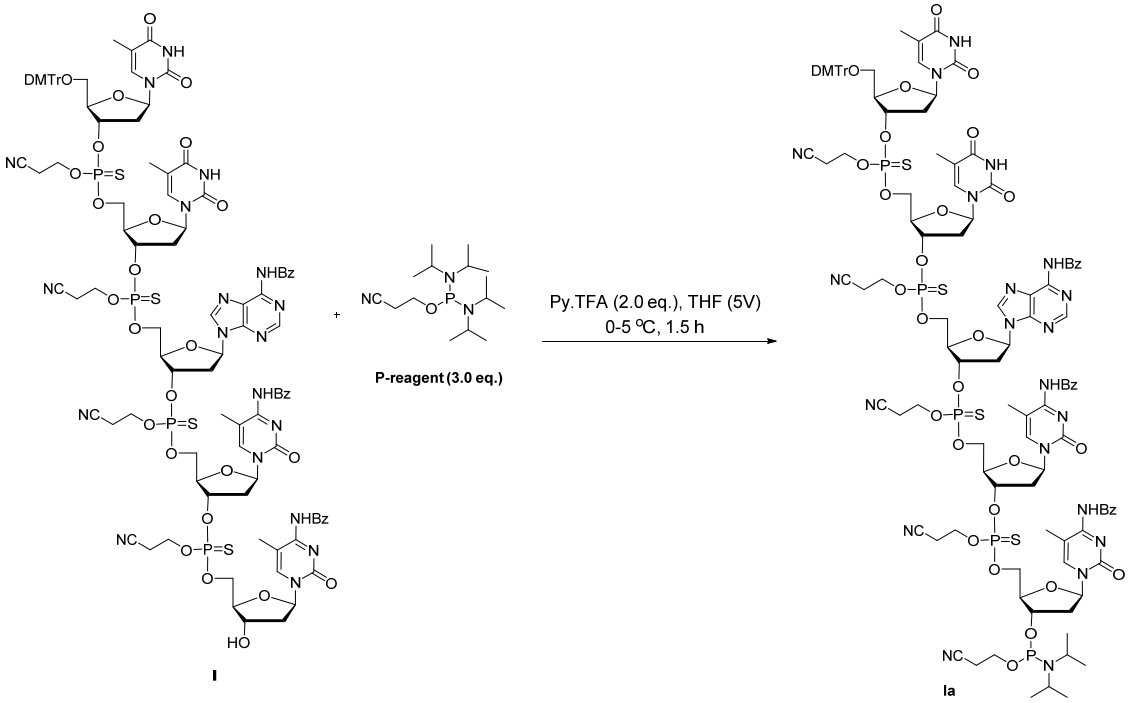


A mixture of **I-5** (235.0 g, 122.5 mmol, 1.00 eq), ACN/DCM (1600 mL, 3:1, v/v), deoxy T amidite (149.0 g, 159.3 mmol, 1.30 eq), and 3 Å MS (165.0 g) was stirred at 20-30 °C for 1 h and DCI (21.8 g, 183.8 mmol, 1.50 eq) was added, and the mixture was stirred for 0.5 h (HPLC indicated the reaction conversion > 99.8%). DDTT (27.8 g, 134.8 mmol, 1.10 eq) was added and the reaction mixture was stirred at 20~30 °C for 0.5 h.

To a separated reactor containing a solution of imidazole (188.4 g, 3.68 mol, 30.00 eq) in anhydrous THF (705 mL) at 0 ± 5 °C was added HF•Py (36.9 g,15.0 eq, ~70% HF in pyridine) slowly and the mixture was stirred at 0 ± 5 °C for 30 min. The freshly prepared HF-imidazole solution was slowly added into the reaction mixture from above step and the resulting mixture was

stirred at 0 ± 5 °C for 2.5 h (HPLC indicated the reaction conversion > 99.8%). EtOAc (5.4 L) was added and the reaction mixture was filtered to remove the 3 Å MS, washed with aqueous $NaHCO_3$ solution (2.5%wt, 2 x 3.5 L), brine (2 x 2.8 L), dried over $MgSO_4$ (100 gram), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in DCM/ACN (2.5 L, 2:1, v/v) and was slowly added to MTBE (11.8 L). The precipitated product was filtered, washed with TBME (2 x 940 mL), and dried under vacuum at 20-30 ºC for 40 h to yield **I** as a light-yellow solid (287.3 g, 99.6% yield). HRMS calcd for $C_{104}H_{110}N_{19}O_{30}P_4S_4^+$ $[M+H]^+$: 2357.5533, found: 2357.5610.

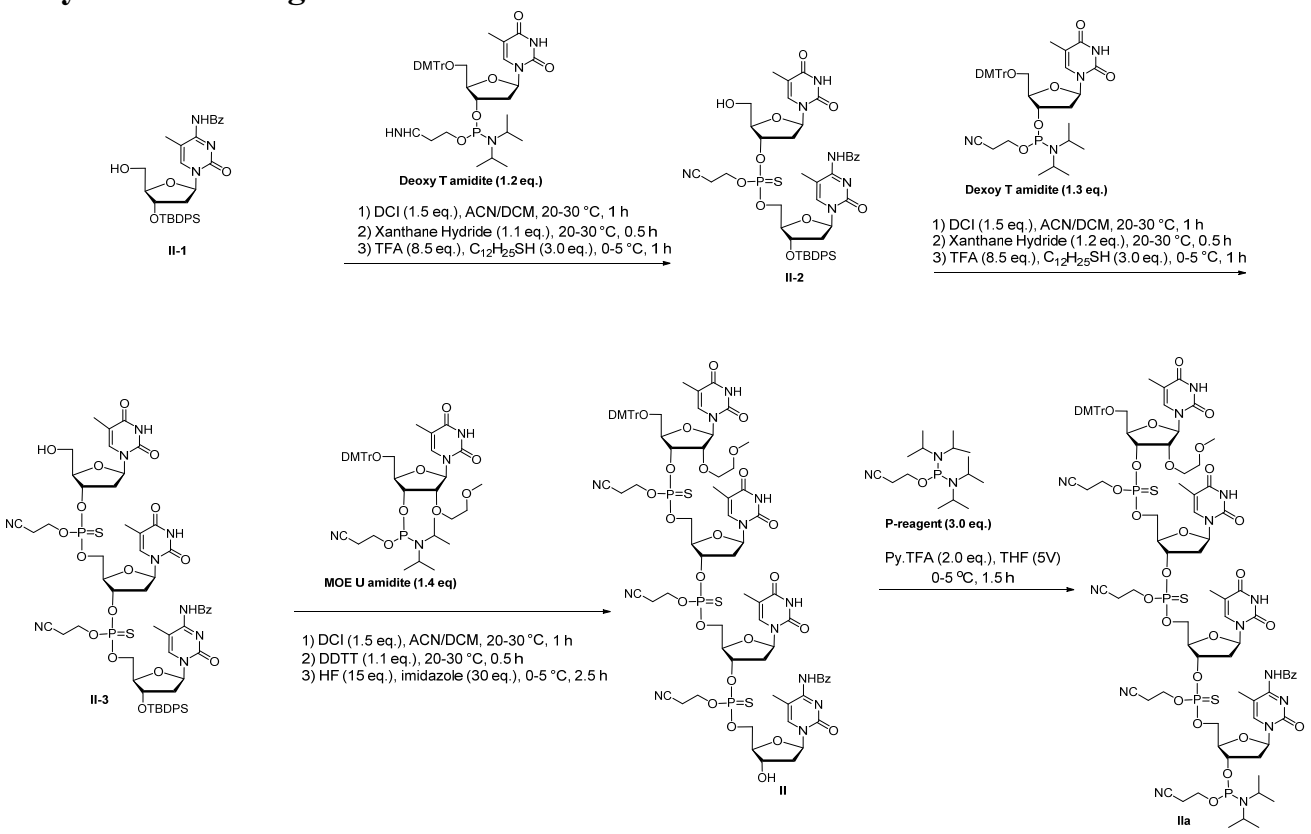
5'-DMTr-Deoxy-TTACC-3'-Phosphoramidite, Ia



A solution of **I** (270 gram, 114.6 mmol) in DCM (1000 mL) was azeotropically distilled on a rotary evaporator with continuous addition of DCM (~2 L) until the water content of the solution was <0.05% by Karl Fischer analysis). After removal of most of the DCM on the rotary evaporator, THF (1350 mL) was added and the mixture was cooled to 0 ± 5 ºC. 2-Cyanoethyl tetraisopropylphosphorodiamidite (104 gram, 344 mmol) and TFA•Py (44.3 gram, 229.2 mmol) were added and the reaction mixture was stirred at 0 ± 5 °C for 1 h until the conversion of compound **I** was >99% (monitored by HPLC).

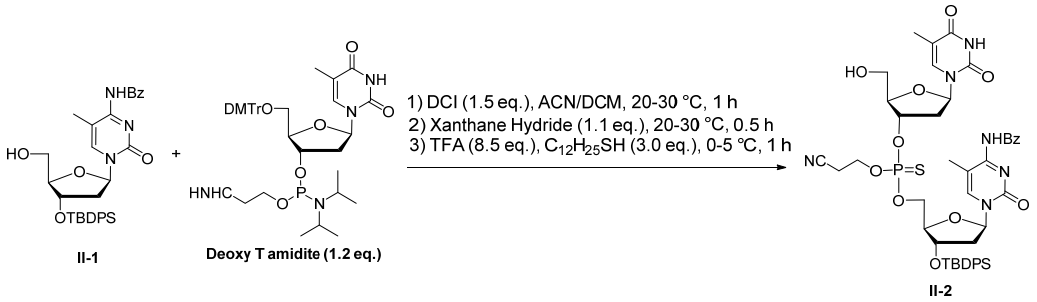
The reaction mixture was added into TBME/heptane (13.5 L, 6:1, v/v) 0 ± 5 ºC in 30 min with vigorous stirring and agitation was continued at the same temperature for 30 min. The precipitated product was filtered, washed with heptane/TBME (2 x 675 mL, 6:1, v/v), dried under vacuum at 20-30 ºC for 24 h to yield **Ia** as a white solid (283.0 gram, yield 96.6%).

2. Synthesis of fragment II



Scheme 2. *Synthesis route of Fragment* ***II***

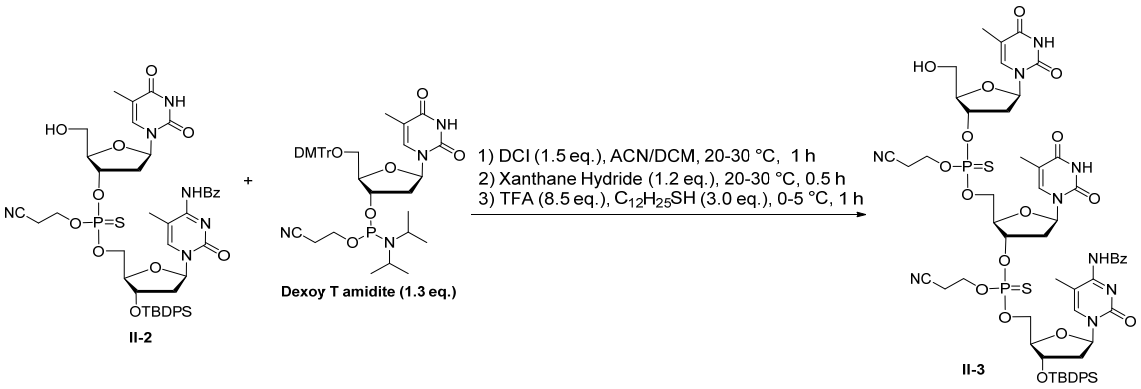
5'-OH-Deoxy-TC-3'-TBDPS, II-2



A mixture of **II-1** (171.5 g, 294.0 mmol, 1.00 eq), ACN/DCM (1700 mL, 1:1, v/v), deoxy T amidite (261.5 g, 352.8 mmol, 1.20 eq) and 3 Å MS (170.0 g) was stirred at 20-30 °C for 1 h and DCI (52.0 g, 441 mmol, 1.50 eq) wad added. The reaction mixture was stirred for 1.0 h at 20-30 °C (HPLC indicated the reaction conversion > 99.9%). IPA (1.8 g, 30.3 mmol, 0.103 eq.) was added, and the reaction mixture was stirred for 0.5 h at 20~30 °C to quench the excess amidites. Xanthane hydride (49.0 g, 326.3 mmol, 1.11 eq) was added and the mixture was stirred at 20~30 °C for 0.5 h. The reaction mixture was cooled to 0 ± 5 °C and dodecane-1-thiol (182.2.8 g, 899.6 mmol, 3.06 eq) and TFA (283.5 g, 2.49 mol, 8.46 eq) were added slowly and the reaction mixture was stirred

at 0 ± 5 °C for 1 h (HPLC indicated the reaction conversion > 99.9%). The reaction mixture was filtered to remove 3 Å MS and added into Na_2CO_3 solution (163.5 g Na_2CO_3 dissolved in 1.80 L DI water) in 30 min under vigorous stirring. EtOAc/TBME (5.1 L, 1:3, v/v) was added and stirred vigorously for 10 min. The organic layer was separated, washed with $NaHCO_3$ solution (3.5%wt, 2 x 1.7 L), brine (2 x 1.7 L), dried over $MgSO_4$ (170 gram), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in EtOAc/DIPE (850 mL, 4:1, v/v) and slowly added to a mixture of DIPE/heptane (6.6 L, 7:32, v/v). The precipitated product was filtered, washed with heptane (2 x 170 mL) and dried under vacuum at 20-30 °C for 40 h to yield **II-2** as a yellowish solid (261.5 g, 93.0% yield). HRMS calcd for $C_{46}H_{54}N_6O_{11}PSSi^+$ $[M+H]^+$: 957.3078, found: 957.3038.

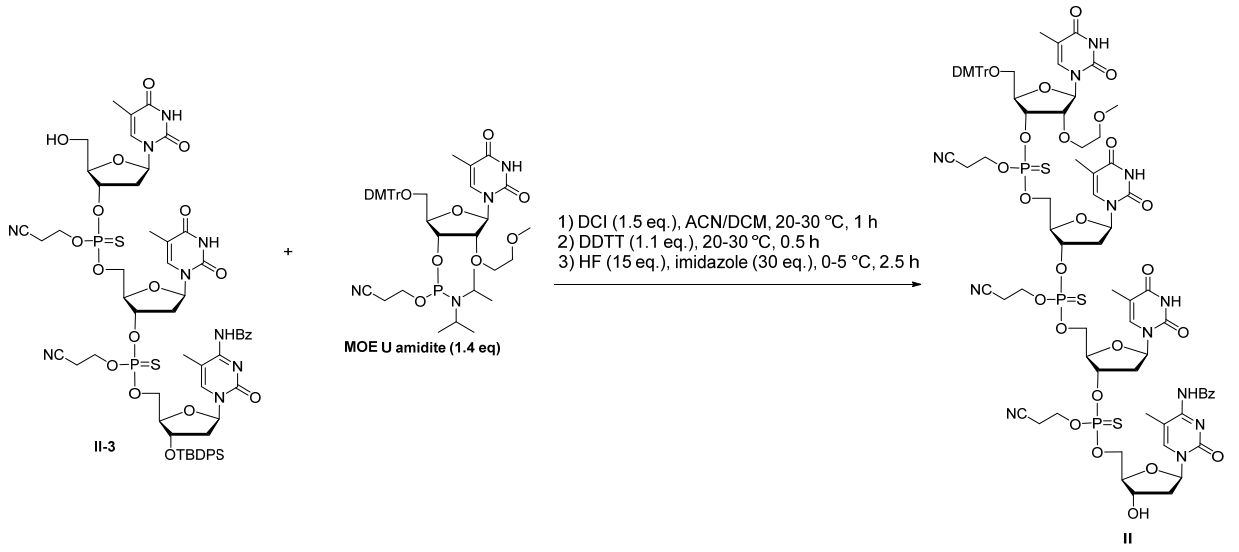
5'-OH-Deoxy-TTC-3'-TBDPS, II-3



A mixture of **II-2** (253.5 g, 265.1 mmol, 1.00 eq), ACN/DCM (1400 mL, 1:1, v/v), deoxy T amidite (256.5 g, 344.6 mmol, 1.30 eq) and 3 Å MS (210.0 g) was stirred at 20-30 °C for 1 h and DCI (47.0 g, 397.6 mmol, 1.50 eq) was added. The reaction mixture was stirred for 1.0 h at 20-30 °C (HPLC indicated the reaction conversion > 99.9%). IPA (1.6 g, 26.6 mmol, 0.103 eq.) was added and stirred for 0.5 h at 20-30 °C to quench the excess amidite. Xanthane hydride (47.8 g, 318.1 mmol, 1.2 eq) was added and the mixture was stirred at 20~30 °C for 0.5 h. The reaction mixture was cooled to 0 ± 5 °C and dodecane-1-thiol (161.0 g, 795.3 mmol, 3.0 eq) and TFA (257.0 g, 2.25 mol, 8.5 eq) were added slowly, and the reaction mixture was stirred at 0 ± 5 °C for 1 h (HPLC indicated the reaction conversion > 99.9%).

The reaction mixture was filtered to remove 3 Å MS and was added into Na_2CO_3 solution (147.5 g Na_2CO_3 in 2.6 L DI water) in 30 min under vigorous stirring. EtOAc/TBME (10 L, 1:2, v/v) was added and stirred vigorously for 10 min. The organic layer was separated, washed with $NaHCO_3$ solution (3.5% wt, 2 x 2.5 L), brine (2 x 2.5 L), dried over $MgSO_4$ (250 gram), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in EtOAc (1300 mL) and added to a mixture of TBME/heptane (7.9 L, 1:1, v/v). The precipitated product was filtered, washed with MTBE/heptane (2 x 250 mL, 1:1, v/v) and dried under vacuum at 20-30 °C for 40 h to yield **II-3** as a lightly yellow solid (315.0 g, 89.4% yield). HRMS calcd for $C_{59}H_{69}N_9O_{17}P_2S_2Si^+$ $[M+H]^+$: 1330.3576, found: 1330.3634.

5'-DMTr-MOE-U-Deoxy TTC-3'-OH, II

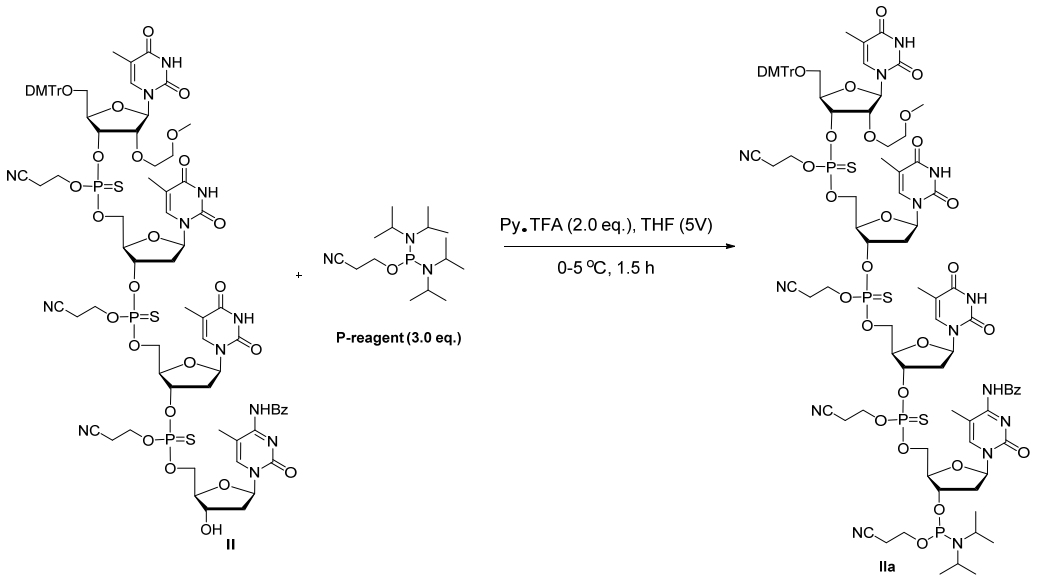


A mixture of **II-3** (301.5 g, 226.8 mmol, 1.00 eq), ACN (1500 mL), MOE U amidite (245.0 g, 299.4 mmol, 1.32 eq) and 3 Å MS (301.0 g) was stirred at 20-30 °C for 1 h and DCI (40.1 g, 340 mmol, 1.50 eq) was added. The mixture was stirred for ~1 h (HPLC indicated the reaction conversion > 99.8%). DDTT (51.5 g, 251.7 mmol, 1.10 eq) was added and the mixture was stirred at 20-30 °C for 0.5 h.

To a separated reactor containing a solution of imidazole (462.9 g, 6.8 mol, 30.00 eq) in anhydrous THF (1200 mL) at 0 ± 5 °C was added HF•Py (97.0 g, 15.0 eq, ~70% HF in pyridine) slowly and the mixture was stirred at 0 ± 5 °C for 30 min. The freshly prepared HF-imidazole solution was slowly added into the reaction mixture from above step and the resulting mixture was stirred at 0 ± 5 °C for 2.5 h (HPLC indicated the reaction conversion > 99.8%).

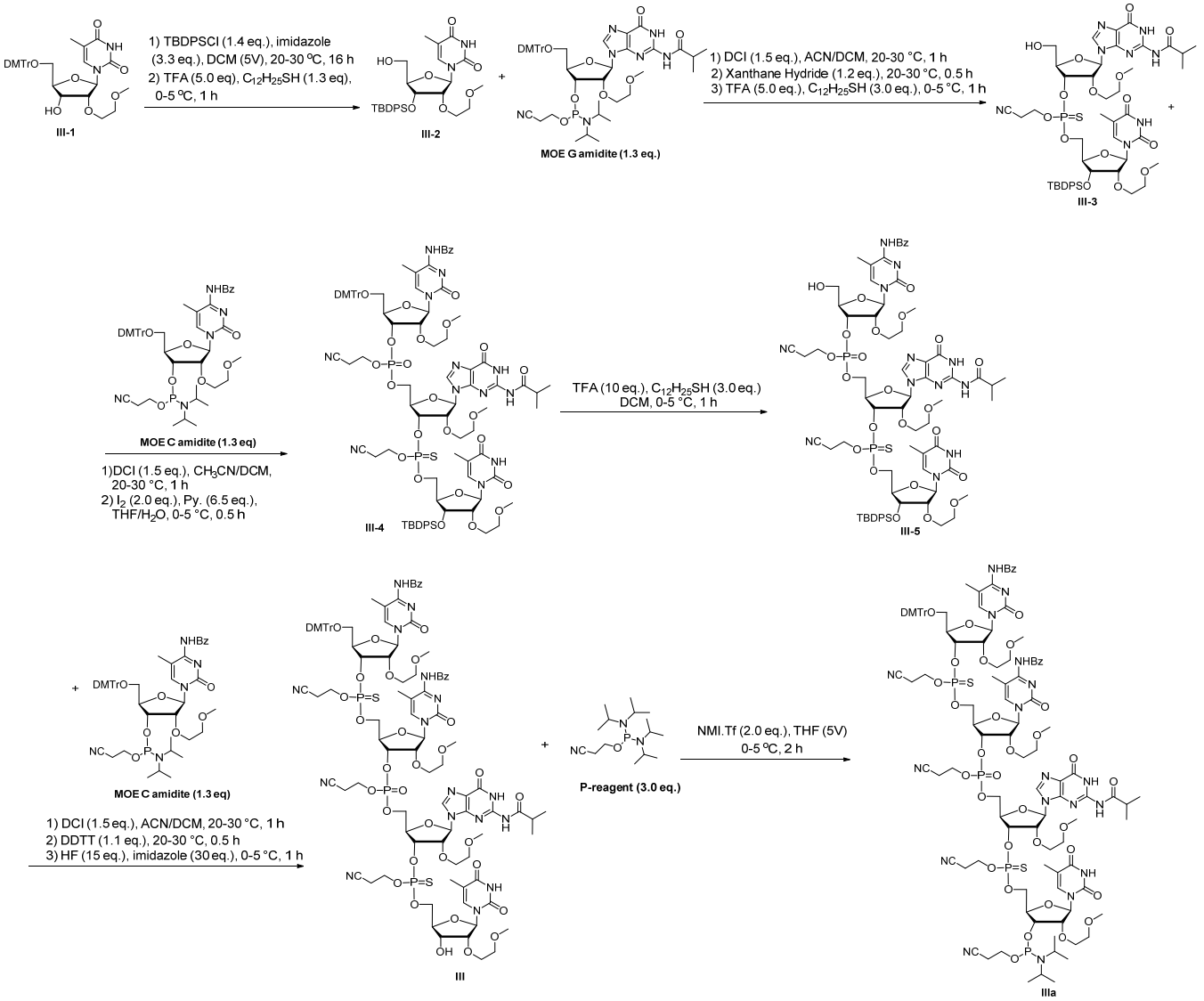
EtOAc (7.5 L) was added and the 3 Å MS was removed by filtration. The filtrate was washed with 3.5% aqueous $NaHCO_3$ solution (2 x 3.0 L), water (3.0 L), brine (3.0 L), dried over $MgSO_4$ (300 gram), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in DCM (2.1 L) and slowly added into EtOH (23.0 L). The precipitated product was filtered, washed with EtOH (2 x 300 mL) and dried under vacuum at 20-30 °C for 40 h to yield **II** as a lightly yellow solid (308.4 g, 74% yield). HRMS calcd for $C_{80}H_{92}N_{12}O_{27}P_3S_3^+$ $[M+H]^+$: 1841.4570, found: 1841.4640.

5'-DMTr-MOE-U-Deoxy TTC-3'- Phosphoramidite, IIa



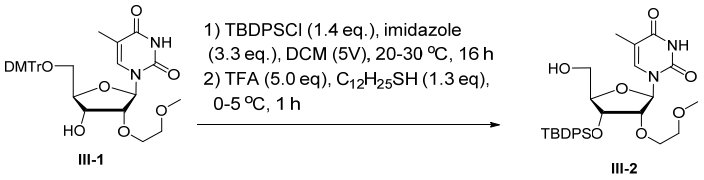
A solution of **II** (210 gram, 114.1 mmol) in DCM (1000 mL) was azeotropically distilled on a rotary evaporator with continuous addition of DCM (2 L) until the water content of the final solution was <0.05% by Karl Fischer analysis. After removal of most of the DCM with the rotary evaporator, the dried **II** was dissolved in THF (1050 mL). The mixture was cooled to 0 ± 5 ºC and 2-cyanoethyl tetraisopropylphosphorodiamidite (103.5 gram, 342 mmol) and TFA•Py (44.0 gram, 228.2 mmol) were added. The reaction mixture was stirred at 0 ± 5 °C for 1 h (the starting material conversion was > 99%, monitored by HPLC). The reaction mixture was then added into TBME/heptane (10.5 L, 6:1, v/v) in 30 min with vigorous stirring and the mixture was agitated at 0 ± 5 ºC for 30 min. The product was filtered, washed with heptane/TBME (2 x 675 mL, 6:1, v/v) and dried under vacuum at 20-30 ºC for 24 h to yield **IIa** as a white solid (230 gram, yield 98.7%).

3. Synthesis of fragment III



Scheme 3**. Synthesis route of fragment **III

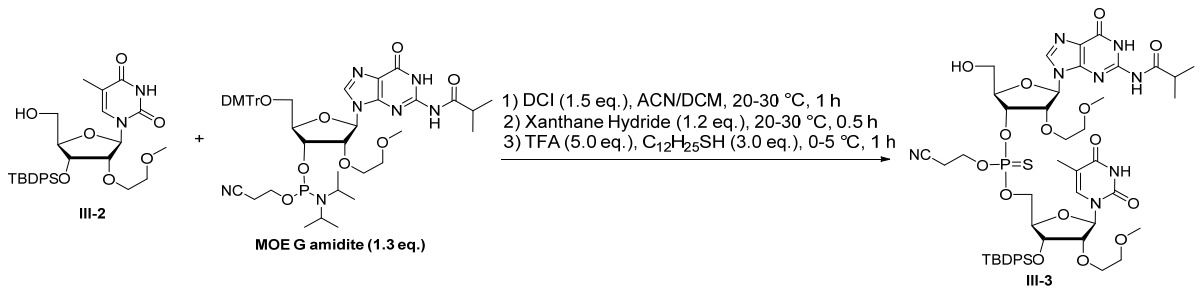
5'-OH-MOE-U-3'-TBDPS, III-2



A mixture of **III-1** (150.0 g, 242.0 mmol, 1.00 *eq*), imidazole (54.6 g, 798 mmol, 3.3 *eq*) and TBDPSCl (93.3 g, 338.8 mmol, 1.40 *eq*) in DCM (750 mL) was stirred at 25°C for 16 h (HLPC indicated compound **III-1** was consumed > 99.9%). After IPA (14.6 g, 18.6 mL, 1.00 eq) was

added, the mixture was stirred at 25 °C for 0.5 h and then cooled to 0 °C. 1-Dodecanethiol (63.8 g, 314.6 mmol, 75.3 mL, 1.30 *eq*) and TFA (138 g, 1210 mmol, 92.5 mL, 5.00 *eq*) were added dropwise and the mixture was stirred at 0 °C for 1 h (HPLC indicated the reaction conversion > 99.9%). The reaction mixture was added into Na_2CO_3 solution (77.1 g Na_2CO_3 in 750 mL DI water) in 30 min with vigorous stirring. The organic layer was separated, washed with brine (2 x 450 mL), dried over $MgSO_4$ (100 g), filtered, and concentrated in vacuo. The crude product was dissolved in ACN/H_2O (1350 mL, 2:1, v/v) and extracted with heptane/TBME (2 x 1350 mL, 4:1, v/v). The organic layer was washed with brine (2 x 450 mL), dried over $MgSO_4$ (100 g), filtered, and concentrated in vacuo. The product was dried under vacuum at 20-30 ºC for 40 h to yield **III-2** as a white foam solid (130.5 g, 97% yield). HRMS calcd for $C_{29}H_{38}N_2O_7Si^+$ $[M+H]^+$: 555.2527, found: 555.2596.

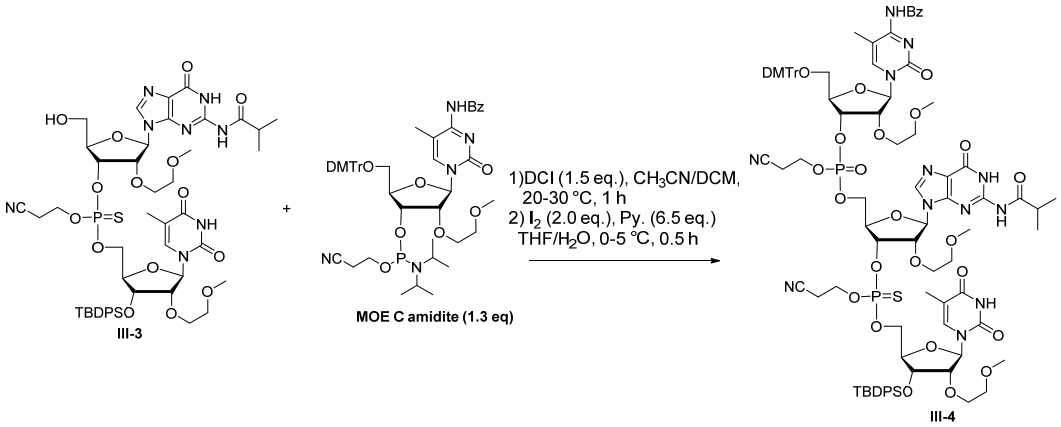
5'-OH-MOE-GU-3'-TBDPS, III-3



A mixture of **III-2** (120.0 g, 216.5 mmol, 1.00 eq), MOE G amidite (257 g, 281.5 mmol, 1.30 eq) and 3Å MS (120.0 g) in ACN (1.2 L) was stirred at 20~30 °C for 1 h and DCI (38.3 g, 324.8 mmol 1.50 eq) was added. The reaction mixture was stirred for 1.0 h at 20~30 °C (HPLC indicated the reaction conversion > 99.9%). IPA (1.3 gram, 21.6 mmol, 0.103 eq.) was added and the reaction mixture was stirred for 0.5 h at 20~30 °C to quench the excess amidites. Xanthane hydride (39 g, 259.8 mmol, 1.2 eq) was added and the reaction mixture was stirred at 20~30 °C for 0.5 h, cooled to 0 ± 5 °C and dodecane-1-thiol (131.4 g, 649.6 mmol, 3.0 eq). TFA (123.3 g, 1.08 mol, 5.0 eq) were added slowly and the reaction mixture was stirred at 0±5 °C for 1.0 h (HPLC indicated the reaction conversion > 99.9%).

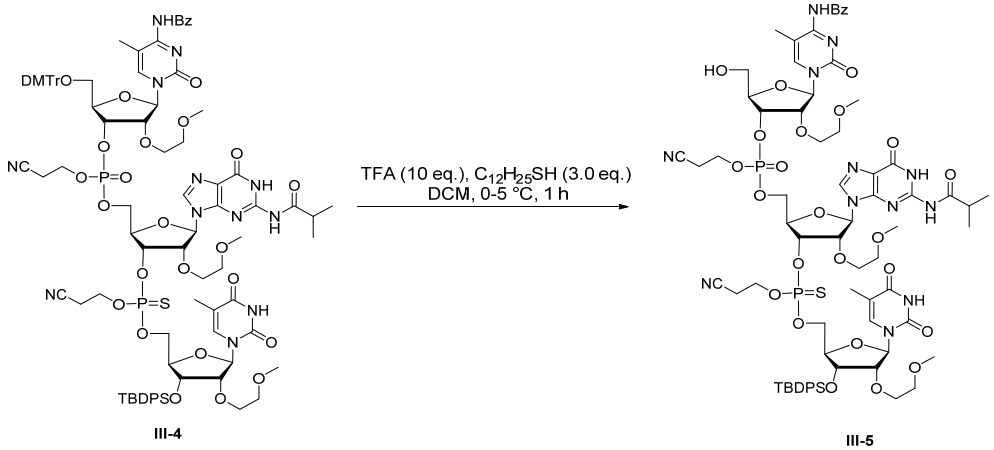
The reaction mixture was filtered to remove 3 Å MS and added into Na_2CO_3 solution (80.3 g Na_2CO_3 in 1.2 L DI water) in 30 min with vigorous stirring. EtOAc/TBME (3.6 L, 1:2, v/v) was added and stirred vigorously for 10 min. The organic layer was separated, washed with $NaHCO_3$ solution (3.5wt %, 2 x 2.4 L), brine (2.4 L), dried over $MgSO_4$ (120 g), filtered, and concentrated in vacuo. The crude product was dissolved in ACN/water (1.8 L, v/v, 2:1) and extracted with heptane/TBME (2 x 1.2 L, 4:1, v/v). The top layer was discarded and TBME (720 mL) and brine (500 mL) were added to the bottom layers. The organic layer was separated, washed with brine (2 x 500 mL), and concentrated. The crude product was dissolved in EtOAc/MTBE (1.2 L, 7:3, v/v) and PPh_3 (22.7 g, 86.6 mmol, 0.40 eq) was added. The solution was slowly added to a mixture of TBME/heptane (5.2 L, 7:36, v/v). The precipitated product was filtered, washed with heptane (2 x 120 mL), dried under vacuum at 20-30 ºC for 40 h to yield **III-3** as a lightly yellow solid (214.4 g, 90.3% yield). HRMS calcd for $C_{49}H_{66}N_8O_{15}PSSi^+$ $[M+H]^+$: 1097.3875, found: 1097.3854.

5'-DMTr-MOE-CoGU-3'-TBDPS, III-4



A mixture of **III-3** (200 g, 182.4 mmol, 1.00 eq), MOE C amidite (231.6 g, 237.2 mmol, 1.30 eq) and 3Å MS (200.0 g, 10 % wt/vol of solvent) in ACN (2.0 L) was stirred at 20-30 °C for 1 h, DCI (32.3 g, 273.6 mmol, 1.50 eq) was added, and the reaction mixture was stirred at 20-30 °C for 1.0 h (HPLC indicated the reaction conversion > 99.9%). A solution of iodine (92.5 g, 364.8 mmol, 2.00 eq) and pyridine (86.5 g, 1094.0 mmol, 6.00 eq) in THF/H_2O (1.2 L, 5:1, v/v) was added to the reaction mixture dropwise at 0-5 °C in 0.5 h and stirred at 0-5 °C for 0.5 h. A 4 wt% aqueous solution of $Na_2S_2O_3$ (90.5 g, 364.8 mmol, 2.00 eq) was added dropwise at 0-5 °C and stirred at 15-25 °C for 10 min. EtOAc/TBME (6.0 L, 1:1, v/v) was added and stirred vigorously for 30 min. The top organic layer was separated, washed with 5 wt% $NaHCO_3$ solution (2 x 2.0 L), brine (2.0 L), dried over $MgSO_4$ (200 g), filtered, and concentrated in vacuo to yield the crude product **III-4** (253.6 g), which was used for the next step without further purification.

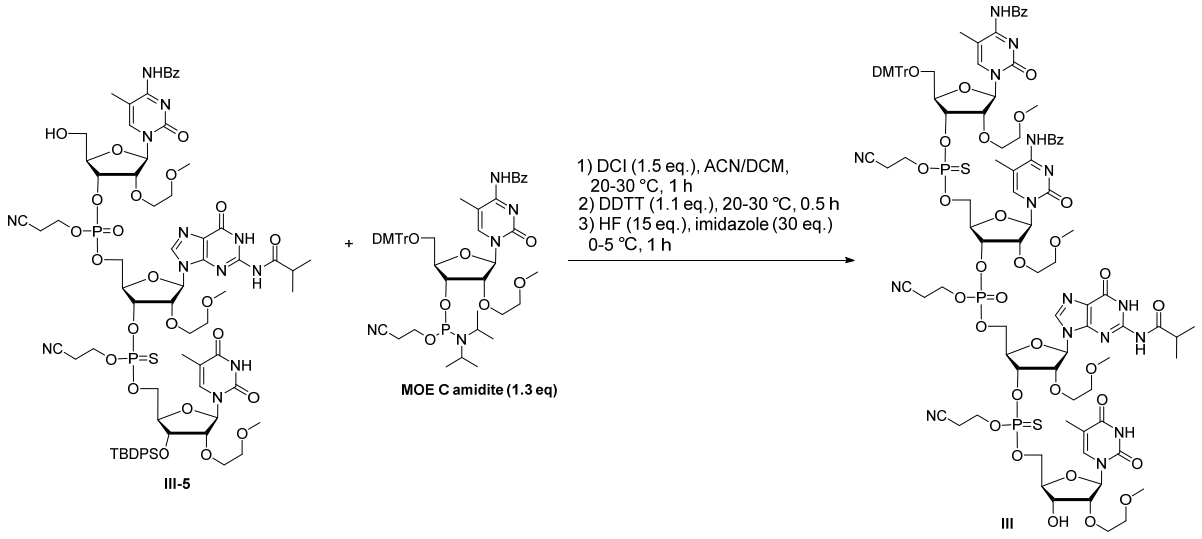
5'-OH-MOE-CoGU-3'-TBDPS, III-5



A solution of **III-4** (325.5 g, 168.4 mmol, 1.00 *eq*) 3Å MS (81 g) in DCM (1.63 L) was stirred at 25 ºC for 1.0 h and cooled down to 0-5 °C. 1-Dodecanethiol (102.0 g, 505.2 mmol, 3.0 *eq*) and TFA (191.8 g, 1684 mmol, 10.00 *eq*) were added dropwise at 0 °C in 0.5 h, the reaction mixture

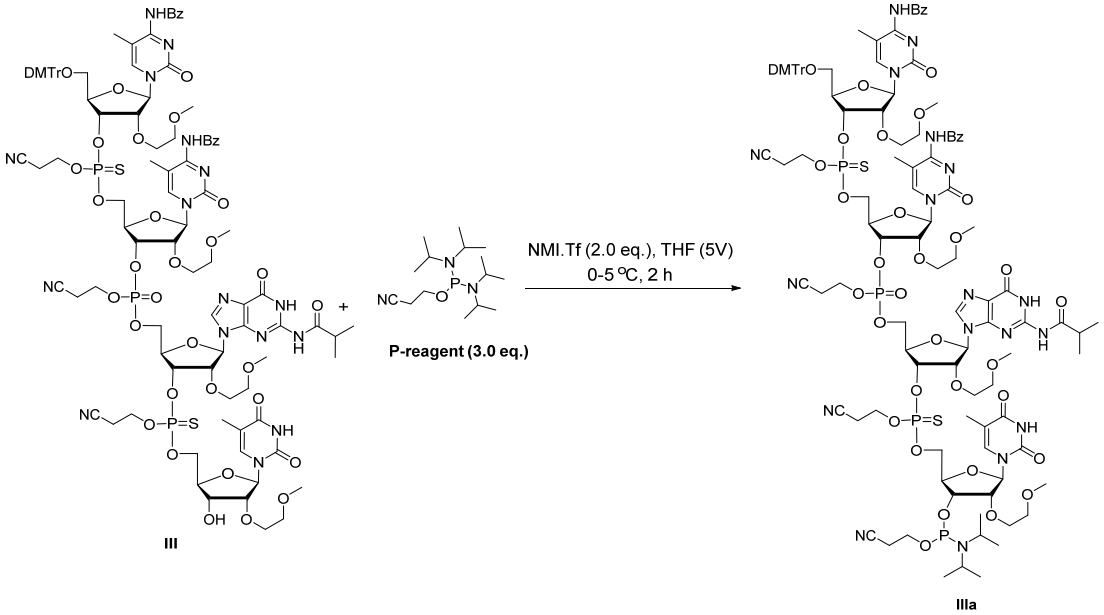
was stirred at 0±5 °C for 1.0 h (HPLC indicated the reaction conversion > 99.9%).
The reaction mixture was filtered to remove 3 Å MS and added into Na_2CO_3 solution (107 g Na_2CO_3 in 1.7 L DI water) with vigorous stirring. EtOAc (4.8 L) and MTBE (4.8 L) were added and stirred vigorously for 10 min. The organic layer was separated, washed with 5wt% aqueous $NaHCO_3$ solution (2 x 1.5 L), brine (1.5 L), dried over $MgSO_4$ (350 g), filtered, and concentrated in vacuo. The crude product was dissolved in EtOAC (650 mL) slowly added to a mixture of heptane/TBME (3.2 L, 1:1, v/v). The precipitated product was filtered, washed with heptane/TBME (2 x 350 mL, 1:1, v/v) and dried under vacuum at 20-30 ºC for 40 h to yield **III-5** as a white solid (245.1 g, 89.2% yield). HRMS calcd for $C_{72}H_{93}N_{12}O_{24}P_2SSi^+$ $[M+H]^+$: 1631.5391, found: 1631.5377.

5'-DMTr-MOE-CCoGU-3'-OH, III



A mixture of **III-5** (250 g, 153.3 mmol, 1.00 eq), MOE C amidite (183.7 g, 199.3 mmol, 1.30 eq) and 3Å MS (250.0 g, 10 % wt/vol of solvent) in ACN (2.5 L) was stirred at 20-30 °C for 1.0 h, DCI (27.5 g, 231.5 mmol, 1.50 eq) was added and the reaction mixture was stirred at 20-30 °C (for 1.0 h HPLC indicated the reaction conversion > 99.9%). DDTT (34.5 g, 168.6 mmol, 1.10 eq) was added and the reaction mixture was stirred at 20~30 °C for 0.5 h.
In a separated reactor, HF-Py (65.8 g, 2.30 mol HF, 15.0 eq, ~70% HF in pyridine) was slowly added into a solution of imidazole (313.1 g, 4.6 mol, 30.00 eq) in anhydrous THF (0.75 L) at -0±5 °C and stirred at 0±5 °C for 30 min. This HF-imidazole solution slowly added to the reaction mixture from above and the reaction mixture was stirred at 0±5 °C for 2.5 h (HPLC indicated the reaction conversion > 99.8%).
The reaction mixture was filtered to remove 3Å MS and EtOAc (5.0 L) was added. The solution was washed with the 2.5 wt% aqueous $NaHCO_3$ solution (2 x 3.7 L), brine (5 wt%, 9.0 L), brine (2 x 3.0 L), dried over $MgSO_4$ (150 g), filtered, and concentrated in vacuo. The crude product was dissolved in EtOAc (1.5 L) and slowly added to IPA (11.3 L). The precipitated product was filtered, washed with IPA (2 x 500 mL) and dried under vacuum at 20-30 ºC for 40 h to yield **III** as a lightly yellow solid (323.5 g, 93.6% yield). HRMS calcd for $C_{100}H_{120}N_{16}O_{34}P_3S_2^+$ $[M+H]^+$: 2246.6841, found: 2246.6826.

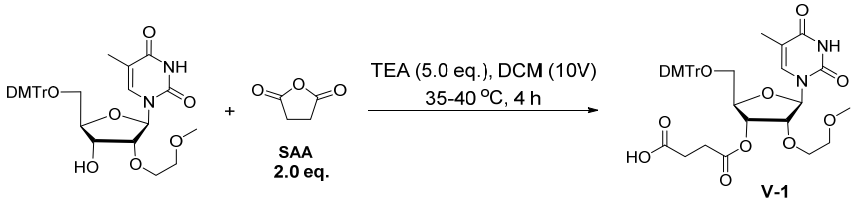
5'-DMTr-MOE-CCoGU-3'- Phosphoramidite, IIIa



A solution of **III** (100 g, 44.5 mmol) in DCM (500 mL) was evaporated azeotropically on a rotary evaporator at 20-35 °C (vapor temperature) with simultaneous addition of DCM to maintain a constant volume until the water content was <0.05% by Karl Fischer analysis and then concentrated. The dried **III** was dissolved in THF (500 mL) and the solution was cooled down to 0±5 ºC. The 2-cyanoethyl tetraisopropylphosphorodiamidite (40.2 gram, 133.5 mmol) and NMI•TFA (20.7 gram, 90.0 mmol) were added. The reaction mixture was stirred at 0±5 °C for 1 h (HPLC indicated the reaction conversion > 99.0%) and added into 5wt % aqueous $NaHCO_3$ solution (2.0 L). EtOAc (3.0 L) was added with vigorous stirring for 10 min. The organic layer was separated and washed with 5 wt% aqueous $NaHCO_3$ solution (2.0 L), brine (2 x 1.0 L), dried over Na_2SO_4 (200 g), filtered, and concentrated in vacuo to about 500 mL. The concentrated crude mixture was added to heptane/MTBE/Pyridine (4.525 L, 2:7:0.025, v/v/v) at 0±5 ºC in 30 min with vigorous agitation. The precipitated product was filtered, washed with heptane/TBME (2 x 250 mL, 2:7, v/v) and dried under vacuum at 20-30 ºC for 36 h to yield **IIIa** as a white solid (92.5 g, 84.9% yield).

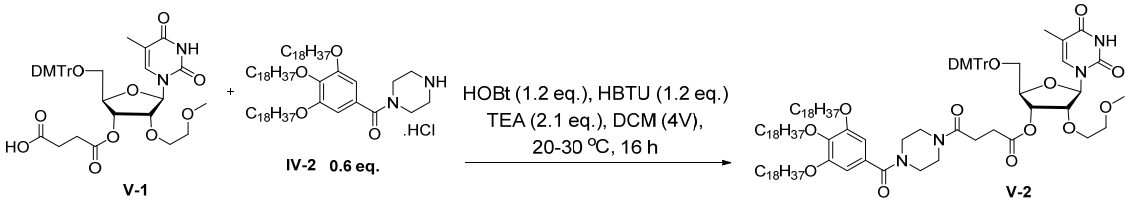
4. Synthesis of fragment V

5'-DMTr-MOE-U-3'-Succinate, V-1



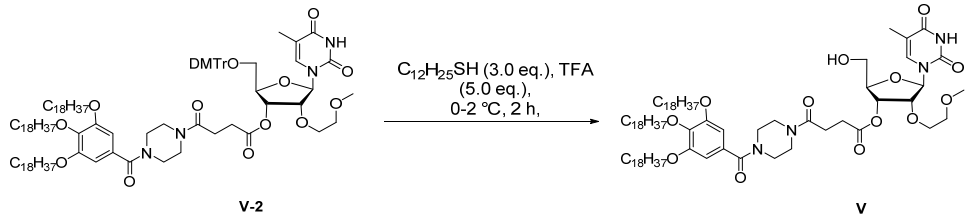
A mixture of 5'-DMT-MOE U (120 g, 194 mmol), TEA (98.16 g, 5.001 eq) and dihydrofuran-2,5-dione (SAA) (38.82 g, 2.0 eq) in DCM (1.20 L, 10V) was stirred for 4.0 h at 35-40 °C (HPLC indicated the reaction conversion > 99.0%). The reaction mixture was washed with the 0.2 M aqueous triethylamine phosphate solution (3 x 1.8 L) and brine (1.8 L). The bottom layer was separated, dried over Na_2SO_4 (120.0 g), filtered, and concentrated in vacuo. The crude product **V-1** was used directly in the next step without further purification.

5'-DMTr-MOE-U-3'-SDG, V-2



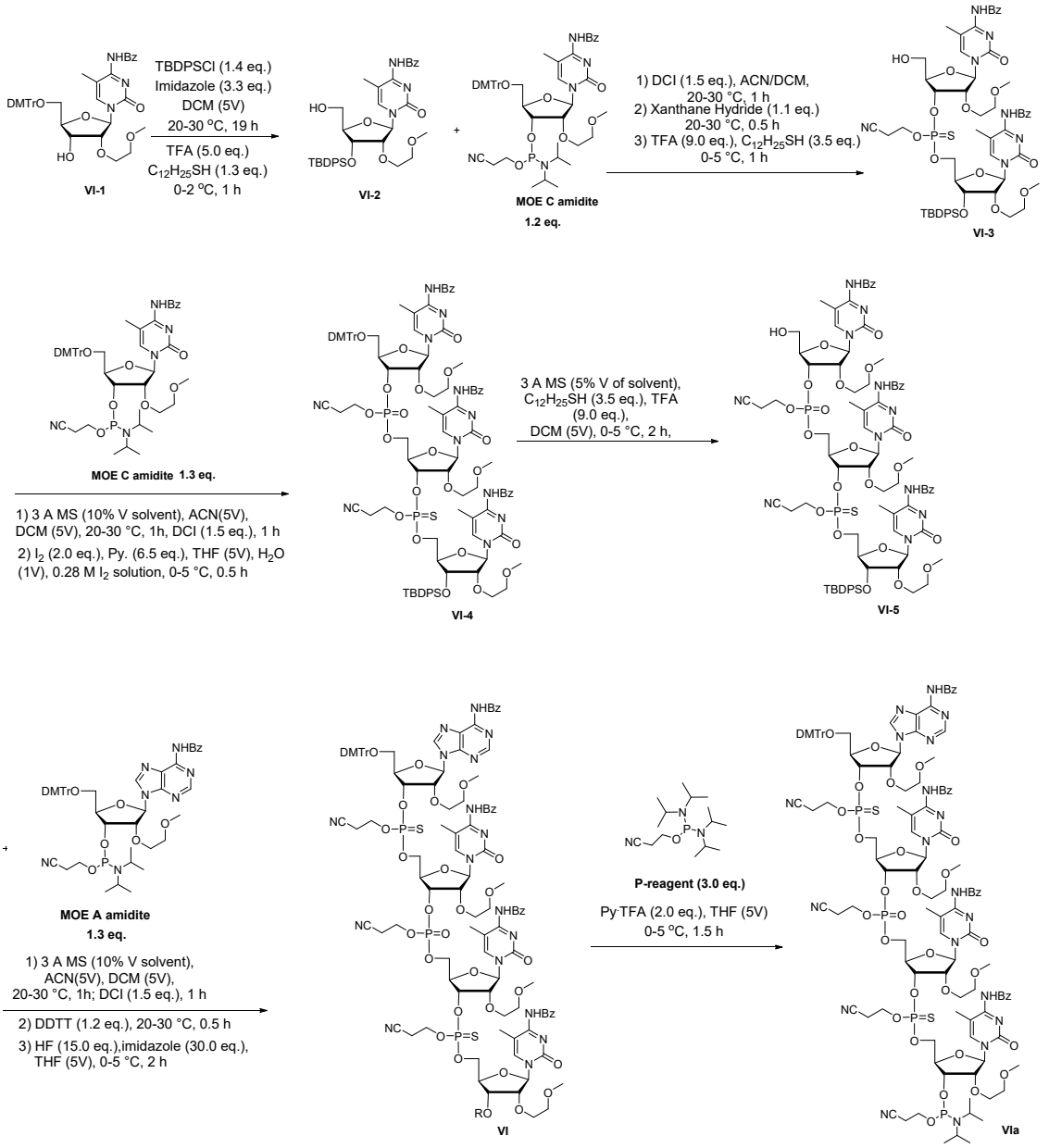
To a solution of **IV-2** (120 g, 115 mmol) in DCM (480 mL), TEA (41.22 g, 2.100 eq.), **V-1** (139.4 g, 191 mmol), HBTU (88.30 g, 1.200 eq) and HOBt (31.44 g, 1.200 eq) were added (Note: the order of reagent addition was essential in this step), the reaction mixture was stirred at 20-30 °C for 16 h (HPLC indicated the reaction conversion > 99.0%), and concentrated to about 250 mL. The concentrated reaction mixture was slowly added to ACN (2.4 L) in 0.5 h with vigorous agitation at 20-30 °C. The precipitated product was filtered, washed with ACN (2 x 120 mL) and dried under vacuum at 27-33 °C for 56 h to give an off-white solid **V-2** (188.16 g, 95.3% yield).

5'-OH-MOE U-3'-SDG, V



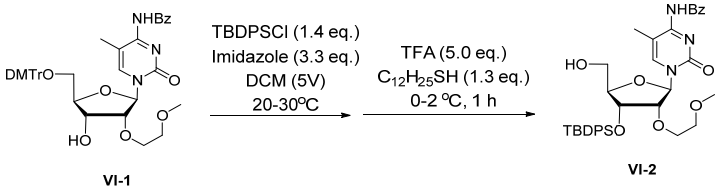
A solution of **V-2** (180.00 g, 106 mmol) in DCM (1.8 L, 10V) was cooled down to 0-5 °C, 1-dodencanethiol (64.43 g, 3.0 eq) and TFA (60.49 g, 5.0 eq.) were added slowly. The reaction mixture was stirred at 0±5 °C for 2 h (HPLC indicated the reaction conversion > 99.0%). Pyridine (58.75 g, 7.0 eq) was added slowly at 0±5 °C and the reaction mixture was added to ACN (5.4 L, 30 V) slowly at 0±5 °C with vigorous agitation. The precipitated product was filtered, washed with ACN (2 x180 m, 2V) and dried under vacuum at 20-30 °C for 40 h to give a white solid **V** (154.09 g, 104.2% yield).

5. Synthesis of fragment VI



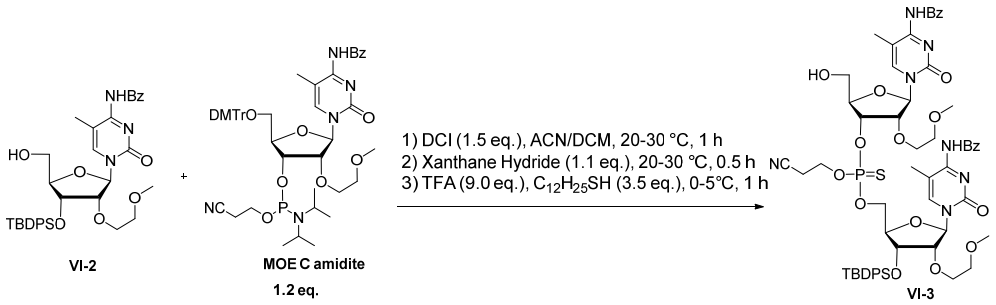
Scheme 4. *Synthesis route of fragment **VI***

5'-OH-MOE C-3'-TBDPS, VI-2



A mixture of compound **VI-1** (100 g, 138.6 mmol, 1.00 *eq*), DCM (500 mL), imidazole (31.1 g, 457 mmol, 3.30 *eq*), and TBDPSCl (53.3 g, 194 mmol, 1.40 *eq*) was stirred at 25 °C for 20 h (HLPC indicated compound **VI-1** was consumed > 99.9%). IPA (8.3 g, 138.6 mmol, 1.00 eq) was added and the mixture was stirred at 25 °C for 0.5 h, cooled to 0 ± 5 °C, and 1-dodecanethiol (36.5 g, 180 mmol, 43.1 mL, 1.30 *eq*) was added. TFA (79 g, 693 mmol, 53.0 mL, 5.00 *eq*) was added dropwise in 0.5 h, and the mixture was stirred at 0°C for 1 h (HPLC indicated the reaction conversion > 99.9%). The reaction mixture was added into aqueous Na_2CO_3 solution (44.1 g Na_2CO_3 dissolved in 500 mL of deionized water) in 30 min with vigorous stirring. The organic layer was separated, washed with brine (2 x 300 mL), dried over anhydrous $MgSO_4$ (100 g), filtered, and concentrated in vacuo. The crude product was dissolved in DCM (150 mL) and added to a mixture of heptane-TBME (2.85 L, 18:1, v/v). The precipitated product was filtered and washed with heptane (2 x 100 mL)), dried under vacuum at 20-30 ºC for 36 h to yield **VI-2** as a white solid (83.10 g, 91.2% yield). HRMS calcd for $C_{36}H_{43}N_3O_7Si+$ $[M+H]^+$: 658.2949, found: 658.2869.

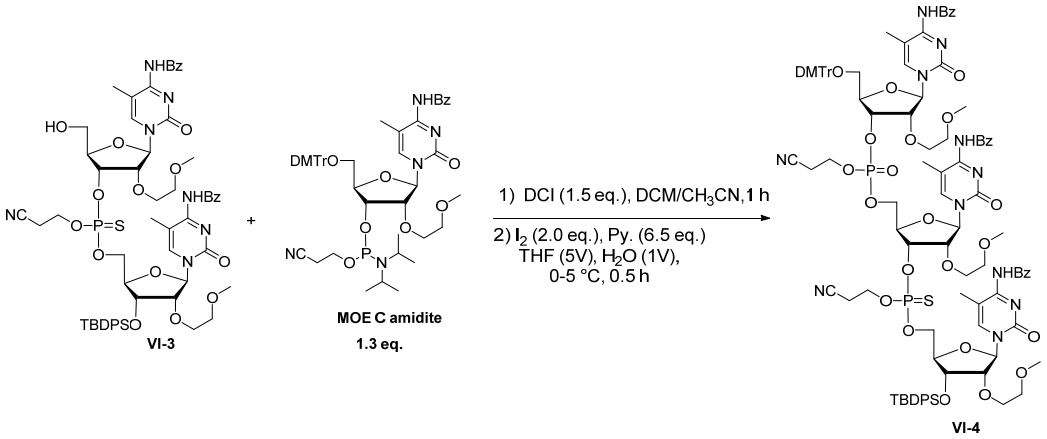
5'-OH-MOE CC-3'-TBDPS, VI-3



A mixture of **VI-2** (120 g, 182.6 mmol, 1.00 eq), ACN/DCM (1200 mL, 1:1, v/v), MOE C amidite (201.8 g, 219.1 mmol, 1.20 eq) and 3Å MS (120.0 g) was stirred at 20-30 °C for 1 h and then DCI (32.3 g, 273.9 mmol, 1.50 eq) was added. The mixture was stirred at 20-30 °C for 1.0 h (HPLC indicated the reaction conversion > 99.9%). After xanthane hydride (30.2 g, 200.9 mmol, 1.10 eq) was added the mixture was stirred at 20-30 °C for 0.5 h. The reaction mixture was cooled to 0 ± 5 °C, dodecane-1-thiol (129.2 g, 639.1 mmol, 3.50 eq) and TFA (187.0 g, 1.64 mol, 9.00 eq) were added sequentially and stirred at 0 ± 5 °C for 2 h (HPLC indicated the reaction conversion > 99.9%). The reaction mixture was filtered to remove 3 Å MS and the filtrate was added into Na_2CO_3 solution (80.0 g Na_2CO_3 dissolved in 1.20 L DI water) in 30 min with vigorous stirring. To the mixture was added a mixture of EtOAc/TBME (3.6 L, 1:2, v/v) at 0-15 °C and stirred vigorously for 10 min. The organic layer was separated and washed with aqueous $NaHCO_3$ solution (5%wt, 2 x 1.2 L), brine (1.2 L) and concentrated on a rotary evaporator. The residue was dissolved in ACN (600 mL) and water (360 mL) was added. The mixture was extracted with a mixture of heptane/TBME (1.2 L, 4:1, v/v). The bottom layers were collected and mixed with TBME (720 mL) and water (300 mL). The organic layer (upper layer) was separated, washed with brine (360 mL) and concentrated. The residue was dissolved in DCM (1.2 L) and filtered through a silica gel pad (300~400 mesh, 600 g) and the pad was washed with a mixture of EtOAc/DCM (7.2 L, 1:1, v/v). All the filtrates were collected and concentrated. The residue was redissolved in EtOAc (360 mL) and slowly added into the mixture of diisopropyl ether/heptane (5.4 L, 1:4, v/v). The product

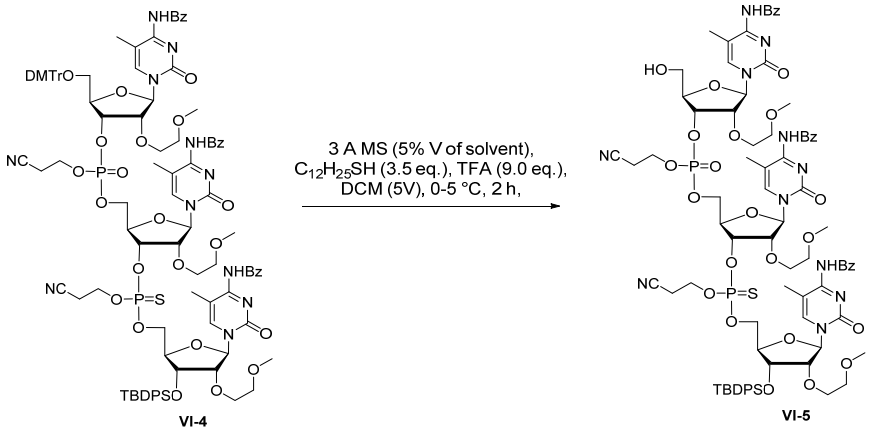
precipitated was filtered, washed with heptane (2 x 120 mL)), dried under vacuum at 20-30 ºC for 40 h to yield **VI-3** as a white solid (225.5 g, 102.3% yield). HRMS calcd for $C_{59}H_{70}N_7O_{15}PSSi^+$ $[M+H]^+$: 1208.4236, found: 1208.4189.

5'-DMTr-MOE CoCC-3'-TBDPS, VI-4



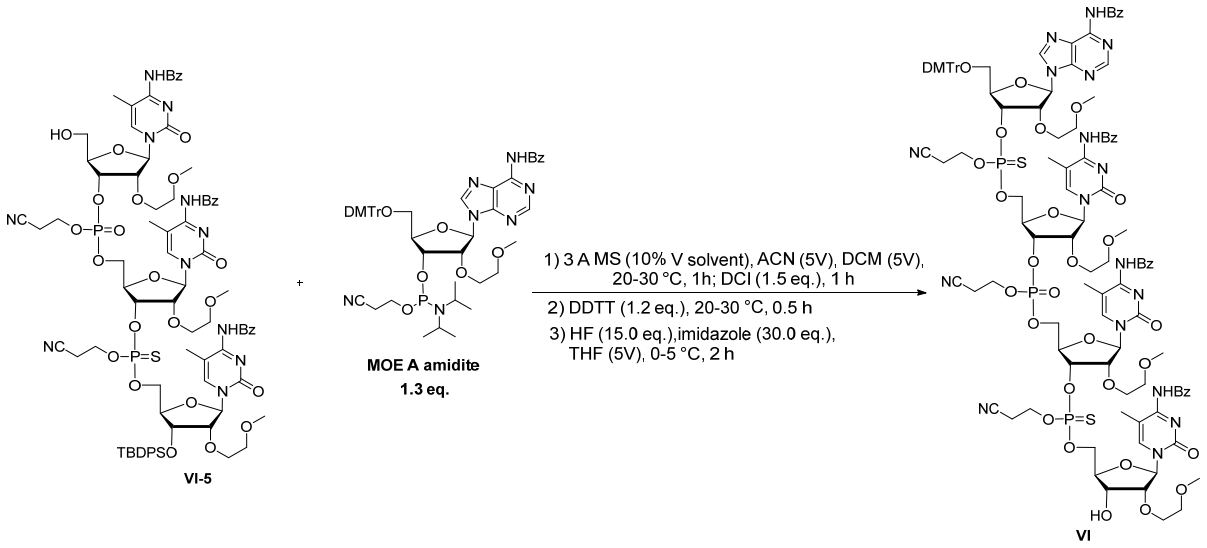
A mixture of **VI-3** (210 g, 174 mmol, 1.00 eq), ACN/DCM (2100 mL, 1:1, v/v), MOE C amidite (208.3 g, 226.2 mmol, 1.30 eq) and 3 Å MS (210.0 g) was stirred at 20-30 °C for 1 h, and then DCI (30.8 g, 161.0 mmol, 1.50 eq) was added and stirred for 1.0 h at 20-30 °C (HPLC indicated the reaction conversion > 99.9%). A solution of iodine (88.2 g, 348.0 mmol, 2.00 eq) and pyridine (89.4 g, 1131.0 mmol, 6.50 eq) in THF/H_2O (1260 mL, 5:1, v/v) was added to the reaction mixture dropwise at 0 ± 5 °C in 30 min and stirred at 0 ± 5 °C for 30 min. An aqueous solution of 4 % $Na_2S_2O_3$ solution (86.3 g, 348.0 mmol, 2.00 eq) was charged dropwise to the reaction mixture at 0 ± 5 °C and stirred at 15-25 °C for 10 min. A mixture of EtOAc/TBME (6.3 L, 1:2, v/v) was added and stirred vigorously for 30 min. The organic layer was separated, washed with 5 % $NaHCO_3$ aqueous solution (2 x 2.10 L) and brine (2.10 L), dried over $MgSO_4$ (210 g), filtered, and concentrated in vacuo to yield the crude product **VI-4** (~335.4 g). **VI-4** was used into the next step without further purification.

5'-OH-MOE CoCC-3'-TBDPS, VI-5



A mixture of compound **VI-4** (335.4 g, 164.2 mmol, 1.00 *eq*), DCM (1.67 L), and 3 Å MS (83.7 g) was stirred at 25 ºC for 1 h, cooled to 0 ± 5 °C and 1-dodecanethiol (116.1 g, 574.7 mmol, 3.5 *eq*) was added. TFA (168.3 g, 1477 mmol, 9.00 *eq*) was added dropwise at 0 ± 5 °C in 0.5 h and the mixture was stirred at 0 ± 5 °C for 2 h (HPLC indicated the reaction conversion > 99.9%).
After removal of the 3 Å MS by filtration, the reaction mixture was slowly added into an aqueous Na_2CO_3 solution (95.6 g Na_2CO_3 dissolved in 1000 mL DI water) with vigorous stirring. The mixture was then extracted with a mixture of EtOAc/MTBE (4200 mL, 1:1, v/v). The organic layer was separated, washed with 5% aqueous $NaHCO_3$ solution (2 x 1000 mL) and brine (1000 mL), dried over anhydrous $MgSO_4$ (210 g), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in EtOAC (630 mL) and slowly added to a mixture of heptane/TBME (9.45 L, 4:1, v/v). The product precipitated was filtered, washed with heptane/TBME, 2 x 210 mL, 4:1, v/v) and dried under vacuum at 20-30 ºC for 40 h to yield **VI-5** as a white solid (253.5 g, 83.7% yield). HRMS calcd for $C_{82}H_{97}N_{11}O_{24}P_2SSi^+$ $[M+H]^+$: 1742.5751, found: 1742.5732.

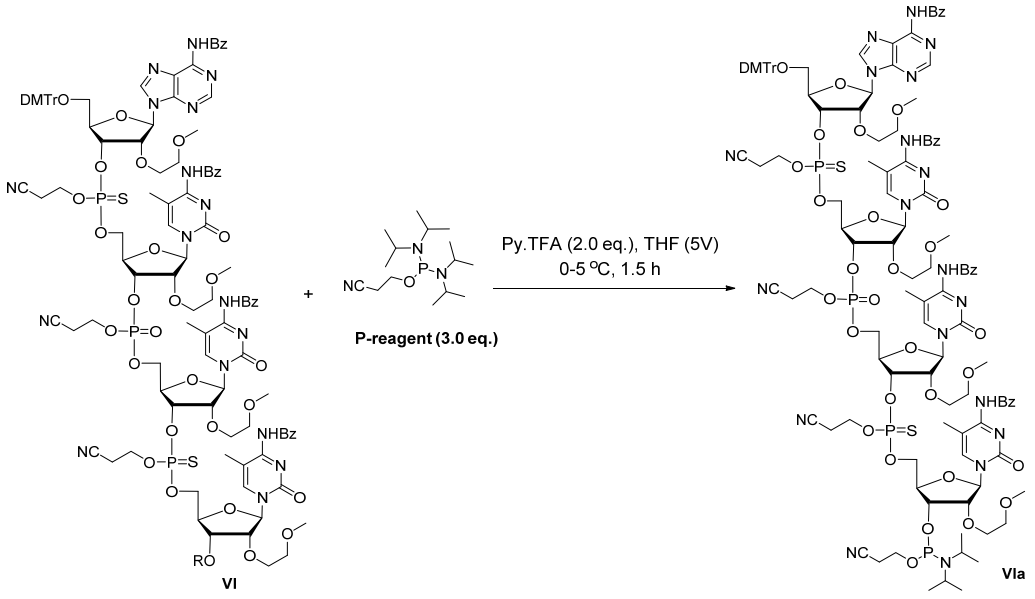
5'-DMTr-MOE ACoCC-3'-OH, VI



A mixture of **VI-5** (245.0 g, 140.0 mmol, 1.00 eq), ACN/DCM (2450 mL, 1:1, v/v), MOE A amidite (170.3 g, 182.0 mmol, 1.30 eq) and 3Å MS (245.0 g) was stirred at 20-30 °C for 1 h and DCI (24.9 g, 210.0 mmol, 1.50 eq) was added. The mixture was stirred at 20-30 °C for 1.0 h (HPLC indicated **VI-5** conversion > 99.8%). IPA (0.85 g, 14.0 mmol, 0.1 eq) was added and the mixture was stirred at 20-30 °C for 10 min. DDTT (34.6 g, 168.0 mmol, 1.20 eq) was added and the mixture was stirred at 20~30 °C for 0.5 h.
To a separated reactor containing the solution of imidazole (287.10 g, 4.2 mol, 30.00 eq) in anhydrous THF (1225 mL) at 0 ± 5 °C was added HF•Py (60.2 g, 15 eq., ~70% HF in pyridine) slowly and the mixture was stirred at 0 ± 5 °C for 30 min to yield a homogeneous solution. This freshly prepared HF-imidazole solution was slowly added into the reaction mixture above and the mixture was stirred at 0 ± 5 °C for 2 h (HPLC indicated the reaction conversion > 99.8%). EtOAc (4.9 L) was added and the 3Å MS was removed by filtration. The filtrate was washed with 2.5%

$NaHCO_3$ aqueous solution (2 x 2.45 L) and brine (2.45 L), dried over anhydrous $MgSO_4$ (210 g), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in EtOAC (1225 mL) and slowly added to a mixture of EtOH/heptane (18.5 L, 2:3, v/v). The precipitated product was filtered, washed with TBME (2 x 245 mL), dried under vacuum at 20-30 ºC for 40 h to yield **VI** as a slightly yellow solid (292.3 g, 87.8% yield). HRMS calcd for $C_{110}H_{123}N_{17}O_{33}P_3S_2^+$ $[M+H]^+$: 2367.7157, found: 2367.7173.

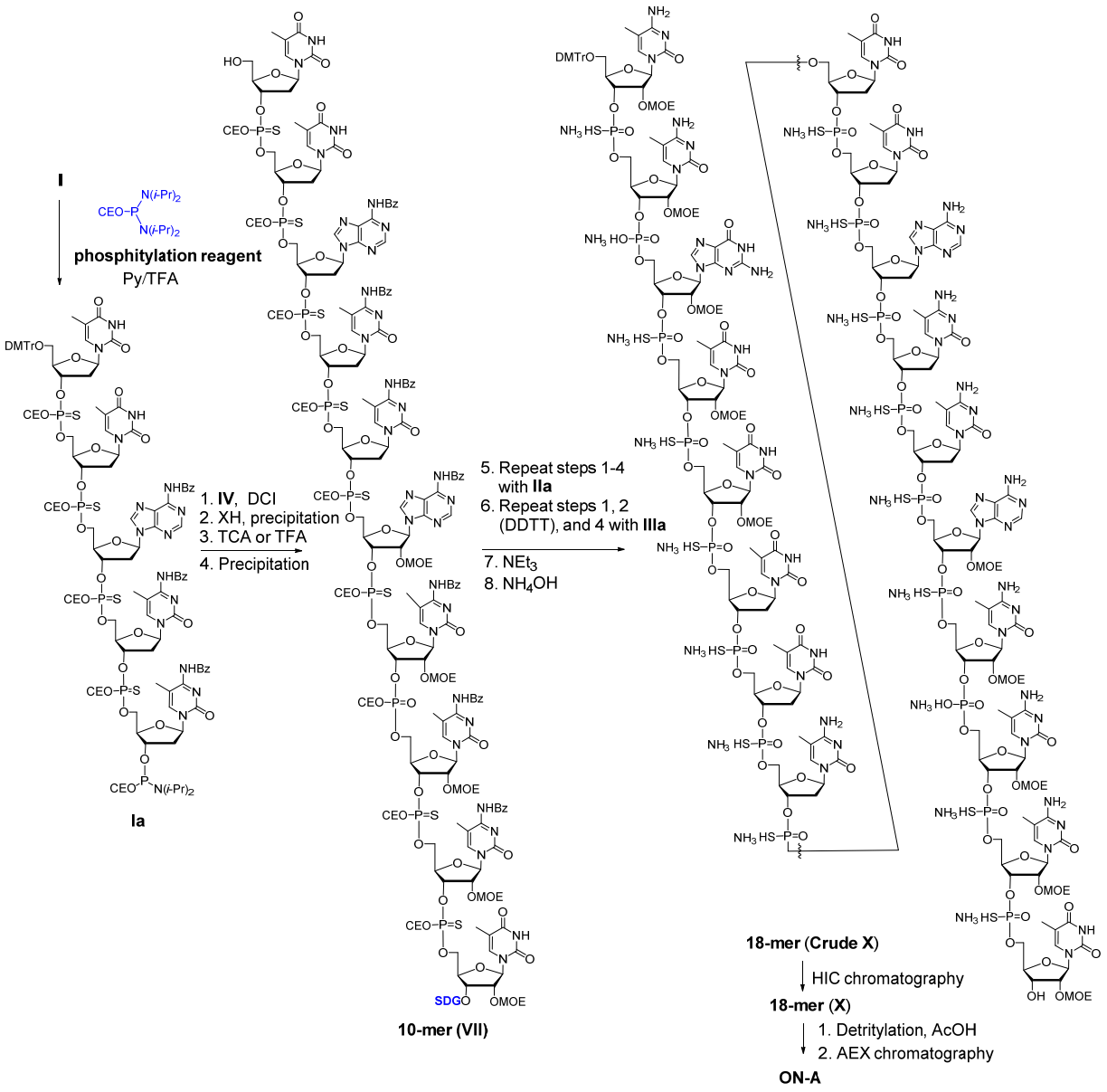
5'-DMTr-MOE ACoCC-3'-Phosphoramidite, VIa



A solution of **VI** (280 gram, 118.4 mmol) in DCM (1000 mL) was azeotropically distilled on a rotary evaporator with continuous addition of DCM (total ~ 2 L) until the water content of the solution was <0.05% by Karl Fischer analysis. After removal of the DCM, THF (1400 mL) was added. The mixture was cooled to 0 ± 5 ºC, and then 2-cyanoethyl tetraisopropylphosphorodiamidite (106.7 gram, 355mmol) and Py•TFA (45.7 gram, 236.8 mmol) were added and stirred at 0 ± 5 °C for 1 h until the conversion of **VI** was > 99% (monitored by HPLC).

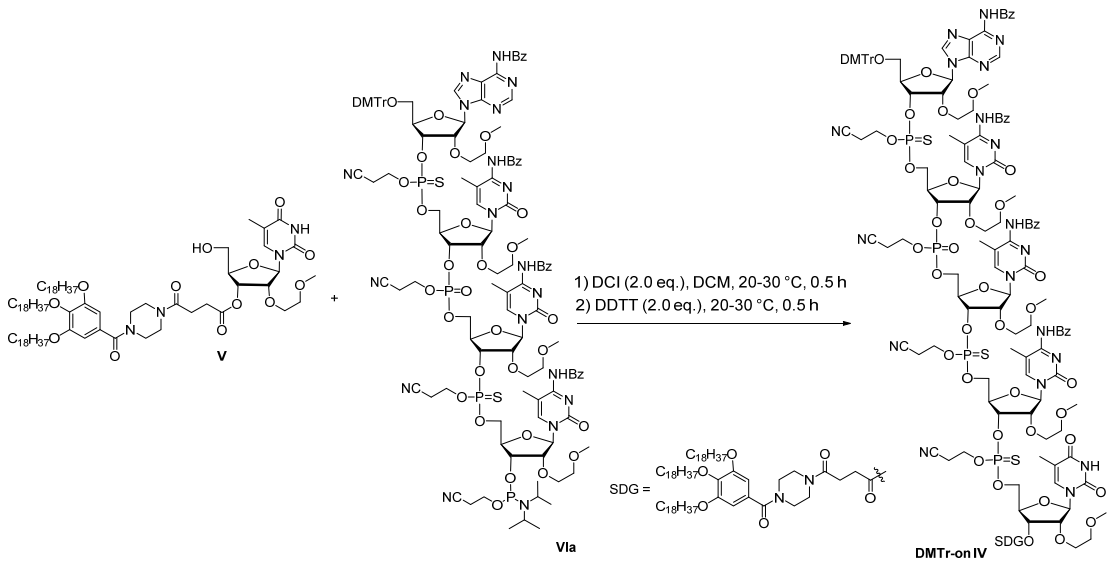
The reaction mixture was added into TBME/heptane (14 L, 5:2, v/v) 0 ± 5 ºC in 30 min under vigorous stirring and stirred for 30 min at the same temperature. Precipitation began immediately when addition started. The product was filtered, washed with heptane/TBME (2 x 700 mL, 5:2, v/v), and dried under vacuum at 20-30 ºC for 24 h to yield **VIa** as a white solid (292.3 gram, yield 96.3%).

6. Synthesis of compounds IV, VII, VIII, and X



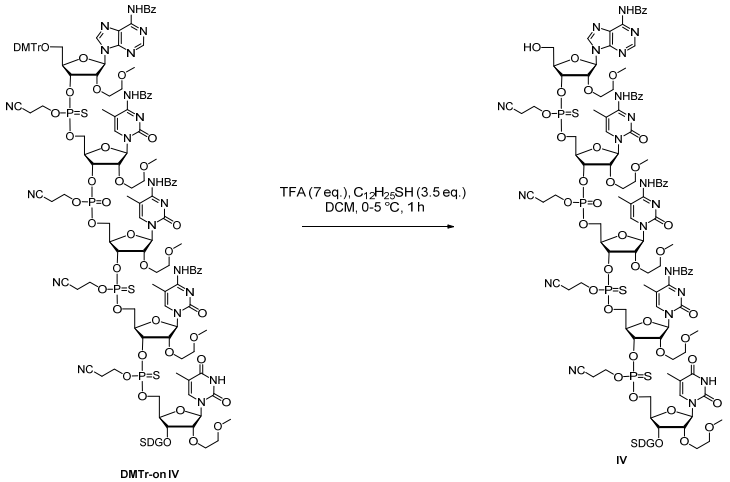
Scheme 5. *Convergent assembly of* ***ON-A***

5'-DMTr-MOE-ACoCCU-3'-SDG, DMT-on IV



A mixture of **V** (120.0 g, 86.1 mmol), **VIa** (280.7 g, 109.4 mmol), 3Å MS (10 g/100mL, 300 g), and DCM (3.0 L) under N_2 atmosphere was stirred at 20-25 ºC for 1.0 h. DCI (20.33 g, 172.2 mmol) was added and the reaction mixture was stirred for 1.0 h at 20-30 °C (TLC and HPLC indicated the reaction conversion > 99.9%). DDTT (35.35 g, 172.2 mmol) was added and the reaction mixture was stirred at 20-25 ºC for 30 min. The reaction mixture was filtrated and 3Å MS was washed with DCM (2 x 300 mL). The combined filtrate was concentrated to about 600 mL on a rotary evaporator and added to ACN (9.0 L) in 30 min with vigorous agitation. The precipitated product was filtered, washed with ACN (2 x 600 mL) and dried under vacuum at 20-30 ºC for 48 h to yield **DMTr-on IV** as a lightly yellow solid (287.1 g, 85.7% yield).

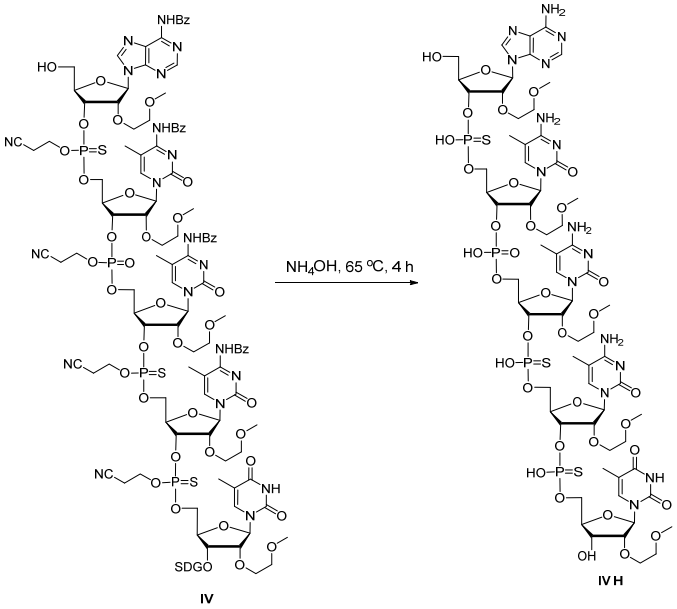
5'-OH-MOE-ACoCCU-3'-SDG, IV



A mixture of **DMTr-on IV** (285.5 g, 79.4 mmol), 3Å MS (142.0 g) and DCM (2855 mL) was stirred at 20-25 ºC for 1.0 h under N_2 atmosphere and cooled to 0±5 ºC. Dodecane-1-thiol (51.9 g, 278 mmol) was added and the reaction mixture was stirred at 0±5 ºC for 10 min. TFA (58.6 g, 555.8 mmol) was added slowly and the reaction mixture was stirred at 0±5 ºC for 1.0 h (TLC and HPLC indicated the reaction conversion > 99.9%). NMI (54.2 g, 714.6 mmol) was added in 10

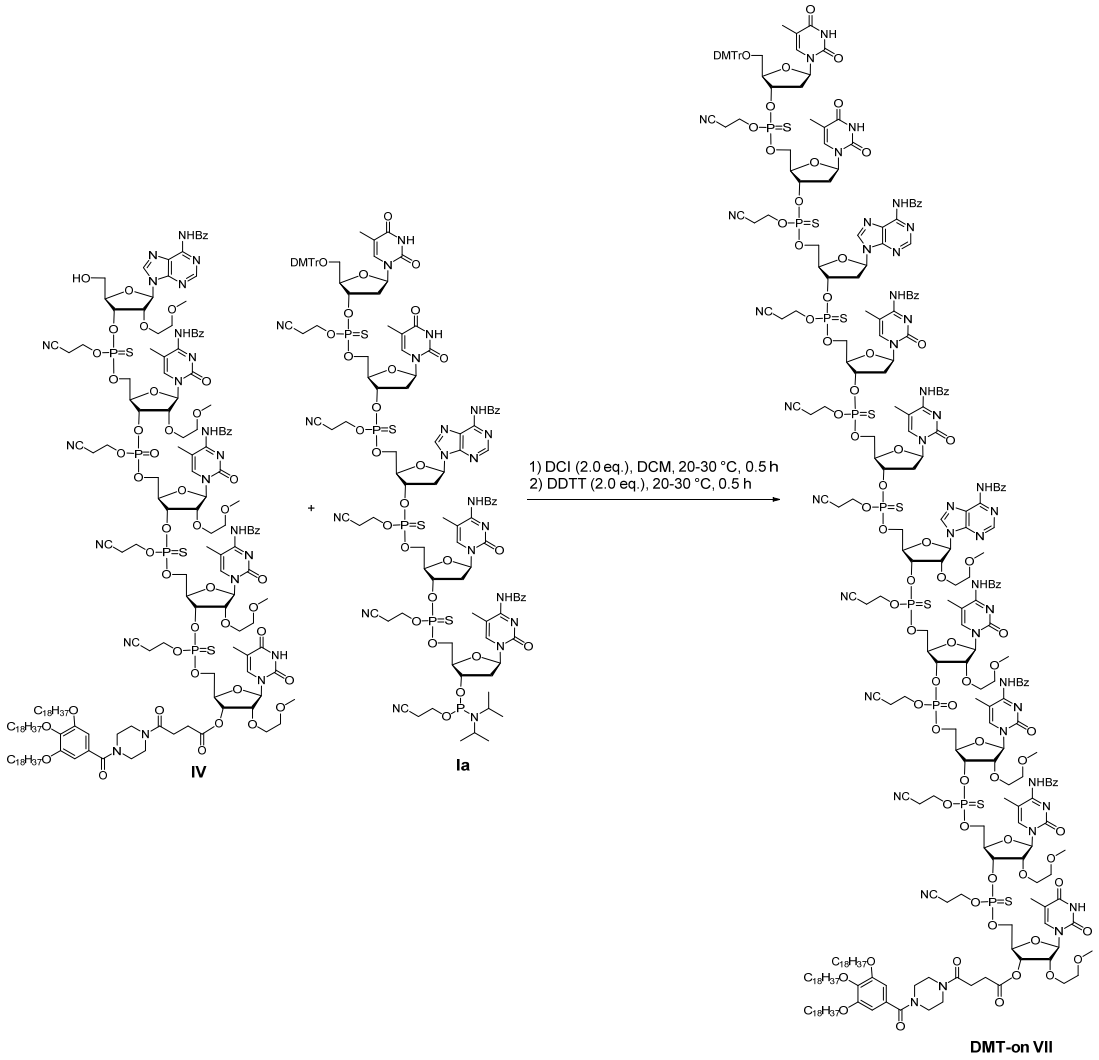
min, and the reaction mixture was filtered to remove 3Å MS, concentrated to about 500 mL by Rotavapor and added to ACN (8.5 L) in 30 min with vigorous agitation at 0±5 ºC. The precipitated product was filtered, washed with ACN (2 x 600 mL) and dried under vacuum at 20-30 ºC for 48 h to yield **IV** as a white solid (239.92 g, 91.1% yield).

IV ammonolysis



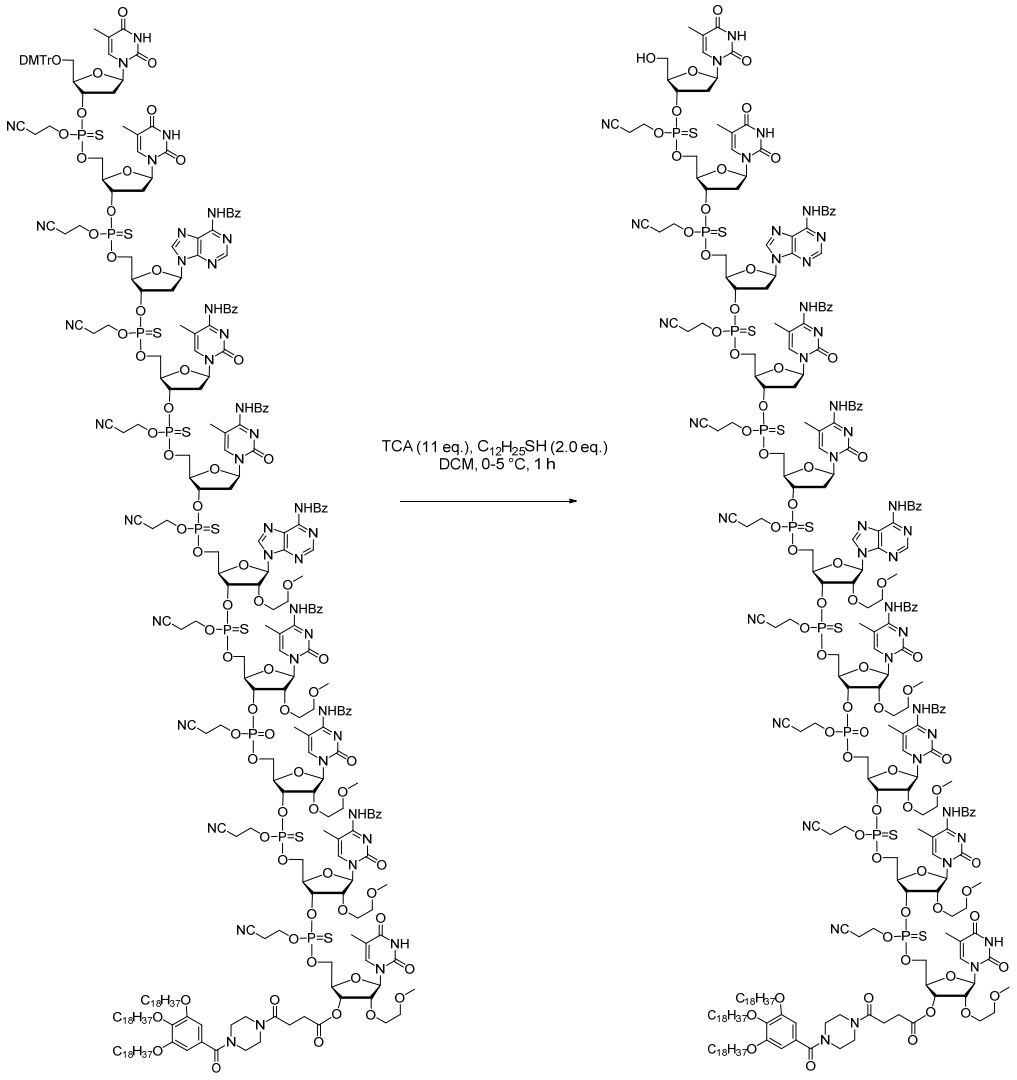
Preparation of **IV H**: The mixture of **IV** (1.0 g) and 30% NH_4OH (10 mL) in a 40 mL pressure flask was stirred for at 65 ºC 4 h. The structure of **IV H** was confirmed by LCMS. HRMS calcd for $C_{65}H_{96}N_{16}O_{35}P_4S_3^-$ [M-2H]/2: 940.2168, found: 940.2239.

5'-DMTr-10-mer-3'-SDG, DMT-on VII



A mixture of **IV** (227.0 g, 63.1 mmol), **Ia** (210.2 g, 82.2 mmol), 3Å MS (255.4 g) and ACN/ DCM (1.7 L, 1:2, v/v) was stirred at 20-25 ºC for 1.0 h under N_2 atmosphere. DCI (18.7 g, 158.1 mmol) was added, and the reaction mixture was stirred for 1.0 h (TLC and HPLC indicated the reaction conversion > 99.9%). DDTT (19.5 g, 94.8 mmol) was added and the reaction mixture was stirred at 20-25 ºC for 30 min. The reaction mixture was filtrated and the 3Å MS filter cake was washed with DCM (2 x 250 mL). The combined filtrate was concentrated to about 1200 mL on a rotary evaporator and added to ACN (10 L) in 30 min with vigorous agitation at 0±5 ºC. The precipitated product was filtered, washed with ACN (2 x 1.0 L) and dried under vacuum at 20-30 ºC for 80 h to yield **DMT-on VII** as a slightly yellow solid (384.7 g, 100.1% yield).

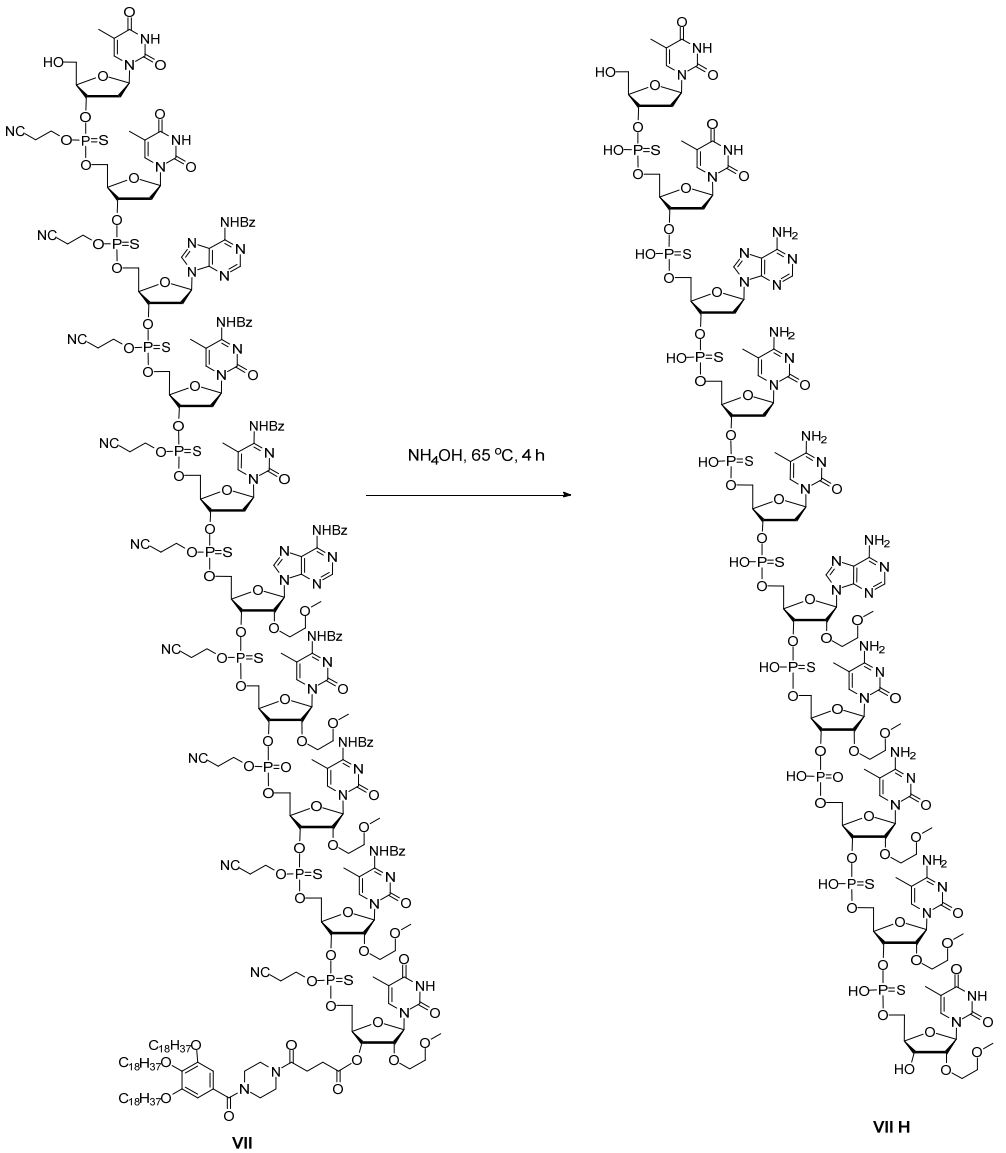
5'-OH-10-mer-3'-SDG, VII



DMT-on VII **VII**

A mixture of **DMT-on VII** (380.0 g, 62.5 mmol), 3Å MS (142.0 g) and DCM (2660 mL) was stirred at 20-25 ºC for 1.0 h under N_2 atmosphere and cooled to 0±5 ºC. Dodecane-1-thiol (26.3 g, 125 mmol) was added and the reaction mixture was stirred at 0 ºC for 10 min. TCA (112.3 g, 687.5 mmol) was added slowly and the reaction mixture was stirred at 0±5 ºC for 1.5 h (TLC and HPLC indicated the reaction conversion > 99.9%). NMI (67.7 g, 825 mmol) was added in 10 min, the reaction mixture was filtered to remove 3Å MS, concentrated to about 1000 mL on rotary evaporator and added to ACN (13.6 L) in 30 min with vigorous agitation at 0±5 ºC. The precipitated product was filtered, washed with ACN (2 x 1000 mL) and dried under vacuum at 20-30 ºC for 48 h to yield **VII** as a white solid (314.5 g, 87.1% yield).

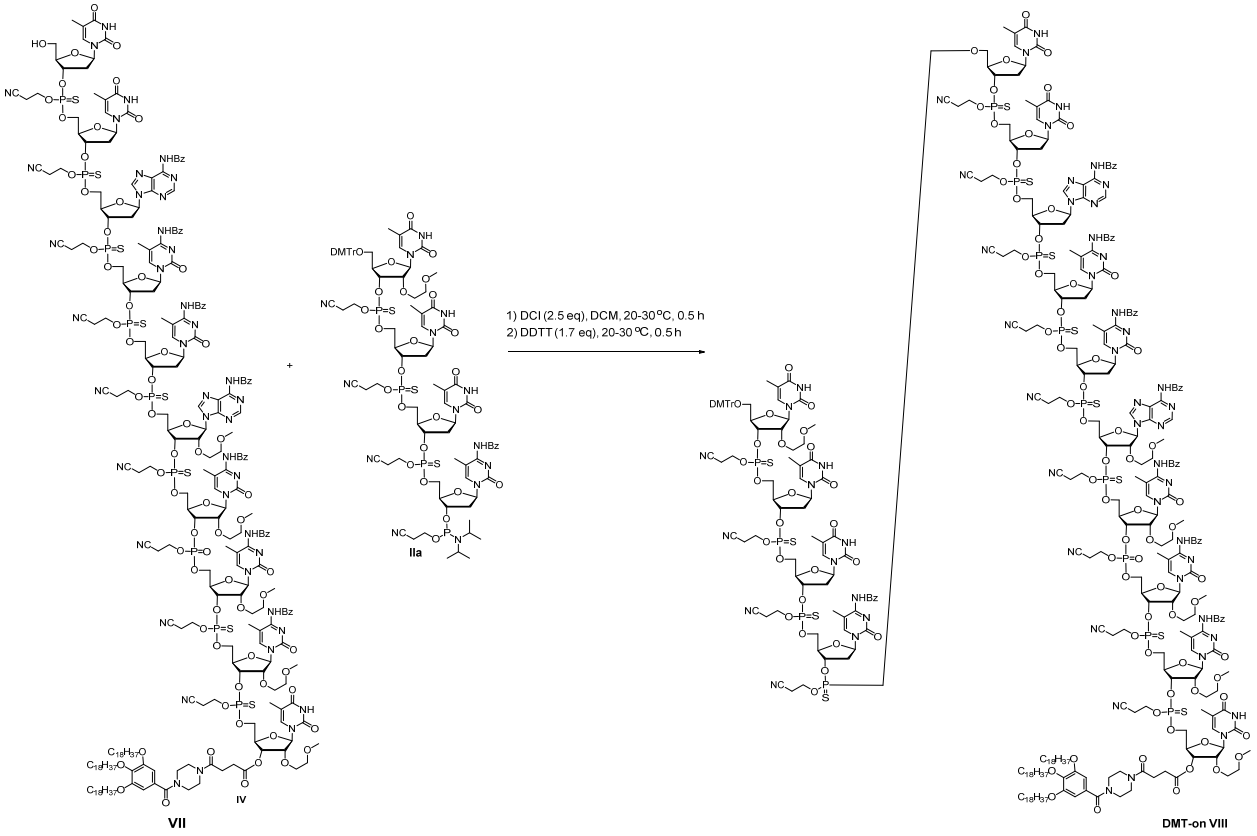
VII ammonolysis



VII VII H

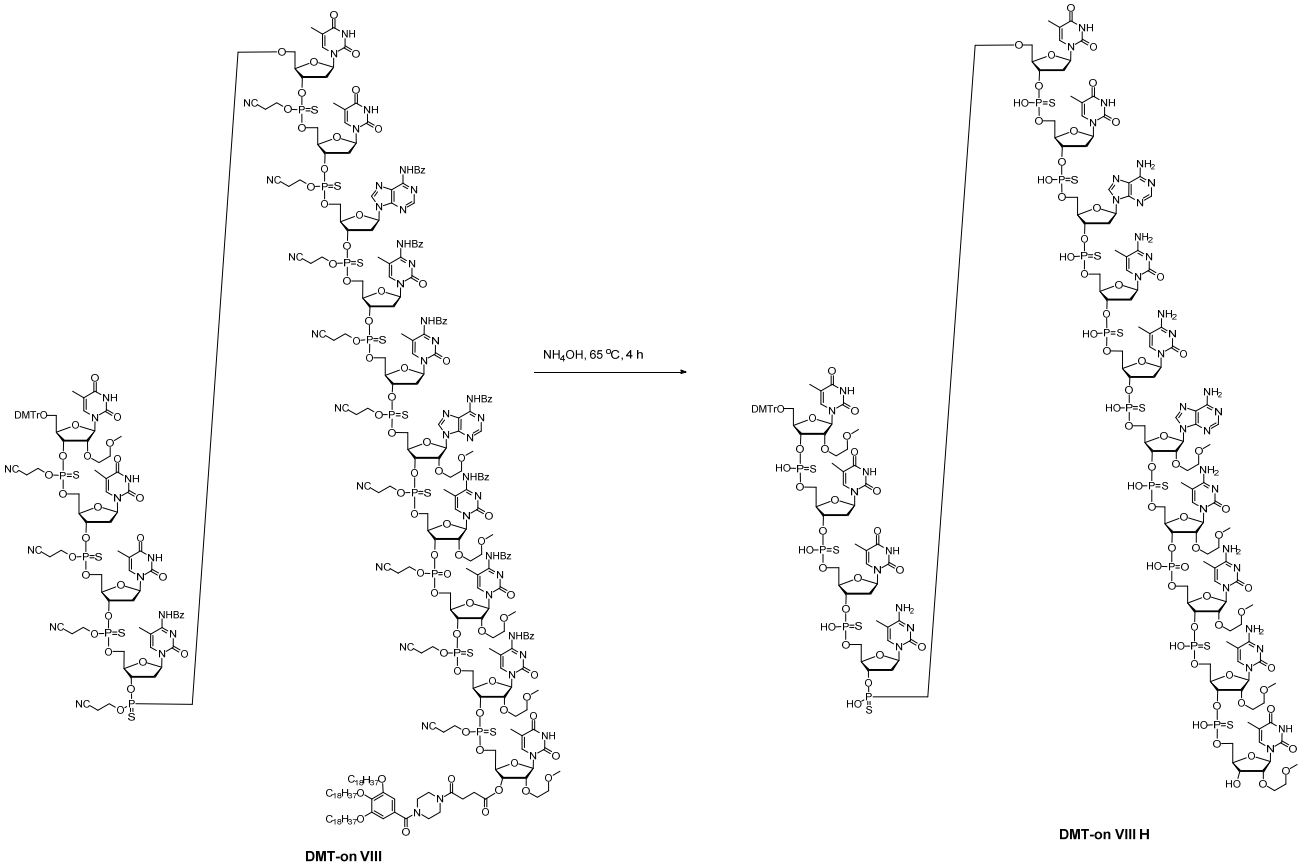
Preparation of **VII H**: The mixture of **VII** (0.2 g) and 30% NH_4OH (2 mL) in a 10 mL pressure flask was stirred at 65 ºC for 4 h. The structure of **VII H** was confirmed by LCMS. HRMS calcd for $C_{115}H_{161}N_{31}O_{61}P_9S_8$/3- [M-3H]/3: 1162.5295, found: 1162.5245.

5'-DMTr-14-mer-3'-SDG, DMT-on VIII



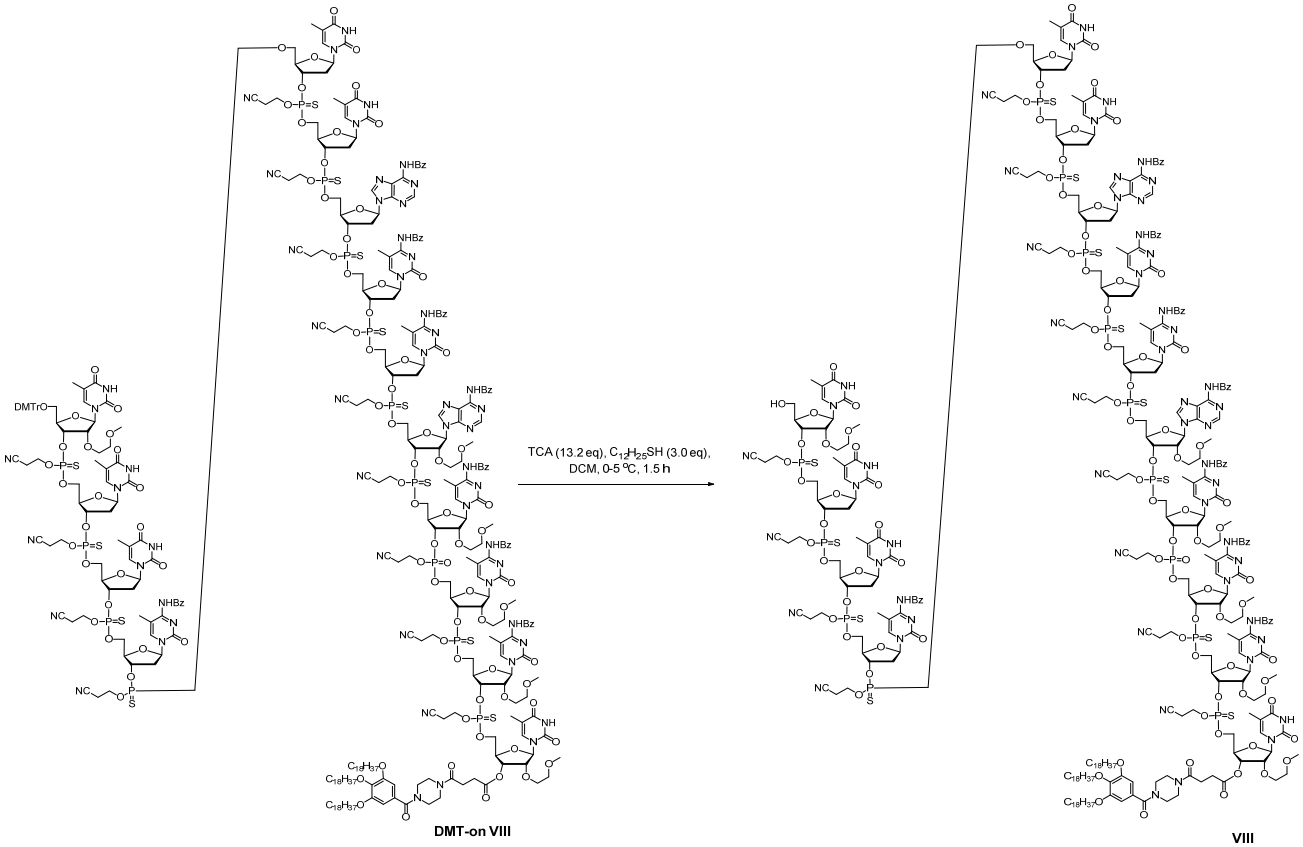
A mixture of **VII** (295.0 g, 51.1 mmol), **IIa** (146.1 g, 71.5 mmol), 3Å MS (331.9 g) and ACN/DCM (2213 mL, 1:2, v/v) was stirred at 20-25 ºC for 1.0 h under N_2 atmosphere. DCI (15.1 g, 127.7 mmol) was added and the reaction mixture was stirred for 1.0 h (TLC and HPLC indicated the reaction conversion > 99.9%). DDTT (17.8 g, 86.9 mmol) was added and the reaction mixture was stirred at 20-25 ºC for 30 min. DCM (885 mL) was added and the reaction mixture was filtrated and the 3Å MS filter cake was washed with DCM (2 x 300 mL). The combined filtrate was concentrated to about 1200 mL on a rotary evaporator and slowly added to ACN (10 L) in 30 min with vigorous agitation at 0±5 ºC. The precipitated product was filtered, washed with ACN (3 x 850 mL) and dried under vacuum at 20-30 ºC for 48 h to yield **DMT-on VIII** as a light yellow solid (336.8 g, 85.1% yield).

DMT-on VIII ammonolysis



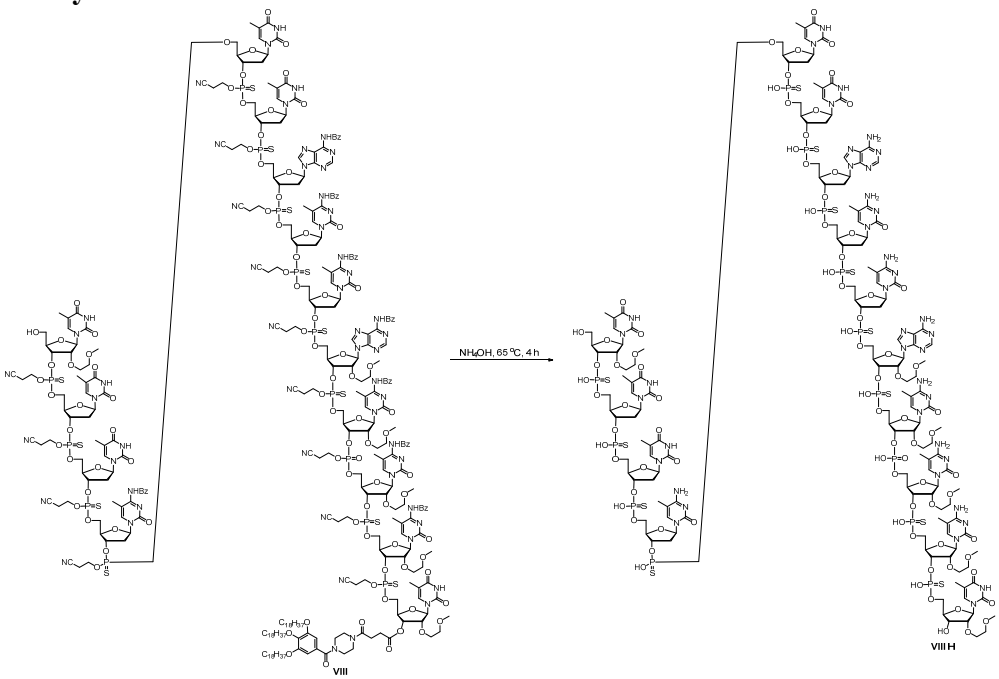
Preparation of **DMT-on VIII H**: a mixture of **DMT-on VIII** (0.2 g) and 30% NH_4OH (2 mL) in a 10 mL pressure flask was stirred at 65 ºC for 4 h. The structure of **DMT-on VIII H** was confirmed by LCMS. HRMS calcd for $C_{179}H_{237}N_{40}O_{88}P_{13}S_{12}/4^-$ [M-4H]/4: 1285.4642, found: 1285.4613.

5'-OH-14-mer-3'-SDG, VIII



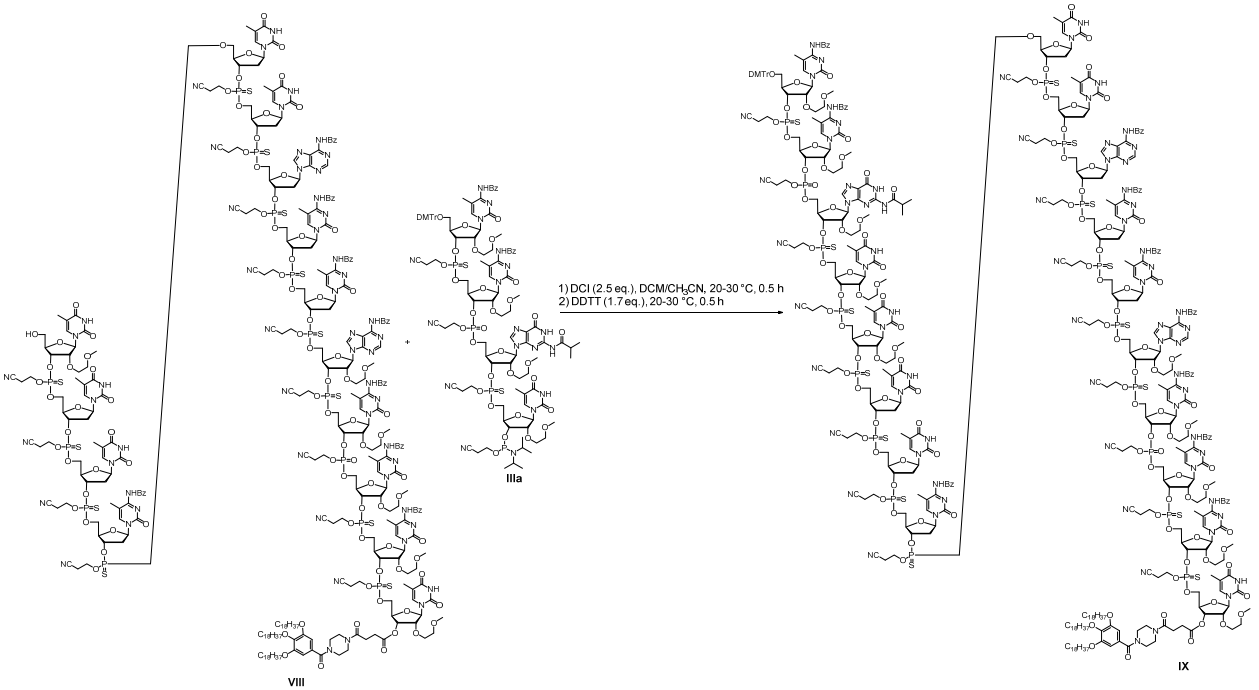
A mixture of **DMT-on VIII** (320.0 g, 41.4 mmol), 3Å MS (112.0 g) and DCM (2240 mL) was stirred at 20-25 ºC for 1.0 h under N_2 atmosphere and cooled down to 0±5 ºC. Dodecane-1-thiol (25.1 g, 124.2 mmol) was added, and the reaction mixture was stirred at 0 ºC for 10 min. TCA (89.1 g, 546.5 mmol) was added slowly and the reaction mixture was stirred at 0±5 ºC for 1.5 h (TLC and HPLC indicated the reaction conversion > 99.9%). NMI (55.9 g, 683.1 mmol) was added in 10 min, and the reaction mixture was filtered, and the 3Å MS filter cake was washed with DCM. The combined foiltrate was concentrated to about 1200 mL on a rotary evaporator and added to ACN (17.0 L) in 30 min with vigorous agitation at 0±5 ºC. The precipitated product was filtered, washed with ACN (2 x 650 mL) and dried under vacuum at 20-30 ºC for 48 h to yield **VIII** as a white solid (286.0 g, 93.0% yield).

VIII ammonolysis



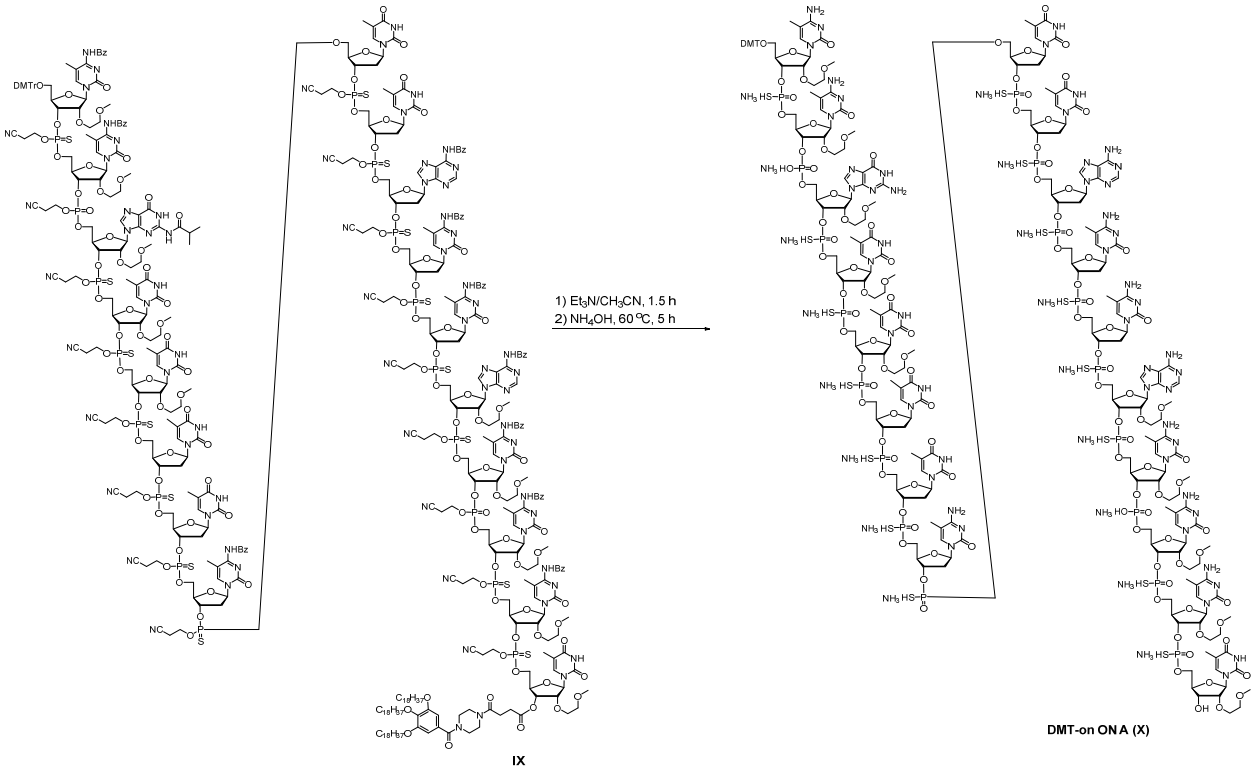
Preparation of **VIII H**. a mixture of **VIII** (0.2 g) and 30% NH_4OH solution (2 mL) in a 10 mL pressure flask was stirred at 65 ºC for 4 h. The structure of **VIII H** was confirmed by LCMS. HRMS calcd for $C_{158}H_{219}N_{40}O_{86}P_{13}S_{12}/4^-$ [M-4H]/4: 1209.9316, found: 1209.9237.

5'-DMTr-18-mer-3'-SDG, IX



A mixture of **VIII** (150.0 g, 20.2 mmol), **IIIa** (74.0 g, 30.3 mmol), 3Å MS (168.7 g) and DCM (1125 mL, 1:2, v/v) was stirred at 20-25 ºC for 1.0 h under N_2 atmosphere. DCI (5.95 g, 50.5 mmol) was added and the reaction mixture was stirred for 1.0 h (TLC and HPLC indicated the reaction conversion > 99.9%). DDTT (7.03 g, 34.3 mmol) was added and the reaction mixture was stirred at 20-25 ºC for 30 min. DCM (450 mL) was added and the reaction mixture was filtrated and 3Å MS filter cake was washed with DCM (2 x 150 mL). The combined reaction mixture was concentrated to about 1000 mL on a rotary evaporator and slowly added to ACN (9.0 L) in 30 min with vigorous agitation at 0±5 ºC. The precipitated product was filtered, washed with ACN (2 x 550 mL) and dried under vacuum at 20-30 ºC for 60 h to yield **IX** as a light yellow solid (181.0 g, 91.5% yield).

DMT-on ON-A, X



A mixture of **IX** (30 g) and EDTA (150 mg) in ACN/TEA (300 mL, 1:1 v/v) was stirred at 20-25 ºC for 90 min, and the reaction mixture was concentrated in vacuo. A 30% NH_4OH water solution (300 mL) was added and the reaction mixture was stirred until all solids dissolved at 25 ºC (20-30 min). The homogenous solution was transferred to a 1.0 L pressure flask and was stirred at 60 ºC for 5 h. The crude product solution was cooled down to room temperature and kept at -20 ºC before purification.

Fragments I-VI and VI Synthesis Data and Critical Impurities

Fragments	I	II		III	IV[1]	VI	
Scale (kg/batch)	0.3	0.3	3	0.3	0.2	0.3	3
Yield (%)	72	59	76	73	77	68	62
Purity (%)	93.4	90.2	93.0	87.6	90.8	86.6	87.7
PO (%)	<0.10	0.7	0.11	0.59	0.87	<0.10	0.15
n-1 (%)	<0.10	0.16	0.44	1.1	0.15	0.29	0.27
n+1 (%)	0.14	<0.10	<0.10	<0.10	<0.10	<0.10	0.19
Deamination (%)	<0.10	<0.10	<0.10	0.10	NT[2]	0.25	<0.10

[1] Fragment IV was analyzed after ammonolysis
[2] NT: not tested

7. Purification of DMTr-on (X) and DMTr-off 18-mer (ON-A)

The DMTr-on **X** and DMTr-off 18-mer purification process consisted of depth filtration, orthogonal chromatography steps (hydrophobic interaction chromatography and anion exchange chromatography) and a low pH detritylation step. An ultra-filtration/dia-filtration step was also employed for final buffer exchange and concentration.

Depth filtration occurred post ammonolysis and required the dilution of the crude. This step removes the insoluble materials in the crude product **X**. The HIC chromatography step was run in a bind and elute mode, where the DMT-on 18-mer **X** was salted out to bind to the hydrophobic resin (buffer of ammonium sulfate at about 1000mM). Reducing the ammonium sulfate to about 800mM provided an optimal buffer for the wash step. Elution of the product was carried out by further reducing the ammonium sulfate to 50mM. The elute containing **X** was subjected detritylation reaction to remove the 5'-DMTr group from **X**. This step was performed at 10 °C ±1 °C by adjusting the pH of HIC eluate to a pH of 2.7 ± 0.2 using 25% citric acid. Once the DMTr removal was complete and filtered, the pH was adjusted to 8.5 in preparation for the AEX step.
The AEX chromatography step was the second purification step. The material from the detritylation reaction was diluted with water prior to loading onto the AEX column. A wash step of 300mM sodium chloride was used to increase the purity of the 18-mer **ON-A**. Elution was carried out by further increasing the sodium chloride to 525mM. The materials and conditions for these steps are provided below.

Depth Filtration:

Materials:

DMTr-on 18-mer **X** crude (post ammonolysis, no adjustments)
Dilution buffer: 1111mM ammonium sulfate
Equilibration buffer: 1000mM ammonium sulfate, 50mM tris, pH 8.5
Depth filter

Method:

Crude preparation- Dilute the crude 10-fold into 1111mM ammonium sulfate (final ammonium sulfate will be 1000mM)
Wet and flush the filter according to manufacturer's guidelines.
Equilibrate the depth filter with 3 bed volumes (BVs) of equilibration buffer.
Run the diluted crude through the filter.

Hydrophobic Interaction Chromatography:

Materials:

Column: HIC resin packed into a 14cm ID column
Equilibration buffer: 1000mM ammonium sulfate, 50mM tris, pH 8.5
Wash buffer: 800mM ammonium sulfate, 50mM tris, pH 8.5
Elution buffer: 50mM ammonium sulfate, 50mM tris, pH 8.5
Strip: deionized water
Cleaning in place: 1N sodium hydroxide
Storage: 0.1N sodium hydroxide

UV monitor: 295nm

Method:

Equilibrate the column with 4 column volumes (CVs) of 1000mM ammonium sulfate, 50mM tris, pH 8.5 at 200 cm/h.
Load the sample at 200 cm/h.
Chase the column with 4 CVs of 1000mM ammonium sulfate, 50mM tris, pH 8.5 at 200 cm/h.
Wash the column with 800mM ammonium sulfate, 50mM tris, pH 8.5 at 100cm/hr until UV gate (2mm flowcell) hits 0.5 AU.
Continue column wash with 800mM ammonium sulfate, 50mM tris, pH 8.5 for 2 CVs at 100 cm/h.
Elute the column with 8 CVs of 50mM ammonium sulfate, 50mM tris, pH 8.5 at 200 cm/h.
Strip the column with 4 CVs of deionized water at 200 cm/h.
Clean the column with 3 CVs of 1N sodium hydroxide at 200 cm/h.
Store the column with 3 CVs of 0.1N sodium hydroxide at 200 cm/h.

Detritylation:

Materials:

Acidification buffer: 25% (w/w) citric acid
Neutralization buffer: 5N sodium hydroxide

Method:

Equilibrate the material pool to 10°C.
Add 25% (w/w) citric acid until the pH is 2.7.
The reaction will take about 4-5 hours.
Once reaction is completed, neutralize the pool with 5N sodium hydroxide to pH 8.5
Filter the pool through a pre-filter and 0.22um sterilizing filter.

Anion Exchange Chromatography:

Materials:

Column: AEX resin packed into a 14cm ID column
Dilution buffer: dH2O
Charge/ Strip: 2000mM sodium chloride, 250mM tris, pH 8.5
Equilibration buffer: 100mM sodium chloride, 50mM tris, pH 8.5
Wash buffer: 300mM sodium chloride, 50mM tris, pH 8.5
Elution buffer: 525mM sodium chloride, 50mM tris, pH 8.5
Cleaning in place: 1N sodium hydroxide
Storage: 0.1N sodium hydroxide

UV monitor: 295nm

Method: -Sample Preparation-Dilute sample into water until conductivity is ~20mS/cm.

Process Method:

Charge the column with 2 CVs of 2000mM sodium chloride, 250mM tris, pH 8.5 at 200 cm/h.
Equilibrate the column with 4 CVs of 100mM sodium chloride, 50mM tris, pH 8.5 at 200 cm/h.
Load the sample at 200 cm/h.
Chase the column with 2 CVs of 100mM sodium chloride, 50mM tris, pH 8.5 at 200 cm/h.
Wash the column with 300mM sodium chloride, 50mM tris, pH 8.5 at 200cm/hr until UV gate (2mm flowcell) hits 0.4 AU at 200 cm/h.
Continue column wash with 300mM sodium chloride, 50mM tris, pH 8.5 for 4 CVs at 200 cm/h.
Elute the column with 7 CVs of 525mM sodium chloride, 50mM tris, pH 8.5 at 200 cm/h.
Strip the column with 3 CVs of 2000mM sodium chloride, 250mM tris, pH 8.5 at 200 cm/h.
Clean the column with 3 CVs of 1N sodium hydroxide at 200 cm/h.
Store the column with 3 CVs of 0.1N sodium hydroxide.

8. Analytical data and spectrums

Compound I LC-MS Conditions, Chromatogram, HRMS, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR spectra

LC-MS Conditions

Column	Acquity UPLC BEH C18（130 Å, 2.1mm*150mm, 1.7 um）
Oven	60ºC
Mobile phase	A: 20 mM NH_4OAc in H_2O, 25% ; B: ACN
Gradient program	Time (min) A% 0.0 60 1.0 60 28 52 30 20 31 60 36 60
Flow rate	0.5 mL/min
Detector	UV 260 nm
Injection volume	2μL (10mg/mL)
Diluent	ACN:H_2O=8:2

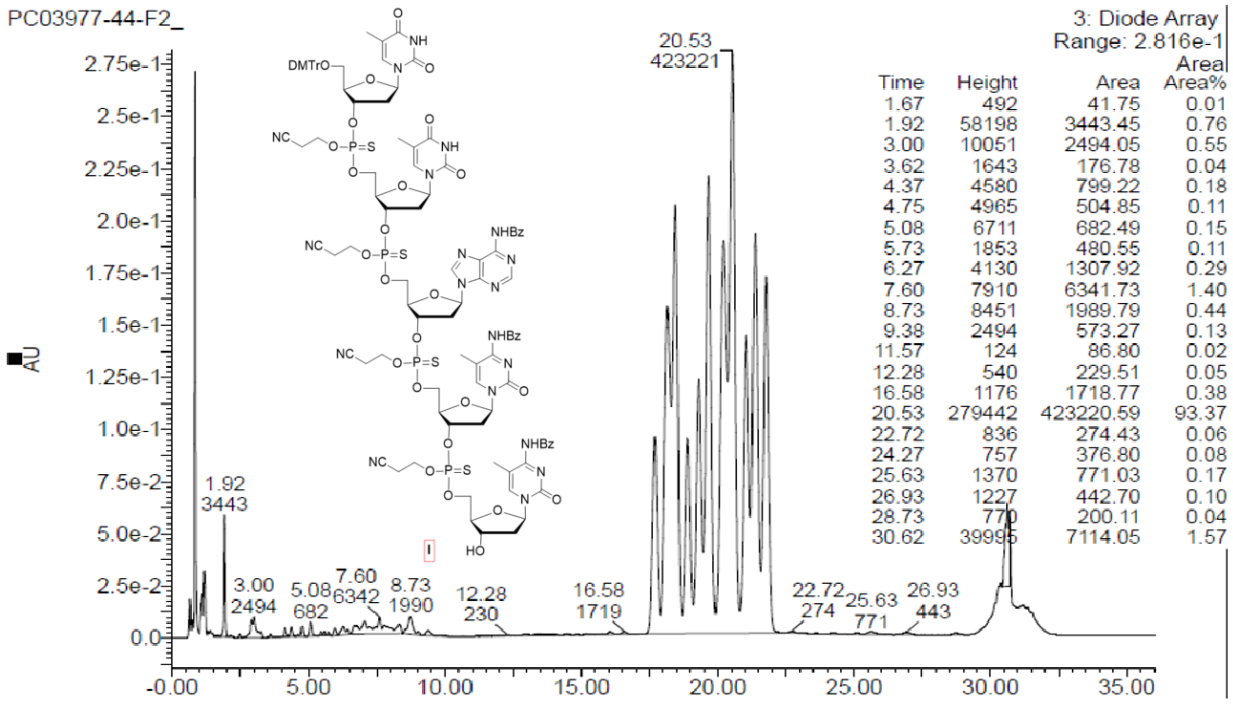


Figure 1. LC chromatogram of ***I***

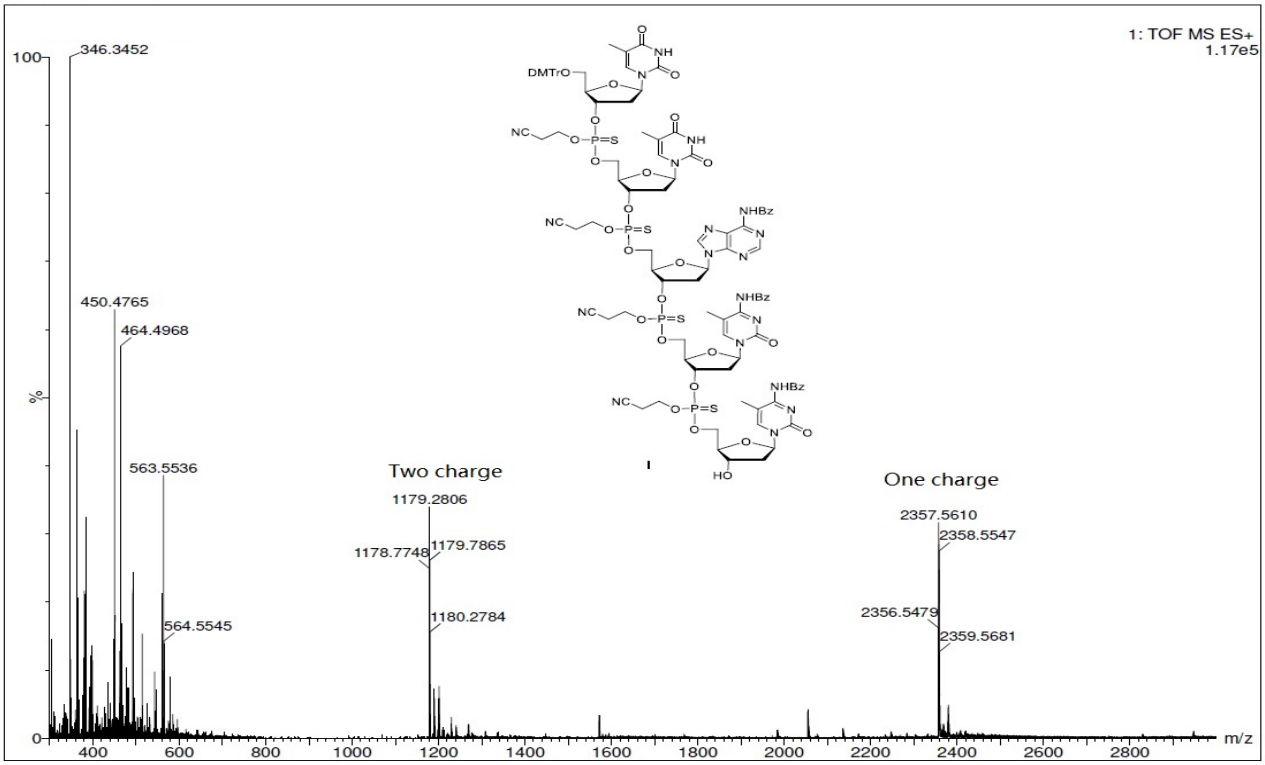


*Figure 2. HRMS spectrum of **I***

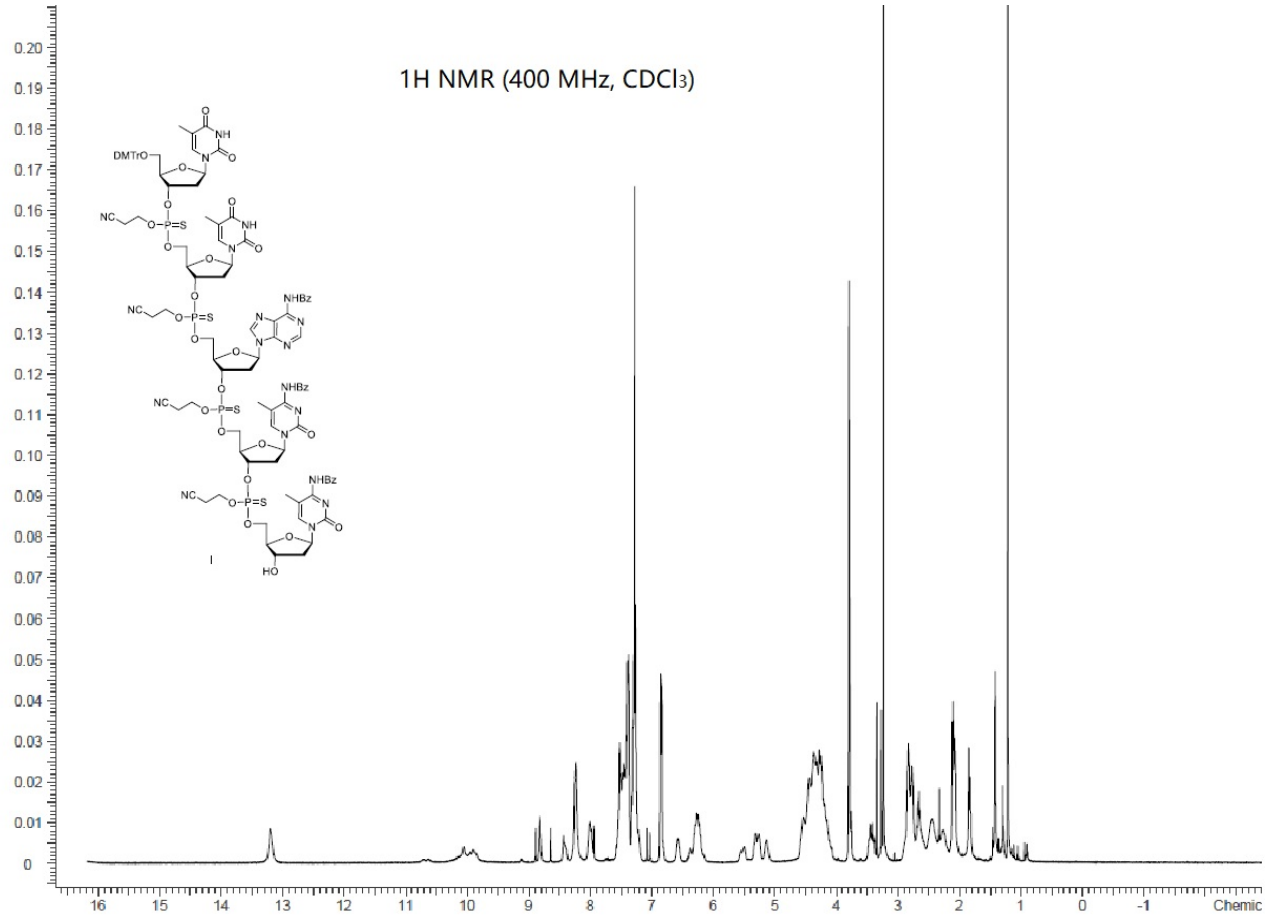


*Figure 3. 1H NMR spectrum of **I***

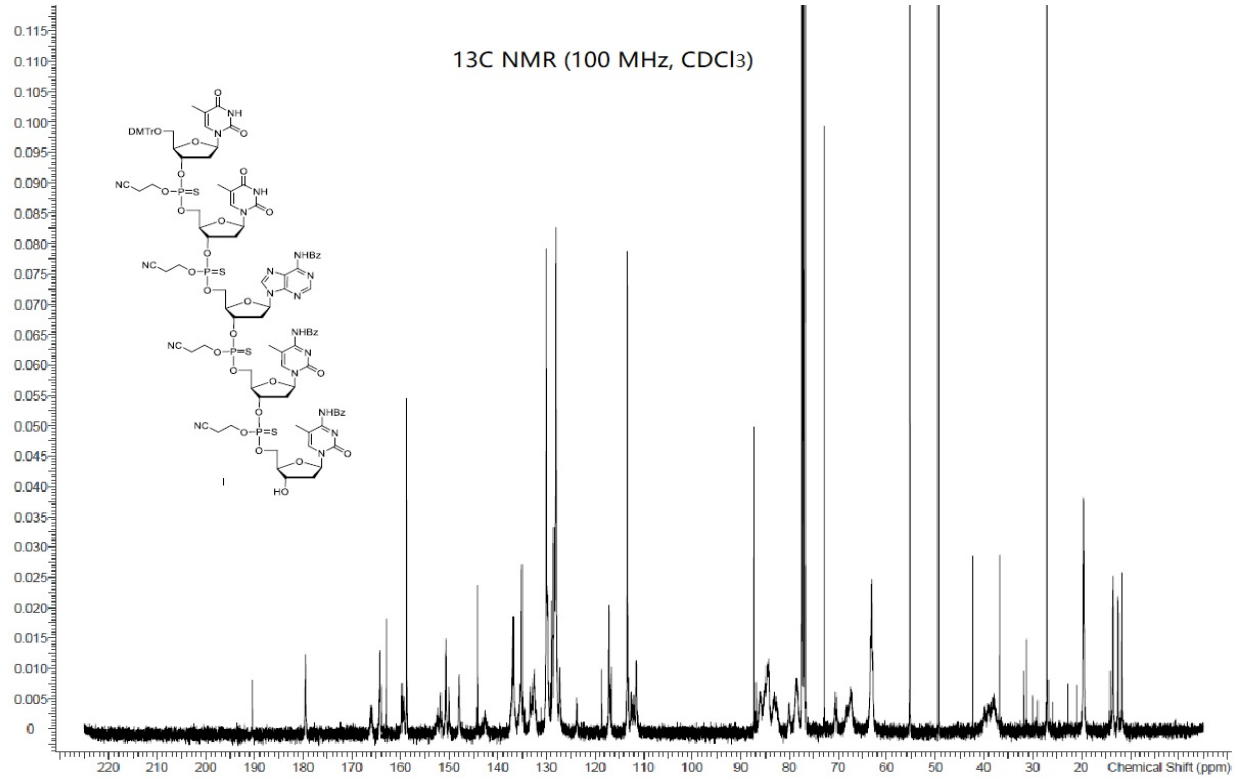


*Figure 4. 13C NMR spectrum of **I***

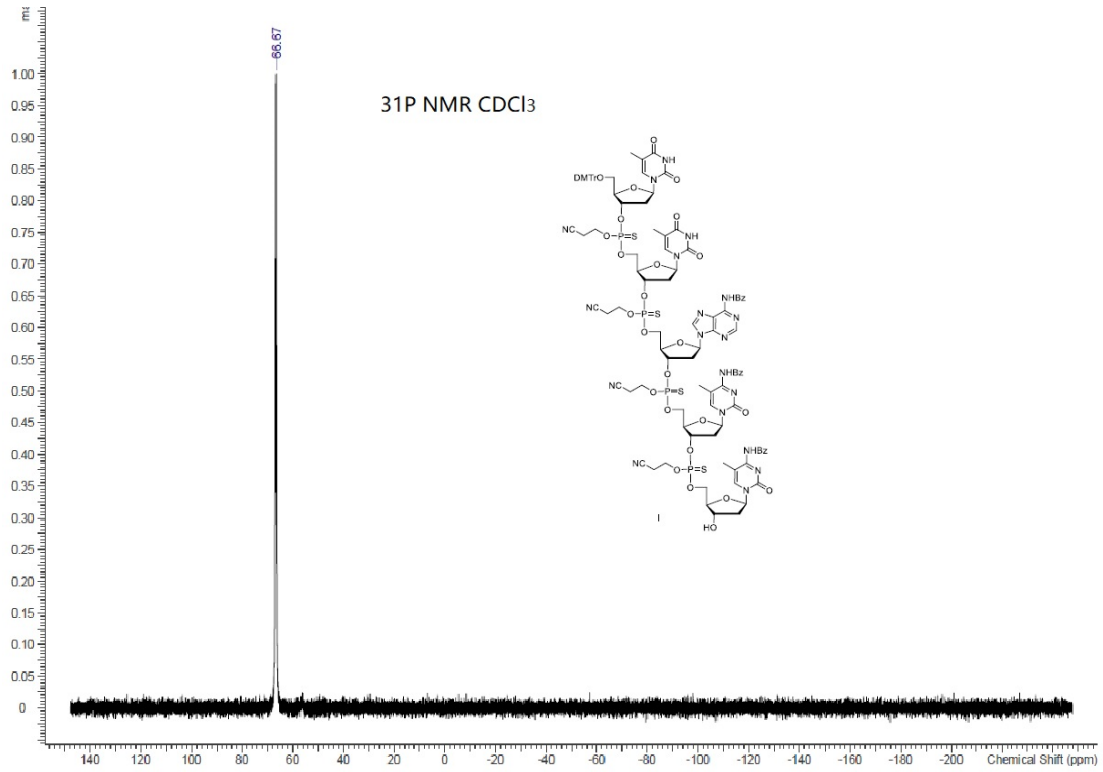


*Figure 5. 31P NMR spectrum of **I***

Compound II LC-MS Conditions, Chromatogram, HRMS, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR spectra

LC-MS Conditions

Column	Acquity UPLC BEH C18（130 Å, 2.1mm*150mm, 1.7 um）
Oven	60ºC
Mobile phase	A: 20 mM NH_4OAc in H_2O, 25% ; B: ACN
Gradient program	Time (min) A% 0.0 70 1.0 70 28 60 29 10 30 10 31 70 36 70
Flow rate	0.5 mL/min
Detector	UV 260 nm
Injection volume	2μL (8mg/mL)
Diluent	ACN:H_2O=8:2

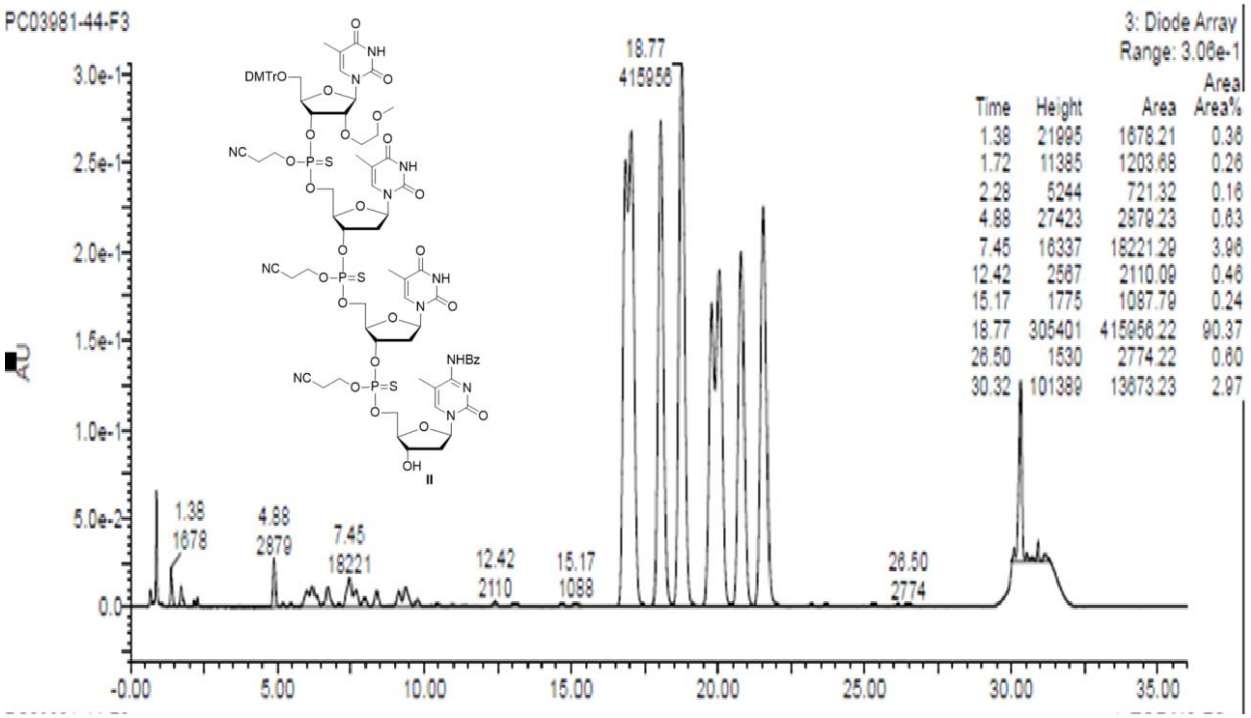


*Figure 6. LC chromatogram of **II***

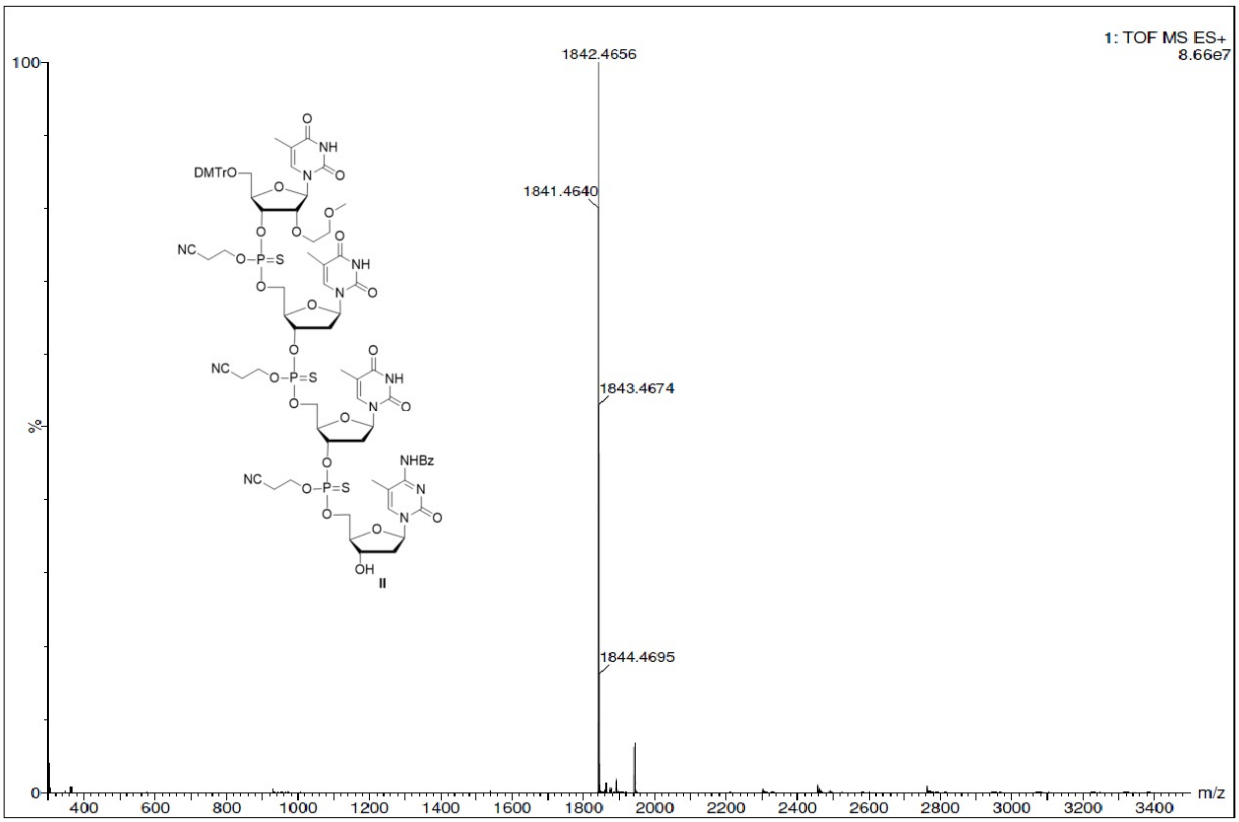


Figure 7. HRMS spectrum of ***II***

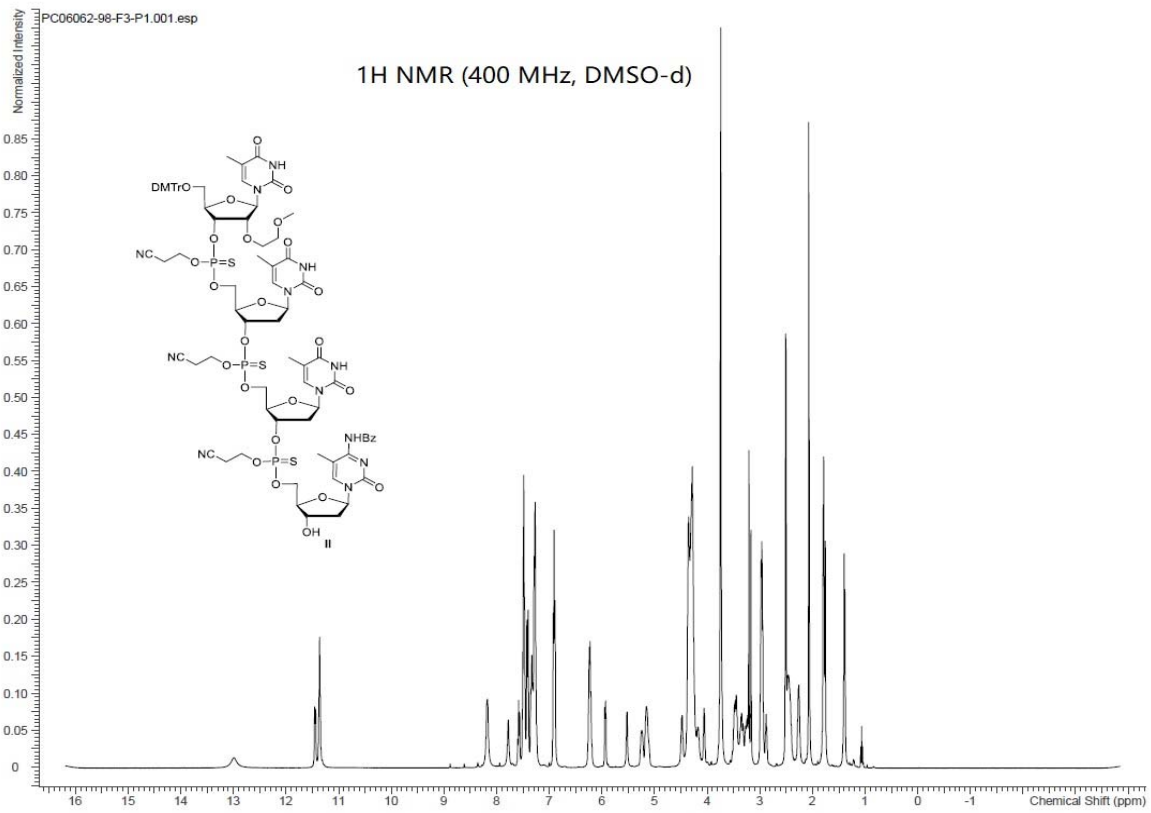


*Figure 8. 1H NMR spectrum of **II***

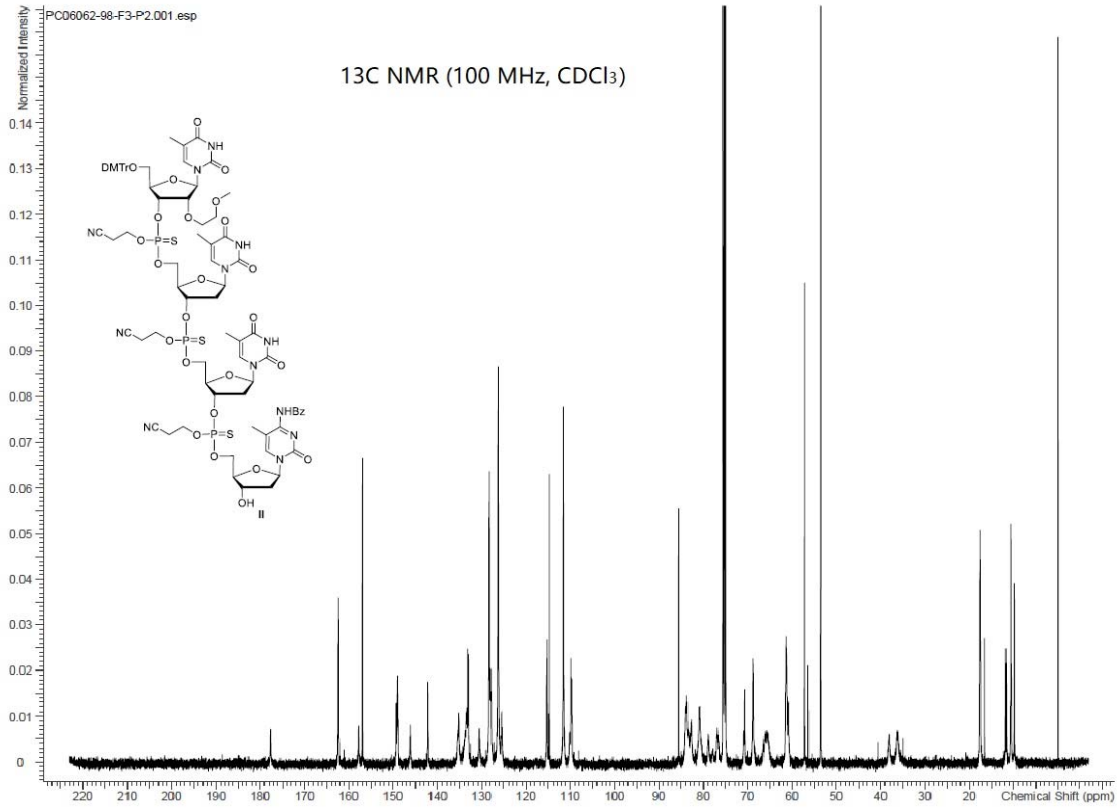


*Figure 9. 13C NMR spectrum of **II***

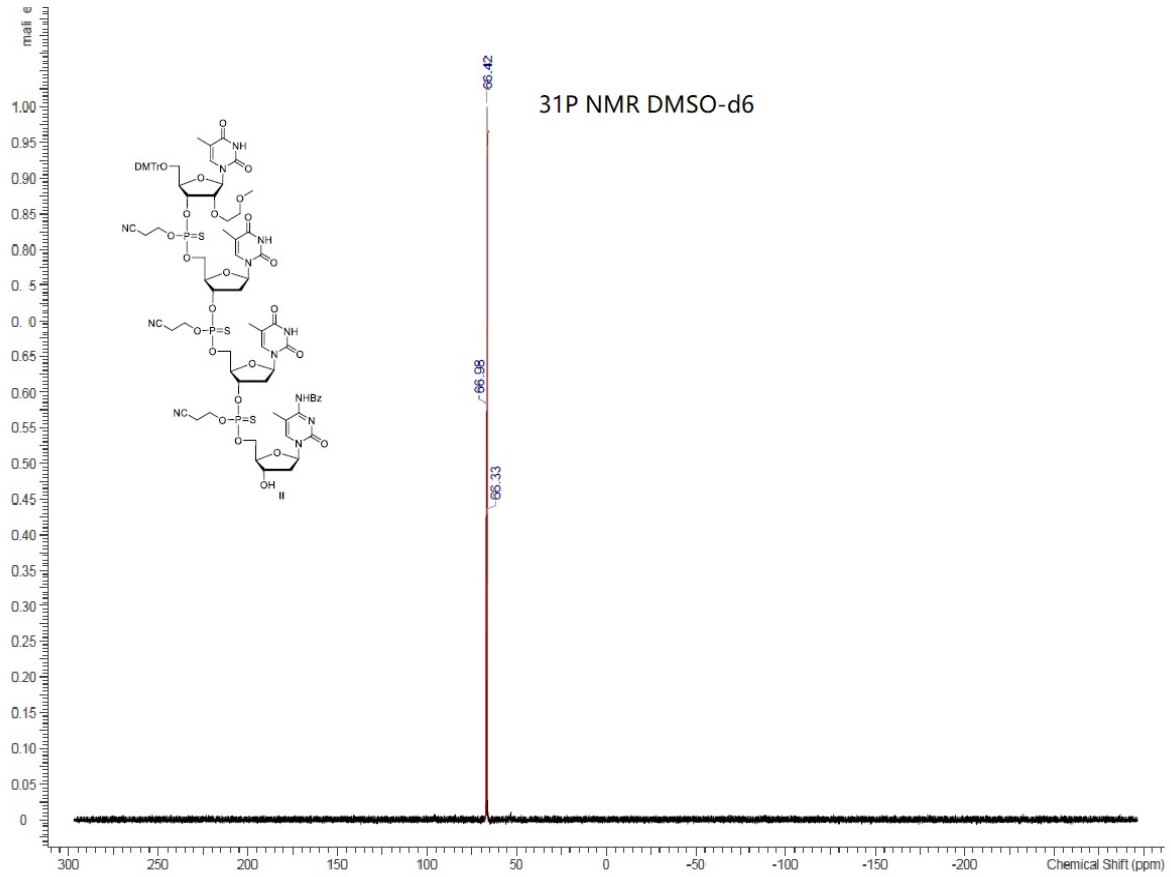


Figure 10. 31P NMR of ***II***

Compound III LC-MS Conditions, Chromatogram, HRMS, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR spectra

LC-MS Conditions

Column	Acquity UPLC BEH C18（130 Å, 2.1mm*150mm, 1.7 um）
Oven	60ºC
Mobile phase	A: 20 mM NH_4OAc in H_2O, 25% ; B: ACN
Gradient program	Time (min) A% 0.0 60 1.0 60 28 35 29 10 30 10 31 60 36 60
Flow rate	0.5 mL/min

Detector	UV 260 nm
Injection volume	2μL (5mg/mL)
Diluent	ACN:H_2O=8:2

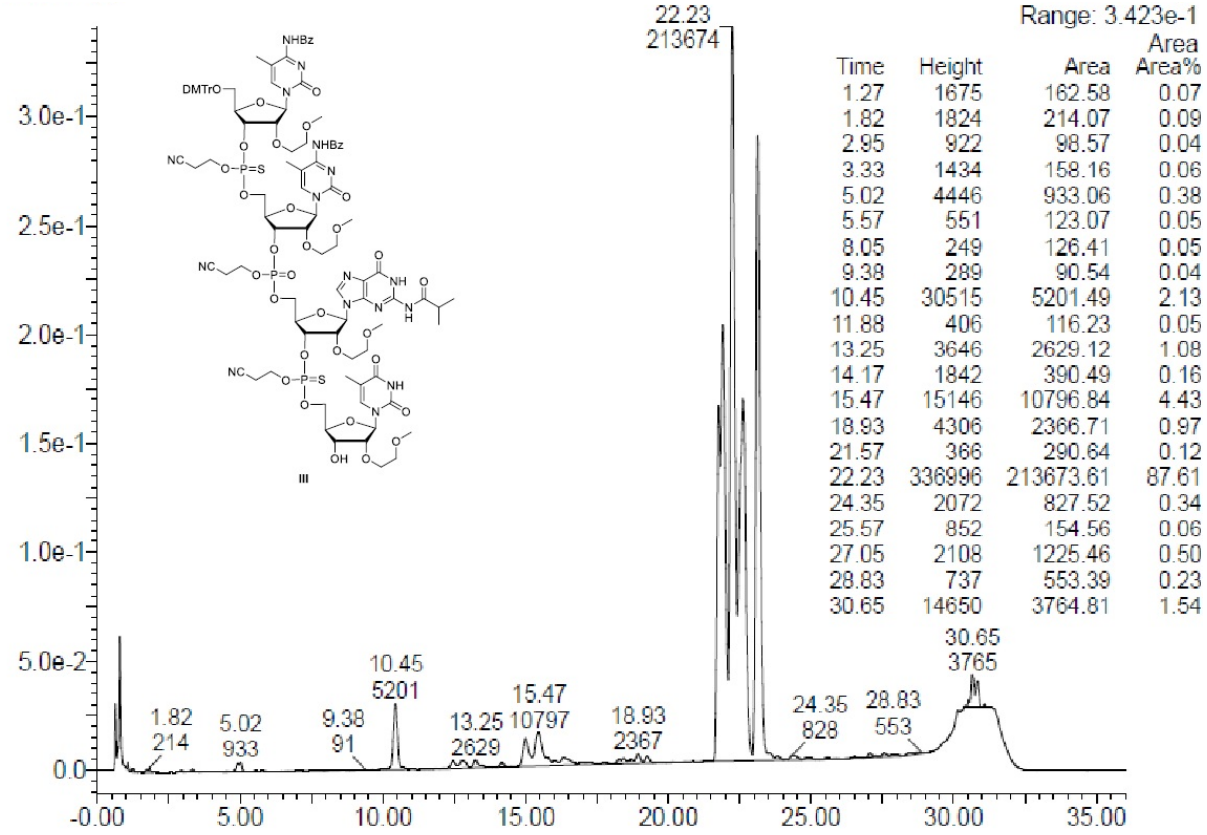


*Figure 11. LC chromatogram of **III***

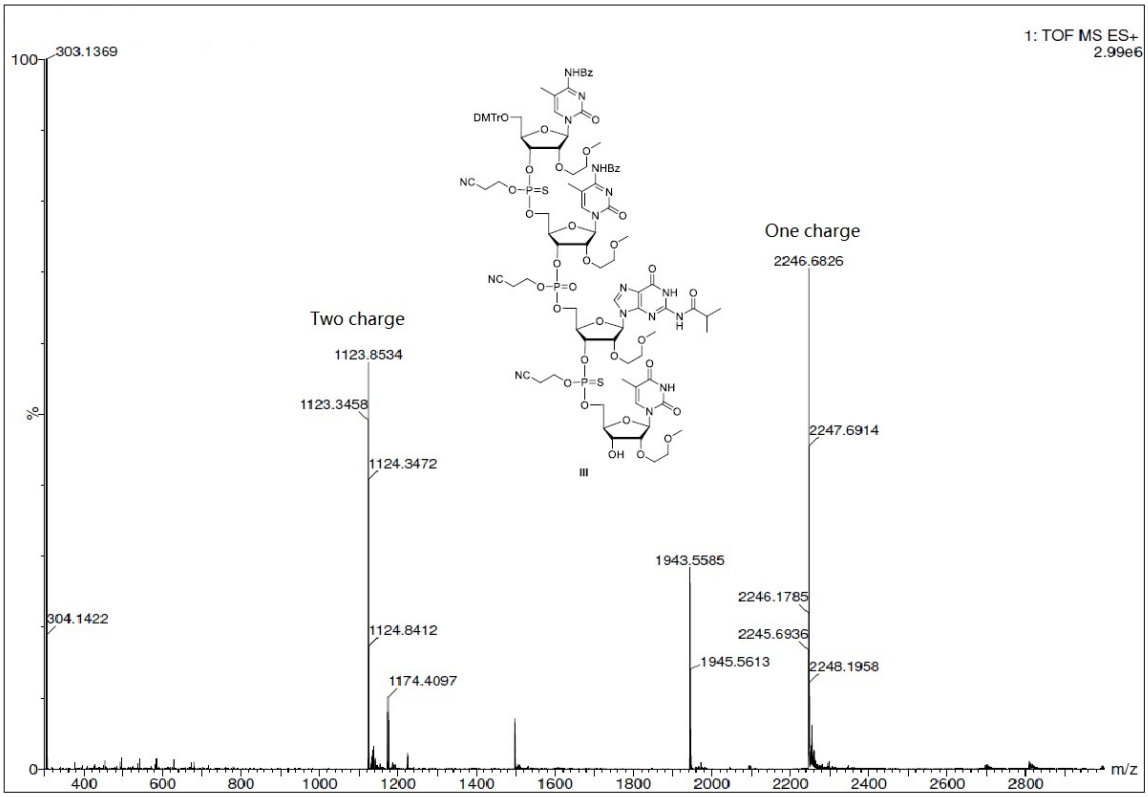


*Figure 12. HRMS spectrum of **III***

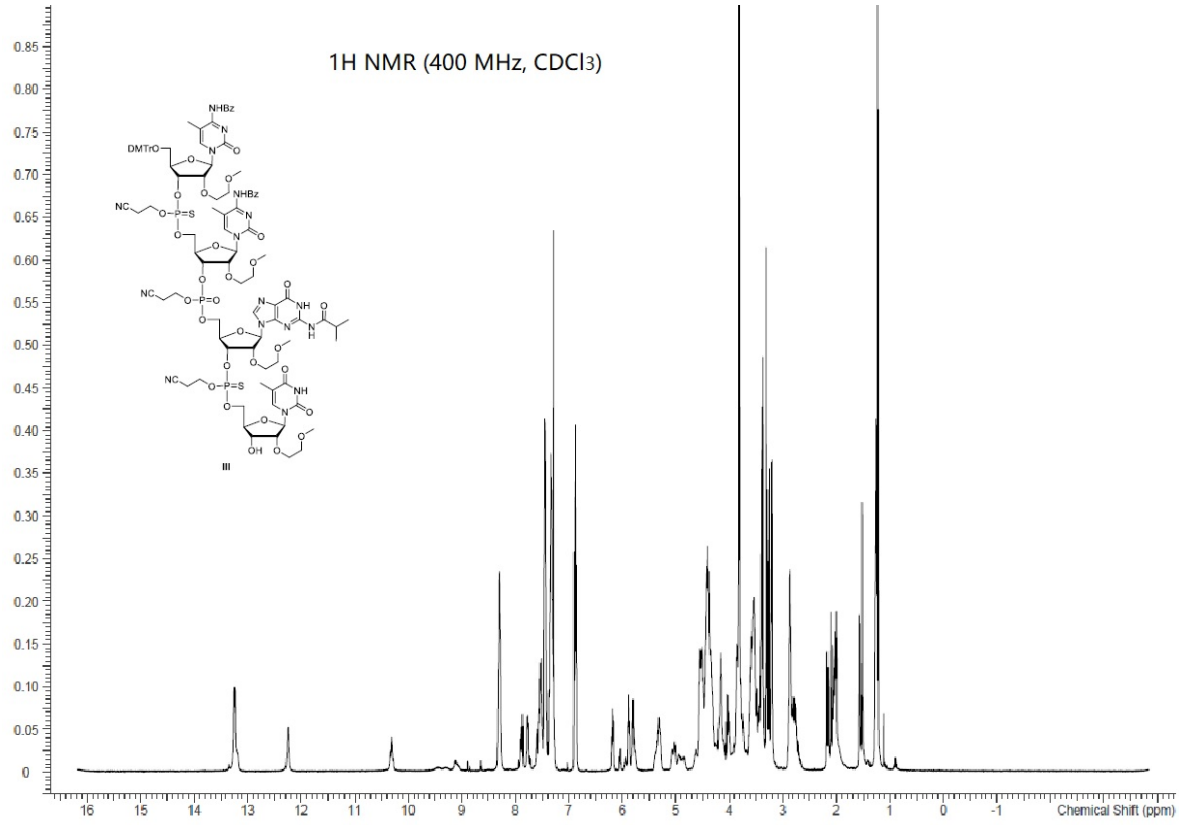


*Figure 13. 1H NMR spectrum of **III***

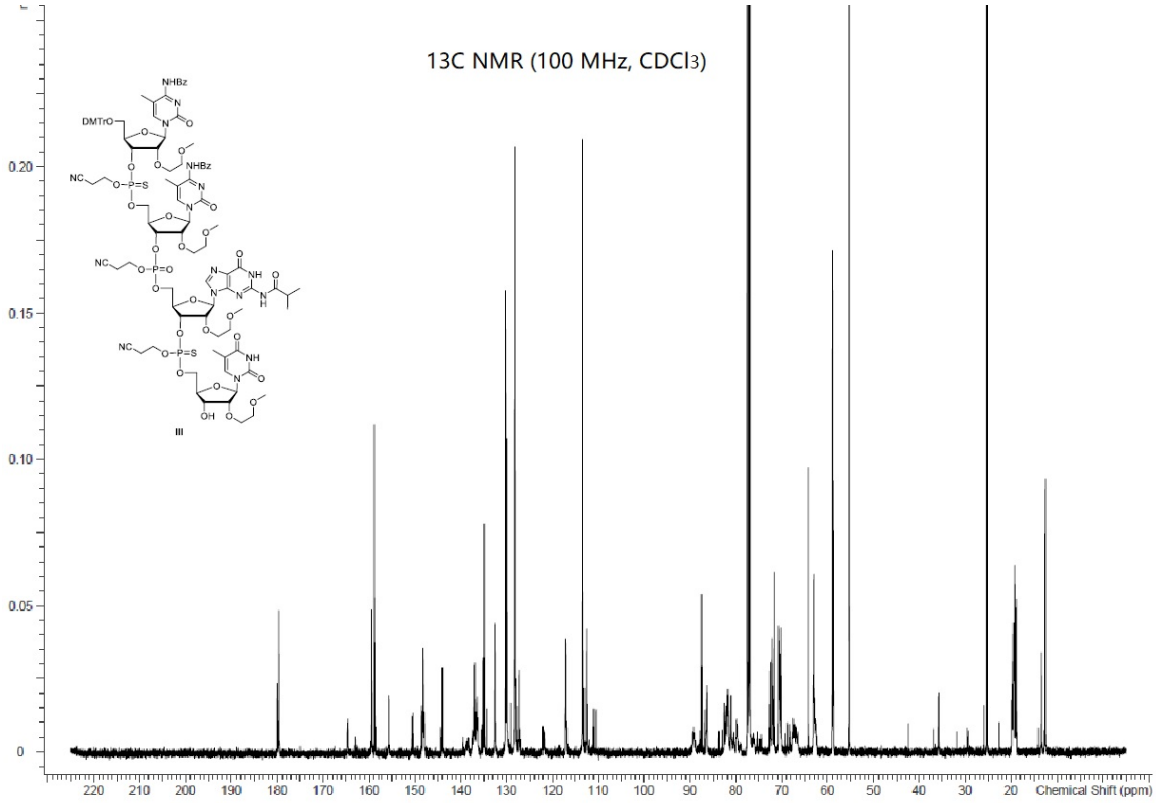


*Figure 14. 13C NMR spectrum of **III***

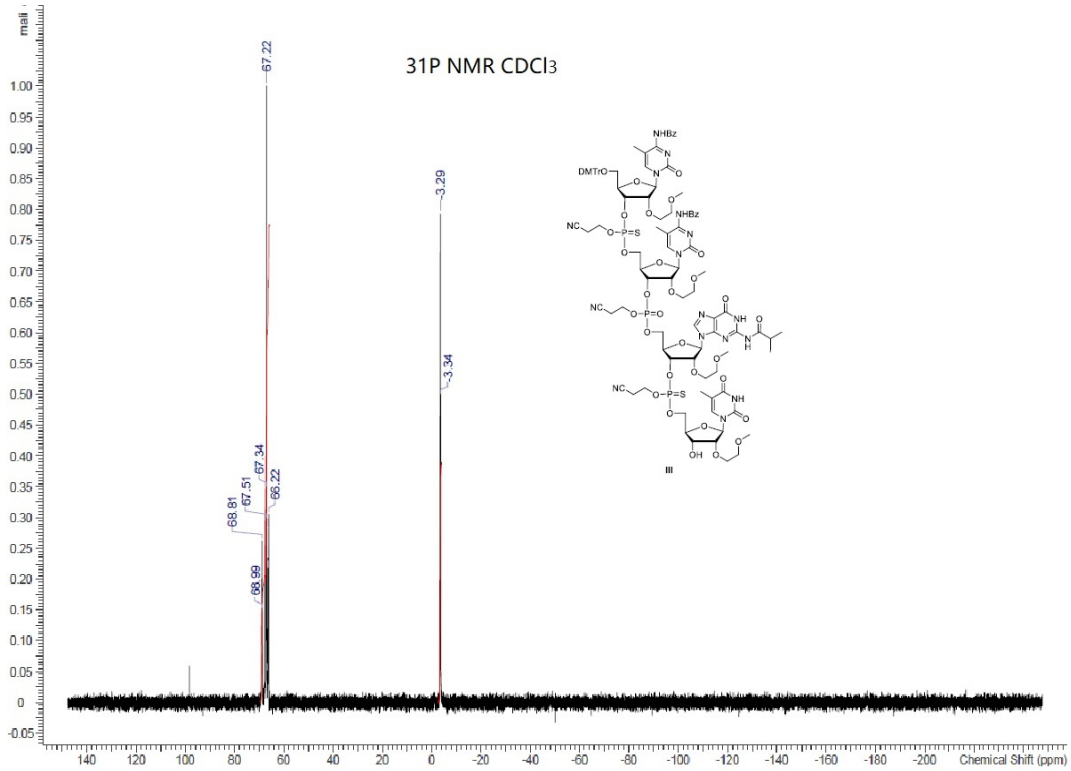


*Figure 15. 31P NMR spectrum of **III***

Compound VI LC-MS Conditions, Chromatogram, HRMS, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR spectra

Column	Acquity UPLC BEH C18（2.1mm*100mm*1.7 um）
Oven	45ºC
Mobile phase	A: 5 mM NH_4OAc in H_2O; B: ACN
Gradient program	Time (min) A% 0 70 8.0 5 15 5 16 70 20 70
Flow rate	0.4 mL/min
Detector	UV 260 nm
Injection volume	1μL (2mg/mL)
Diluent	ACN:H_2O=8:2

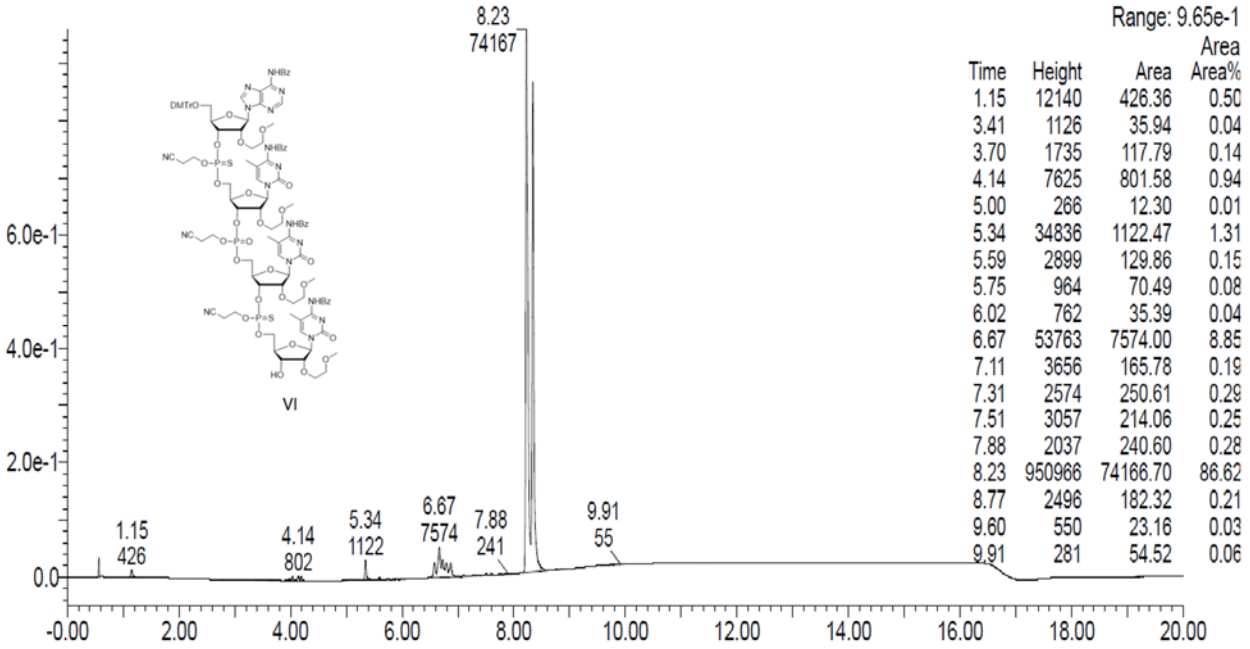


*Figure 16. LC chromatogram of **VI***

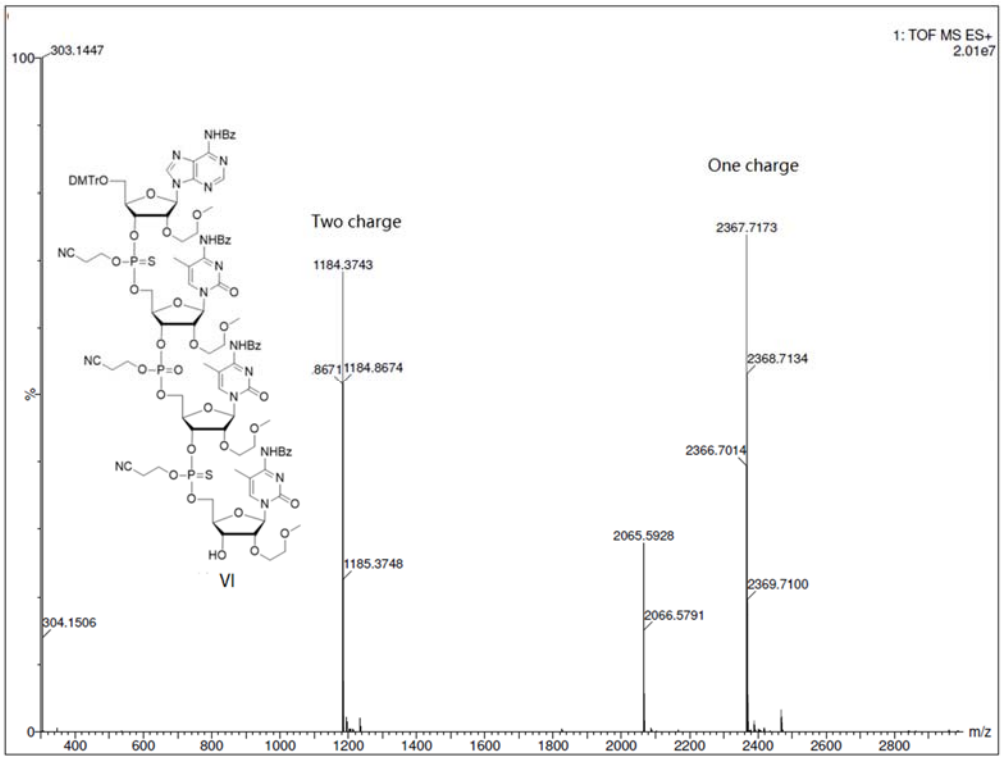


*Figure 17. HRMS spectrum of **VI***

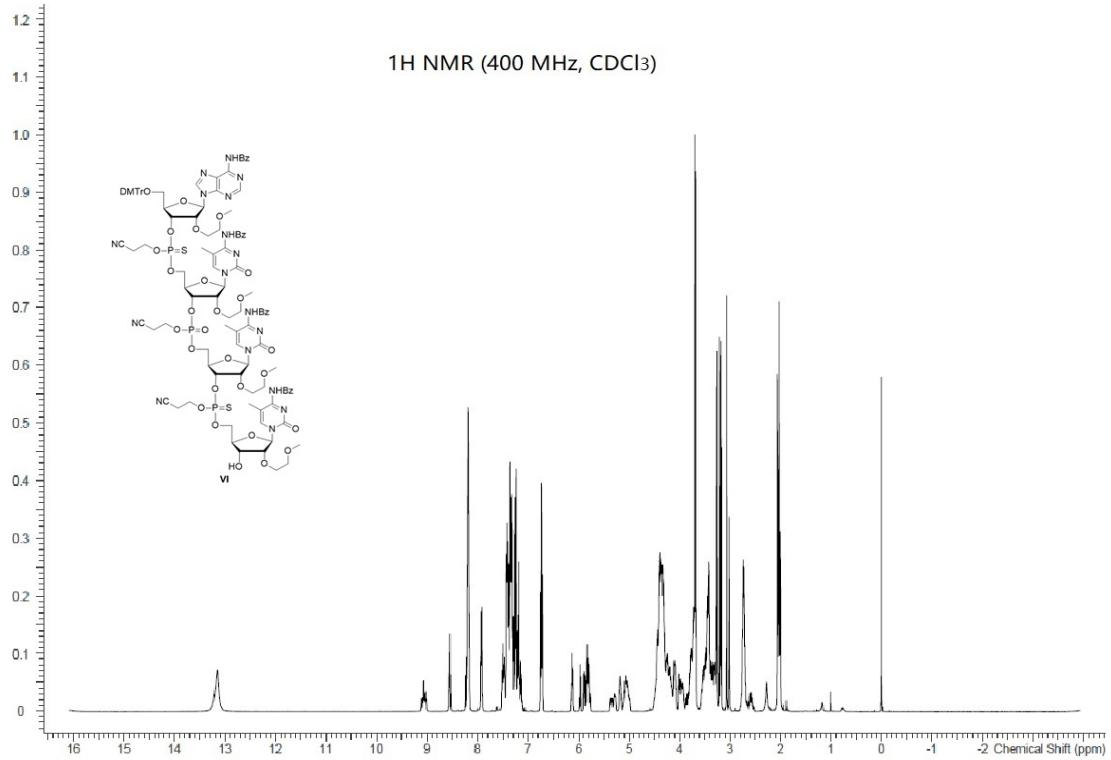


*Figure 18. 1H NMR spectrum of **VI***

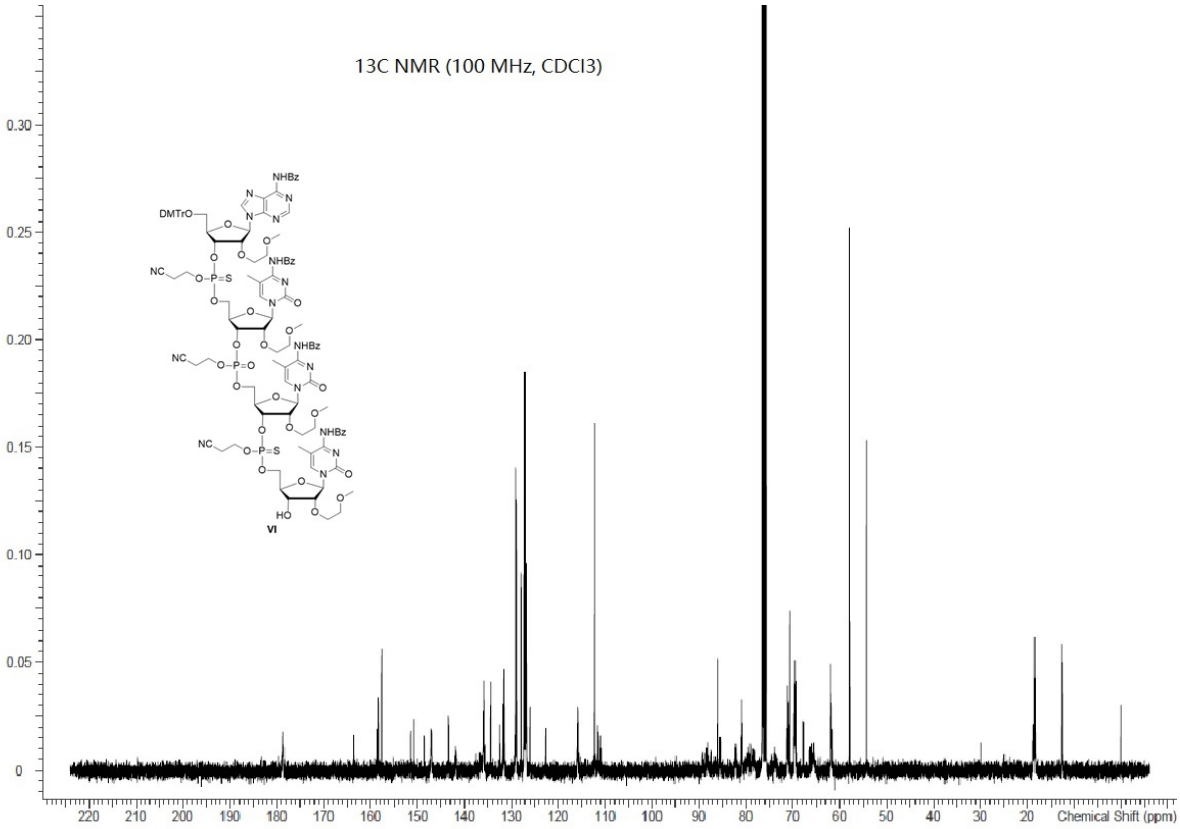


*Figure 19. 13C NMR spectrum of **VI***

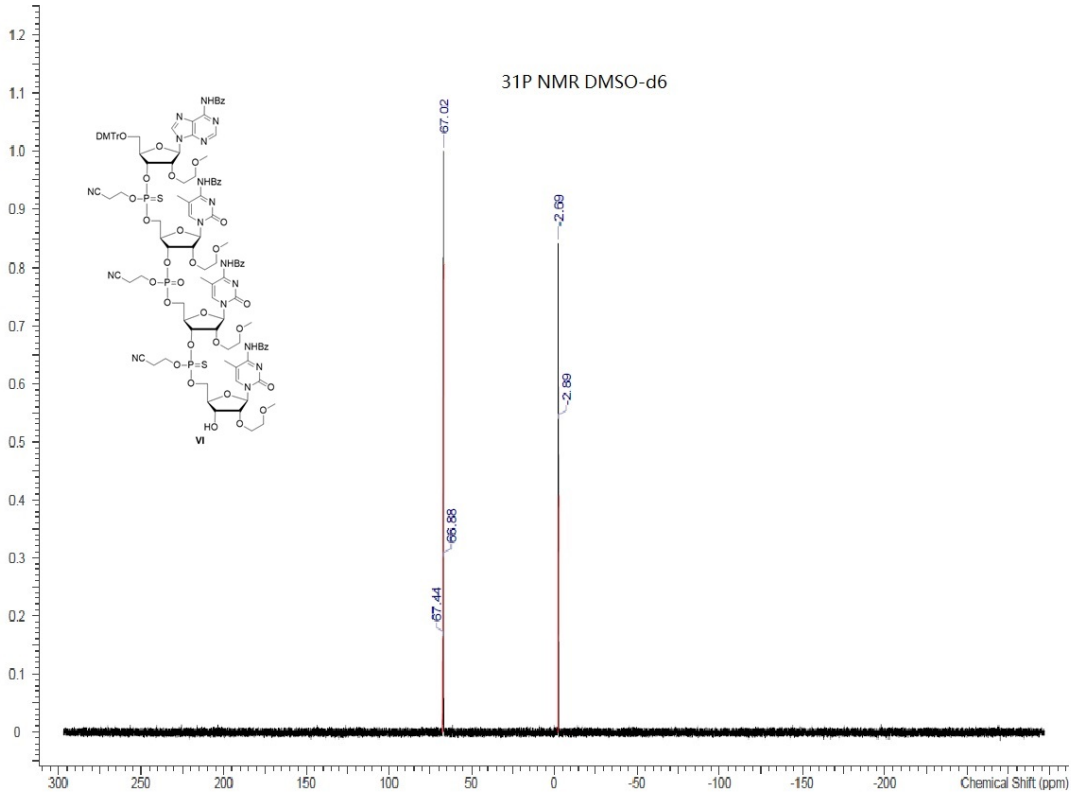


*Figure 20. 31P NMR spectrum of **VI***

Compound IV-H (after ammonolysis) UPLC-MS Conditions, Chromatogram, and HRMS Spectra

LC-MS Conditions

Column	Acquity UPLC BEH C18（2.1mm*150mm, 1.7 um）
Oven	45ºC
Mobile phase	A: 1% HFIP, 0.1% HA in water; B: MeOH
Gradient program	Time (min) A% 0.0 98 1.0 98 13 50 16 15 16.5 15 17 98 21 98
Flow rate	0.3 mL/min
Detector	UV 260 nm
Injection volume	3μL (5mg/mL)
Diluent	H_2O

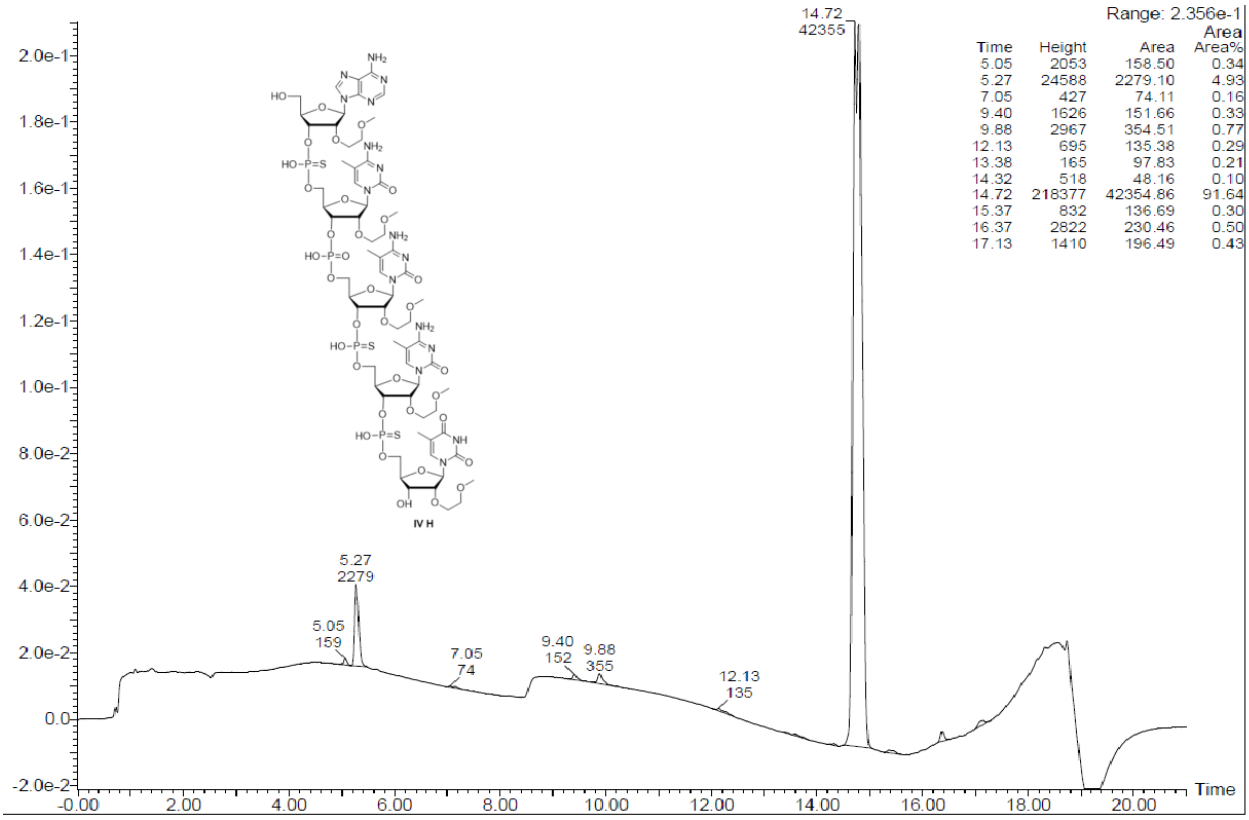


*Figure 21. LC chromatogram of **IV-H** (after ammonolysis of **IV**)*

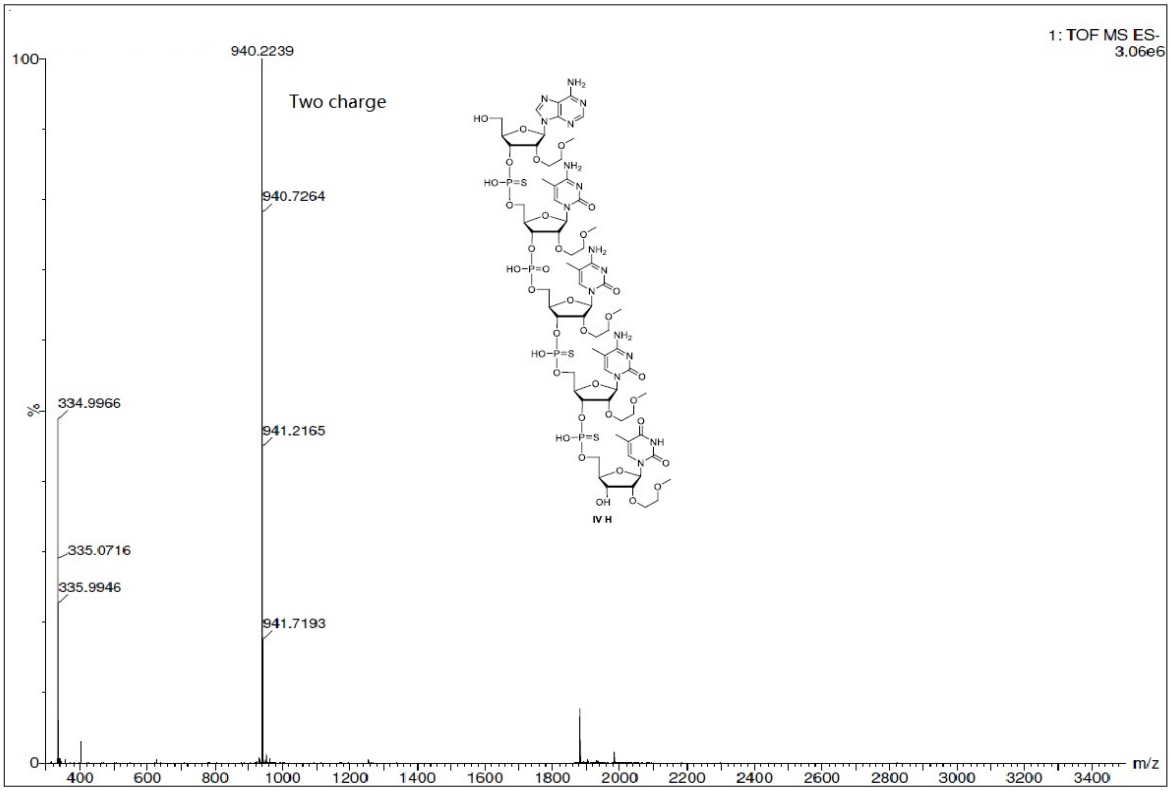
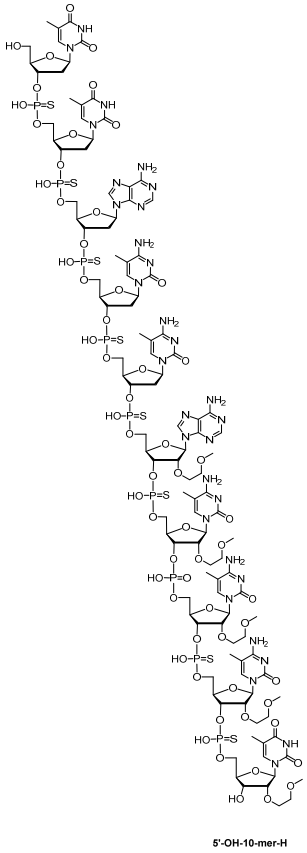


*Figure 22. HRMS spectrum of **IV-H (**after ammonolysis of **IV**)*

Compound 5'-HO-10-mer-H (VII-H). UPLC Condition, Chromatogram, and HRMS Spectrum:



5'-OH-10-mer-H

Column	Acquity UPLC CSH Fluoro-Phenyl column （2.1mm*150mm, 1.7 um）
Oven	55°C
Mobile phase	A: 10 mM TEA + 100 mM HFIP in water B: 10 mM TEA + 100 mM HFIP in MeOH
Gradient program	Time (min) A% 0.0 90 1.0 90 10 50 11 15 12 90 16 90
Flow rate	0.2 mL/min
Detector	UV 260 nm
Injection volume	0.5 µL
Diluent	H_2O

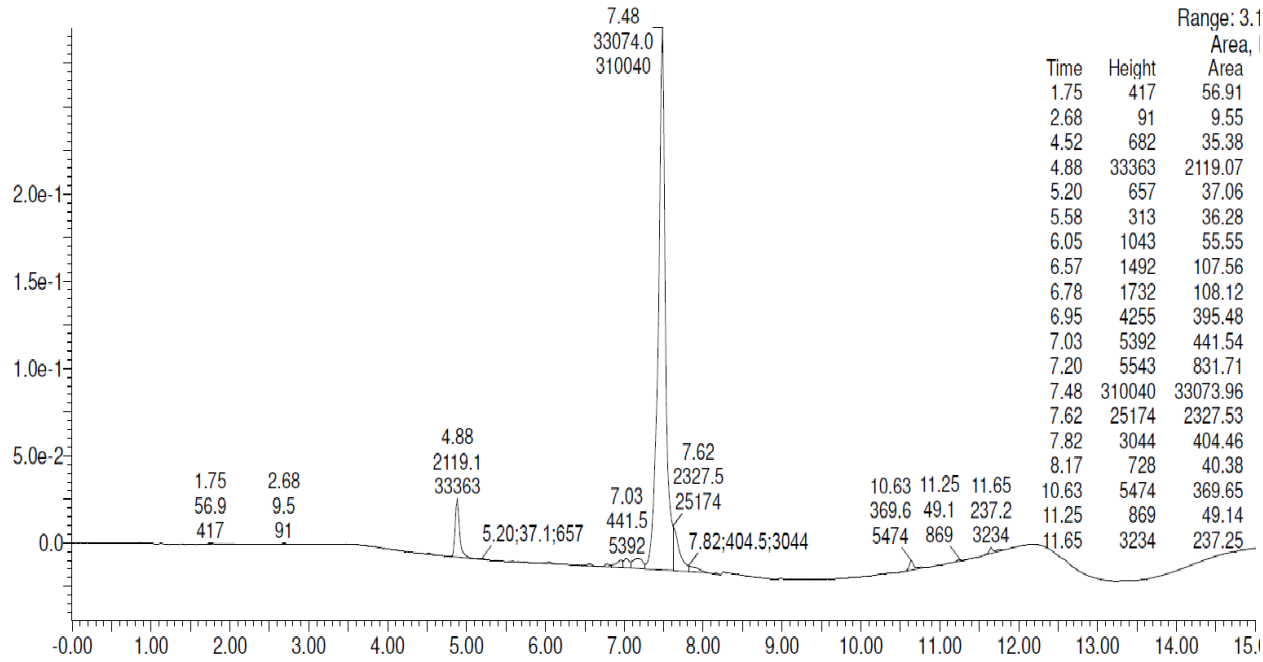


*Figure 23. LC chromatogram of **VII-H** (after ammonolysis of **VII**)*

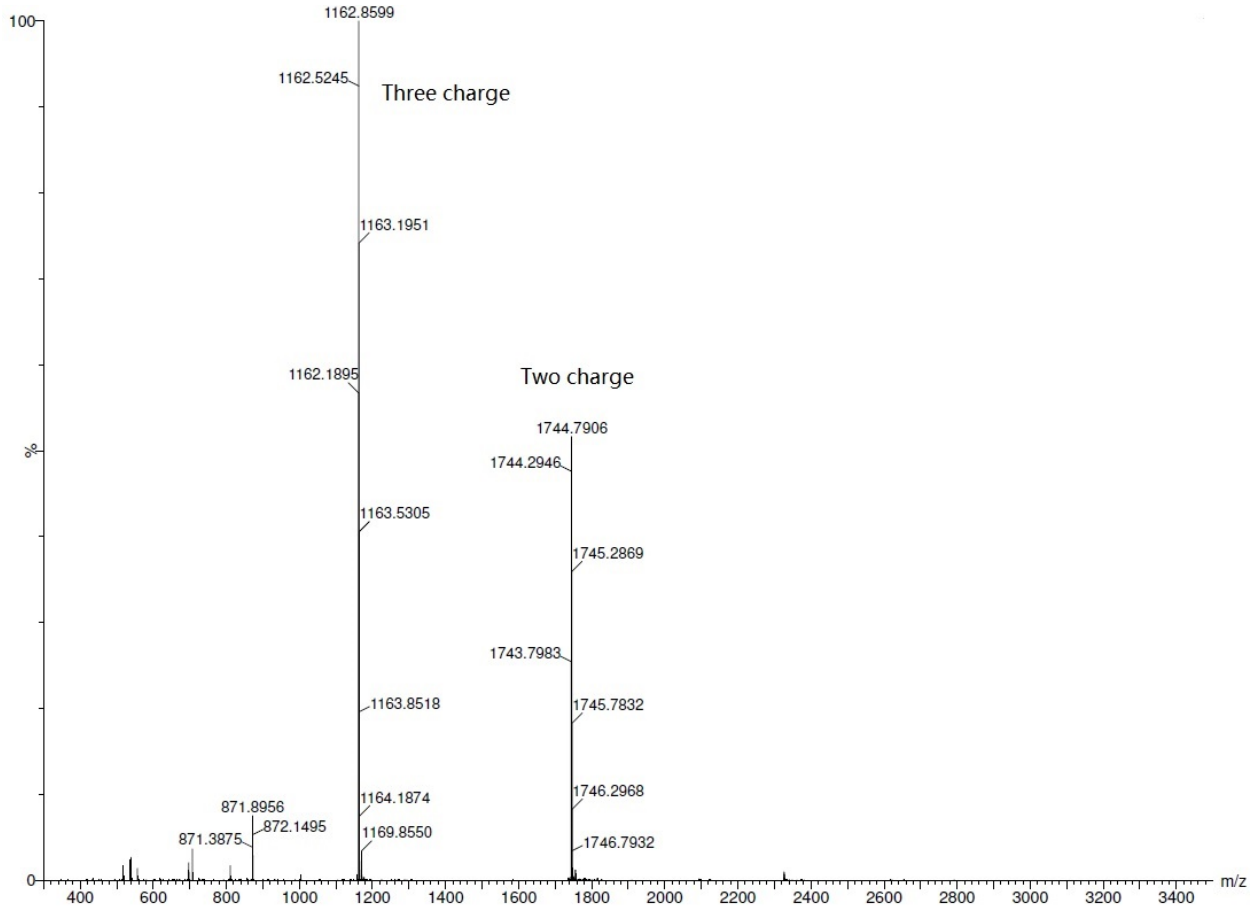
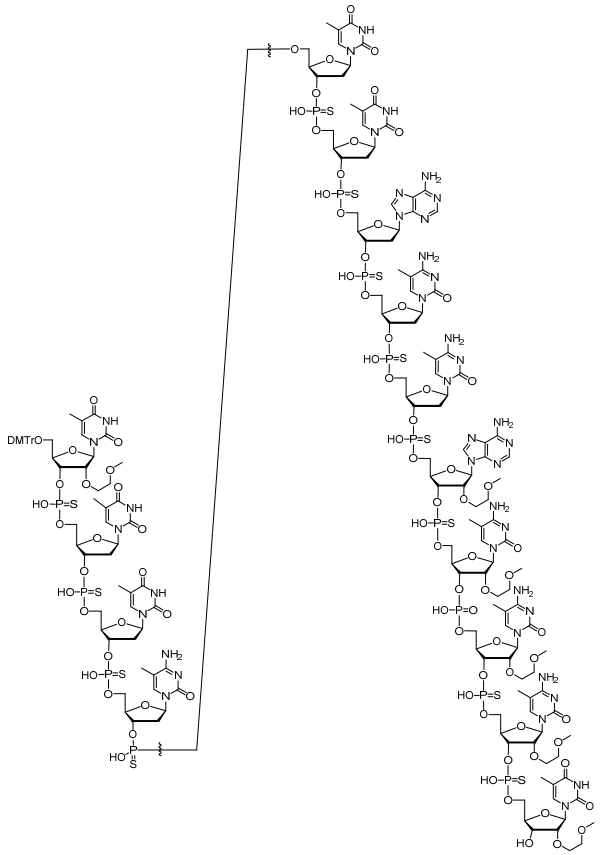


*Figure 24. HRMS spectrum of **VII-H** (after ammonolysis of **VII***

Compound 5'-DMT-14-mer-H (DMT-on VIII-H) UPLC Condition, Chromatogram, and HRMS Spectrum:



5'-DMTr-14-mer-H

Column	Acquity UPLC CSH Fluoro-Phenylcolumn（2.1mm*150mm, 1.7 um）
Oven	55°C
Mobile phase	A: 10 mM TEA + 100 mM HFIP in water B: 10 mM TEA + 100 mM HFIP in MeOH
Gradient program	Time (min) A% 0.0 90 1.0 90 10 50 11 15 12 90 16 90
Flow rate	0.2 mL/min
Detector	UV 260 nm
Injection volume	0.5 μL
Diluent	H_2O

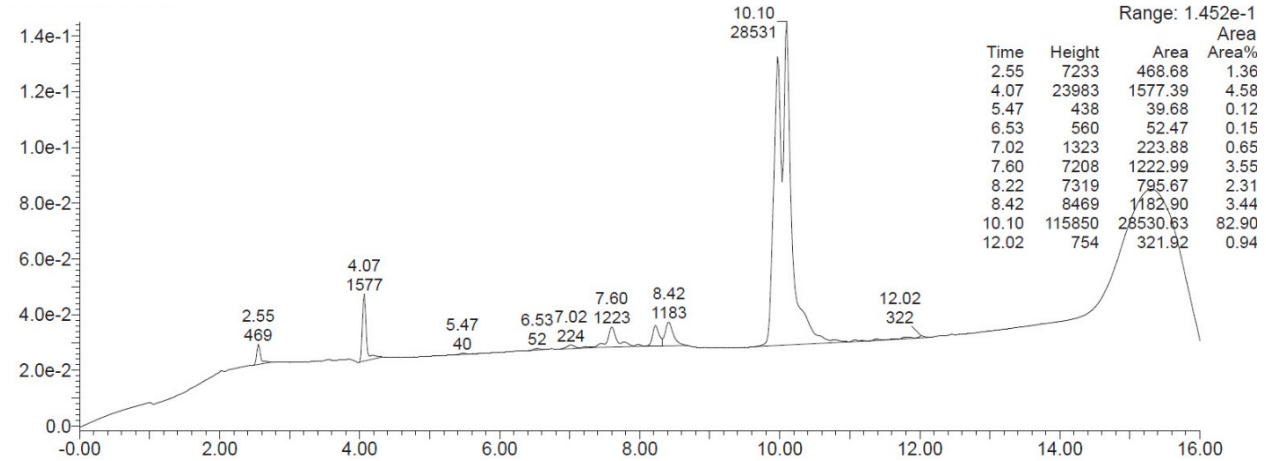


*Figure 25. LC chromatogram of **DMT-on VIII-H***

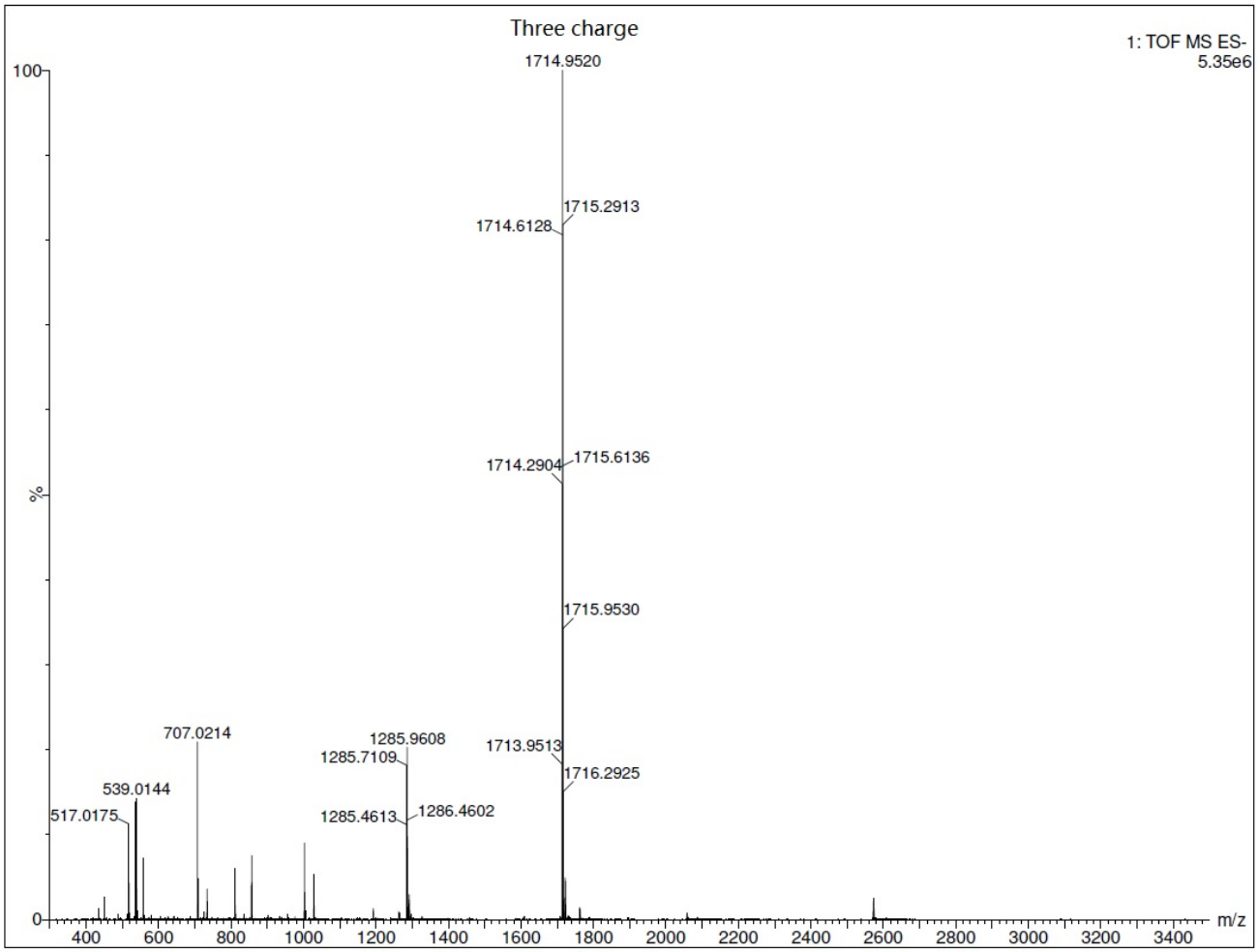
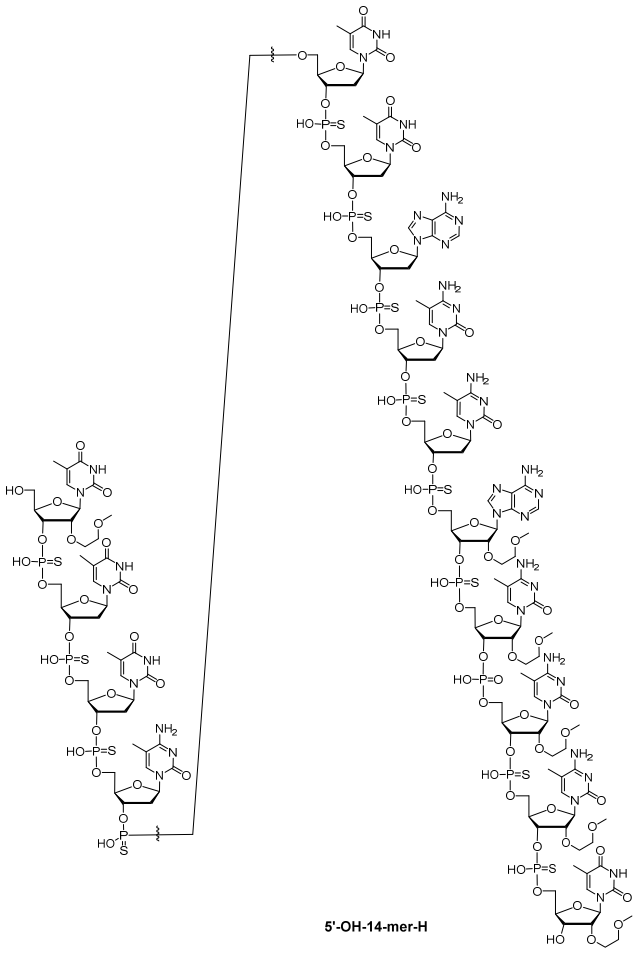


*Figure 26. HRMS spectrum of **DMT-on VIII-H***

Compound 5'-HO-14-mer-H (VIII-H) UPLC Condition, Chromatogram, and HRMS Spectrum:



Column	Acquity UPLC CSH Fluoro-Phenylcolumn（2.1mm*150mm, 1.7 um）
Oven	55ºC
Mobile phase	A: 10 mM TEA + 100 mM HFIP in water B: 10 mM TEA + 100 mM HFIP in MeOH
Gradient program	Time (min) A% 0.0 90 1.0 90 10 50 11 15 12 90 16 90
Flow rate	0.2 mL/min
Detector	UV 260 nm
Injection volume	0.5 μL
Diluent	H_2O

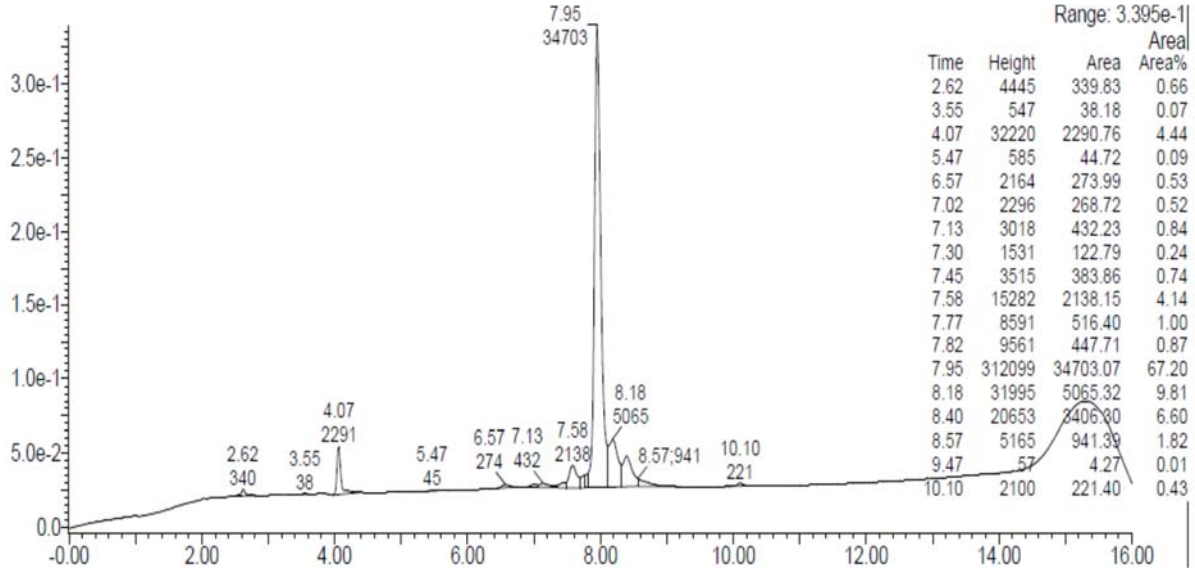


*Figure 27. LC chromatogram of **VIII-H** (after ammonolysis of **VIII**)*

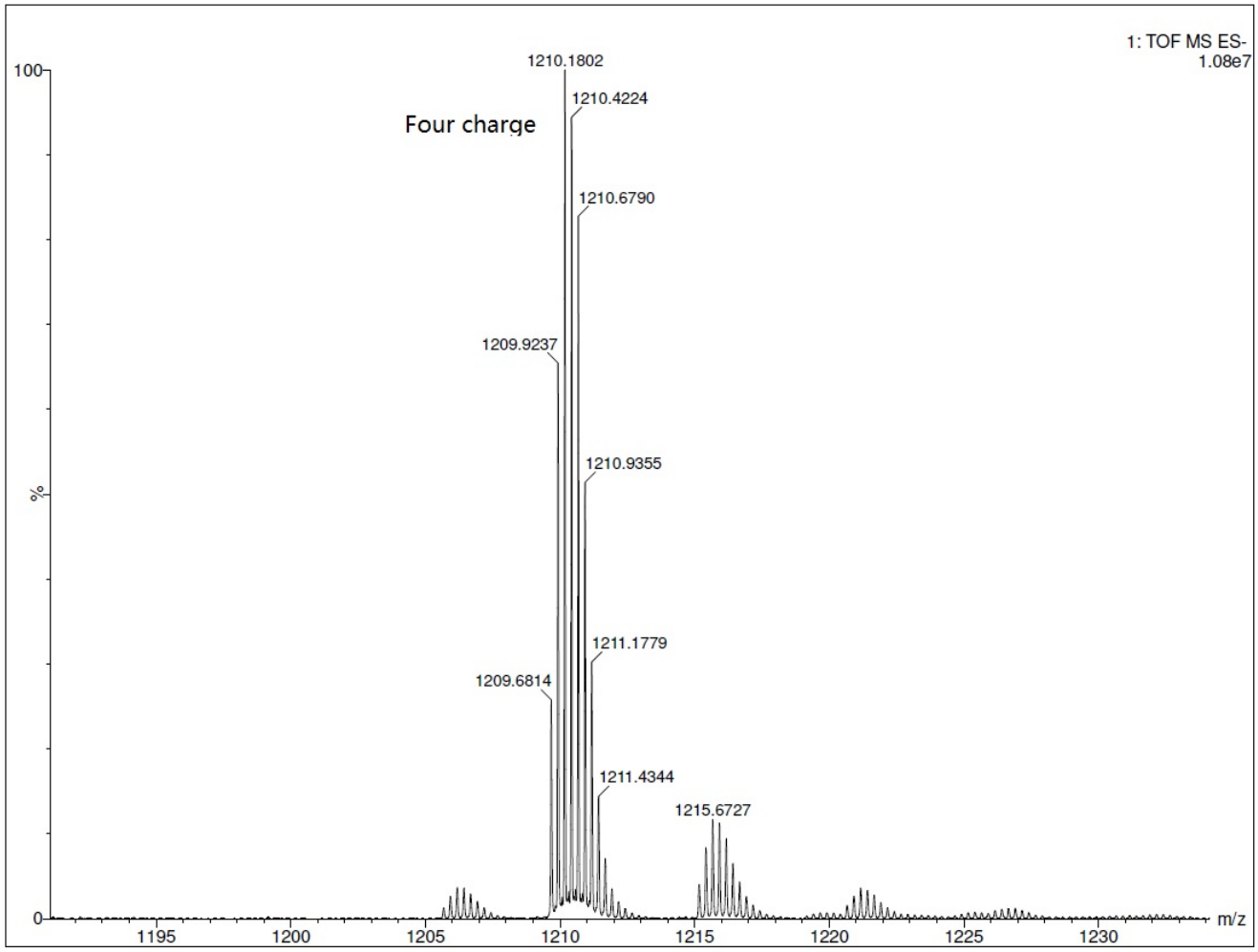


*Figure 28. HRMS spectrum of **VIII-H** (after ammonolysis of **VIII**)*

HPLC condition of DMTr-on crude X and purified ON-A

Column	Waters Acquity BEH C18, 1.7 µ 2.1x100 mm
Oven	50ºC
Mobile phase	A: 10% ACN in water, 5 mM TBuAA, 1µM EDTA B: 80% ACN in water, 5 mM TBuAA, 1µM EDTA
Gradient program	Time (min) A% 0.0 55 14.67 55 16.67 20 17.33 55 20 55
Flow rate	0.25 mL/min
Detector	UV 260 nm
Injection volume	25 µL

MS condition of DMTr-on crude X and purified ON-A

Equipment	Agilent single quadrupole MS	
Parameter	Standard	Harsh
Mass range (m/z)	1500-2000	
Ionization mode	API-ES	
Scan mode	Negative polarity	
Needle voltage (V)	4000	
Nebulizer press (psig)	30	
Drying gas flow (L/min)	12	13
Drying gas temperature (°C)	275	350
Fragmentor voltage (V)	100	
Gain	2	
Threshold	50	
Step size [amu]	0.1	
Data acquisition time (min)	2-20	
Data storage	Full	
Peak width (min)	0.05	
MS extraction window for quantification	-0.3 - +0.7	

The purity of the full-length DMTr-on crude X and Purified ON-A

The results of IP-RP-UHPLC-UV-MS analysis of crude and post purification ON-A are shown in the tables below. The upper table shows the UV response percentage of components in each sample based on their retention time range in the UHPLC analysis and the full-length product (FLP) purity determined by the combination of UV and MS response. The difference in FLP purity between crude X from SPOS and LPOS was mainly due to the different levels of early and late eluting impurities which were effectively removed by purification process. The lower table shows the MS response percentage of the components coeluting with FLP in the main peak. Purification process decreased the full-length (P=O) impurity level by 50%. The apparent post purification increase of the 2'-OEOE/dithioate/thioate impurity in the SPOS is likely due to interference by the sodium adduct of the FLP rather than a real increase in this impurity's abundance. A separate analysis in the QC lab with a slightly different method reported a lower 2'-OEOE/dithioate/thioate impurity level at 1.5%.

Component (%)	SPOS[1] Crude X	SPOS[1] ON-A	LPOS Crude X[2]	LPOS ON-A[2]
Full-length product purity	88.3	92.4	67.9	91.4
UV purity	93.7	99.1	76.8	97.0
Early eluting impurities	5.3	0.81	17.6	2.5
Late eluting impurities	1.0	0.10	5.6	0.46

For impurities coeluting with FLP under the main peak, MS% is reported as below.

MS purity	94.3	93.2	88.4	94.2
Full-length (P=O)1	0.56	0.48	4.0	2.2
Total n-1	2.6	2.9	1.4	1.2
Total abasic	0.11	<0.10[3]	0.19	<0.10
2'-OEOE/dithioate/thioate	1.3	1.9	0.46	0.55
Total n+1	0.11	0.18	0.87	0.33

1, At 1.2 kg/batch scale. 2. At 180 g/batch scale using the fragments made at ~300 g scales. [3]Limit of detection is 0.10%

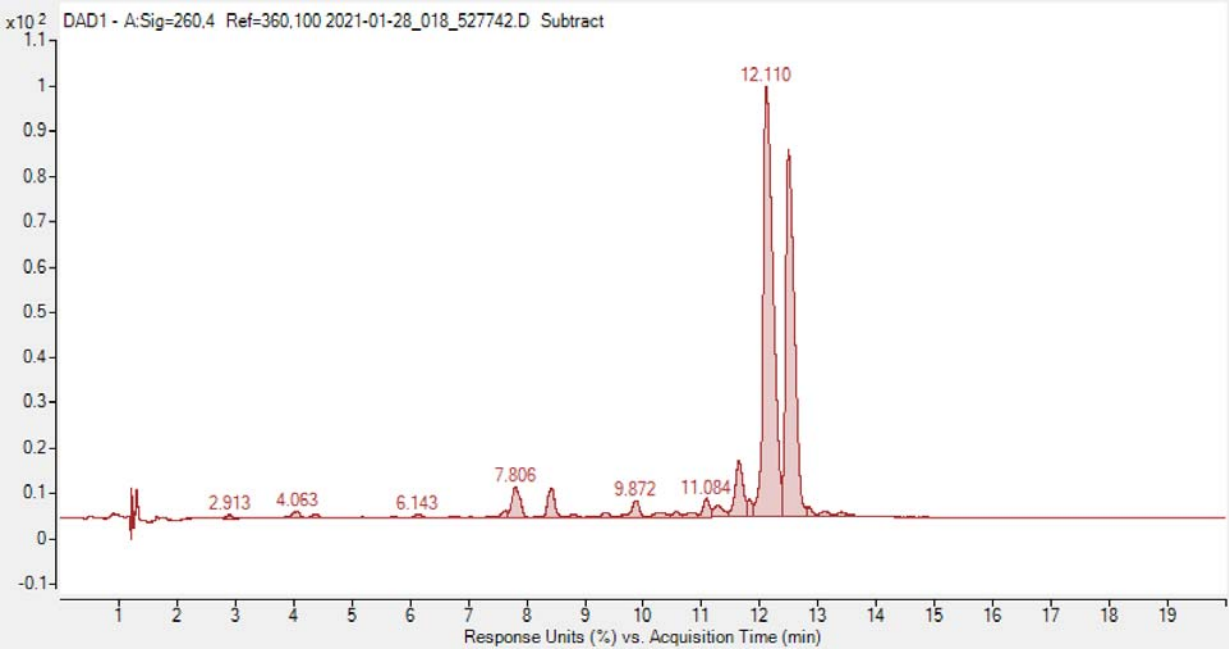


*Figure 29. HPLC chromatogram of **crude X***

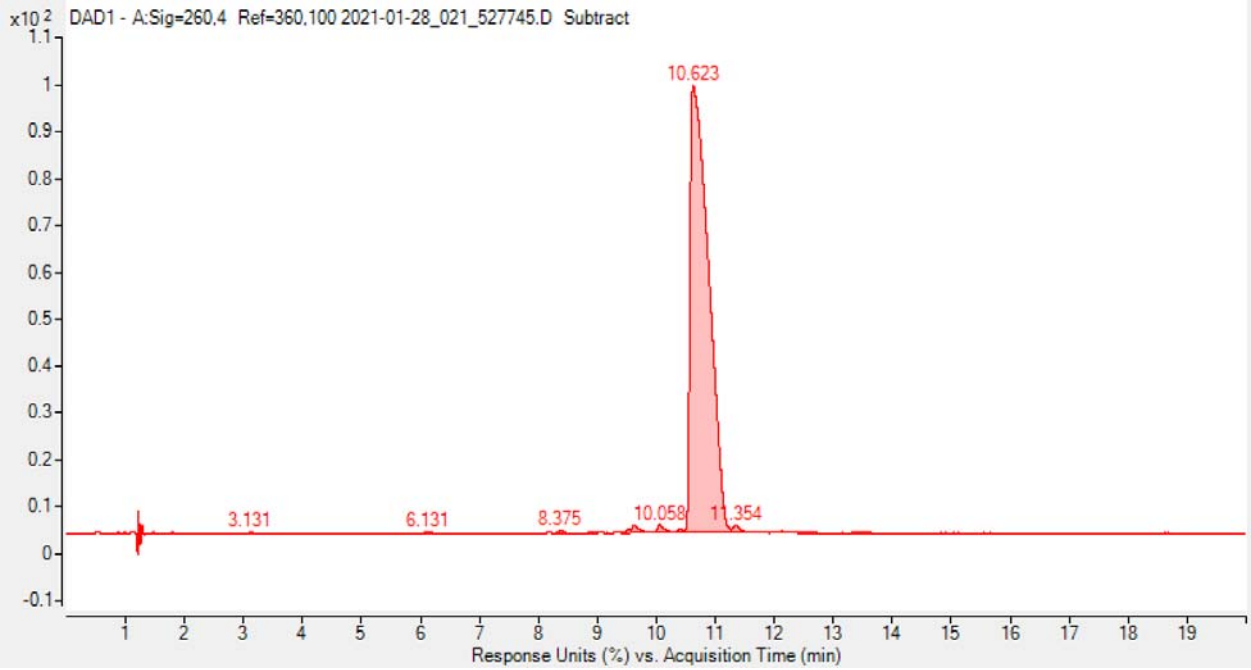


*Figure 30.LC chromatogram of **X** (after purification)*

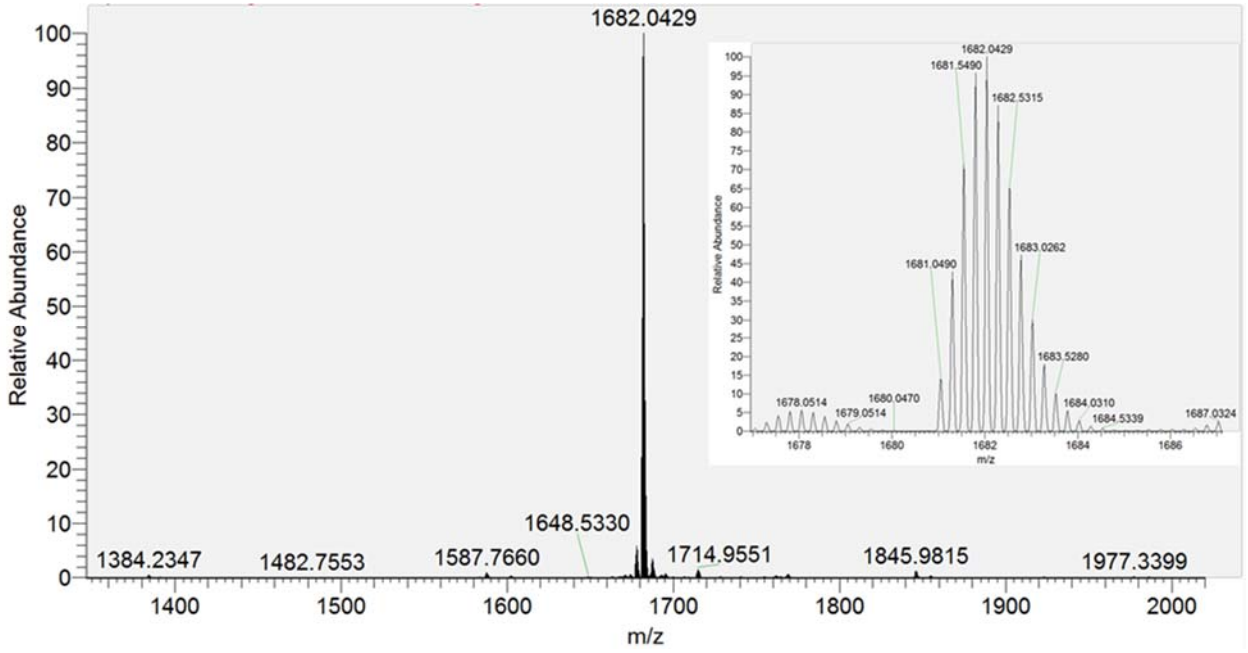


*Figure 31.HRMS of Crude **X***

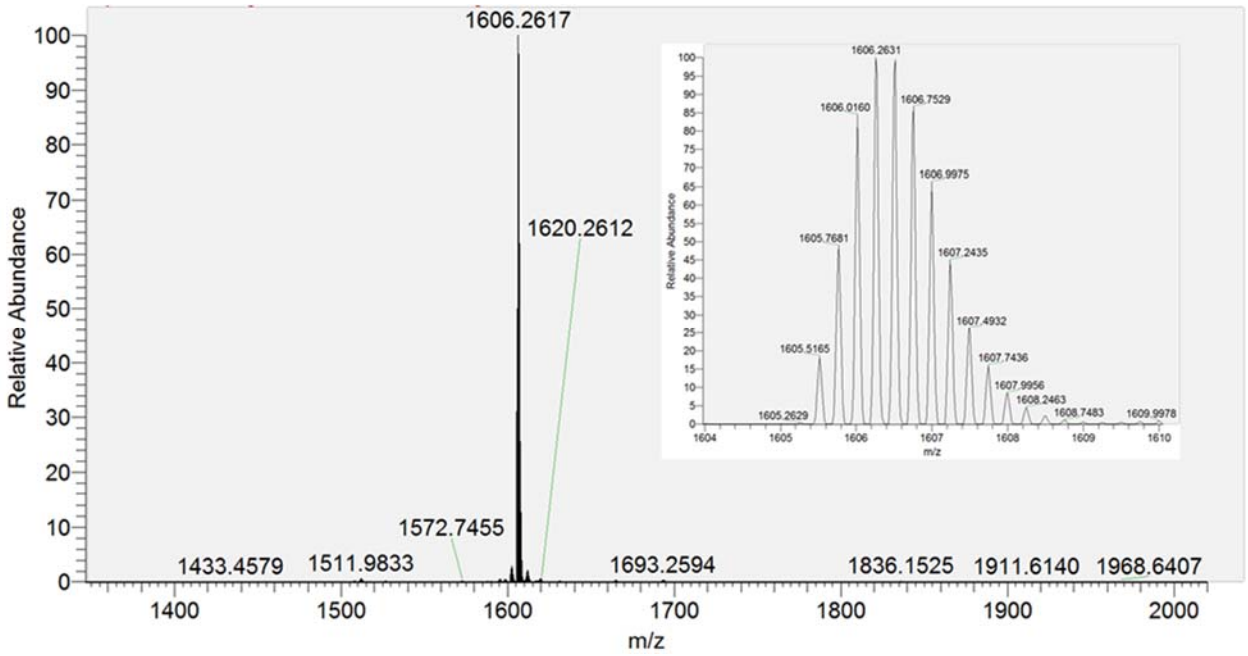


*Figure 32.HRMS of **ON-A***