Supplementary Materials Part II for

Scalable convergent synthesis of therapeutic oligonucleotides

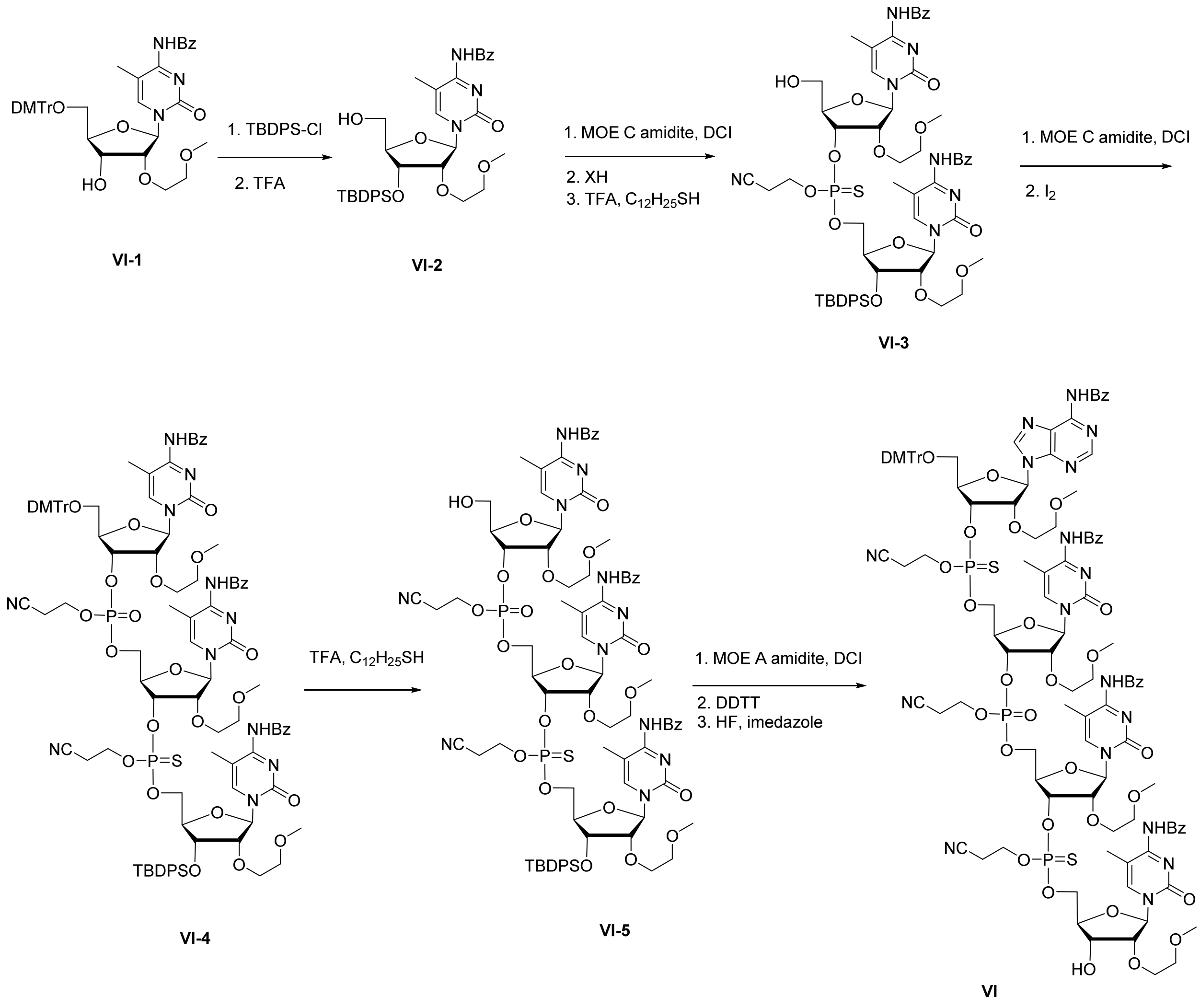
Xuan Zhou, Wuming Yan, William F. Kiesman, Yannick A. Fillon, Hong Jiang, Firoz D. Antia,
Li Xiao, Jing Yang, Armin Delavari, Robert S. Gronke, Xianglin Shi*

Correspondence to: xianglin.shi@biogen.com

Procedure for syntheses of Fragment VI and II at ~ 3-kg scale

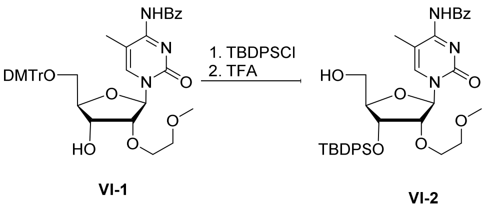
Contents

Synthesis of Fragment VI



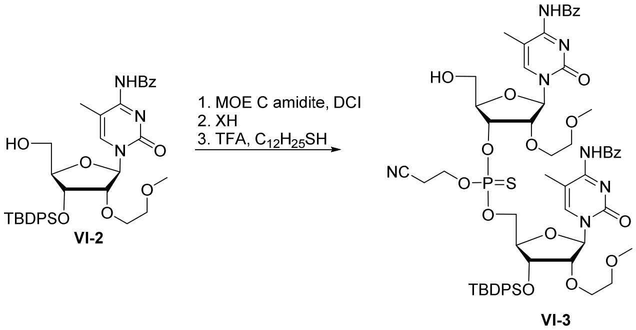
Scheme 1. *Synthesis route of fragment* ***VI***

5′-HO-MOE-C-3′-OTBDPS, VI-2



A mixture of compound **VI-1** (1485.0 g, 2.06 mol, 1.00 equiv), imidazole (462.0 g, 6.79 mol, 3.30 equiv), TBDPSCl (792.0 g, 2.88 mol, 1.40 equiv) and CH_2Cl_2 (7.4 L) was stirred at 20±5 °C for 14.0 h (HLPC indicated > 99.9% conversion of compound **VI-1**). IPA (124.0 g, 2.06 mol, 1.00 equiv) was added and the mixture was stirred at 20±5 °C for 0.5 h, cooled to 0±5 °C, and $C_{12}H_{25}SH$ (541.0 g, 2.68 mol, 1.30 equiv) was added. TFA (1173 g, 10.3 mol, 5.00 equiv) was added in 1.0 h, and the mixture was stirred at 0±5 °C for 1.0 h (HPLC indicated > 99.9% completion of the reaction). The reaction mixture was added into 8% Na_2CO_3 solution (654.0 g Na_2CO_3 dissolved in 7.4 L of deionized water) at 0±5°C in 1.5 h with vigorous stirring. MTBE (7.4 L) was added, and the mixture was stirred for 0.5 h at 25±5 °C. The organic layer was separated, washed with saturated brine (4.5 L), dried over $MgSO_4$ (1500 g), filtered, and concentrated. The crude product was dissolved in CH_2Cl_2 (2.0 L) and added to heptane/MTBE (42.4 L, 18:1, v/v). The precipitated product was filtered and washed with heptane (2 x 1.5 L), dried under vacuum at 25±5 °C for 40 h to yield **VI-2** as a white solid (1190 g, 88.0% yield). HRMS calcd for $C_{36}H_{43}N_3O_7Si^+$ $[M+H]^+$: 658.2949, found: 658.2869.

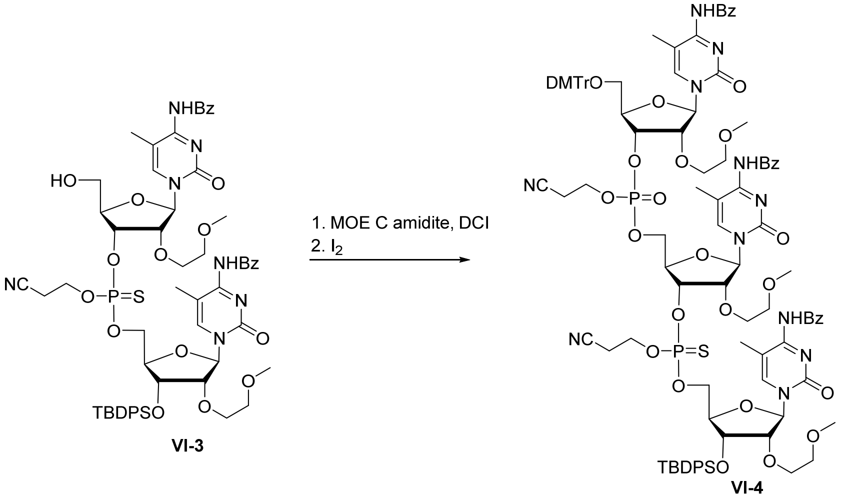
5′-HO-MOE-CC-3′-OTBDPS, VI-3



A mixture of compound **VI-2** (1180.0 g, 1.80 mol, 1.00 equiv), CH_3CN/CH_2Cl_2 (7.1 L, 1:1, v/v), MOE C phosphoramidite (1743.0 g, 1.89 mol, 1.05 equiv) was stirred at 25±5 °C until all solids were dissolved, and the solution was circulated through a column packed with 3Å MS (1180.0 g) for 3.0 h at 25±5 °C. The column was washed with CH_3CN/CH_2Cl_2 (4.7 L, 1:1, v/v) and the resulting solution was added to the mixture. DCI (318.0 g, 2.70 mol, 1.50 equiv) was added and the mixture was stirred at 25±5 °C for 1.0 h (HPLC indicated > 99.9% conversion of **VI-2**). $HOC_5H_{10}CO_2H$ (23.8 g, 0.18 mol, 0.10 equiv) was added and the mixture was stirred at 25±5 °C for 0.5 h. XH (275.0 g, 2.16 mol, 1.20 equiv) was added and the mixture was stirred at 25±5 °C for 0.5 h. The reaction mixture was cooled to 0±5 °C, $C_{12}H_{25}SH$ (1275.0 g, 6.30 mol, 3.50 equiv) and TFA (1841.0 g, 16.2 mol, 9.00 equiv) were added sequentially and the mixture was stirred at 0±5 °C for 1.0 h (HPLC indicated > 99.9% reaction completion). NMI (1473.0 g, 18.0 mol, 10.0 equiv) was added at 0±5 °C in 1.5 h. To the mixture was added a mixture of EtOAc/MTBE (35.4 L, 1:2, v/v) at 5±5 °C and the mixture was stirred vigorously for 15 min. The mixture was washed with 5% $NaHCO_3$ aqueous solution (2 x 11.8 L), saturated brine (11.8 L) and concentrated. The residue was dissolved in CH_3CN (11.8 L) at ambient temperature, and MTBE (2.4 L), heptane (9.5 L) and water (5.9 L) were added, and the mixture was stirred for 15 min. The bottom and middle layers were collected and extracted with MTBE/heptane (2 x11.8 L, 1:4, v/v). To the bottom layers were added MTBE (2.4 L) and saturated brine (0.6 L) and the mixture was mixed thoroughly. The top organic layer was separated and washed with saturated brine (3.6 L) and concentrated. The residue was dissolved in CH_2Cl_2 (11.8 L) and filtered through a silica gel pad (300~400 mesh, 5900 g) and the pad was washed with EtOAc/CH_2Cl_2 (70.8 L, 1:1, v/v).

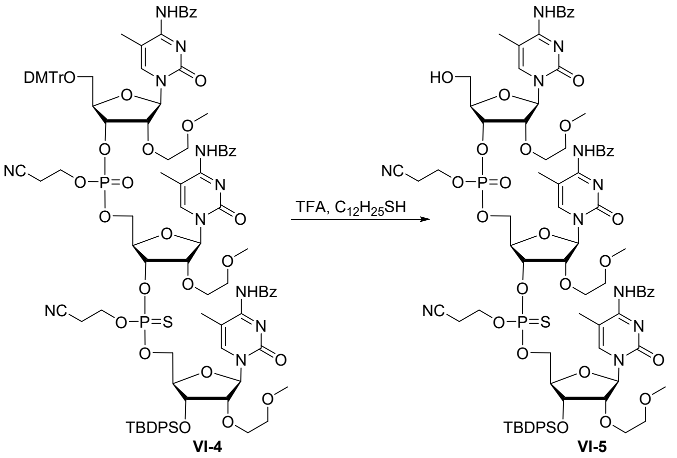
All the filtrates were combined and concentrated. The residue was dissolved in EtOAc (3.54 L) and concentrated (repeated three times). The residue was redissolved in EtOAc and PPh_3 (94.2 g, 0.36 mol, 0.20 equiv) was added. The mixture was stirred at 15±5 °C for 0.5 h and slowly added into the mixture of *i*Pr_2O/heptane (53.1 L, 6:39, v/v). The product precipitated was filtered, washed with heptane (2 x 5.6 L), dried under vacuum at 25±5 °C for 40 h to yield **VI-3** as a white solid (2139 g, 98.7% yield). HRMS calcd for $C_{59}H_{70}N_7O_{15}PSSi^+$ $[M+H]^+$: 1208.4236, found: 1208.4189.

5′-DMTrO-MOE-CoCC-3′-OTBDPS, VI-4



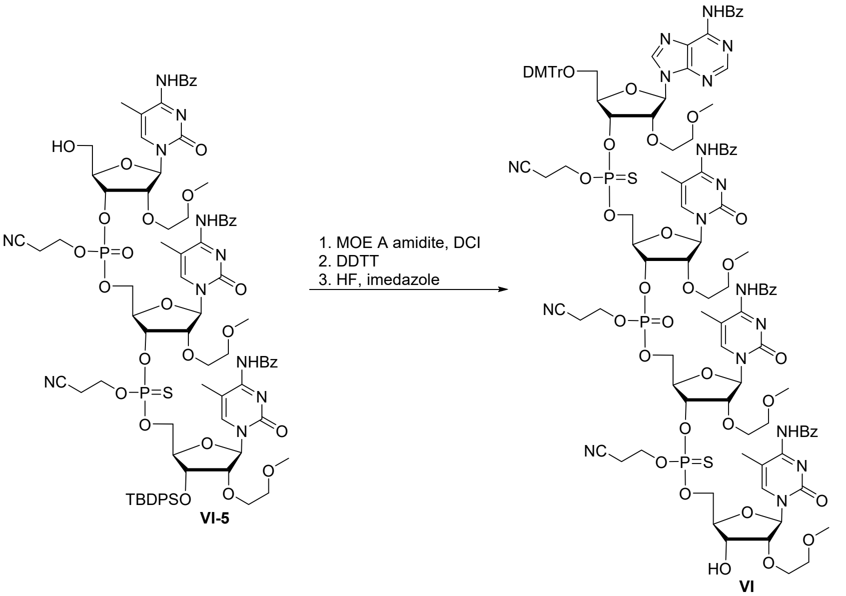
A mixture of compound **VI-3** (2100 g, 1.74 mol, 1.00 equiv), CH_3CN (12.6 L) was stirred at 25±5 °C until all solids were dissolved, and then circulated through a column packed with 3Å MS (2100 g) for 6.0 h at 25±5 °C. The column was washed with CH_3CN (4.2 L) and the resulting solution was added to the dried solution. In a separated reactor a mixture of MOE C phosphoramidite (1460 g, 1.58 mol, 0.91 equiv) and CH_3CN (8.2 L) was stirred at 25±5 °C until all solids dissolved, and the solution was then circulated through a column packed with 3Å MS (1050 g) for 4.0 h at 25±5 °C. The column was washed with CH_3CN (4.2 L) and the resulting solution was added to the dried solution. This phosphoramidite solution was then added to the above solution of **VI-3**. DCI (308.0 g, 1.61 mol, 1.50 equiv) was then added and the reaction mixture was stirred at 25±5 °C for 1.0 h (HPLC indicated > 99.9% conversion of **VI-3**). Pyridine (206.0 g, 3.48 mol, 2.00 equiv) was added and the reaction mixture was cooled to 0±5 °C. A solution of iodine (883.0 g, 3.48 mol, 2.00 equiv) and pyridine (687.0 g, 8.70 mol, 5.00 equiv) in THF/H_2O (12.6 L, 5:1, v/v) was added slowly to the reaction mixture and stirred at 0±5 °C for 0.5 h. The reaction mixture was divided into three equal portions for quench and workup following the same procedure: An aqueous solution of 4% $Na_2S_2O_3$ solution (288.0 g, 1.16 mol, 2.00 equiv) was charged slowly to one portion of the reaction mixture at 0±5 °C and stirred at 0±5 °C for 30 min. A mixture of EtOAc/MTBE (21.0 L, 1:2, v/v) was added and stirred vigorously at 10±5 °C for 0.5 h. The organic layer was separated, washed with 5% $NaHCO_3$ aqueous solution (2 x 7.0 L) and saturated brine (7.0 L), dried over $MgSO_4$ (700 g) for 0.5 h, filtered, and concentrated to yield the crude product **VI-4**. After all the reaction mixture was worked up, the crude product **VI-4** (~3354 g) were combined and used in the next step without further purification.

5′-HO-MOE-CoCC-3′-OTBDPS, VI-5



The crude product **VI-4** (3354 g, 1.64 mol, 1.00 equiv) was co-evaporated with CH_2Cl_2 (2 x 21.0 L) and then dissolved in CH_2Cl_2 (4.2 L), and 3 Å MS (1050 g) were added and the mixture was stirred at 25±5 ºC for 1.0 h, cooled to 0±5 °C and $C_{12}H_{25}SH$ (1232 g, 6.09 mol, 3.50 equiv) was added. TFA (1784 g, 15.7 mol, 9.00 equiv) was added at 0±5 °C in 1.0 h and the mixture was stirred at 0±5 °C for 2.0 h (HPLC > 99.9% reaction completion). NMI (1427 g, 17.4 mol, 10.0 equiv) was added at 0±5 °C in 1.0 h and the mixture was stirred at 0±5 °C for 0.5 h. A mixture of EtOAc/MTBE (4.2 L, 1:1, v/v) was added and the reaction mixture was stirred at 10±5 °C for 0.5 h. After removal of the 3Å MS by filtration, the reaction solution was divided into two equal portions for workup and precipitation following the same procedure: The mixture was washed with water (5.3 L), water/DMF (5.3 L, 9:1, v/v), 5% $NaHCO_3$ aqueous solution (2 x 5.3 L) and saturated brine (5.3 L), dried with $MgSO_4$ (1050 g), filtered, and concentrated. The concentrated crude product was dissolved in EtOAc (2.1 L) and slowly added to a mixture of heptane/MTBE (30.0 L, 4:1, v/v). The product precipitated was filtered, washed with heptane (2 x 1.2 L). After completion of the whole reaction mixture, the combined product was dried under vacuum at 25±5 ºC for 40 h to yield **VI-5** as a white solid (2732 g, 90.2% yield). HRMS calcd for $C_{82}H_{97}N11O_{24}P_2SSi^+$ $[M+H]^+$: 1742.5751, found: 1742.5732.

5′-DMTrO-MOE-ACoCC-3′-OH, VI

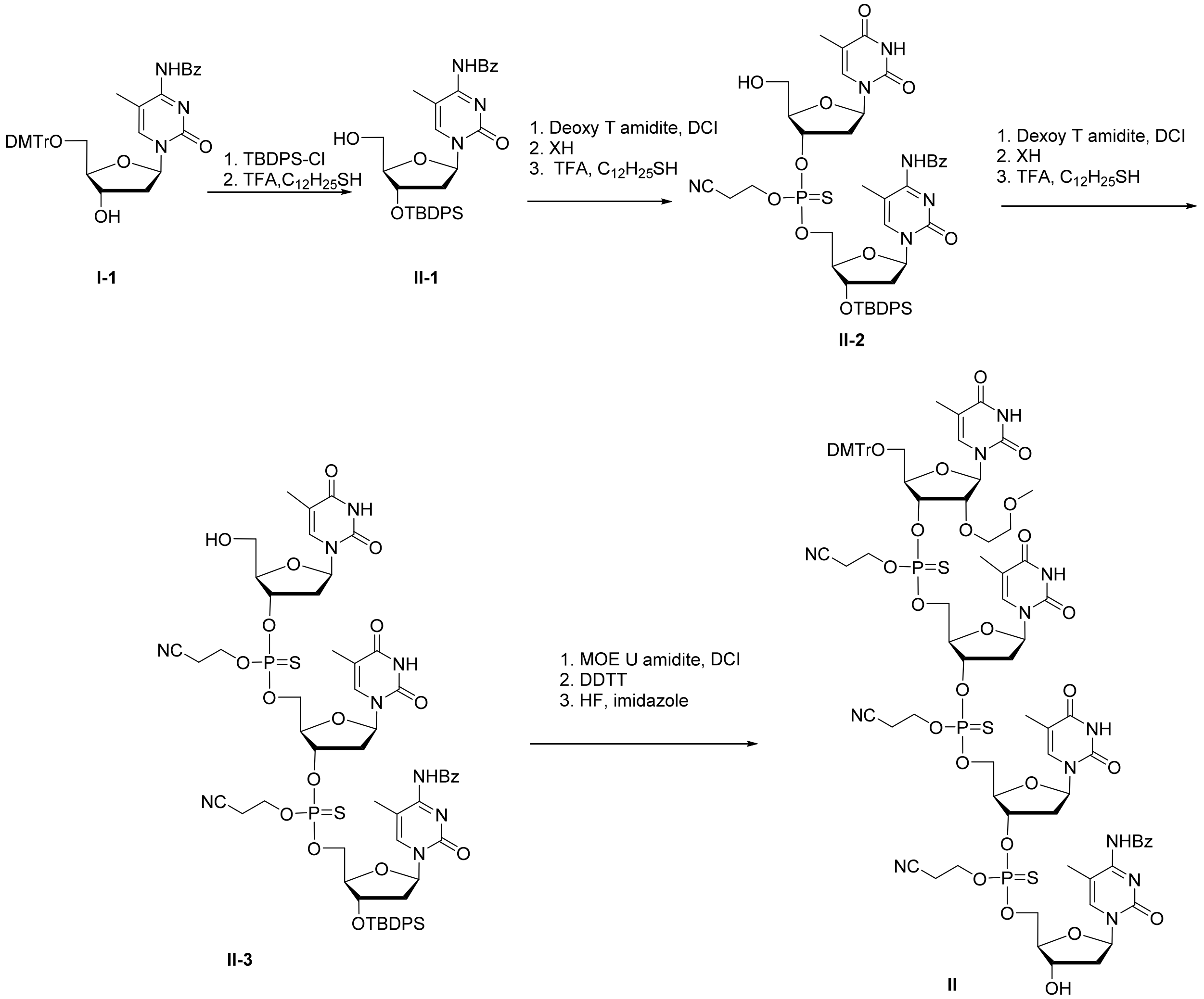


A mixture of compound **VI-5** (2700 g, 1.55 mol, 1.00 equiv), CH_3CN/CH_2Cl_2 (10.4 L, 1:1, v/v) was stirred at 25±5 °C until all solids dissolved, and then circulated through a column packed with 3Å MS (4050 g) for 5.5 h at 25±5 °C. The column was washed with CH_2Cl_2 (8.1 L) and the resulting solution was added to the mixture.

In a separated reactor a mixture of MOE A phosphoramidite (1438 g, 1.55 mol, 1.00 equiv) and CH_3CN (7.1 L) was stirred at 25±5 °C until all solids dissolved, and the solution was circulated through a column packed with 3Å MS (1350 g) for 3.0 h at 25±5 °C. The column was washed with CH_2Cl_2 (2.7 L) and CH_3CN (1.35 L) and the resulting solutions were added to the mixture. To the combined solutions of **VI-5** and MOE A was added DCI (274.5 g, 2.33 mol, 1.50 equiv) and the mixture was stirred at 25±5 °C for 1.0 h (HPLC indicated > 99.8% conversion of **VI-5**). Water (11.2 g, 0.62 mol, 0.40 equiv) was added and the mixture was stirred at 25±5 °C for 0.5 h. IPA (9.3 g, 0.16 mol, 0.10 equiv) was added and the mixture was stirred at 25±5 °C for 10 min. DDTT (350.0 g, 1.71 mol, 1.10 equiv) was added and the mixture was stirred at 25±5 °C for 1.0 h and cooled to 0±5 °C.

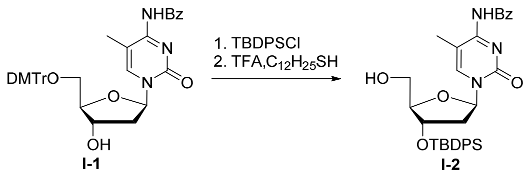
To a separate reactor containing a solution of imidazole (3164 g, 46.5 mol, 30.0 equiv) in anhydrous THF (13.5 L) at 0±5 °C was slowly added HF/pyridine (664.0 g, 23.3 mol, 15.0 equiv, ~70% HF in pyridine) and the mixture was stirred at 0±5 °C for 0.5 h to give a homogeneous solution. This freshly prepared HF/imidazole solution was slowly added into the reaction mixture above and the mixture was stirred at 0±5 °C for 2.0 h (HPLC indicated > 99.8% reaction completion). The reaction mixture was divided into three equal portions for workup and precipitation: EtOAc (18.0 L) was added to one portion of reaction mixture at 0±5 °C and the mixture was stirred at 0±5 °C °C for 0.5 h, washed with 2.5% $NaHCO_3$ aqueous solution (2 x 9.0 L), 5% brine (9.0 L) and saturated brine (9.0 L), dried over anhydrous $MgSO_4$ (900 g), filtered, and concentrated in vacuo. The concentrated crude product was dissolved in EtOAc (3.8 L) and slowly added to a mixture of IPA/heptane (45.0 L, 4:1, v/v). The precipitated product was filtered, washed with heptane (2 x 0.9 L). After workup and precipitation of the other two portions, the products were combined and dried under vacuum at 25±5 °C for 71 h to yield **VI** as a slightly yellow solid (2906 g, 79.3% yield). HRMS calcd for $C_{110}H_{123}N_{17}O_{33}P_3S_2^+$ $[M+H]^+$: 2367.7157, found: 2367.7173.

Synthesis of Fragment II



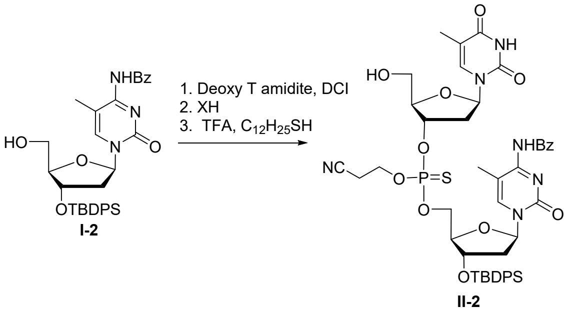
Scheme 22. *Synthesis route of fragment* ***II***

5′-HO-Deoxy-C-3′-OTBDPS, I-2



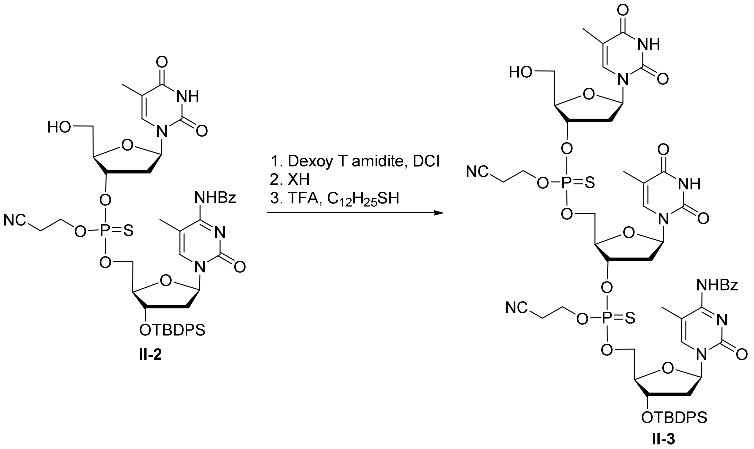
Made from the nucleoside **I-1** using the same procedure as compound **VI-2**. *Silylation*: compound **I-1** (1540 g, 2.38 mol, 1.00 equiv), TBDPSCl (980.0 g, 4.28 mol, 1.50 equiv) and imidazole (486.0 g, 7.14 mol, 3.00 equiv) in CH_2Cl_2 (7.7 L), 25±5 °C, 14 h; IPA (143.0 g, 2.38 mol, 1.00 equiv), 25±5 °C, 0.5 h. *Detritylation*: $C_{12}H_{25}SH$ (637.0 g, 3.14 mol, 1.32 equiv), TFA (1385 g, 12.2 mol, 5.11 equiv), 0±5 ºC, 1.0 h. *Workup*: added slowly into 10% aqueous Na_2CO_3 solution (7.7 L), stirred at 0±5 °C for 1.5 h; added MTBE (7.4 L), stirred at 25±5 ºC for 0.5 h; organic layer separated and washed with saturated brine (4.7 L), dried with $MgSO_4$ (1500 g), filtered, and concentrated. *Precipitation*: Dissolved in EtOH (3.8 L), added to MTBE/heptane (43.9 L, 18:1, v/v). Product **I-2** as a white solid (1241 g, 89.4%). HRMS calcd for $C_{36}H_{43}N_3O_7Si^+$ $[M+H]^+$: 658.2949, found: 658.2869.

5′-HO-Deoxy-TC-3′-OTBDPS, II-2



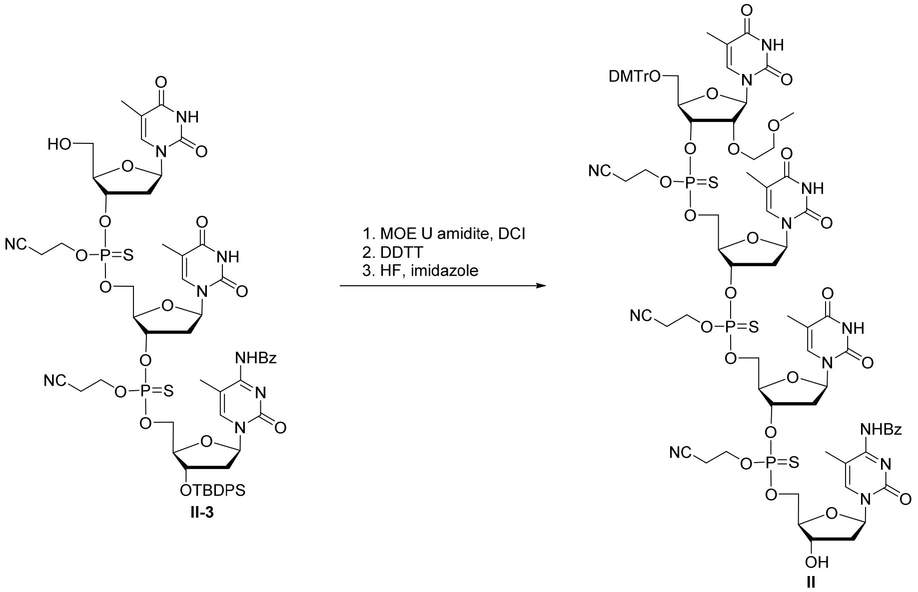
A mixture of compound **I-2** (1214.0 g, 2.08 mol, 1.00 equiv), deoxy T phosphoramidite (1705.0 g, 2.29 mol, 1.10 equiv), CH_3CN/CH_2Cl_2 (15.2 L, 1:1, v/v) was stirred at 25±5 °C until all solids were dissolved, and the solution was circulated through a column packed with 3Å MS (1215.0 g) for 4.0 h at 25±5 °C. The column was washed with CH_3CN/CH_2Cl_2 (3.0 L, 1:1, v/v) and the resulting solution was added to the dried solution of **I-2** and T phosphoramidite. DCI (318.0 g, 2.70 mol, 1.50 equiv) and 3Å MS (240.0 g) were added and the mixture was stirred at 25±5 °C for 1.0 h (HPLC indicated > 99.9% conversion of **I-2**). Water (7.5 g, 0.42 mol, 0.20 equiv) was added and the mixture was stirred at 25±5 °C for 0.5 h. IPA (12.5 g, 0.21 mol, 0.10 equiv) was added and the mixture was stirred at 25±5 °C for 0.5 h. XH (344.0 g, 2.50 mol, 1.20 equiv) was added and the mixture was stirred at 25±5 °C for 0.5 h. The reaction mixture was cooled to 0±5 °C, $C_{12}H_{25}SH$ (1263.0 g, 6.24 mol, 3.00 equiv) and TFA (2016.0 g, 17.7 mol, 8.50 equiv) were added sequentially and the mixture was stirred at 0±5 °C for 1.5 h (HPLC indicated > 99.9% reaction completion). NMI (1623.0 g, 19.8 mol, 9.50 equiv) was added at 0±5 °C in 0.5 h. To the mixture was added a mixture of EtOAc/MTBE (36.5 L, 1:3, v/v) at 5±5 °C and the mixture was stirred vigorously for 15 min and filtered. The mixture was equally divided into two portions for the following washing: The mixture was washed with 5% brine (6.0 L), 3.5% $NaHCO_3$ aqueous solution (3 x 6.0 L), water (6.0 L) and saturated brine (6.0 L). After the second portion was washed, the organic layers were combined, dried over $MgSO_4$ (1200 g), filtered, and concentrated. The concentrated crude products were dissolved in EtOAc–*i*Pr_2O (6.1 L, 4:1, v/v) and PPh_3 (109.0 g, 0.42 mol, 0.20 eq.) was added. The mixture was stirred for 0.5 h and divided into two equal portions for precipitation: The solution was slowly added to a stirred mixture of *i*Pr_2O/heptane (23.7 L, 4.7:1, v/v) and the precipitated product was filtered, washed with heptane (2 x 0.75 L). The products obtained from both portions were combined and dried under vacuum at 25±5 ºC for 60 h to yield product **II-2** as a light yellow solid (2212 g, 111.1%). HRMS calcd for $C_{46}H_{54}N_6O_{11}PSSi^+$ $[M+H]^+$: 957.3078, found: 957.3038.

5′-HO-Deoxy-TTC-3′-OTBDPS, II-3



Use the same procedure for synthesis of **II-2** with compound **II-2** as the starting material, except **II-2** and T phosphoramidite were dried separately through the columns of 3Å MS. *Coupling*: compound **II-2** (2190 g, 2.29 mol, 1.00 equiv) in CH_2Cl_2/CH_3CN (13.2 L, 1:1, v/v), circulated through a column of 3Å MS (2190 g), 25±5 °C, 4.0 h; T phosphoramidite (1728 g, 2.32 mol, 1.01 equiv) in CH_2Cl_2/CH_3CN (10.0 L, 1:1, v/v), circulated through a column of 3Å MS (1100 g), 25±5 °C, 0.5 h; 3Å MS (438 g), 25±5 °C, 0.5 h; DCI (405.0 g, 3.44 mol, 1.50 equiv), 25±5 °C, 1.0 h; water (16.5 g, 0.92 mol, 0.40 equiv), 25±5 °C, 0.5 h. *Sulfurization*: XH (413.0 g, 2.75 mol, 1.20 equiv), 25±5, 1.0 h. *Detritylation*: $C_{12}H_{25}SH$(1389 g, 6.87 mol, 3.00 equiv), TFA (2218 g, 19.5 mol, 8.50 equiv), 0±5ºC, 1.5 h; NMI (1785 g, 21.8 mol, 9.50 equiv). *Workup*: added EtOAc/MTBE (43.8 L, 1:1, v/v), stirred at 25±5 °C for 15 min; washed with 5% brine (22.1 L), 3.5% $NaHCO_3$ aqueous solution (2 x 22.1 L), water (22.1 L) and saturated brine (22.1 L), dried with $MgSO_4$ (2190 g), filtered, and concentrated; dissolved in EtOAc (11.0 L), added PPh_3 (120.0 g, 0.20 equiv), stirred at 25±5 °C for 0.5 h. *Precipitation*: added to MTBE/heptane (74.5 L, 1:1, v/v). Product **II-3** as a light yellow solid (2614 g, 85.9%). HRMS calcd for $C_{59}H_{69}N_9O_{17}P_2S_2Si^+$ $[M+H]^+$: 1330.3576, found: 1330.3634.

5′-DMTrO-MOE-U-Deoxy-TTC-3′-OH, II



Used the same procedure as for compound **VI**. *Coupling*: compound **II-3** (2468 g, 1.86 mol, 1.00 equiv) in CH_3CN (11.7 L), 3Å MS (2000 g), 25±5°C, 3.0 h; MOE U phosphoramidite (1663 g, 2.41 mol, 1.30 equiv) in CH_3CN (8.5 L), 3Å MS (1000 g), 25±5 °C, 3.0 h; DCI (329.0 g, 2.79 mol, 1.50 equiv), 25±5 °C, 0.5 h; H_2O (13.5 g, 0.74 mol, 0.40 equiv), 25±5 °C, 0.5 h. *Sulfurization*: DDTT (420.0 g, 2.05 mol, 1.10 equiv), 25±5 °C, 1.0 h. *Desilylation*: imidazole (3800 g, 55.8 mol, 30.0 equiv), HF (557.0 g, 27.9 mol, 15.0 equiv) in THF (5.0 L), 15±5 °C, 0.5 h; 0±5 °C, 2.0 h. *Workup*: added EtOAc (25.0 L), stirred at 0±5 °C for 0.5 h; The mixture was divided into two equal portions for workup using following procedure: one portion was washed with 3.5% $NaHCO_3$ aqueous solution (2 x 12.5 L), water (12.5 L) and saturated brine (12.5 L); After workup of the other portion, the organic layers were combined, dried over $MgSO_4$ (2500 g), filtered, and concentrated; *Precipitation*: the residue was dissolved in CH_2Cl_2 (12.0 L) and the resulting solution was divided into four equal portions for precipitation. One portion was added to EtOH/heptane (43.2 L, 6:1, v/v) at 10±5 °C and the product was filtered and washed with EtOH (2 x 1.3 L). After precipitations of other three portions, the products were combined and dried under vacuum at 25±5 °C for 84 h to yield **II** as a light-yellow solid (3073 g, 89.9%). HRMS calcd for $C_{80}H_{92}N_{12}O_{27}P_3S_3^+$ $[M+H]^+$: 1841.4570, found: 1841.4640.