Supporting Information

Triazole-linked Analogue of Deoxyribonucleic Acid (^{TL}DNA): Design, Synthesis and Double Strand Formation with Natural DNA

Hiroyuki Isobe,* Tomoko Fujino, Naomi Yamazaki, Marine Guillot-Nieckowski, and Eiichi Nakamura

Table of contents

1. General

Microwave reactions were carried out in sealed vessels in Discover (CEM) equipped with an IR temperature monitor. Analysis of reaction mixtures and purification of products with high pressure liquid chromatography (HPLC) was performed on HPLC systems equipped with triazole-modified HILIC column (COSMOSIL® HILIC 20 × 250 mm, Nacalai Tesque). Analytical thin-layer chromatography (TLC) was performed on a glass plate coated with silica gel (230-400 mesh, 0.25 mm thickness) containing a fluorescent indicator (silica gel 60F_{254}, Merck). Flash silica gel column chromatography was performed on silica gel 60N (spherical and neutral gel, 40-140 μm, Kanto).[1] IR spectra were recorded on JASCO FT-IR 240 (pellet) and Applied Systems REACT IR1000 equipped with an attenuated total reflection (ATR; neat), and were reported as wavenumbers (ν) in cm^{-1}. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on JEOL ECA-500 (^{1}H: 500 MHz; ^{13}C: 125 MHz), ECX-400 (^{1}H: 400 MHz; ^{13}C: 100 MHz), AL 400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) and LA 400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) spectrometers. Methyl (CH_3), methylene (CH_2),

1. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

and methyne (CH) signals in ^{13}C NMR spectra were assigned by DEPT spectra. Proton NMR spectra in $CDCl_3$ were referenced internally to tetramethylsilane, and spectra in CD_3OD and ^{13}C NMR spectra were referenced to the solvent resonances. Mass spectra were obtained on a JEOL JMS-T100LC instrument (APCI-TOF MS) and on an Applied Biosystems Voyager DE STR SI-3 instrument (MALDI-TOF MS). Thermal melting curves and mixing curves were obtained on a UV-visible spectrometer (JASCO, V-660) equipped with a water-circulated temperature-controlled cell holder (JASCO, ETC-717). The melting temperature was determined using a melting program (JASCO, VWTP-780). CD spectra were obtained on a spectropolarimeter (JASCO, J-820) equipped with a water-circulated temperature controlled cell holder (JASCO, PTC-423L).

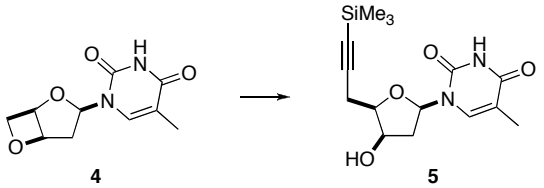
2. Materials

Trimethylsilyl acetylene was purchased from GFS Chemicals. Triphenylphosphine polystyrene resin and NovaSyn TG amino resin were obtained from Novabiochem. Natural oligonucleotides ($d(T)_{10}$, $d(A)_{10}$, $d(T)_2(A)_{10}(T)_2$) were obtained from Operon. Anhydrous THF (stabilizer free, Wako), dichloromethane and toluene (Kanto) were purified by a solvent purification system (GlassContour) equipped with columns of activated alumina and supported copper catalyst (Q-5). Water was purified by Milli-Q ultrapure water system (Millipore). Other solvents were purified by distillation and dried over 4-Å molecular sieves.

3. Synthesis

3-1. Solution phase synthesis of ^{TL}DNA oligomer

2',5'-Dideoxy-5'-(trimethylsilyl)ethynyl-3'-*epi*-thymidine 5

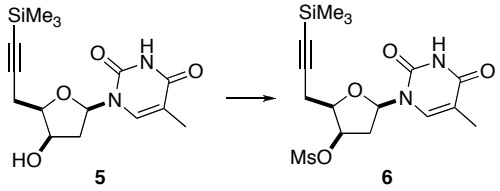


4 **5**

To a solution of trimethylsilylacetylene (13.0 mL, 82.5 mmol) in THF (150 mL) was dropwise added butyllithium (1.60 M in hexane, 56.7 mL, 90.8 mmol) at –78 °C over 10 min. The reaction mixture was stirred at –78 °C for 30 min and warmed to 0 °C for 45 min. The mixture was then cooled to –78 °C, and boron trifluoride diethyl ether complex (11.4 mL, 82.5 mmol) was added. After 10 min, a solution of 1-(3,5-anhydro-2-deoxy-*β*-D-*threo*-pentofuranosyl)thymine **4** (6.15 g, 27.5 mmol) in THF (300 mL) was added and the mixture was stirred at –78 °C for 16 h. After addition of saturated aqueous solution of sodium bicarbonate (500 mL), the mixture was warmed to ambient temperature and extracted with

chloroform (3 × 500 mL). The organic layer was washed with brine (500 mL), dried over magnesium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 90% ethyl acetate/chloroform) to give the title compound **5** (7.97 g, 90%) as a white solid. IR (powder) 3395, 3042, 1695, 1667, 1651, 1540, 1472, 1418, 1276, 1250, 1227, 1033, 995, 947, 839 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.14 (s, 9H), 1.92 (d, *J* = 1.3 Hz, 3H), 2.13 (dd, *J* = 2.9, 15.3 Hz, 1H), 2.64-2.74 (m, 3H), 2.77 (dd, *J* = 5.7, 16.6 Hz, 1H), 4.00 (ddd, *J* = 2.9, 5.7, 8.7 Hz, 1H), 4.45-4.49 (m, 1H), 6.13 (dd, *J* = 2.9, 8.7 Hz, 1H), 7.58 (d, *J* = 1.3 Hz, 1H), 7.96-8.04 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 0.04 (3C, CH_3), 12.7 (CH_3), 20.3 (CH_2), 40.1 (CH_2), 70.7 (CH), 81.8 (CH), 85.2 (CH), 87.8, 101.4, 110.9, 137.2 (CH), 151.2, 163.2; HRMS (MALDI-TOF) calcd for $C_{15}H_{23}N_2O_4Si$ $[M + H]^+$ 323.1422, found 323.1417.

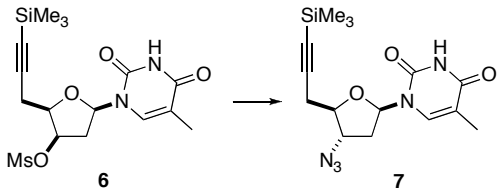
2',5'-Dideoxy-3'-*O*-methansulfonyl-5'-(trimethylsilyl)ethynyl-3'-*epi*-thymidine 6



5 **6**

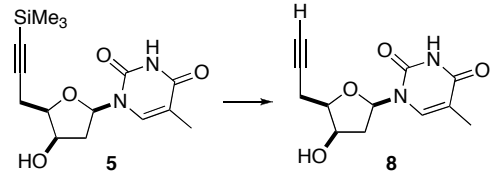
To a solution of alcohol **5** (9.24 g, 28.7 mmol) in anhydrous pyridine (100 mL) at 0 °C was added dropwise methansulfonyl chloride (17.0 mL, 43.1 mmol), and the mixture was allowed to warm to ambient temperature and stirred for 4 h. After addition of saturated aqueous solution of sodium bicarbonate (100 mL), the mixture was extracted with ethyl acetate (3 × 100 mL), and the organic layer was washed with saturated aqueous solution of sodium bicarbonate, dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 30% ethyl acetate/chloroform) to give the title compound **6** (9.75 g, 85%) as a white solid. IR (powder) 3184, 3037, 2960, 2358, 2180, 1688, 1470, 1351, 1273, 1171, 1079, 1028, 907, 843, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 9H), 1.89 (s, 3H), 2.41 (dd, *J* = 3.5, 16.6 Hz, 1H), 2.68 (dd, *J* = 8.6, 16.6 Hz, 1H), 2.78 (dd, *J* = 5.2, 16.6 Hz, 1H), 2.83 (ddd, *J* = 5.2, 8.6, 16.6 Hz, 1H), 3.11 (s, 3H), 4.10 (ddd, *J* = 2.9, 5.2, 8.6 Hz, 1H), 5.18 (dd, *J* = 2.9, 5.2 Hz, 1H), 6.29 (dd, *J* = 3.5, 8.6 Hz, 1H), 7.34 (s, 1H), 9.84-9.91 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –0.21 (3C, CH_3), 12.6 (CH_3), 20.0 (CH_2), 38.5 (CH_3), 39.4 (CH_2), 79.3 (CH), 80.0 (CH), 83.3 (CH), 88.0, 100.4, 111.8, 135.2 (CH), 150.7, 163.7; HRMS (APCI-TOF) calcd for $C_{16}H_{25}O_6N_2SSi$ $[M + H]^+$ 401.1203, found 401.1183.

3'-Azido-2',3',5'-trideoxy-5'-(trimethylsilyl)ethynylthymidine 7



A mixture of mesylate **6** (0.401 g, 1.00 mmol) and sodium azide (0.195 g, 3.00 mmol) in *N*,*N*-dimethylformamide (DMF; 7.5 mL) was stirred at 100 °C for 2 h. After addition of water (20 mL), the mixture was extracted with chloroform (6 × 20 mL). The organic layer was washed with water (4 × 10 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 20% ethyl acetate/chloroform) to give title compound **7** (0.310 g, 89%). IR (powder) 3184, 3045, 2960, 2177, 2099, 1683, 1468, 1268, 1248, 1075, 839, 758, 729 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.17 (s, 9H), 1.95 (d, *J* = 1.2 Hz, 3H), 2.30 (ddd, *J* = 7.3, 7.6, 13.8 Hz, 1H), 2.45 (ddd, *J* = 3.7, 6.0, 13.8 Hz, 1H), 2.71 (dd, *J* = 4.4, 17.6 Hz, 1H), 2.72 (dd, *J* = 5.1, 17.6 Hz, 1H), 4.02 (ddd, *J* = 3.4, 4.4, 5.1 Hz, 1H), 4.24 (ddd, *J* = 3.4, 3.6, 7.0 Hz, 1H), 6.15 (dd, *J* = 6.0, 7.6 Hz, 1H), 7.39 (d, *J* = 1.2 Hz, 1H), 8.42 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -0.06 (CH_3), 12.8 (CH_3), 24.9 (CH_2), 37.5 (CH_2), 63.0 (CH), 81.6 (CH), 84.7 (CH), 88.8, 101.1, 111.2, 135.0 (CH), 149.9, 163.3; HRMS (APCI-TOF) calcd for $C_{15}H_{22}O_3N_5Si$ $[M + H]^+$ 348.1492, found 348.1477. Representative spectra are shown in Figure S5 and S6.

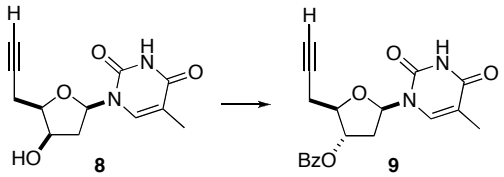
2',5'-Dideoxy-5'-ethynyl-3'-*epi*-thymidine 8



To a solution of trimethylsilyl-protected acetylene **5** (161 mg, 0.500 mmol) in THF (2.5 mL) was added tetra-*n*-butylammonium fluoride (TBAF; 1.00 M in THF, 0.550 mL, 0.550 mmol) at 0 °C. After 10 min, the mixture was filtrated through a pad of silica gel, and the solvent was removed in vacuo. The crude material was purified by silica gel column chromatography (eluent: 8% methanol/chloroform) to give title compound **8** (123 mg, 98%). IR (powder) 3375, 3290, 3190, 3041, 1694, 1667, 1652, 1470, 1285, 1229, 1030, 962, 845, 754 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.92 (d, *J* = 1.1 Hz, 3H), 2.07 (t, *J* = 2.8 Hz, 1H), 2.42 (dd, *J* = 1.7, 15.2 Hz, 1H), 2.57 (ddd, *J* = 5.1, 8.0, 15.2 Hz, 1H), 2.73 (ddd, *J* = 2.8, 7.5, 16.6 Hz, 1H), 2.76 (ddd, *J* = 2.8, 6.9, 16.6 Hz, 1H), 3.65-3.72 (br s, 1H), 4.06 (ddd, *J* = 2.9, 6.9, 7.5 Hz, 1H), 4.40-4.45 (m, 1H), 6.07 (dd, *J* = 1.7, 8.0 Hz, 1H), 7.55 (d, *J* = 1.1 Hz, 1H), 9.86-10.0 (br s,

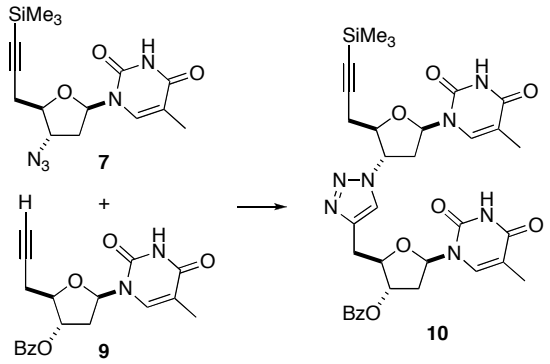
1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.6 (CH_3), 18.9 (CH_2), 40.9 (CH_2), 69.9 (CH), 70.4 (CH), 80.0, 83.1 (CH), 86.3 (CH), 109.9, 137.7 (CH), 151.0, 164.4; HRMS (APCI-TOF) calcd for $C_{12}H_{13}N_2O_4$ [M – H]$^-$ 249.0875, found 249.0865.

3'-*O*-Benzoyl-2',5'-dideoxy-5'-ethynylthymidine 9



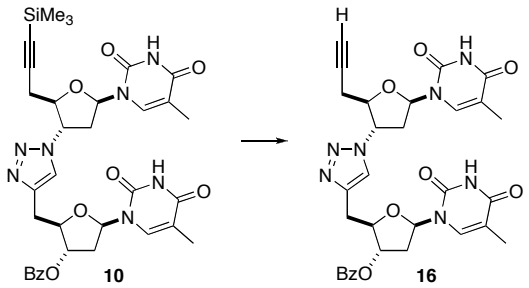
A mixture of alcohol **8** (30.7 mg, 0.123 mmol), benzoic acid (16.5 mg, 0.135 mmol), triphenylphosphine resin (1.00 mmol/g, 185 mg, 0.185 mmol) and diisopropyl azodicarboxylate (DIAD; 1.90 M in THF, 71.7 μL, 0.135 mmol) was stirred in THF (0.90 mL) at 0 °C for 2 h. The resin was removed by filtration, and the filtrate was washed with saturated aqueous solution of sodium bicarbonate (3 × 2 mL) and brine (3 × 2 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography to give the title compound **9** (32.9 mg, 75%) as a white solid. IR (powder) 3275, 3159, 3045, 2929, 2821, 1700, 1673, 1476, 1410, 1366, 1270, 1254, 1111, 1094, 1054, 1005, 971, 890, 836, 712, 687 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.16 (t, J = 2.9 Hz, 1H), 2.42 (m, 1H), 2.62 (m, 1H), 2.83 (ddd, J = 2.9, 4.6, 17.2 Hz, 1H), 2.85 (ddd, J = 2.9, 4.6, 17.2 Hz, 1H), 4.28 (m, 1H), 5.47 (m, 1H), 6.42 (m, 1H), 7.47 (t, J = 7.3 Hz, 2H), 7.61 (t, J = 7.3 Hz, 1H), 7.62 (s, 1H), 8.03 (d, J = 7.3 Hz, 2H), 9.29-9.41 (br s, 1H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 12.7 (CH_3), 23.4 (CH_2), 37.7 (CH_2), 71.7 (CH), 76.6 (CH), 80.3, 82.0 (CH), 84.4 (CH), 111.6, 128.7 (2C, CH), 129.1, 130.0 (2C, CH), 133.8 (CH), 135.2 (CH), 150.1, 163.2, 166.2; HRMS (APCI-TOF) calcd for $C_{19}H_{18}N_2O_5Na$ [M + Na]$^+$ 377.1108, found 337.1126.

Trimethylsilyl-protected ^{TL}DNA dimer 10



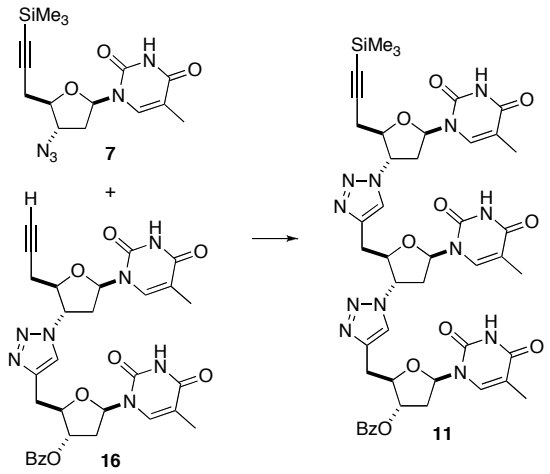
A solution of azide **7** (9.67 mg, 27.9 μmol), acetylene **9** (10.6 mg, 30.0 μmol), and copper bromide dimethyl sulfide complex (7.40 mg, 36.0 μmol) in THF (1.2 mL) was stirred at ambient temperature for 15 h. The solvent was removed in vacuo, and the crude material was purified by silica gel column chromatography (eluent: 7% methanol/chloroform) to give the title compound **10** (16.0 mg, 82%) as a white solid. IR (powder) 3865, 3751, 3672, 3252, 1717, 1698, 1684, 1654, 1559, 1542, 1507, 1472, 1457, 1270, 1071, 843, 712 cm^{-1}; ^1H NMR (400 MHz, 10% v/v $CD_3OD/CDCl_3$) δ 0.08 (s, 9H), 1.87 (d, *J* = 1.2 Hz, 3H), 1.88 (d, *J* = 0.8 Hz, 3H), 2.40 (ddd, *J* = 6.8, 7.2, 14.2 Hz, 1H), 2.47 (ddd, *J* = 3.1, 6.8, 14.2 Hz, 1H), 2.55 (ddd, *J* = 7.5, 8.9, 14.2 Hz, 1H), 2.66 (dd, *J* = 4.4, 17.6 Hz, 1H), 2.75 (dd, *J* = 4.9, 17.6 Hz, 1H), 2.87 (ddd, *J* = 4.3, 6.4, 14.2 Hz, 1H), 3.16 (dd, *J* = 7.3, 15.2 Hz, 1H), 3.24 (dd, *J* = 5.4, 15.2 Hz, 1H), 4.32-4.36 (overlapped m, 2H), 5.16 (ddd, *J* = 4.3, 4.4, 8.9 Hz, 1H), 5.34 (ddd, *J* = 3.1, 3.3, 7.2 Hz, 1H), 6.20 (dd, *J* = 6.4, 7.5 Hz, 1H), 6.30 (t, *J* = 6.8 Hz, 1H), 7.33 (d, *J* = 0.8 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 2H), 7.42 (d, *J* = 1.2 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.67 (s, 1H), 7.92 (d, *J* = 7.7 Hz, 2H); ^{13}C NMR (100 Hz, 10% v/v $CD_3OD/CDCl_3$) δ -0.3 (CH_3), 12.2 (CH_3), 12.4 (CH_3), 24.5 (CH_2), 29.3 (CH_2), 36.5 (CH_2), 37.9 (CH_2), 62.0 (CH), 75.9 (CH), 81.7 (CH), 82.5 (CH), 85.1 (CH), 85.6 (CH), 88.7, 100.8, 111.1, 111.4, 122.1 (CH), 128.4 (CH), 128.9, 129.5 (CH), 133.5 (CH), 135.5 (CH), 135.9 (CH), 143.3, 150.3, 150.4, 164.2, 164.2, 165.9; HRMS (APCI-TOF) calcd for $C_{34}H_{40}N_7O_8Si$ $[M + H]^+$ 702.2708, found 702.2696. Representative spectra are shown in Figure S7-S9.

^{TL}DNA dimer 16



To a solution of trimethylsilyl-protected dimer **10** (60.5 mg, 86.2 μmol) in THF (0.862 mL) was added TBAF (1.00 M in THF, 103 mL, 103 μmol) at 0 °C, and the mixture was stirred for 40 min. After addition of saturated aqueous solution of ammonium chloride (2 mL), the crude mixture was extracted with chloroform (3 × 2 mL). The organic layer was washed with saturated aqueous solution of sodium bicarbonate (3 × 2 mL) and brine (3 × 2 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 7% methanol/chloroform) to give the deprotected dimer **16** (40.2 mg, 74%). IR (powder) 3840, 3672, 3253, 1696, 1679, 1636, 1559, 1542, 1522, 1472, 1457, 1266, 1071, 1027, 714 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (d, J = 1.1 Hz, 3H), 1.94 (d, J = 1.1 Hz, 3H), 2.15 (t, J = 2.6 Hz, 1H), 2.42-2.58 (m, 2H), 2.62-2.86 (m, 3H), 2.99-3.09 (m, 1H), 3.27 (dd, J = 7.0, 15.4 Hz, 1H), 3.31 (dd, J = 5.1, 15.4 Hz, 1H), 4.38-4.45 (m, 2H), 5.21-5.30 (m, 1H), 5.39-5.48 (m, 1H), 6.25 (t, J = 7.3 Hz, 1H), 6.31 (t, J = 6.2 Hz, 1H), 7.35 (d, J = 1.1 Hz, 1H), 7.44 (t, J = 7.3 Hz, 2H), 7.50 (d, J = 1.1 Hz, 1H), 7.58 (t, J = 7.3 Hz, 1H), 7.70 (s, 1H), 8.00 (d, J = 7.3 Hz, 2H), 9.31-9.40 (br s, 1H), 9.36-9.44 (br s, 1H, NH); ^{13}C NMR (100 Hz, $CDCl_3$) δ 12.7 (CH_3), 12.7 (CH_3), 22.8 (CH_2), 29.6 (CH_2), 36.9 (CH_2), 38.2 (CH_2), 61.4 (CH), 76.1, 77.3 (CH), 79.1 (CH), 81.6 (CH), 82.8 (CH), 85.8 (CH), 85.9 (CH), 111.5, 111.7, 122.4 (CH), 128.7 (2C, CH), 129.2, 130.0 (2C, CH), 133.8 (CH), 136.0 (CH), 136.2 (CH), 143.5, 150.4, 150.5, 163.9, 163.9, 166.1; HRMS (ESI-TOF) calcd for $C_{31}H_{31}N_7O_8Na$ [M + Na]+ 652.2132, found 652.2145.

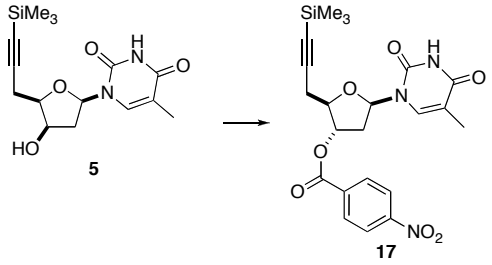
Trimethylsilyl-protected ^{TL}DNA trimer 11



A solution of azide **7** (12.8 mg, 36.9 μmol), deprotected dimer **16** (21.1 mg, 33.5 μmol), and copper bromide dimethyl sulfide complex (1.72 mg, 8.38 μmol) in THF/*tert*-butanol/water (1:3:3 v/v, 0.30 mL) was stirred at ambient temperature for 2 h. After the solvent was removed in vacuo, the crude material was purified by silica gel column chromatography (eluent: 10% methanol/chloroform) to give the title compound **11** (24.8 mg, 76%) as a white solid. IR (powder) 3191, 3068, 2960, 2925, 2852, 1679, 1468, 1451, 1318, 1264, 1071, 1044, 1027, 953, 899, 841, 758, 714 cm^{-1}; ^1H NMR (400 MHz, 10% v/v $CD_3OD/CDCl_3$) δ 0.08 (s, 9H), 1.85 (br s, 3H), 1.88 (br s, 6H), 2.37-2.56 (overlapped m, 3H), 2.69 (dd, *J* = 4.4, 17.6 Hz, 1H), 2.74-2.80 (overlapped m, 2H), 2.84-3.96 (overlapped m, 2H), 3.06 (dd, *J* = 5.2, 15.6 Hz, 1H), 3.11-3.24 (overlapped m, 3H), 4.32-4.36 (overlapped m, 2H), 4.42 (dt, *J* = 5.2, 7.2 Hz, 1H), 5.11 (dt, *J* = 7.2, 9.2 Hz, 1H), 5.18 (dt, *J* = 3.8, 8.8 Hz, 1H), 5.34 (dt, *J* = 3.2, 6.4 Hz, 1H), 5.98 (dd, *J* = 5.4, 7.6 Hz, 1H), 6.20 (dd, *J* = 6.4, 7.6 Hz, 1H), 6.33 (dd, *J* = 6.4, 7.6 Hz, 1H), 7.13 (br s, 1H), 7.34 (d, *J* = 0.8 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.45 (d, *J* = 0.8 Hz, 1H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.63 (s, 1H), 7.67 (s, 1H), 7.92 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (100 Hz, 10% v/v $CD_3OD/CDCl_3$) δ -0.3 (CH_3), 12.1 (CH_3), 12.2 (CH_3), 12.5 (CH_3), 24.8 (CH_2), 27.8 (CH_2), 29.3 (CH_2), 36.4 (CH_2), 36.5 (CH_2), 38.1 (CH_2), 60.9 (CH), 62.3 (CH), 76.0 (CH), 81.8 (CH), 81.9 (CH), 82.6 (CH), 85.1 (CH), 85.5 (CH), 87.5 (CH), 88.8, 100.8, 111.0, 111.3, 111.4, 122.5 (CH), 123.0 (CH), 128.4 (CH), 128.9, 129.5 (CH), 133.5 (CH), 135.1 (CH), 135.9 (CH), 138.0 (CH), 142.3, 143.0, 150.0, 150.4, 150.4, 164.1, 164.2, 164.3, 165.9; HRMS (ESI-TOF) calcd for $C_{46}H_{52}N_{12}O_{11}SiNa$ $[M + Na]^+$ 999.3545, found 999.3541. Representative spectra are shown in Figure S10-S13.

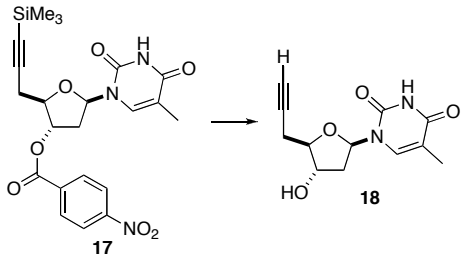
3-2. Solid phase synthesis of ^{TL}DNA oligomer

2',5'-Dideoxy-3'-*O*-(4-nitrobenzoyl)-5'-(trimethylsilyl)ethynylthymidine 17



A mixture of alcohol **5** (1.09 g, 3.38 mmol), *p*-nitrobenzoic acid (0.622 g, 3.72 mmol), triphenylphosphine resin (1.00 mmol/g, 4.00 g, 4.00 mmol) and dimethyl azodicarboxylate (40% solution in toluene, 1.37 mL, 3.72 mmol) was stirred at ambient temperature for 4 h. The resin was removed by filteration, and the filtrate was washed with saturated aqueous sodium bicarbonate (3 × 20 mL), saturated aqueous ammonium chloride (3 × 20 mL) and brine (3 × 20 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography to give the title compound **17** (0.856 g, 63%). IR (powder) 3184, 3045, 2960, 2929, 2900, 2829, 2177, 2099, 1683, 1468, 1364, 1324, 1268, 1248, 1135, 1075, 907, 839, 758, 729, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.16 (s, 9H), 1.96 (d, J = 1.3 Hz, 3H), 2.38 (ddd, J = 6.5, 9.4, 14.2 Hz, 1H), 2.64 (dd, J = 5.2, 14.2 Hz, 1H), 2.79 (dd, J = 4.2, 17.4 Hz, 1H), 2.80 (dd, J = 3.7, 17.4 Hz, 1H), 4.27-4.31 (m, 1H), 5.51 (d, J = 6.5 Hz, 1H), 6.40 (dd, J = 5.2, 9.4 Hz), 7.62 (d, J = 1.3 Hz, 1H), 8.21-8.24 (m, 2H), 8.29-8.34 (m, 2H), 9.08-9.15 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 0.03 (CH_3), 12.9 (CH_3), 24.9 (CH_2), 37.7 (CH_2), 78.1 (CH), 82.2 (CH), 84.6 (CH), 88.8, 102.1, 111.6, 123.8 (2C, CH), 131.0 (2C, CH), 134.5, 134.9 (CH), 150.5, 151.0, 163.6, 164.3; HRMS (APCI-TOF) calcd for $C_{22}H_{26}N_3O_7Si$ $[M + H]^+$ 472.1540, found 472.1533.

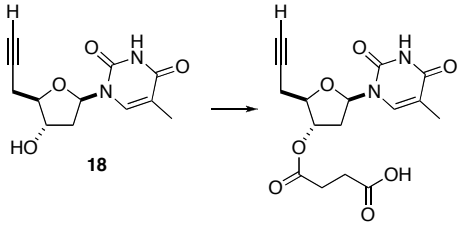
2',5'-Dideoxy-5'-ethynylthymidine 18



To a solution of trimethylsilyl-protected acetylene **17** (1.15 g, 2.91 mmol) in methanol (30 mL) was added 10% aqueous potassium carbonate (14.0 mL), and the mixture was stirred at ambient

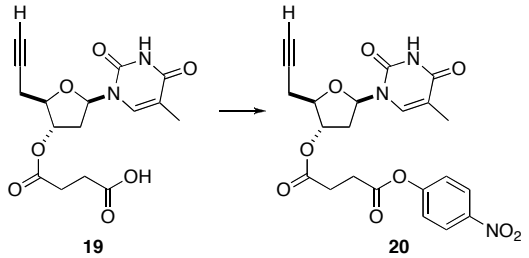
temperature for 2.5 h. After addition of 5% aqueous citric acid (20 mL), the mixture was extracted with chloroform (8 × 30 mL). The organic layer was washed with brine (30 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified by recrystallization from chloroform to give the title compound **18** (0.560 g, 77%). IR (powder) 3494, 3301, 3153, 3091, 3029, 2821, 1702, 1656, 1478, 1416, 1301, 1254, 1189, 1131, 1069, 1057, 1034, 982, 967, 951, 870, 843, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.93 (s, 3H), 2.13 (t, *J* = 2.5 Hz, 1H), 2.12-2.16 (m, 1H), 2.27 (ddd, *J* = 6.8, 7.1, 13.8 Hz, 1H), 2.41 (ddd, *J* = 3.9, 6.4, 13.8 Hz, 1H), 2.62-2.74 (m, 2H), 4.02-4.40 (m, 1H), 4.42-4.52 (m, 1H), 6.29 (dd, *J* = 6.4, 6.8 Hz, 1H), 7.49 (s, 1H), 8.27-8.33 (br s, 1H); ^{13}C NMR (100 Hz, $CDCl_3$) δ 12.7 (CH_3), 23.0 (CH_2), 40.5 (CH_2), 71.5 (CH), 73.6 (CH), 79.9, 83.5 (CH), 84.6 (CH), 111.2, 135.6 (CH), 150.0, 163.3; HRMS (APCI-TOF) calcd for $C_{12}H_{13}N_2O_4$ $[M - H]^-$ 249.0875, found 249.0887.

2',5'-Dideoxy-5'-ethynyl-3'-*O*-hydroxysuccinylthymidine 19



A mixture of alcohol **18** (256 mg, 1.02 mmol), succinic anhydride (150 mg, 1.50 mmol), 4-dimethylaminopyridine (61.1 mg, 0.500 mmol) and triethylamine (277 μL, 2.00 mmol) in 1,2-dichloroethane (4.0 mL) was stirred at ambient temperature for 12 h. After addition of saturated aqueous ammonium chloride (10 mL), the mixture was extracted with ethyl acetate (3 × 10 mL). The organic layer was washed with brine (3 × 10 mL), dried over sodium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 8% methanol/chloroform) to give the title compound **19** (330 mg, 94%). IR (powder) 3521, 3186, 3037, 2929, 1683, 1663, 1472, 1409, 1366, 1270, 1200, 1158, 1081, 1046, 956, 753 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.91 (d, *J* = 1.1 Hz, 3H), 2.12 (t, *J* = 2.3 Hz, 1H), 2.27 (ddd, *J* = 6.9, 8.6, 14.6 Hz, 1H), 2.48 (ddd, *J* = 1.8, 5.2, 14.6 Hz, 1H), 2.58-2.74 (m, 6H), 4.14-4.18 (m, 1H), 5.23-5.27 (m, 1H), 6.29 (dd, *J* = 5.2, 8.6 Hz, 1H), 7.62 (d, *J* = 1.1 Hz, 1H), 9.72-9.78 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.7 (CH_3), 23.2 (CH_2), 28.9 (CH_2), 29.3 (CH_2), 37.6 (CH_2), 71.7 (CH), 76.3 (CH), 80.1, 81.2 (CH), 84.3 (CH), 111.4, 135.7 (CH), 150.5, 164.5, 171.8, 176.0; HRMS (APCI-TOF) calcd for $C_{16}H_{17}N_2O_7$ $[M - H]^-$ 349.1036, found 349.1029.

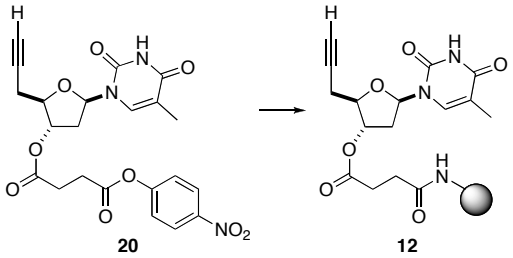
2',5'-Dideoxy-5'-ethynyl-3'-*O*-(4-nitrophenoxysuccinyl)thymidine 20



19 **20**

To a solution of carboxylic acid **19** (1.03 g, 2.94 mmol) and 4-nitrophenol (0.491 g, 3.53 mmol) in pyridine/THF (1:8 v/v, 13 mL) was added a solution of *N*,*N*'-dicyclohexylcarbodiimide (0.910 g, 4.41 mmol) in THF (3.4 mL), and the mixture was stirred at ambient temperature for 4 h. After addition of saturated aqueous sodium bicarbonate (20 mL), precipitates were filtrated and rinsed with chloroform (20 mL). The filtrate was washed with saturated aqueous sodium bicarbonate (3 × 20 mL) and brine (3 × 20 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent: 60% ethyl acetate/chloroform) to give the title compound **20** (1.03 g, 74%). IR (powder) 3277, 2930, 1701, 1685, 1522, 1348, 1205, 1130, 864, 752 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.92 (d, *J* = 1.4 Hz, 3H), 2.13 (t, *J* = 3.4 Hz, 1H), 2.30 (ddd, *J* = 8.3, 11.5, 17.9 Hz, 1H), 2.45 (ddd, *J* = 1.9, 6.9, 17.9 Hz, 1H), 2.74 (dd, *J* = 3.4, 5.0 Hz, 2H), 2.76-2.80 (m, 2H), 2.92-2.96 (m, 2H), 4.12 (dt, *J* = 2.2, 5.0 Hz, 1H), 5.27 (ddd, *J* = 1.9, 2.2, 8.3 Hz, 1H), 6.32 (dd, *J* = 6.9, 11.5 Hz, 1H), 7.27-7.31 (m, 2H), 7.60 (d, *J* = 1.4 Hz, 1H), 8.24-8.28 (m, 2H), 8.68-8.74 (br s, 1H); ^{13}C NMR (125 Hz, $CDCl_3$) 12.7 (CH_3), 23.3 (CH_2), 28.9 (CH_2), 29.2 (CH_2), 37.5 (CH_2), 71.7 (CH), 76.8 (CH), 80.1, 81.8 (CH), 84.3 (CH), 111.6, 122.4 (2C, CH), 125.4 (2C, CH), 135.0 (CH), 145.5, 150.3, 155.2, 163.5, 170.0, 171.7; HRMS (APCI-TOF) calcd for $C_{22}H_{22}N_3O_9$ $[M + H]^+$ 472.1356, found 472.1364.

Terminal ^{TL}DNA monomer on a solid support 12

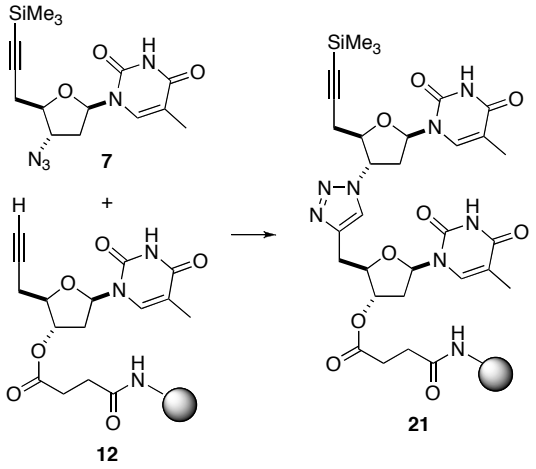


20 **12**

A mixture of activated ester **20** (127 mg, 270 μmol) and NovaSyn TG amino resin (270 μmol/g, 333 mg, 89.9 μmol) in triethylamine/DMF (1:9 v/v, 1.8 mL) was shaken gently at ambient temperature for

24 h. The resin was collected by filtration, washed successively with DMF (3 × 1 mL), methanol (3 × 1 mL), and dichloromethane (3 × 1 mL), and dried in vacuo to give resin bearing the terminal monomer **12** (366 mg). The resin was used in the next step without further purification. The amount of loaded monomer was determined by quantification of the monomer after cleavage: The resin **12** (2.23 mg) in 28% aqueous ammonia (0.20 mL) was shaken gently at ambient temperature for 20 min to give 5'-ethynyl-2',5'-dideoxythymidine **18**. The crude material was analyzed by ^{1}H NMR in the presence of CH_2Br_2 as an internal standard. The amount of monomer on the resin was 213 μmol/g. The loading reaction was carried out several times, and the amount of loaded monomer was determined each time (209-247 μmol/g). The HPLC, NMR and MS data of the product were identical with those of an authentic sample **18** that was prepared by solution phase synthesis.

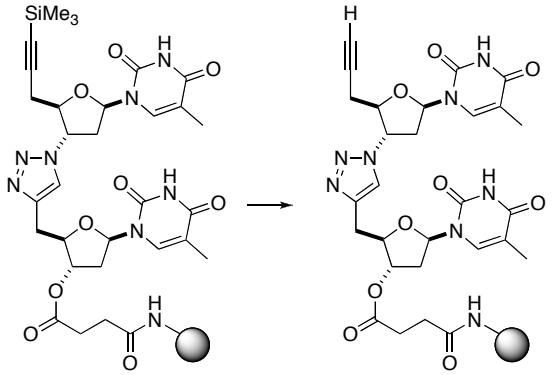
^{TL}DNA dimer on a solid support 21



A mixture of azide **7** (347 mg, 403 μmol), ^{TL}DNA monomer **12** (226 μmol/g, 178 mg, 40.3 μmol) and copper bromide dimethyl sulfide complex (2.08 mg, 10.1 μmol) in THF (1.0 mL) was shaken gently at ambient temperature for 12 h. The resin was collected by filtration, rinsed successively with DMF (3 × 1.0 mL), methanol (3 × 1.0 mL), and dichloromethane (3 × 1.0 mL), and dried in vacuo to give the title compound **21** (230 mg). The yield was determined by quantification of the ^{TL}DNA dimer after cleavage: A small amount of **21** (7.13 mg) in 28% aqueous ammonia (0.40 mL) was gently shaken at ambient temperature for 20 min, and the dimer was obtained as a desilylated form after the cleavage reaction. The crude material was analyzed by ^{1}H NMR in the presence of CH_2Br_2 as an internal standard. The amount of dimer on the resin was 175 μmol/g (100% yield). The elongation reaction was carried out several times, and the amount of loaded dimer was determined each time (161-185 μmol/g). ^{1}H NMR (500 MHz, CD_3OD) δ 1.93 (s, 3H), 1.95 (s, 3H), 2.29-2.32 (m, 2H), 2.55 (t, J = 2.3 Hz, 1H), 2.76 (ddd, J = 2.8, 4.6, 17.4 Hz, 1H), 2.76-2.87 (m, 2H), 2.95 (td, J = 6.9, 13.8 Hz, 1H), 3.11 (dd, J =

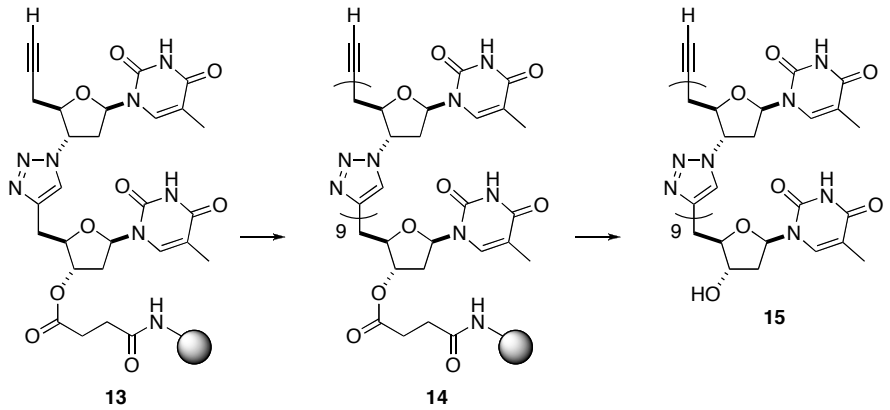
7.3, 15.1 Hz, 1H), 3.16-3.22 (m, 1H), 4.07-4.12 (m, 1H), 4.32-4.37 (m, 1H), 4.44 (dd, J = 5.5, 10.5 Hz, 1H), 5.37-5.42 (m, 1H), 6.23 (t, J = 6.6 Hz, 1H), 6.48 (t, J = 6.2 Hz, 1H), 7.45 (s, 1H), 7.78 (s, 1H), 8.01 (s, 1H); MS (APCI-TOF) calcd for $C_{24}H_{28}N_7O_7$ $[M + H]^+$ 526, found 526. Analysis of the crude material by HPLC is shown in Figure S14.

Deprotected ^{TL}DNA dimer on a solid support 13



The desilylation of **21** (170 μmol/g, 10.7 mg, 1.82 μmol) was carried out with copper chloride (1.80 mg, 18.2 μmol) in *N*,*N*′-dimethylimidazolidinone (0.12 mL) at ambient temperature for 1 h. The resin was collected by filtration, rinsed with aqueous hydrochloride (1 M, 3 × 0.5 mL), DMF (3 × 0.5 mL), methanol (3 × 0.5 mL) and dichloromethane (3 × 0.5 mL), and dried in vacuo to give the title compound **13**. We could not determine the yield simply by quantification of the dimer after cleavage from the resin, because the desilylation reaction also proceeds under the cleavage conditions. The yield was thus determined after elongation of **13** with 5'-azido-2',5'-dideoxythymidine (AZT) and cleavage: Thus, ^{TL}DNA dimer **13** was mixed with AZT (2.43 mg, 9.10 μmol) and copper bromide dimethyl sulfide complex (0.18 mg, 1.82 μmol) in THF (0.15 mL), and the mixture was shaken gently at ambient temperature for 19 h. The resin was collected by filtration, rinsed successively with DMF (3 × 0.5 mL), methanol (3 × 0.5 mL), and dichloromethane (3 × 0.5 mL), and dried in vacuo. The 3-mer was cleaved from the resin in 28% aqueous ammonia (0.10 mL), and the crude material was analyzed by ^{1}H NMR in the presence of CH_2Br_2 as an internal standard. The amount of 3-mer was 1.72 μmol (95% yield). The amount of deprotected dimer on the resin **13** was thus determined as 161 μmol/g. The deprotection reaction was carried out several times, and the amount of dimer was determined each time (140-176 μmol/g). A copper acetylide intermediate that could also participate in the Huisgen cycloaddition was generated during the desilylation reaction. However, the elongation reaction directly from the desilylation step was not successful.

^{TL}DNA decamer 15



The elongation reaction from deprotected 2-mer ^{TL}DNA **13** was carried out as follows: A mixture of 2-mer **13** (140 μmol/g, 51.7 mg, 7.24 μmol), monomer azide **7** (25.2 mg, 72.4 μmol) and copper bromide dimethyl sulfide complex (0.37 mg, 1.81 μmol) in THF (0.24 mL) was irradiated with microwave in a sealed vessel and maintained at 50 °C for 1 h with a power setting of 5 W. The progress of the reaction was checked by HPLC analysis of the crude material after cleavage in a small portion. After completion of the reaction, the resin was collected by filtration, rinsed repeatedly with acetonitrile (1 mL) and aqueous solution of sodium *N*,*N*-diethylaminodithiocarbamate (10 mM, 1 mL). The resin was washed further with methanol (3 × 1 mL) and dichloromethane (3 × 1 mL), and dried in vacuo. The subsequent desilylation was carried out with copper chloride (7.17 mg, 72.4 μmol) in *N*,*N*′-dimethylimidazolidinone (0.31 mL) at ambient temperature for 6 h. The resin was collected by filtration, rinsed with aqueous hydrochloride (1 M, 3 × 1 mL), acetonitrile (1 mL) and aqueous solution of sodium *N*,*N*-diethylaminodithiocarbamate (10 mM, 1 mL). The resin was washed further with methanol (3 × 1 mL) and dichloromethane (3 × 1 mL), and dried in vacuo. The elongation and desilylation processes were further repeated six times to give 10-mer ^{TL}DNA on a solid support (**14**; 20.0 mg). The 10-mer ^{TL}DNA was then cleaved from a part of the resin (1.83 mg) in 28% aqueous ammonia (0.10 mL) under microwave irradiation at 50 °C for 10 min. The resin was removed by filtration and rinsed with methanol (3 × 3 mL), and the filtrate was concentrated in vacuo to give crude material. The crude material was dissolved in acetonitrile/aqueous ammonia (1:1 v/v, 200 μL). Analysis of the crude material by HPLC showed the presence of shorter and longer oligonucleotides as byproducts. The crude material was purified by HPLC with a triazole-HILIC column to give 10-mer ^{TL}DNA **15** (15.5 nmol from 60 μL of crude solution; 0.64% yield from **12**).[2,3] The amount of the

2. The total yield of 10-mer **15** from monomer ^{TL}DNA **12** is 0.61%.
3. As is often the case in the purification of natural oligonucleotides, the yield also suffered from the low recovery through

isolated 10-mer **15** was determined by the absorbance at 260 nm (A_{260} = 0.239, 270 μL in SSPE buffer) and the molar absorption coefficient of 10-mer ($\varepsilon_{260} = 8.39 \times 10^4$ M^{-1}•cm^{-1}).[4] HRMS (MALDI-TOF) calcd for $C_{120}H_{131}N_{47}O_{31}Na$ $[M + Na]^+$ 2749.0011, found 2749.0043. Analysis of the crude materials and the isolated 10-mer **15** by HPLC are shown in Figure S15 and S16. The mass spectrum is shown in Figure S17.

4. Formation and Melting of Double Strand

The double strand formation of polythymine 10-mer ^{TL}DNA **15** with 14-mer DNA ($d(T)_2(A)_{10}(T)_2$) was analyzed by UV-visible spectra. The double strand was formed in a SSPE buffer (pH 7.0) containing 100 mM NaCl, 10 mM sodium phosphate and 0.10 mM ethylenediamine tetraacetic acid (EDTA). The 1:1 binding stoichiometry of the strands in the complexed form was determined by the mixing curve (Job plot) as shown in Figure S1. The hypochromicity at 260 nm upon duplex formation was ca. 20%. The melting curve of the duplex was obtained by following the temperature dependencies of the absorption at 260 nm using a 1:1 mixture of 10-mer ^{TL}DNA **15** and $d(T)_2(A)_{10}(T)_2$ at the total concentration of 8.0 μM in a 700 μL cell with a 1 cm light length under nitrogen atmosphere. Thus, the mixture was heated at 80 °C for 10 min and allowed to cool to 5 °C over a period of 150 min, and the melting curve (Figure 2a) was obtained as the temperature was increased at the rate of 0.5 °C/min.[5]

---

the HPLC purification.

4.The molar absorption coefficient of 10-mer **15** at 260 nm was estimated by nearest-neighbor approximation (Puglisi, J. D.; Tinoco Jr., I. *Methods in Enzymology*; Dahlberg, J. E.; Abelson, J. N., Eds.; Academic Press: San Diego, 1989, *180*, pp. 304-325). The molar absorption coefficients of 1-mer ^{TL}DNA and 2-mer ^{TL}DNA were determined as 8720 M^{-1}•cm^{-1} and 17076 M^{-1}•cm^{-1}, respectively. We thus estimated the molar absorption coefficient of 10-mer ^{TL}DNA **15** as 83924 M^{-1}•cm^{-1} (= 17076 × 9 – 8720 × 8), assuming that the coefficient of oligomers deviates linearly.

5. When we used $d(A)_{10}$ as a complementary strand, T_m measurement did not afford a clear sigmoid curve. Introduction of mismatch thymine residues at each end gave clearer T_m curves, and we think that concatemer formation might have been the problem. See for example: Nguyen, H. -K.; Fournier, O.; Asseline, U.; Dupret, D.; Thuong, N. T. *Nucleic Acids Res.* **1999**, *27*, 1492-1498.

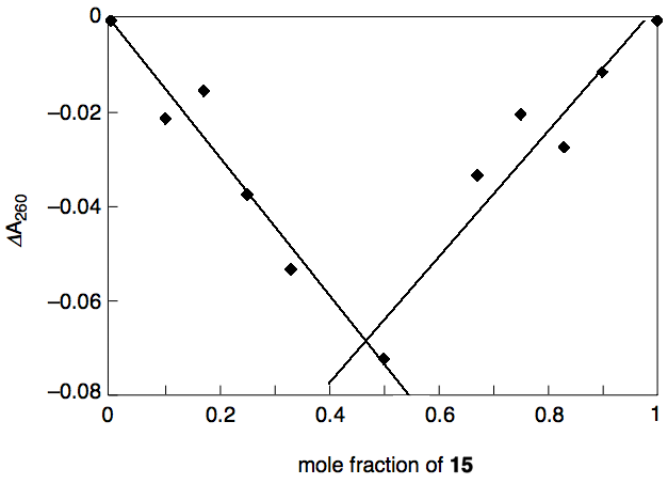


Figure S1. Mixing curve (Job plot) for 10-mer ^{TL}DNA **15** and $d(A)_{10}$ at 5 °C. Their molar ratio was varied while maintaining the total concentration at 4.0 μM. The plot showed the 1:1 stoichiometry of the two strands upon complexation.

5. Analysis of Double Strand by CD Spectroscopy

Analysis of the complex between ^{TL}DNA **15** and natural DNA $d(T)_2(A)_{10}(T)_2$ with CD spectra showed the formation of double helix. Thus, a mixture of **15** (4.0 μM) and $d(T)_2(A)_{10}(T)_2$ (4.0 μM) in a SSPE buffer (pH 7.0) containing 6% acetonitrile (v/v) was annealed under nitrogen atmosphere. We followed the hybridization by UV-vis measurement as described above and obtained a solution of the double strand at 5 °C. The sample was then analyzed by CD spectroscopy while the temperature was increased from 5 °C to 75 °C (Figure S2). The spectra of the double strand indicated the formation of a B-form structure.[6] Comparison of of CD change at 275 nm with the UV melting curve confirmed the melting temperature (Figure S3).

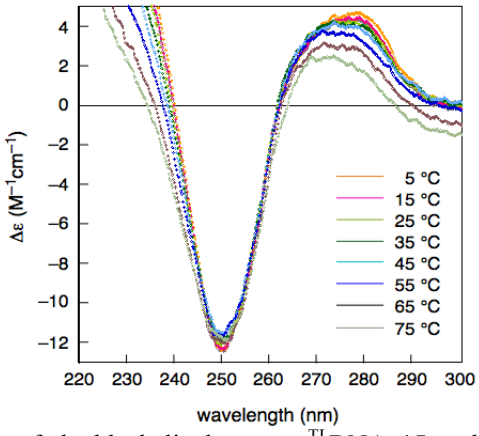


Figure S2. Circular dichroism spectra of double helix between ^{TL}DNA **15** and $d(T)_2(A)_{10}(T)_2$. The temperature was increased from 5 °C to 75 °C.

6. W. C. Johnson in *Circular Dichroism: Principles and Applications,* 2nd ed.; Berova, N.; Nakanishi, K.; Woody, R. W., Eds.; Wiley: New York, 2000, 703-718.

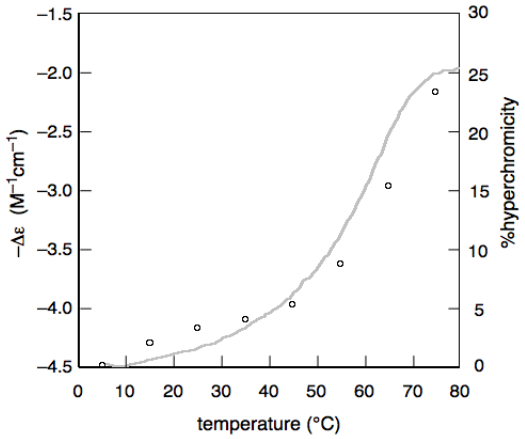


Figure S3. Comparison of the temperature-dependent CD change of double helix between ^{TL}DNA **15** and $d(A)_2(T)_{10}(A)_2$ with the UV melting curve. Open circles shows the differential CD intensity (–Δε) at 275 nm. A gray curve shows the UV melting curve (also shown in Figure 2a).

6. Molecular Dynamics Simulation

The initial duplex structure was built using the helical single strands of $d(T)_{10}$ and $d(A)_{10}$. After replacement of the phosphate linkers with triazole linkers, the structure was obtained by performing stochastic dynamics (SD) using the Amber* force field of the MacroModel (v8.6). The solvent effect of water was simulated using the generalized-Born/surface area (GB/SA) method implemented in the program. A cluster of 1000 structures was obtained by SD calculations performed at 300 K for 1000 ps after an equilibration time of 1000 ps. The cluster was analyzed with XCluster to give an average structure shown in Figure 2. The averaged structure of natural duplex was also obtained by the same procedure, and the structures are shown in Figure S4. The molecular model of the duplex containing ^{TL}DNA showed that the pitch of the helix is a little longer than the natural duplex.

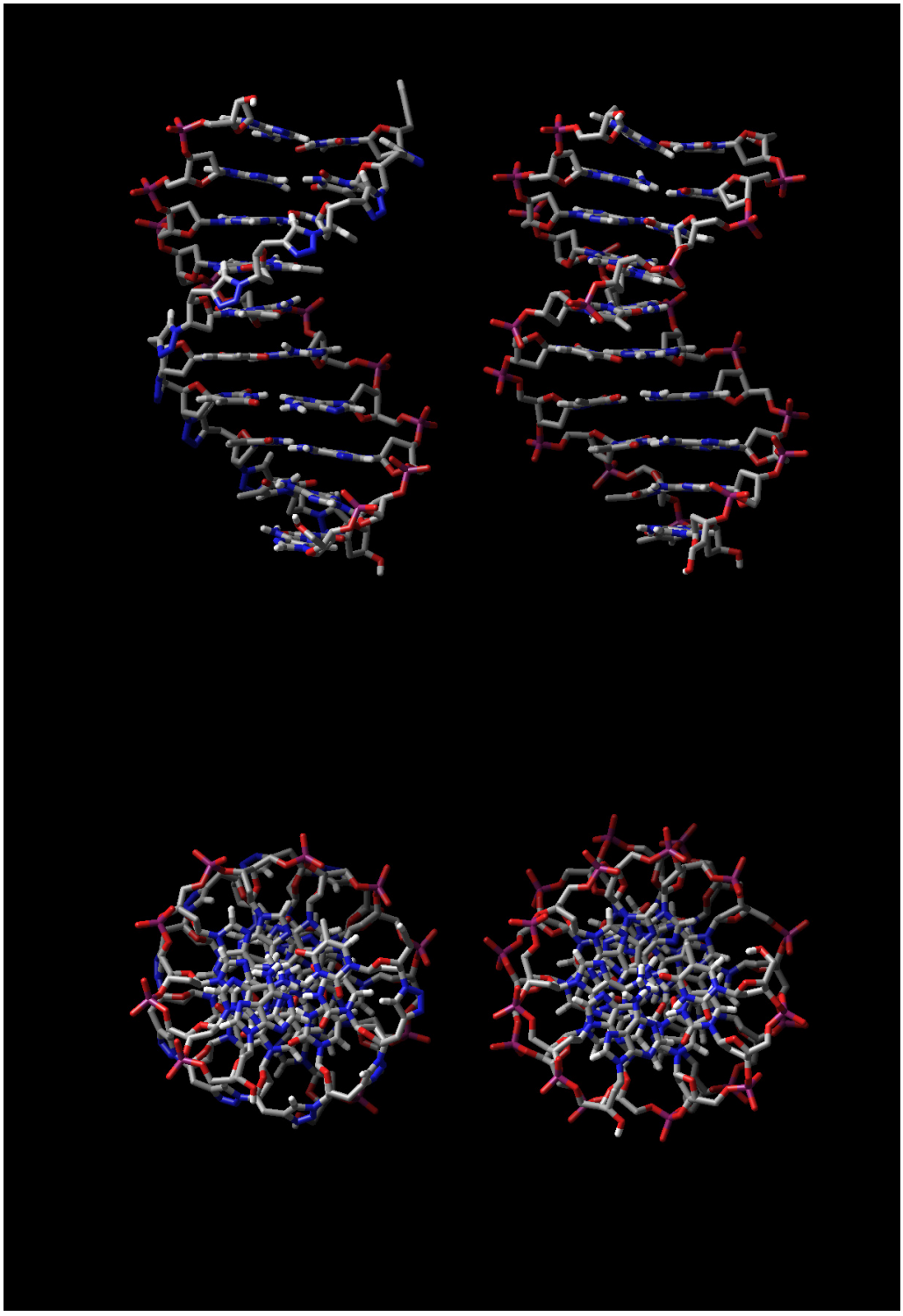


Figure S4. Molecular models of the double strand between 10-mer ^{TL}DNA and $d(A)_{10}$ (left upper; the same model was also shown in Figure 2) and the natural double strand between $d(T)_{10}$ and $d(A)_{10}$ (right). Gray: C, red: O, white: H, blue: N, purple: P.

7. Charts of Spectra (NMR and MS) and HPLC Analysis

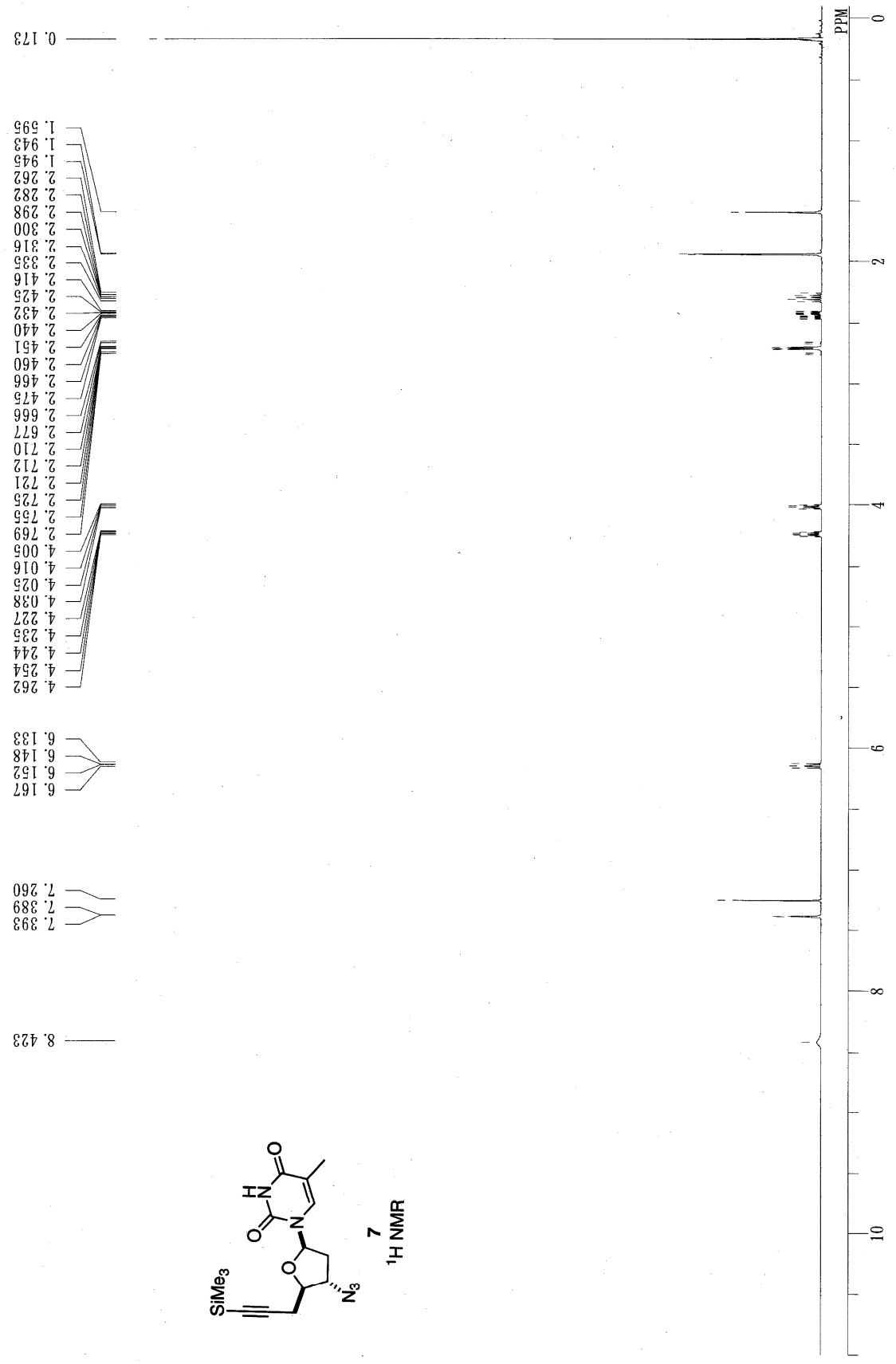


Figure S5. A ^{1}H NMR spectrum of monomer **7** in $CDCl_3$

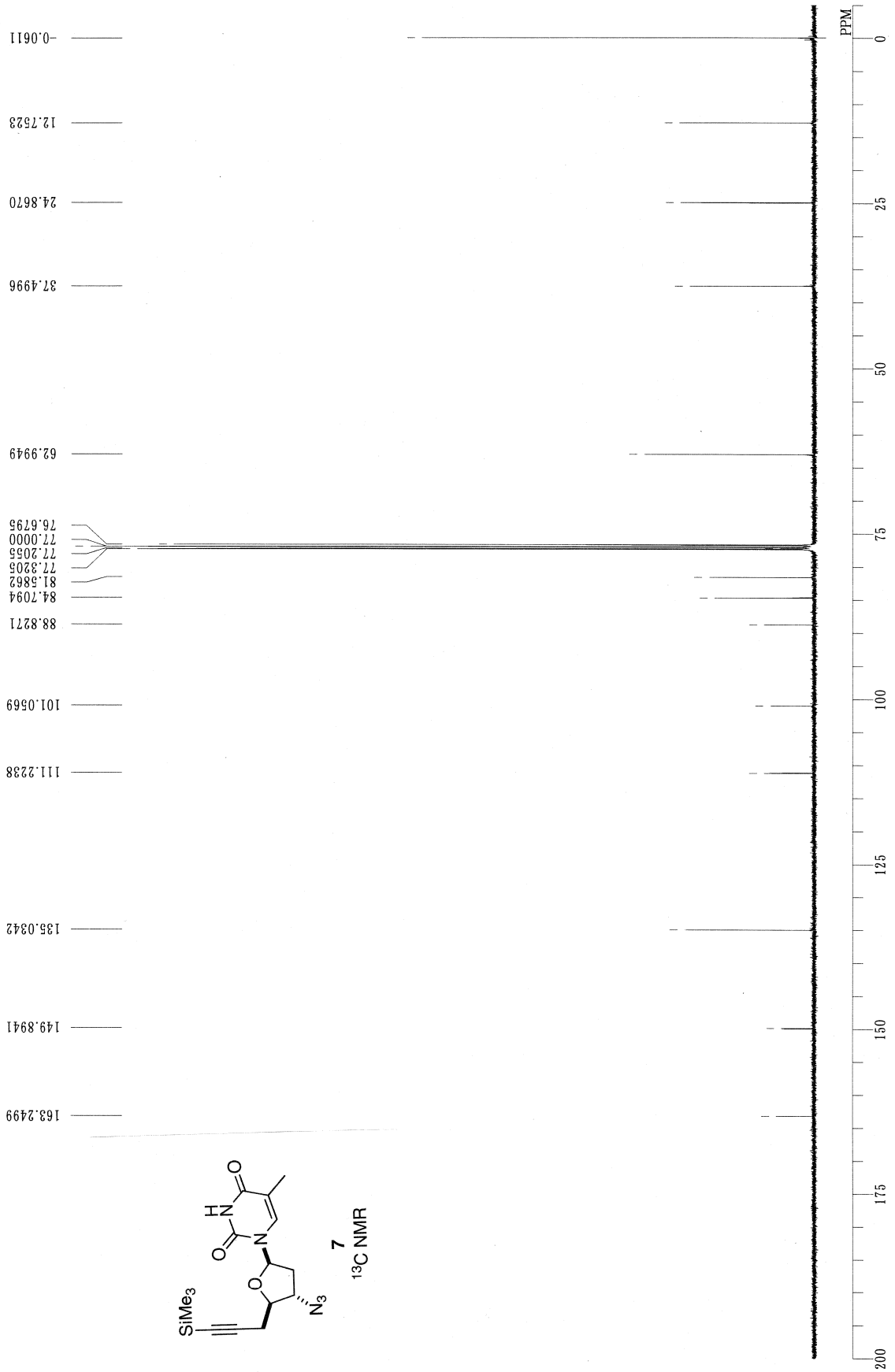


Figure S6. A ^{13}C NMR of spectrum monomer **7** in $CDCl_3$

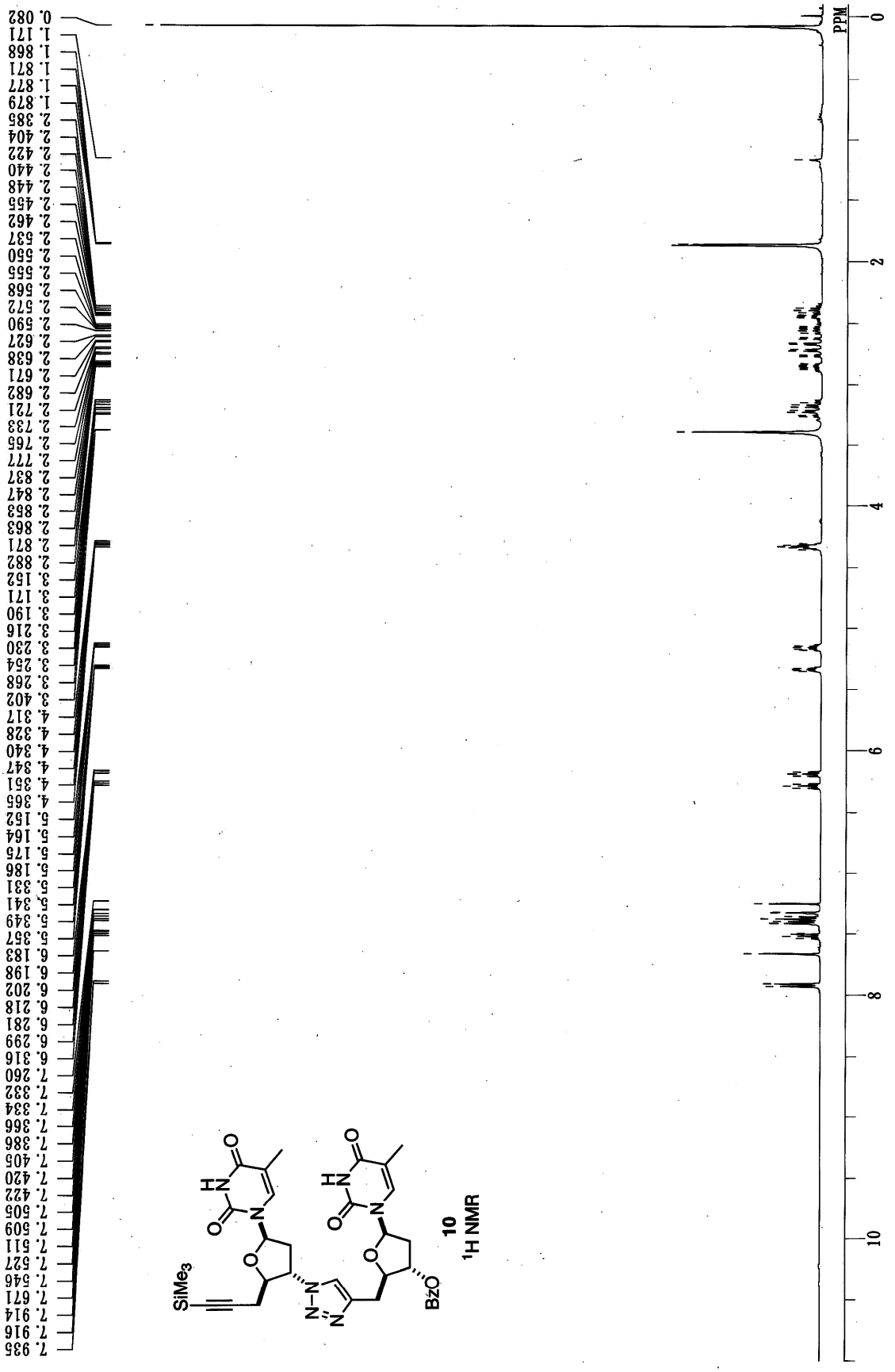


Figure S7. A ^{1}H NMR spectrum of dimer **10** in 10% v/v $CD_3OD/CDCl_3$

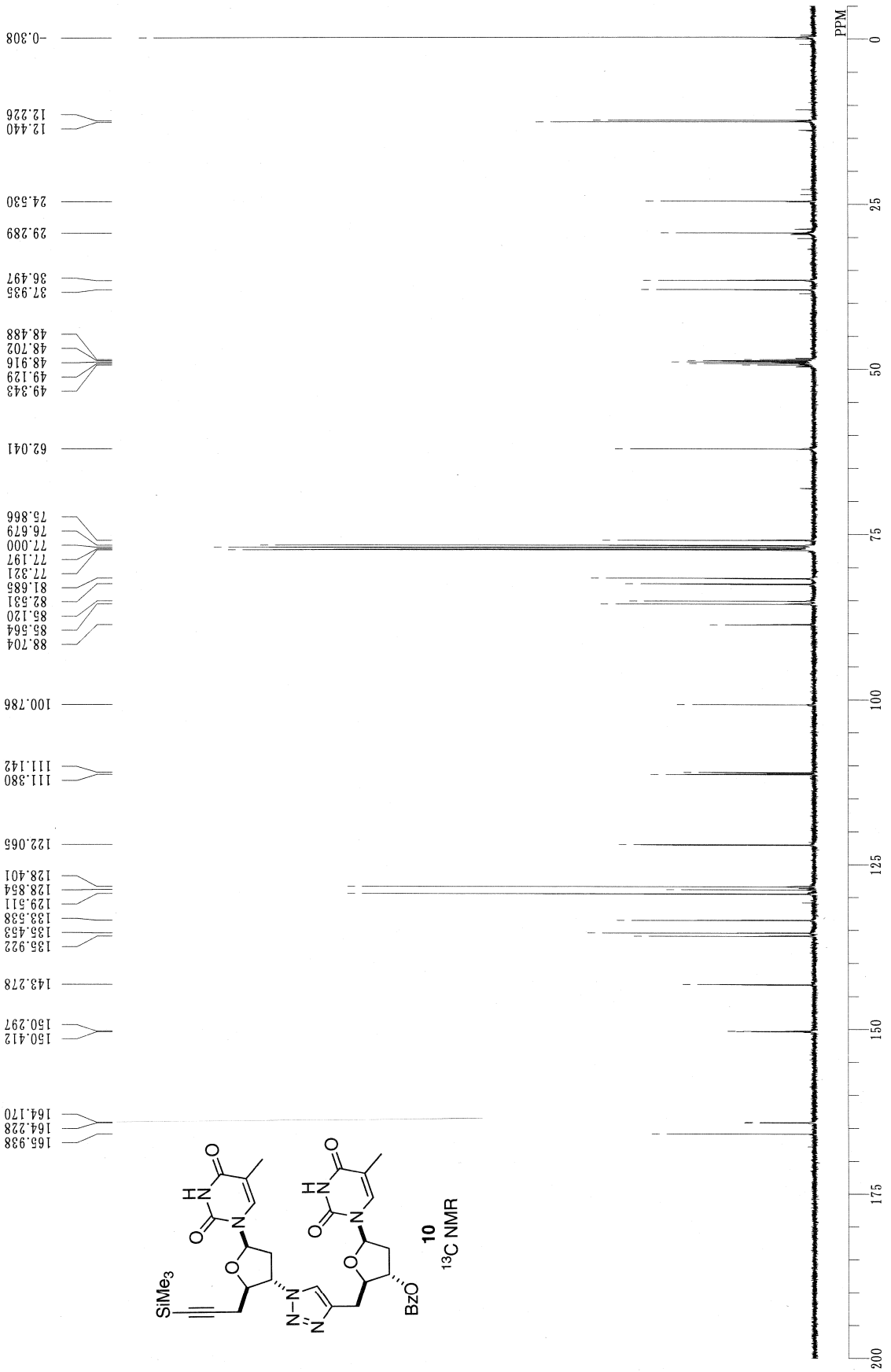


Figure S8. A ^{13}C NMR of spectrum dimer **10** in 10% v/v $CD_3OD/CDCl_3$

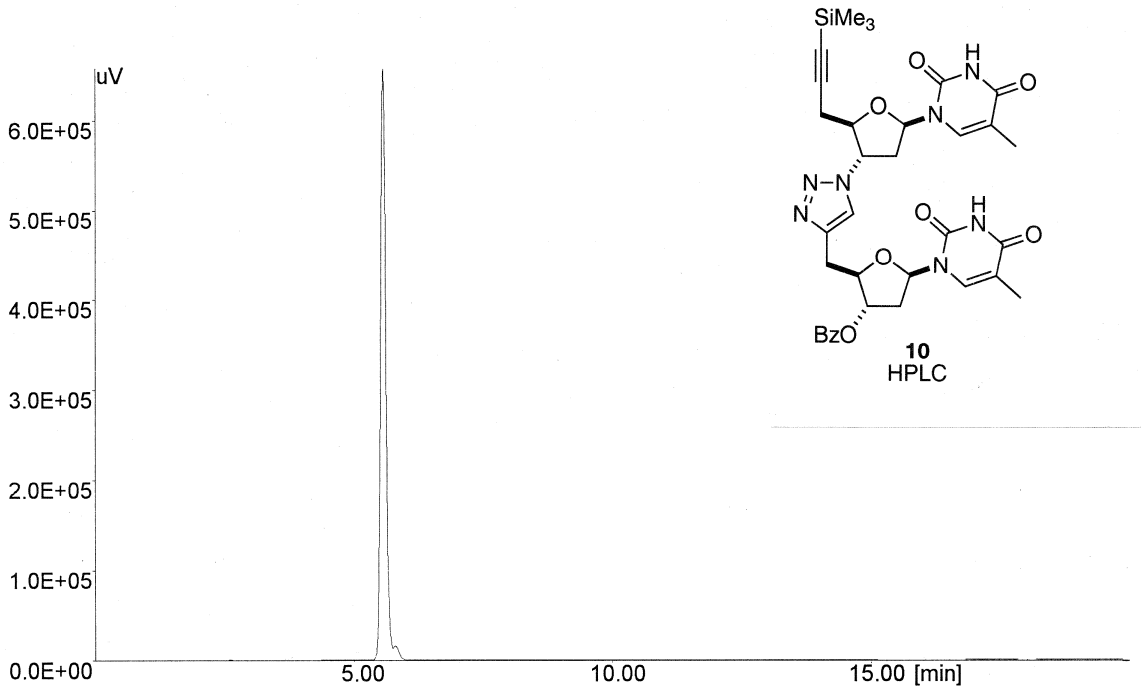


Figure S9. A HPLC chart of of dimer **10**. Column: COSMOSIL HILIC (4.6 × 250 mm); eluent: a linear gradient from 10% to 30% v/v aqueous ammonium acetate (10 mM, pH 7)/acetonitrile during 30 min, flow rate: 1.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.

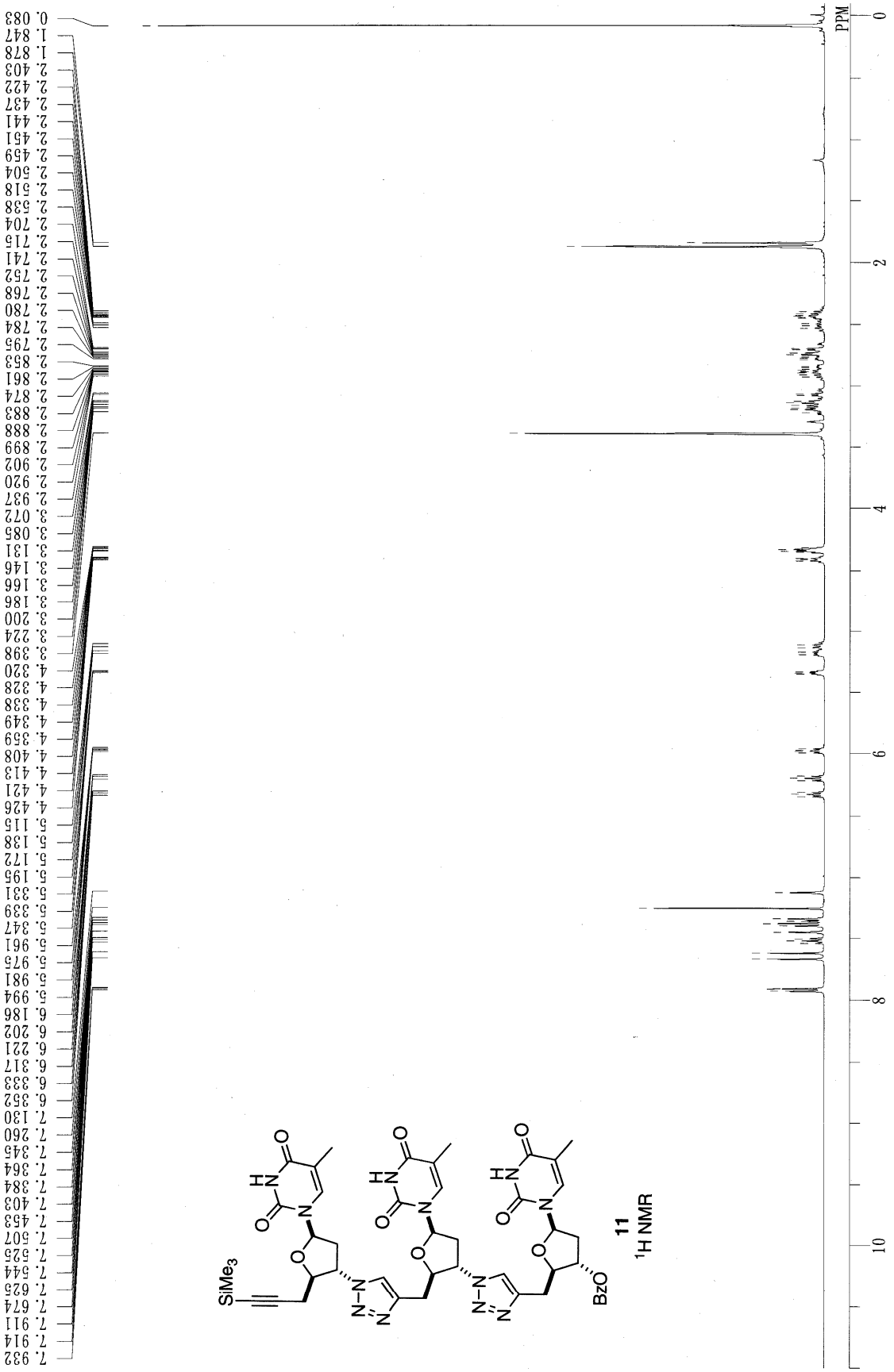


Figure S10. A ^{1}H NMR spectrum of trimer **11** in 10% v/v $CD_3OD/CDCl_3$

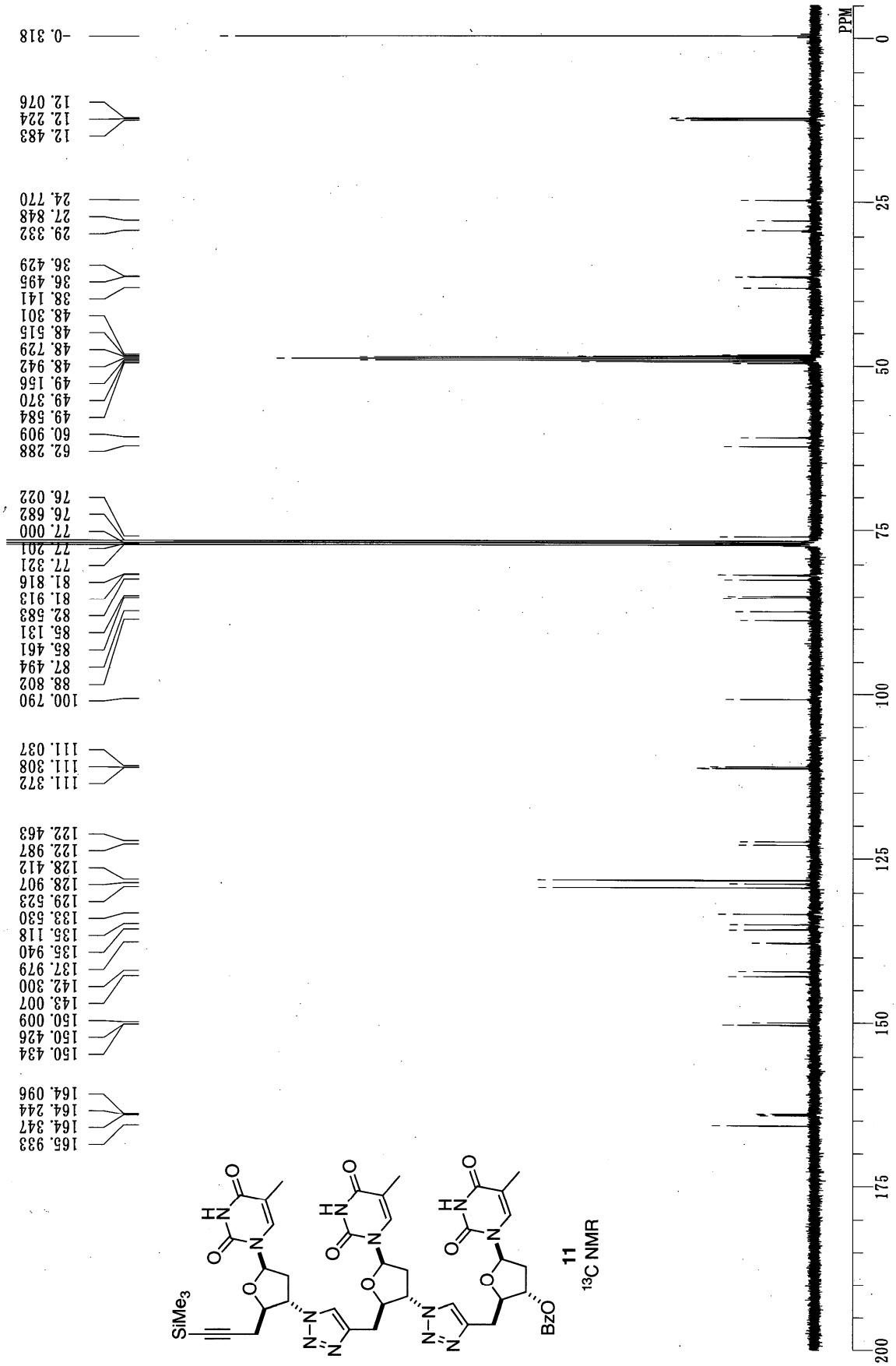


Figure S11. A ^{13}C NMR spectrum of trimer **11** in 10% v/v $CD_3OD/CDCl_3$

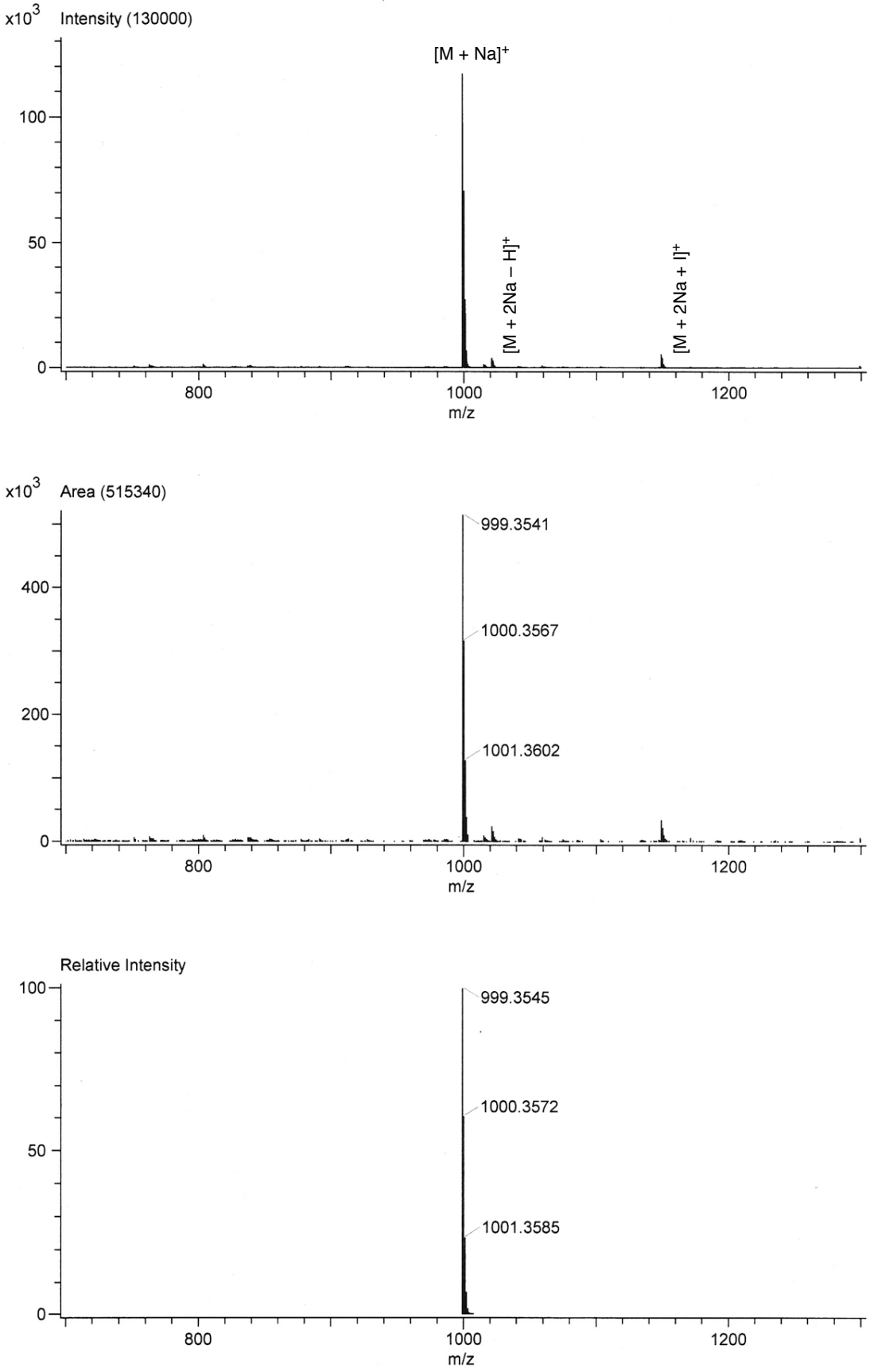


Figure S12. A HRMS spectra of trimer **11** ionized from $CH_3OH/CHCl_3$ containing NaI. Top: Raw data with assignments, middle: Peak data, bottom: calculated data.

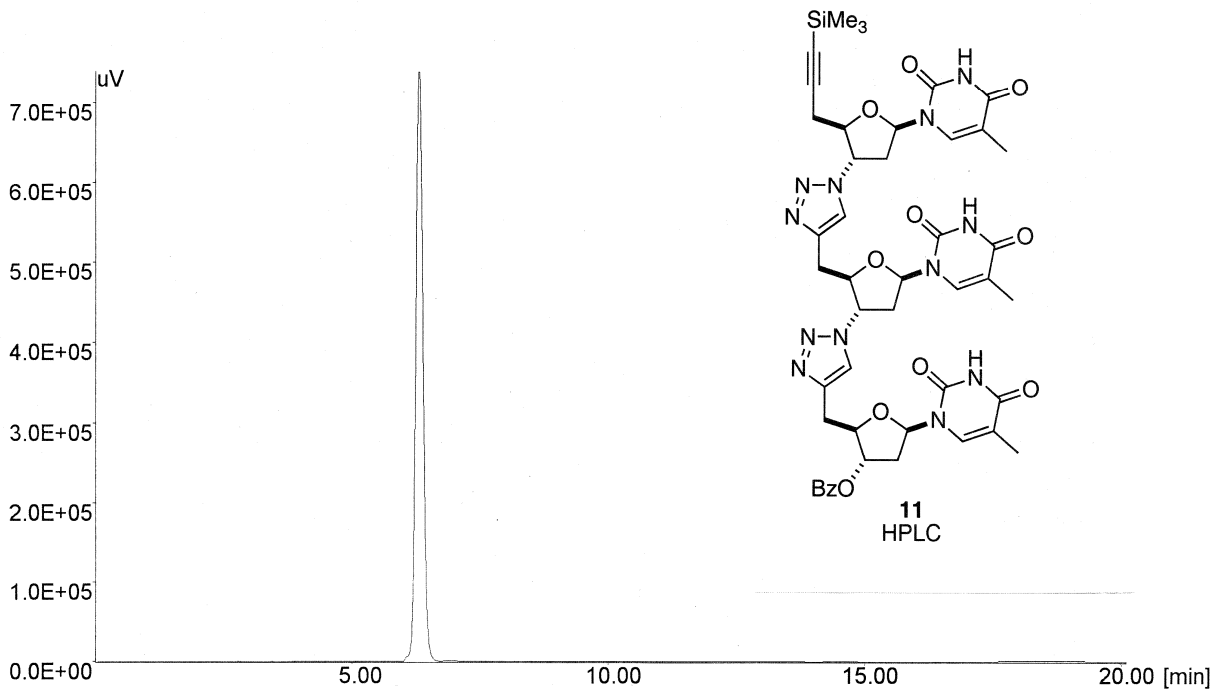


Figure S13. A HPLC chart of trimer **11**. Column: COSMOSIL HILIC (4.6 × 250 mm); eluent: a linear gradient from 10% to 30% v/v aqueous ammonium acetate (10 mM, pH 7)/acetonitrile during 30 min, flow rate: 1.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.

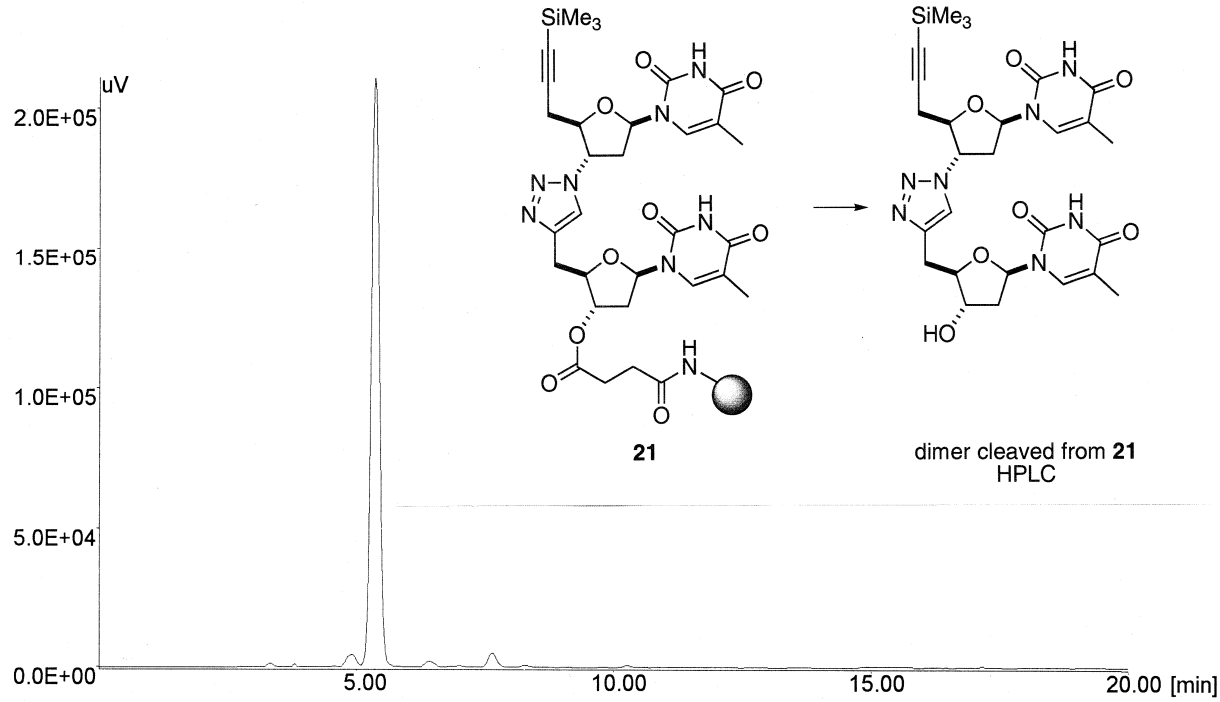


Figure S14. A HPLC chart of crude material cleaved from **21**. Column: COSMOSIL HILIC (4.6 × 250 mm); eluent: a linear gradient from 10% to 30% v/v aqueous ammonium acetate (10 mM, pH 7)/acetonitrile during 30 min, flow rate: 1.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.

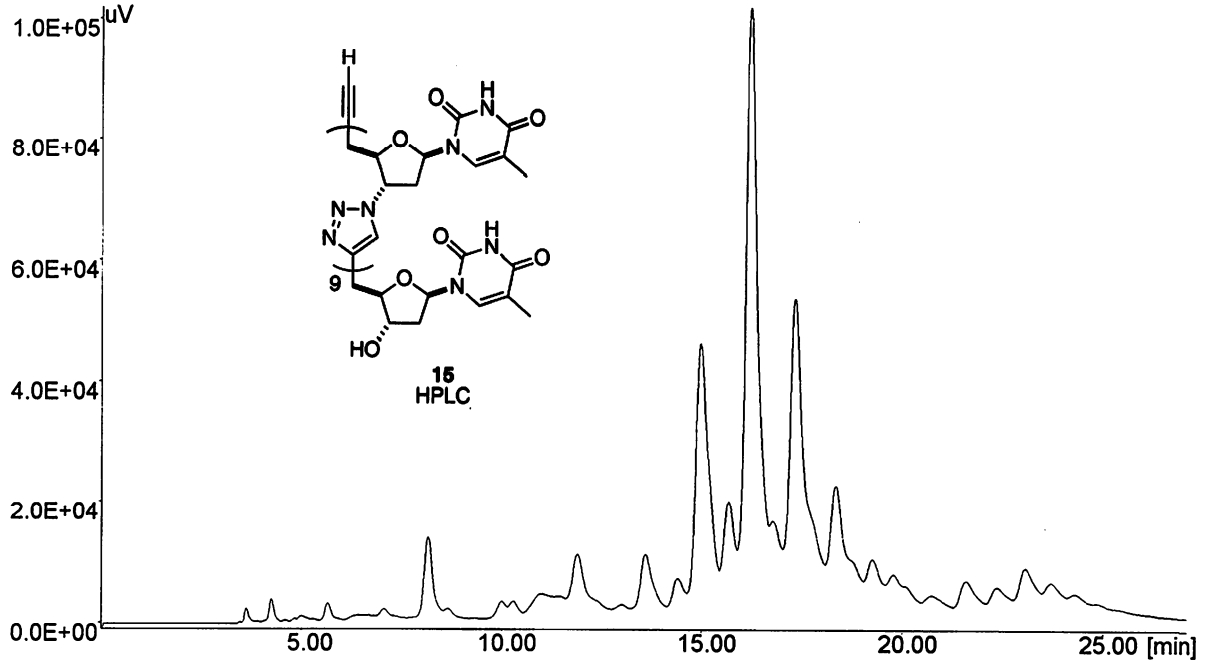


Figure S15. A HPLC chart of crude 10-mer **15** showing the presence of 10-mer (16 min) as the major product. The other oligonucleotides such as 9-mer (15 min), 11-mer (17 min) and 12-mer (18 min) were present as byproducts. Column: COSMOSIL HILIC (4.6 × 250 mm); eluent: a linear gradient from 10% to 30% v/v aqueous ammonium acetate (10 mM, pH 7)/acetonitrile during 30 min, flow rate: 2.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.

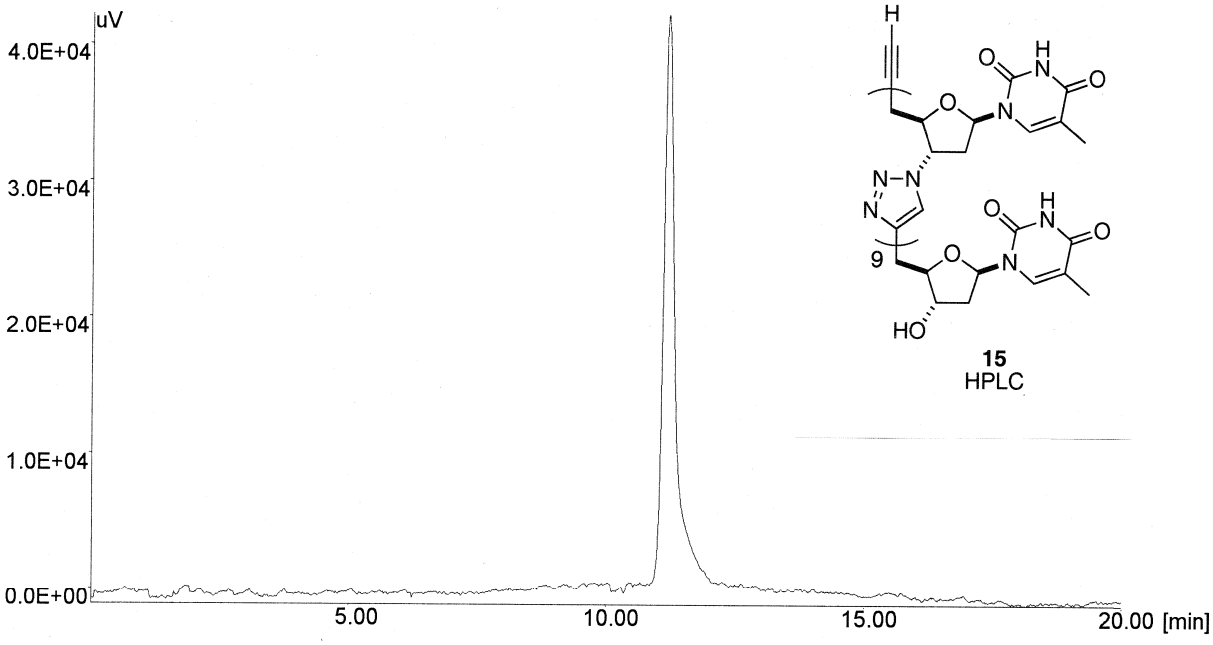


Figure S16. A HPLC chart of isolated 10-mer **15**. Column: COSMOSIL HILIC (4.6 × 250 mm); eluent: a linear gradient from 10% to 30% v/v aqueous ammonium acetate (10 mM, pH 7)/acetonitrile during 20 min, flow rate: 3.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.

Figure S17. A MALDI-TOF MS spectrum of 10-mer **15**