Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supporting Information

Cationic Nucleolipids as efficient siRNA carrier

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Experimental details

General

The reagents are purchased from Sigma–Aldrich, Fluka, Lancaster, Proligo, or Glen Research and used without further purification. All solvents were dried and distilled (PS-MD-3, C&S Specialty Inc.) prior to use. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded using an FT-300 MHz Bruker Aspect 3000 spectrometer. Mass spectra (FAB) were obtained using a Jeol JMS700 high-resolution mass spectrometer at the Korea Basic Science Center, Daegu, Korea. MALDI-TOF mass spectra were recorded using a Kratos Shimadzu AXIMA-CFR MALDI-TOF mass spectrometer at the Bioneer Corporation. IR spectra were recorded using a Bruker FTIR PS55+ spectrometer. Purification of oligonucleotides was performed using an Agilent 1100 HPLC (VyDAC C18 column; 10 × 250 mm; 5 μm; pore size: 120 Å). The RT-PCR was performed using Corbert Research RG-6600 model. The siRNAs were synthesized using an Expedite 8909 synthesizer and primer oligonucleotides were purchased from Bionics Co., Ltd. (Korea). All reactions were performed in flame-dried glassware under Ar. Flash column chromatography was performed using Merck silica gel 60 (230–400 mesh).

5'-*O*-(4,4'-dimethoxytrityl)uridine (1)

See reference 16.

M.p. : 121.2-122.6 °C; ^{1}H NMR (300 MHz, DMSO-d_6) : δ 11.35 (s, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.37~6.87 (m, 13H), 5.73 (d, J = 2.5 Hz, 1H), 5.50 (br, 1H), 5.28 (d, J = 8.1 Hz, 1H), 5.15 (br, 1H), 4.06 (s, 2H), 3.91 (br, 1H), 3.72 (s, 6H), 3.44 ~ 3.20 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 163.0, 158.1, 150.5, 144.7, 140.6, 137.3, 135.4, 135.2, 129.8, 128.9, 128.2, 127.9, 127.7, 126.8, 125.3, 113.3, 101.5, 88.9, 85.9, 82.4, 73.4, 69.6, 63.0, 55.0; IR (neat) : 3412, 3060, 3018, 2917, 2837, 1695, 1608, 1509, 1463, 1301, 1220 cm^{-1}; HRMS-FAB (m/z) : calcd for $C_{30}H_{30}N_2NaO_8^+$ $[M+Na]^+$, 569.1894; found, 569.1897.

2',3'-Di-*O*-alkylcarbamyl-5'-*O*-(4,4'-dimethoxytrityl)uridine (2a-c)

A solution of **1** (500 mg, 0.92 mmol) in dry pyridine was evaporated and dried *in vacuo* to remove moisture. After adding dry DMF (10 mL), CDI (223 mg, 1.37 mmol), and DMAP (11 mg, 0.092 mmol), the mixture was stirred for 1 h under Ar(g) condition. And then alkylamine (3 eq.) was added and the mixture was stirred for additional 12 h. After completion of the reaction, the mixture was evaporated under reduced pressure and resolved in CH_2Cl_2 and washed with 5% citric acid (aq.), water, and brine. The organic layer was dried (Na_2SO_4) and evaporated and purified through flash column chromatography (SiO_2, Hexane /Ethyl acetate, 3:2 to 1:1, v/v), then, a mixture of singly substituted compounds at 2' or 3' position was isolated (2'-*O*-alkylcarbamyl compound: 3'-*O*-alkylcarbamyl compound ≈ 7:3).

The singly substituted compound was solvated in dry pyridine and dissolved in DMF (10 ml). After adding dry DMF (10 mL), carbonyldiimidazole (196 mg, 1.21 mmol), and 4-dimethylaminopyridine (10 mg, 0.081 mmol), the mixture was stirred for 1 h under Ar(g) condition. And then alkylamine (3 eq.) was added and the mixture was stirred for additional 12 h. After completion of the reaction, the mixture was evaporated under reduced pressure and resolved in CH_2Cl_2 and washed with 5% citric acid (aq.), water, and brine. The organic layer was dried (Na_2SO_4) and evaporated and purified through flash column chromatography (SiO_2, Hexane /Ethyl acetate, 3:1 to 1:1, v/v), provided **2a** (70 %), **2b** (56 %), and **2c** (70 %) as a white solid.

Compound **2a**: M.p. : 63.3-64.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) : δ 11.45(s, 1H), 7.68 (d, J=8.1 Hz, 1H), 7.40 ~ 6.88(m, 13H), 5.96 (d, J=5.4 Hz, 1H), 5.69 (t, J=5.6 Hz, 1H), 5.41 (d, J=8.0 Hz, 1H), 5.38 ~ 5.33(m, 1H), 4.11 ~ 4.10(m, 1H), 3.74 (s, 6H), 3.22 ~ 3.19 (m, 2H), 2.97 ~ 2.89 (m, 4H), 1.38 ~ 1.23 (m, 24H), 0.85 ~ 0.82 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 162.8, 158.2, 154.7, 154.5, 150.4, 144.4, 135.1, 134.9, 129.7, 127.9, 127.7, 126.8, 113.3, 102.2, 86.2, 81.1, 72.4, 70.4, 55.1, 31.2, 30.0, 29.3, 29.2, 28.7, 28.6, 26.4, 26.2, 22.1, 13.9; IR (neat) : 3337, 2953, 2927, 2855, 1694, 1538, 1510, 1251 cm^{-1}; HRMS-FAB (m/z) : calcd for $C_{48}H_{64}N_4NaO_{10}^+$ $[M+Na]^+$, 879.4515; found, 879.4524.

Compound **2b**: M.p. : 71.8-72.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 8.17(s, 1H, N3), 7.68 (d, *J*=8.2 Hz, 1H), 7.42 ~ 6.83(m, 13H), 6.23 (d, *J*=6.9 Hz, 1H), 5.54 ~ 5.50 (m, 1H), 5.29 (dd, *J*=8.2, 2.0 Hz, 1H), 4.82 ~ 4.79 (m, 1H), 4.22 (br, 1H), 3.79 (s, 6H), 3.45 (dd, *J*=38.8, 9.9 Hz, 2H), 3.24 ~ 3.16 (m, 4H), 1.50 ~ 1.26 (m, 40H), 0.90 ~ 0.85(t, *J*=6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 162.7, 160.0, 154.7, 144.1, 140.3, 135.3, 135.0, 130.5, 130.4, 128.5, 128.3, 127.4, 113.6, 103.1, 87.7, 83.1, 72.8, 63.4, 55.5, 41.6, 41.4, 32.1, 30.1, 30.0, 29.9, 29.6, 29.5, 27.0 , 22.9, 14.3; IR (neat) : 3341, 2925, 2853, 1707, 1609, 1541, 1510, 1462, 1383, 1252 cm^{-1}; HRMS-FAB (m/z) : calcd for $C_{56}H_{80}N_4NaO_{10}^+$ $[M+Na]^+$, 991.5767; found, 991.5775.

Compound **2c**: M.p. : 61.5-62.8 °C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 8.20 (br, 1H), 7.68 (d, *J*=8.2 Hz, 1H), 7.41 ~ 6.83(m, 13H), 6.22 (d, *J*=6.9 Hz, 1H), 5.53 ~ 5.50 (m, 1H), 5.38 ~ 5.34 (m, 4H), 5.29 (d, *J*=8.0 Hz, 1H), 4.81(br, 1H), 4.22 (br, 1H), 3.79 (s, 6H), 3.46 (dd, *J*=38.6, 10.1 Hz, 2H), 3.17 ~ 3.11 (m, 4H), 2.08 ~ 2.00 (m, 8H), 1.50 ~ 1.25 (m, 48H), 0.88 (t, *J*=6.5 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 162.8, 159.1, 155.3, 154.8, 150.9, 144.3, 140.4, 135.4, 135.1, 130.6, 130.5, 130.4, 130.1, 129.8, 128.6, 128.5, 127.6, 115.8, 113.7, 103.2, 87.9, 85.4, 83.3, 77.8, 77.6, 77.4, 76.9, 73.8, 72.9, 72.7, 63.6, 55.6, 41.7, 41.6, 41.1, 33.0, 32.3, 32.1, 30.6, 30.3, 30.1, 30.0, 29.9, 29.7, 29.3, 29.2, 27.6, 27.3, 27.1, 23.0, 14.5; IR (neat) : 3339, 2924, 2853, 1707, 1613, 1543, 1510, 1462, 1382, 1252 cm^{-1}; HRMS-FAB (m/z) : calcd for $C_{68}H_{100}N_4NaO_{10}^+$ $[M+Na]^+$, 1155.7332; found, 1155.7344.

2',3'-Di-*O*-alkylcarbamyluridine (3a-c)

A solution of **2a-c** in CH_2Cl_2 (0.5 M) was cooled to -15 °C in ice-acetone bath for 1 h. A 4% trichloroacetic acid (in CH_2Cl_2) was dropwisely added upto concentration was 0.05 M. After stirring at the room temperature for 12 h, the reaction was quenched with methanol (1 mL) and the mixture was evaporated under reduced pressure and purified through flash column chromatography (SiO_2, CH_2Cl_2 /Ethyl acetate, 5:1 to 1:2, v/v). A white product **3a** (93 %), **3b** (87 %), and **3c** (quant.) was obtained.

Compound **3a**: M.p. : 61.7-63 °C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 9.86(s, 1H), 7.92 (d, *J*=7.9 Hz, 1H), 6.17 (d, *J*=7.0 Hz, 1H), 5.81 ~ 5.78 (d, *J*=7.9 Hz, 1H), 5.50 (s, 2H), 5.39 ~ 5.37 (m, 1H), 5.30 ~ 5.26 (m, 1H), 4.18 (s, 1H), 3.92 ~ 3.87 (m, 3H), 3.12 (br, 4H), 1.48 ~ 1.24 (m, 24H), 0.86 ~ 0.82 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 163.8, 155.6, 155.1, 151.3, 140.9, 103.5, 86.0, 84.6, 74.1, 72.7, 62.2, 42.1, 41.4, 31.9, 30.0, 29.8, 29.4, 27.0, 26.9, 22.8, 14.2; IR (neat) : 3336, 2956, 2927, 2856, 1706, 1541, 1464, 1385, 1261; HRMS-FAB (m/z) : calcd for $C_{27}H_{46}N_4NaO_8^+$ $[M+Na]^+$, 577.3208; found, 577.3218.

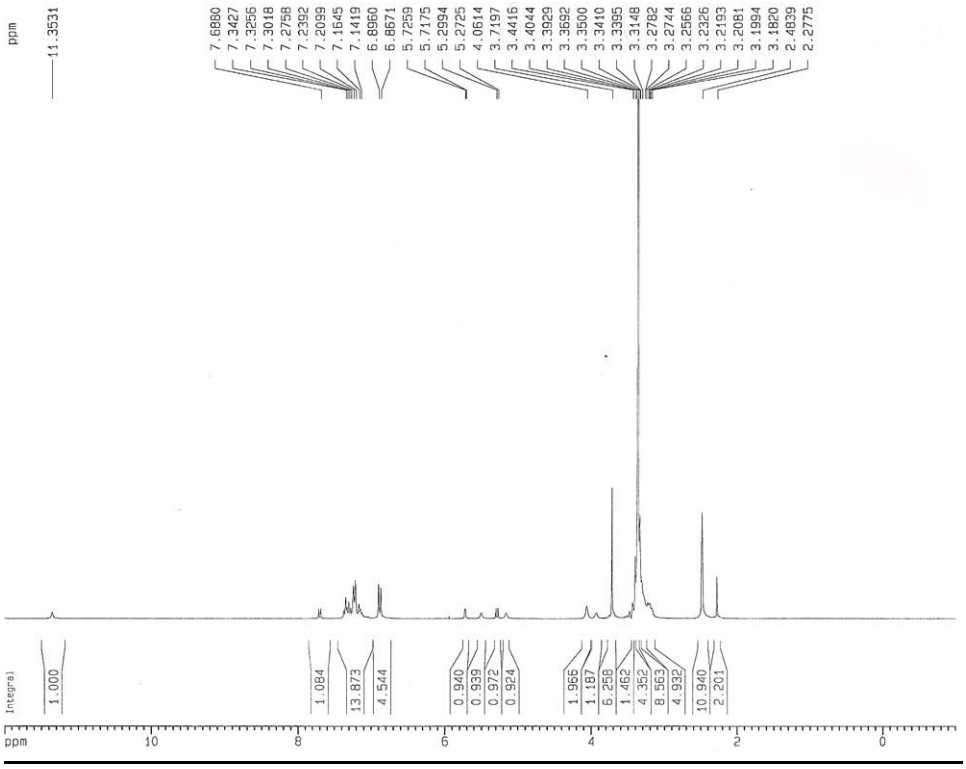
Compound **3b**: M.p. : 122.1-123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 8.29(br, 1H), 7.79 (d, *J*=8.2 Hz, 1H), 6.09 (d, *J*=5.2 Hz, 1H), 5.78 (dd, J= 8.2, 1.9 Hz), 5.36 ~ 5.33 (m, 1H), 4.92 ~ 4.85 (m, 2H), 4.19 (s, 1H), 3.91 (br, 2H), 3.18 ~ 3.11 (m, 4H), 1.50 ~ 1.26 (m, 40H), 0.88 (t, *J*=6.6 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 162.9, 154.8, 154.5, 150.6, 140.2, 102.6, 84.6, 83.7, 72.6, 71.4, 61.0, 31.3, 29.5, 29.3,29.2, 29.1, 29.0, 28.8, 28.7, 26.3, 26,1, 22.1, 13.9; IR (neat) : 3448, 3346, 2924, 2853, 1706, 1629, 1537, 1464, 1386, 1259, 1217 cm^{-1}; HRMS-FAB (m/z) : calcd for $C_{35}H_{62}N_4NaO_8^+$ $[M+Na]^+$, 689.4460; found, 689.4468.

Compound **3c**: M.p. : 91-92.1 °C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 9.28(s, 1H), 7.80 (d, *J*=8.1 Hz, 1H), 6.08 (d, *J*=6.8 Hz, 1H), 5.72 (d, *J*=8.1 Hz, 1H), 5.31 ~ 5.16 (m, 7H), 4.12 (s, 1H), 3.82 (br, 2H), 3.09 ~ 3.02 (m, 4H), 1.95 ~ 1.93 (m, 8H), 1.41 ~ 1.19 (m, 48H), 0.81 (t, *J*=6.5 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 163.6, 155.7, 155.1, 151.2, 147.1, 140.9, 130.5, 130.3, 130.1, 128.0, 103.6, 86.5, 84.6, 84.2, 74.2, 72.7, 62.3, 62.0, 42.2, 41.6, 32.9, 32.2, 32.1, 31.8, 30.2, 30.1, 30.0, 29.8, 29.6, 29.5, 29.3, 27.5, 27.1, 23.0, 22.9, 14.4; IR (neat) : 3337, 3093, 3064, 3006, 2925, 2854, 1707, 1543, 1465, 1386, 1324, 1261, 1162, 1109, 1057, 967, 933, 877, 833; HRMS-FAB (m/z) : calcd for $C_{47}H_{82}N_4NaO_8^+$ $[M+Na]^+$, 853.6025; found, 853.6035.

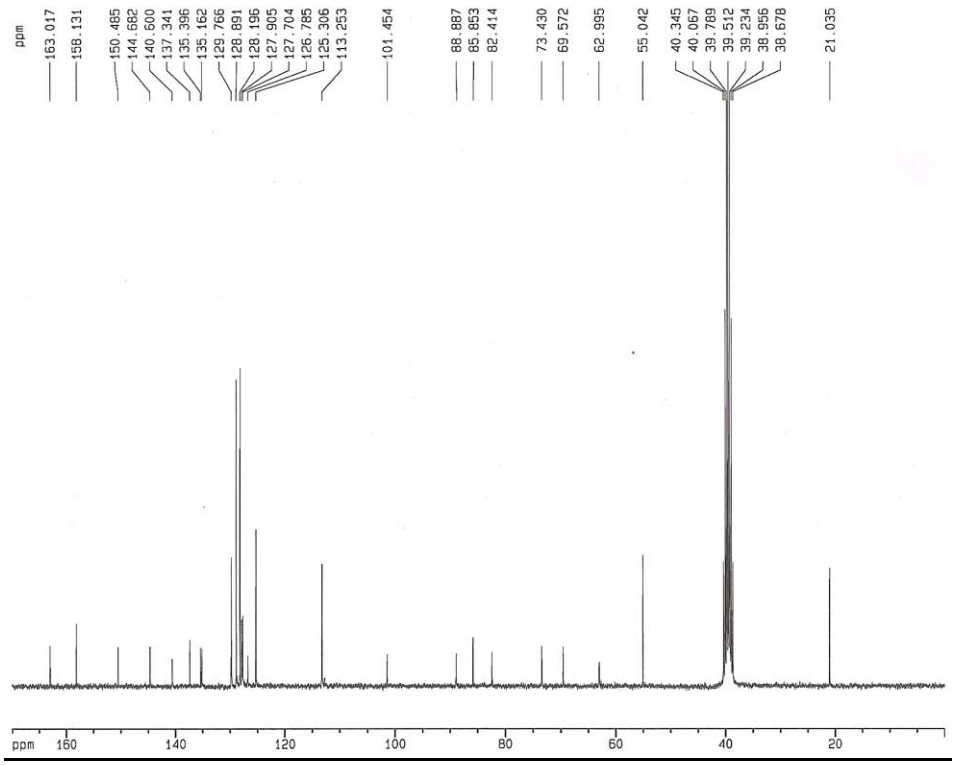
NMR spectra

5'-*O*-(4,4'-dimethoxytrityl)uridine (1)

^{1}H NMR

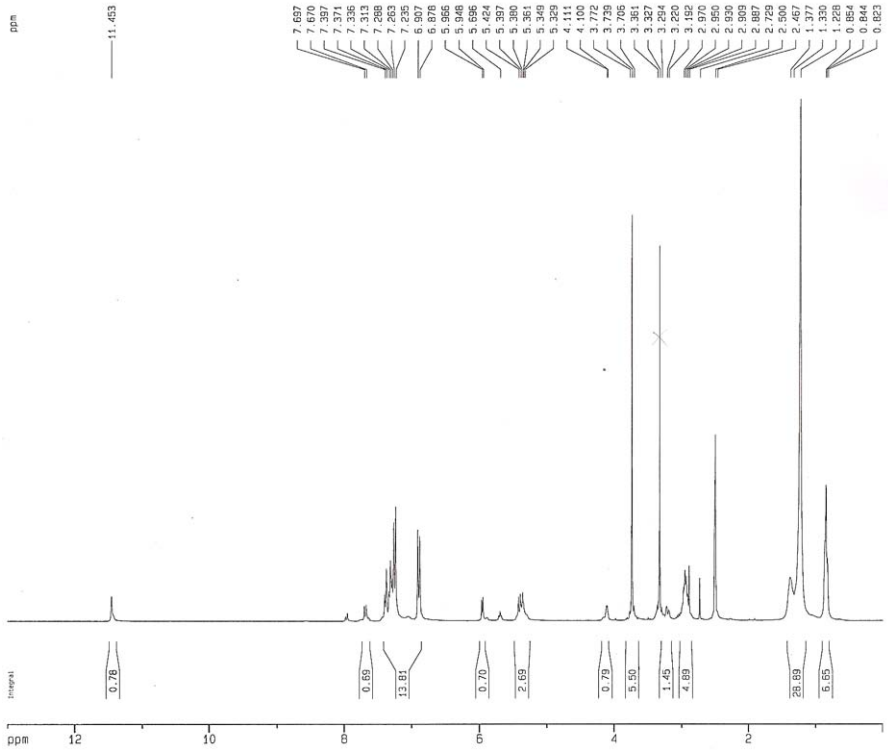


^{13}C NMR

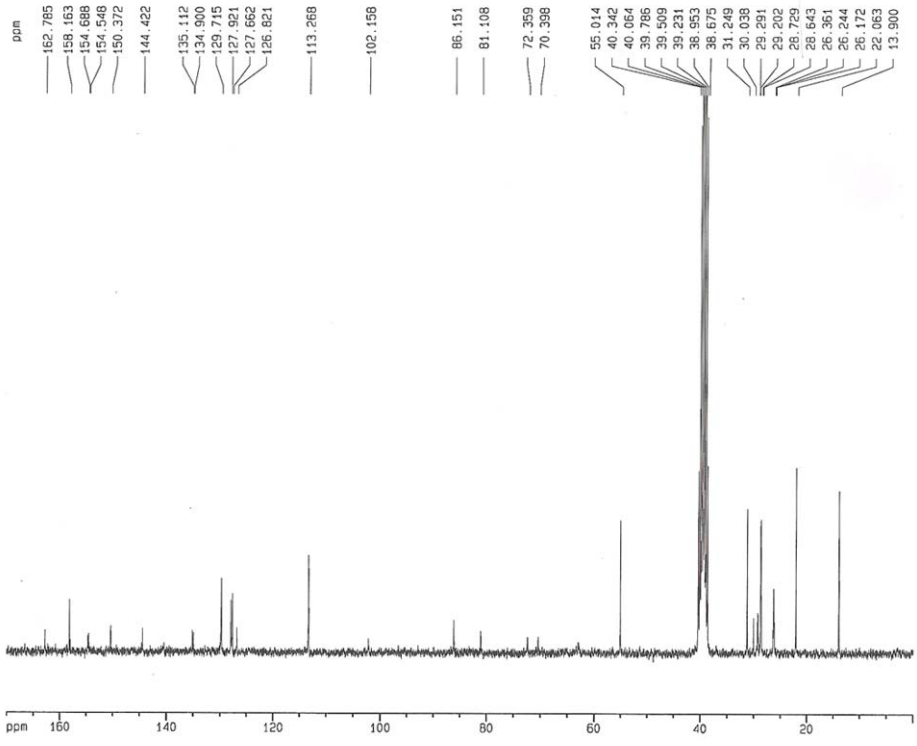


2',3'-Di-*O*-octylcarbamyl-5'-*O*-(4,4'-dimethoxytrityl)uridine (2a)

^{13}H NMR

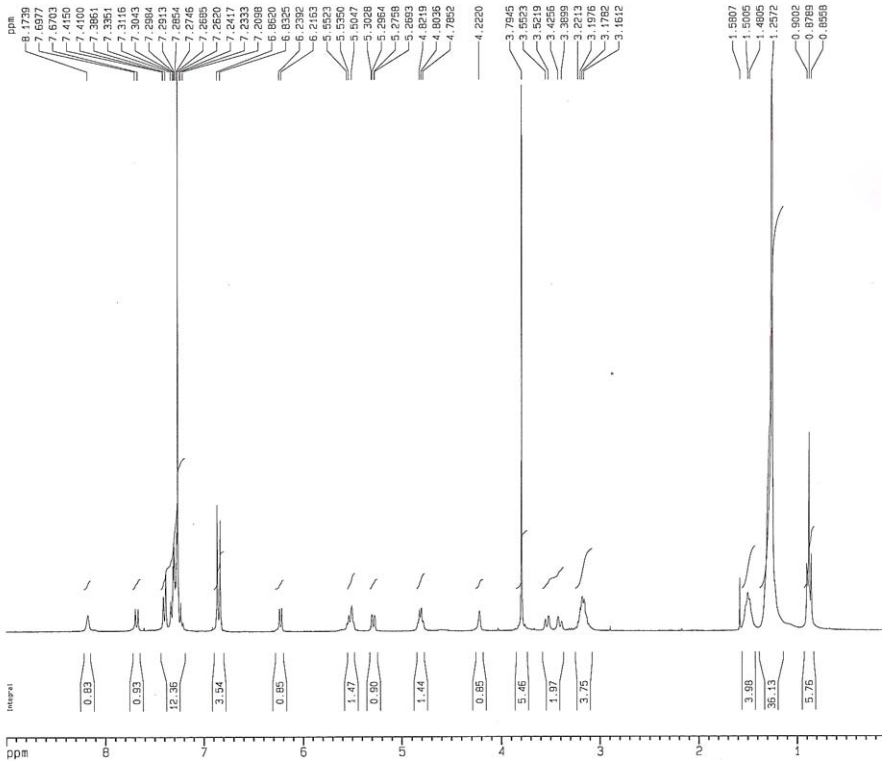


^{1}C NMR

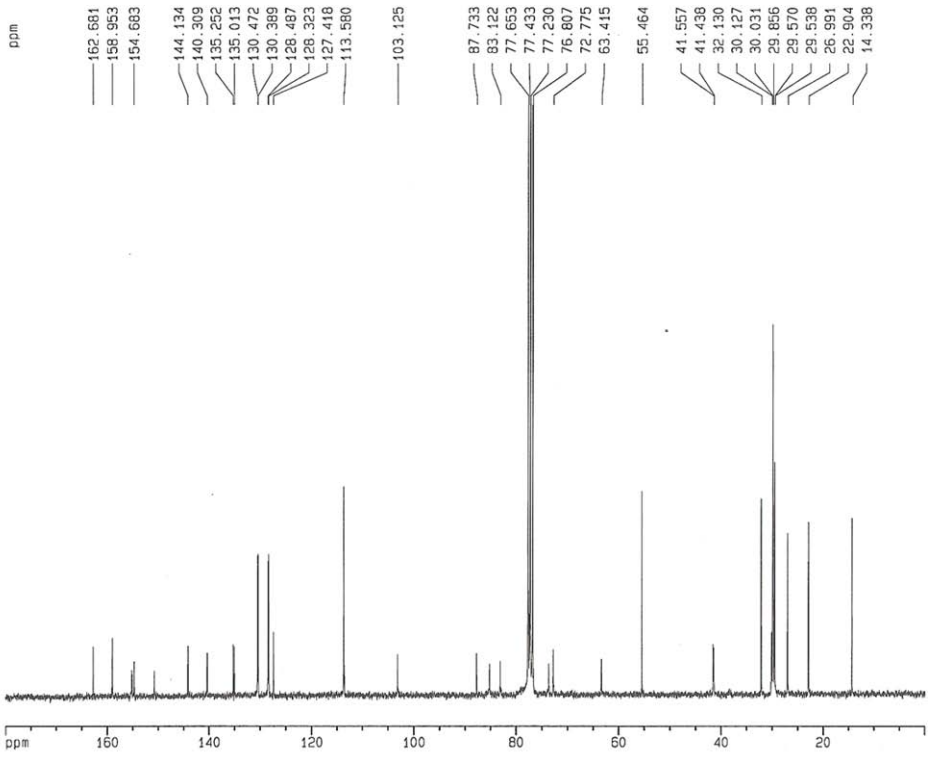


2',3'-Di-*O*-dodecylcarbamyl-5'-*O*-(4,4'-dimethoxytrityl)uridine (2b)

^{13}H NMR

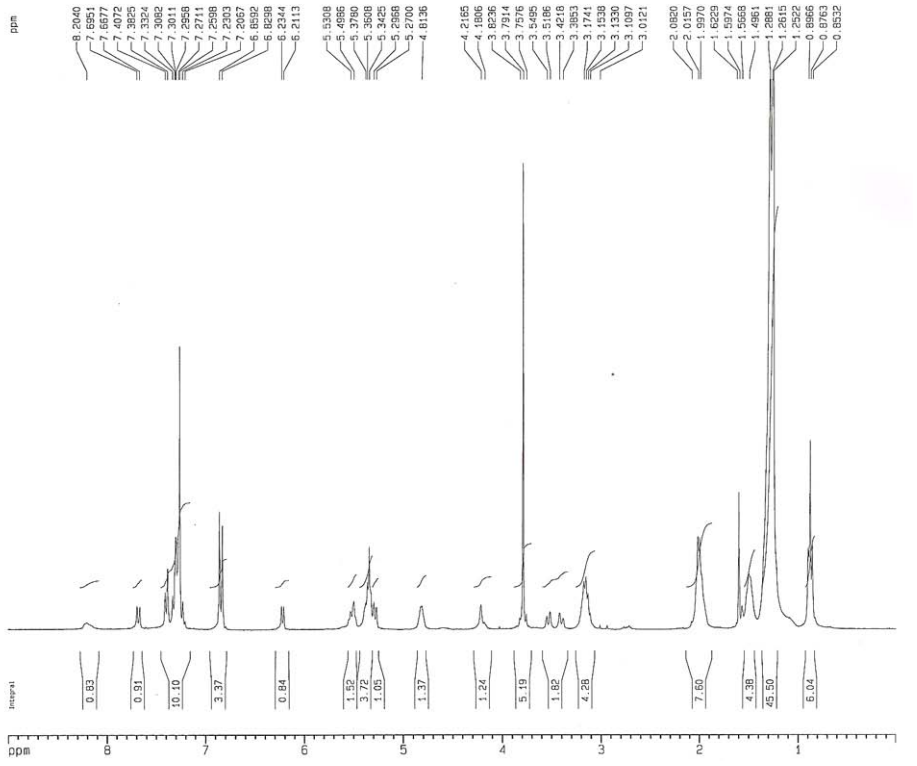


^{1}C NMR

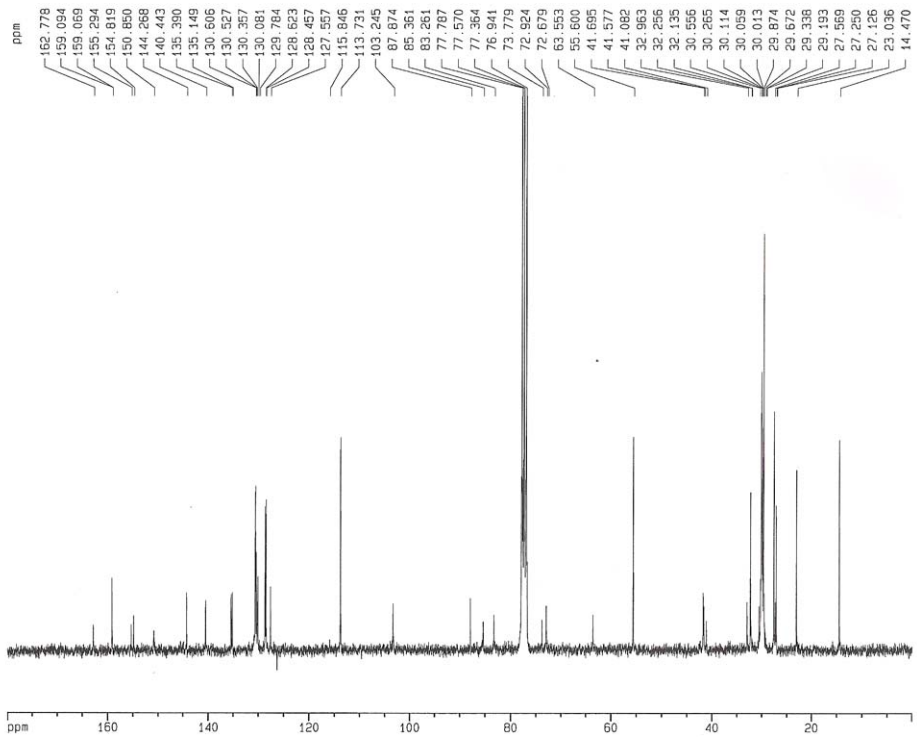


2',3'-Di-*O*-oleylcarbamyl-5'-*O*-(4,4'-dimethoxytrityl)uridine (2c)

^{13}H NMR

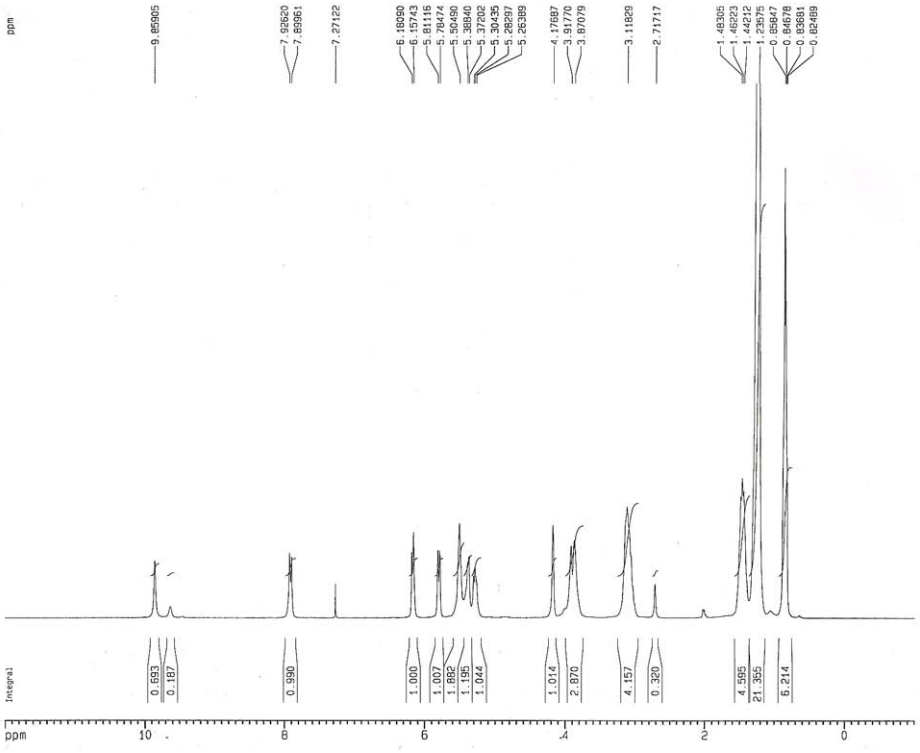


^{1}C NMR

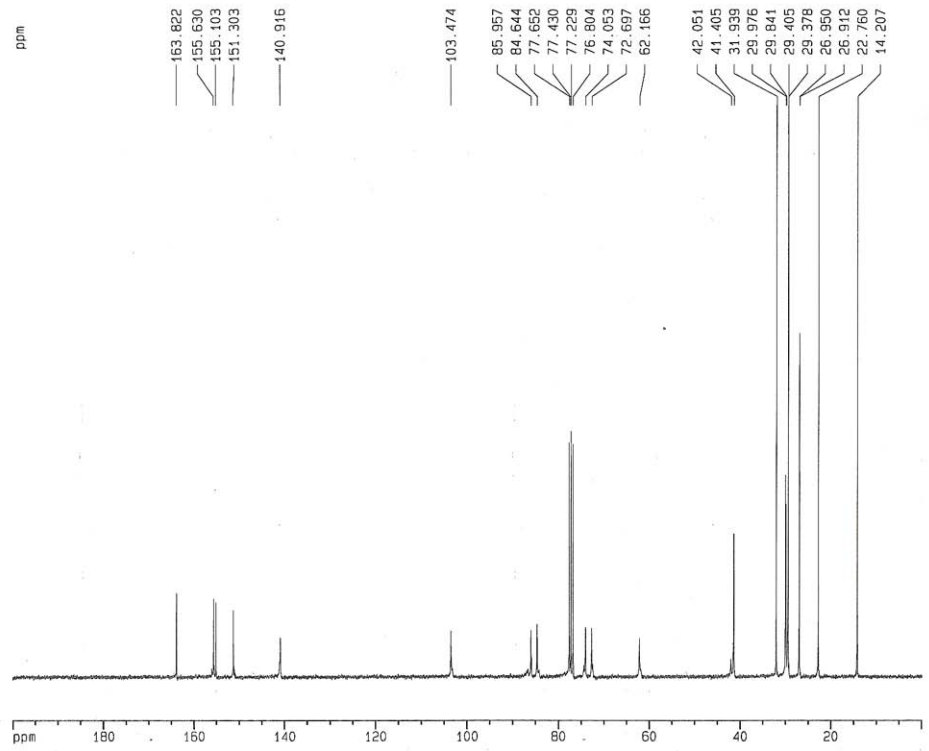


2',3'-Di-*O*-octylcarbamyluridine (3a)

^{13}H NMR

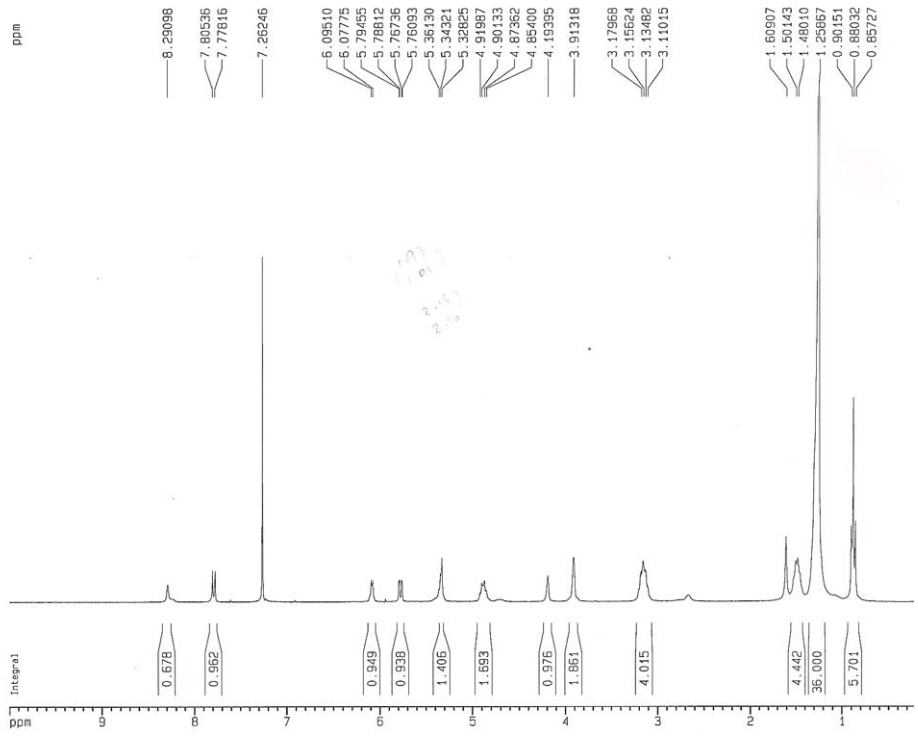


^{1}C NMR

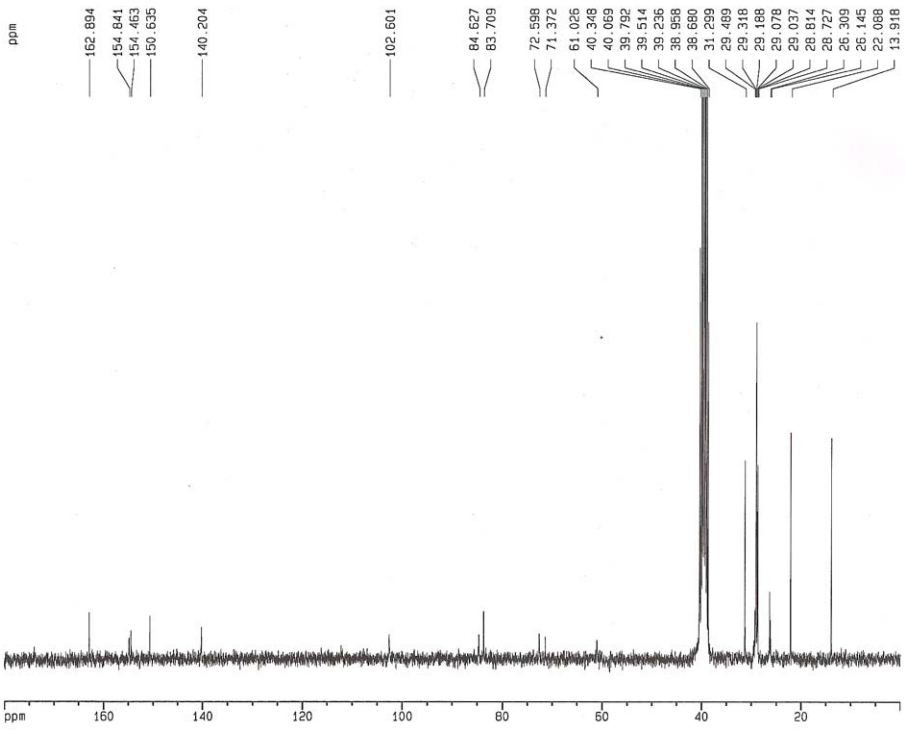


2',3'-Di-*O*-dodecylcarbamyluridine (3b)

^{13}H NMR

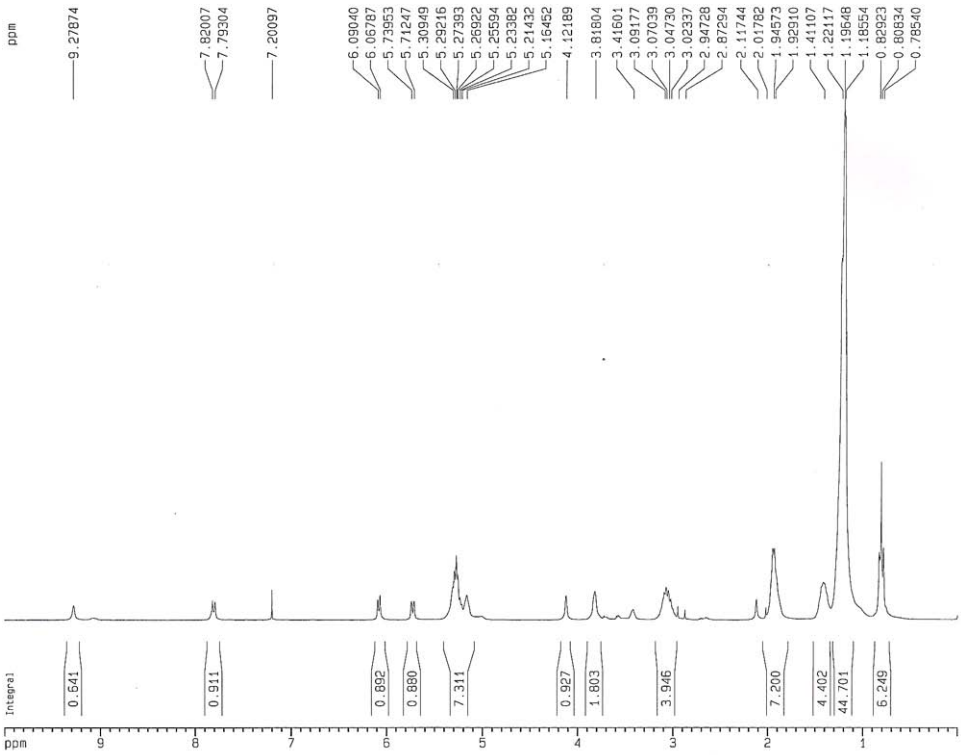


^{1}C NMR

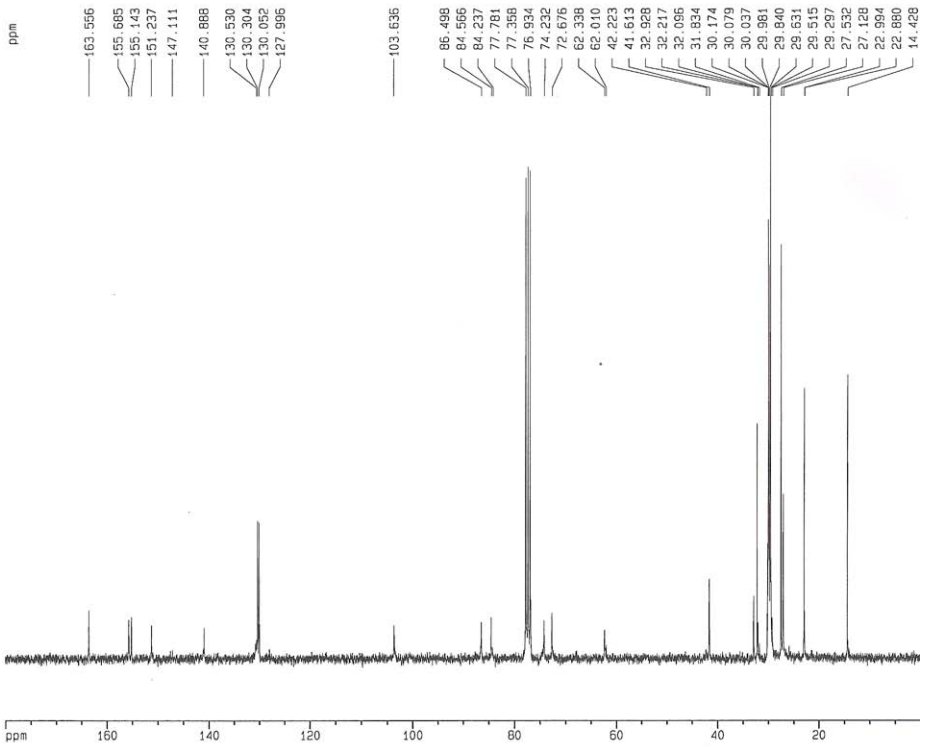


2',3'-Di-*O*-oleylcarbamyluridine (3c)

^{13}H NMR

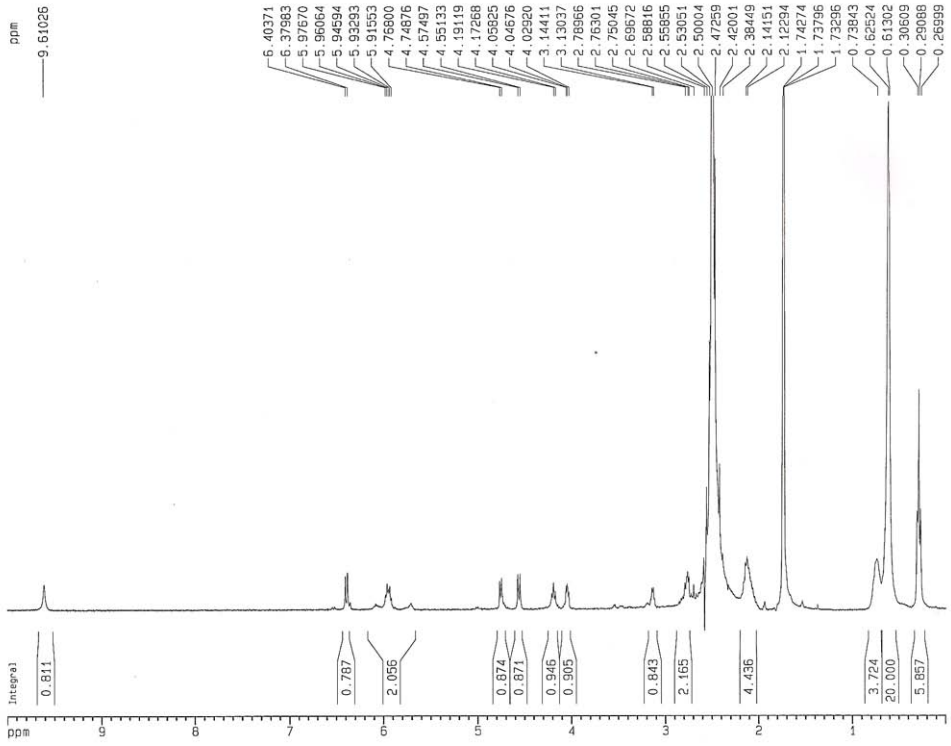


^{1}C NMR

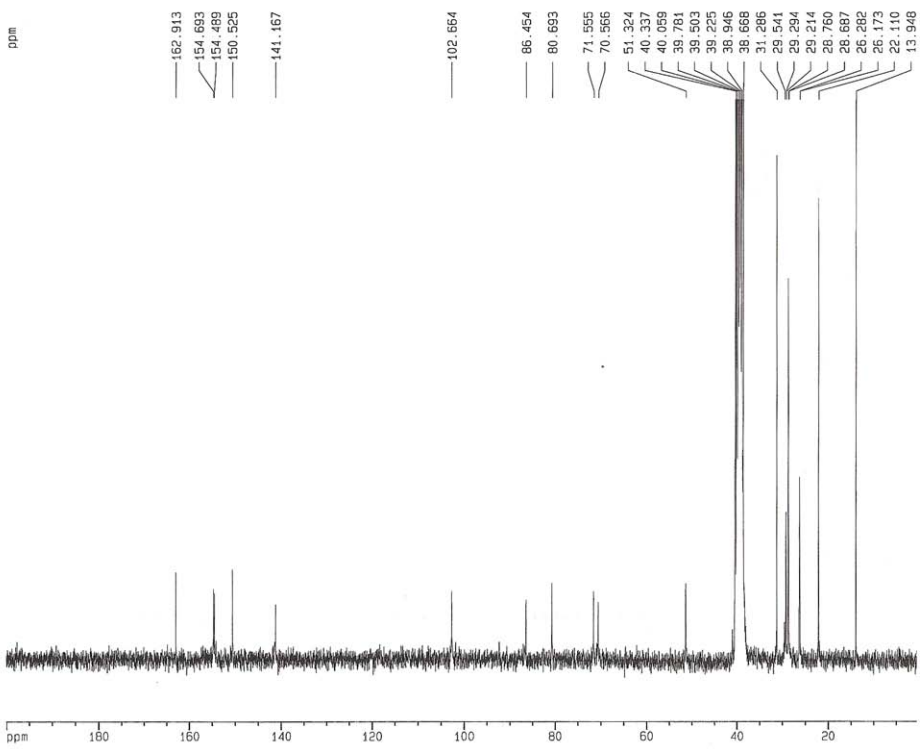


2',3'-Di-*O*-octylcarbamyl-5'-azido-5'-deoxyuridine (4a)

^{13}H NMR

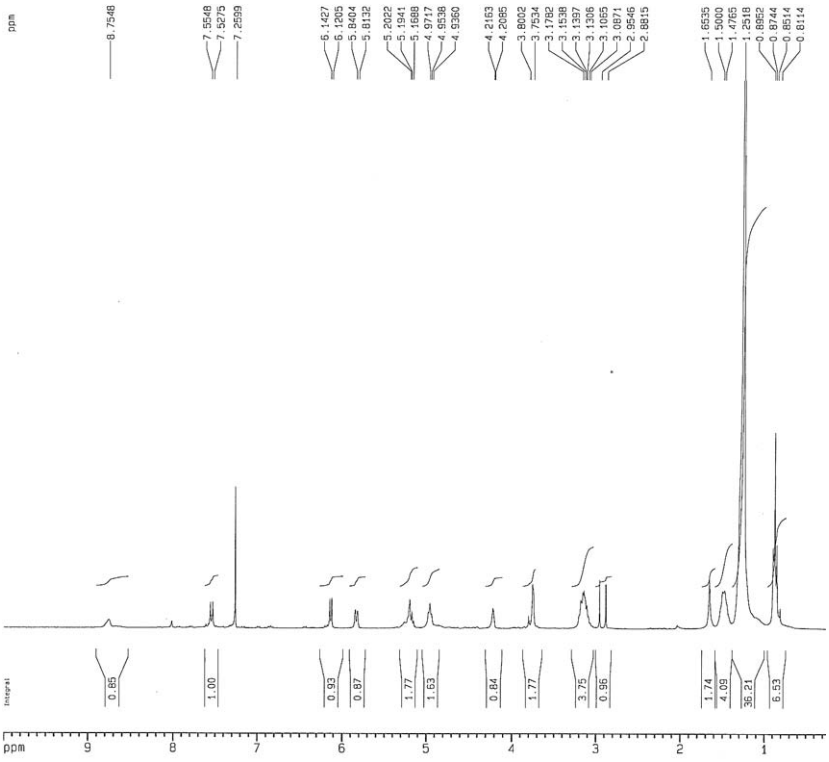


^{1}C NMR



2',3'-Di-*O*-dodecylcarbamyl-5'-azido-5'-deoxyuridine (4b)

^{13}H NMR



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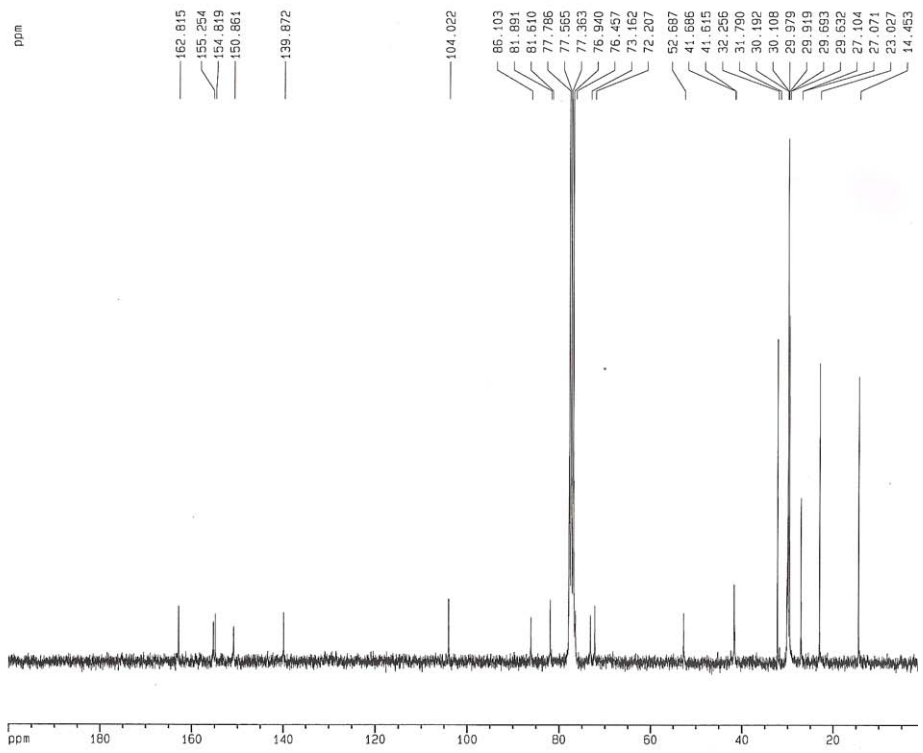
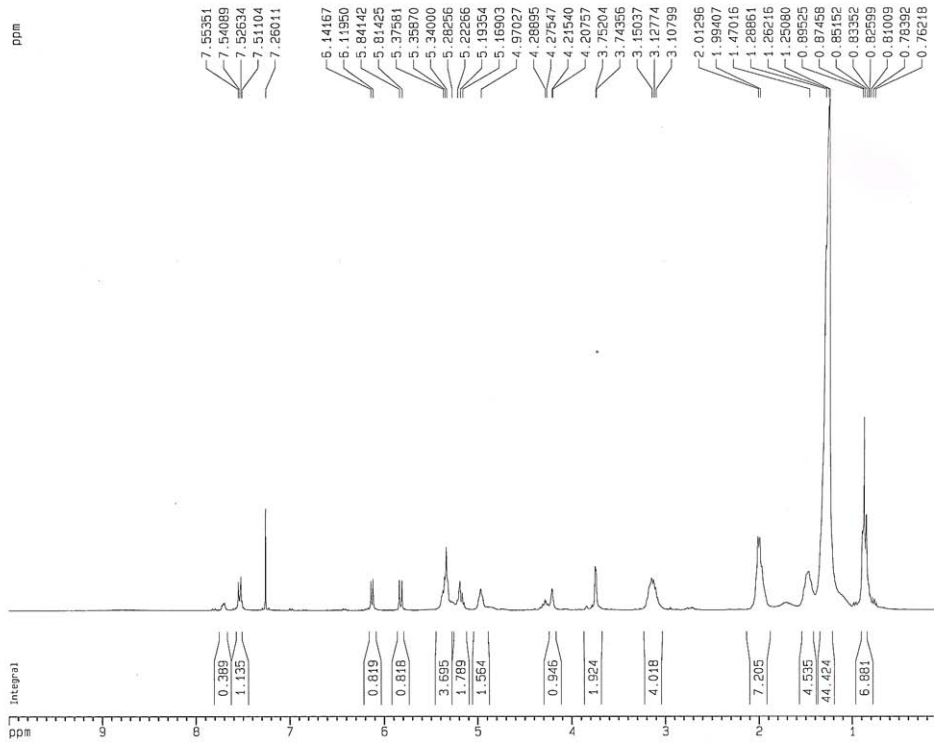
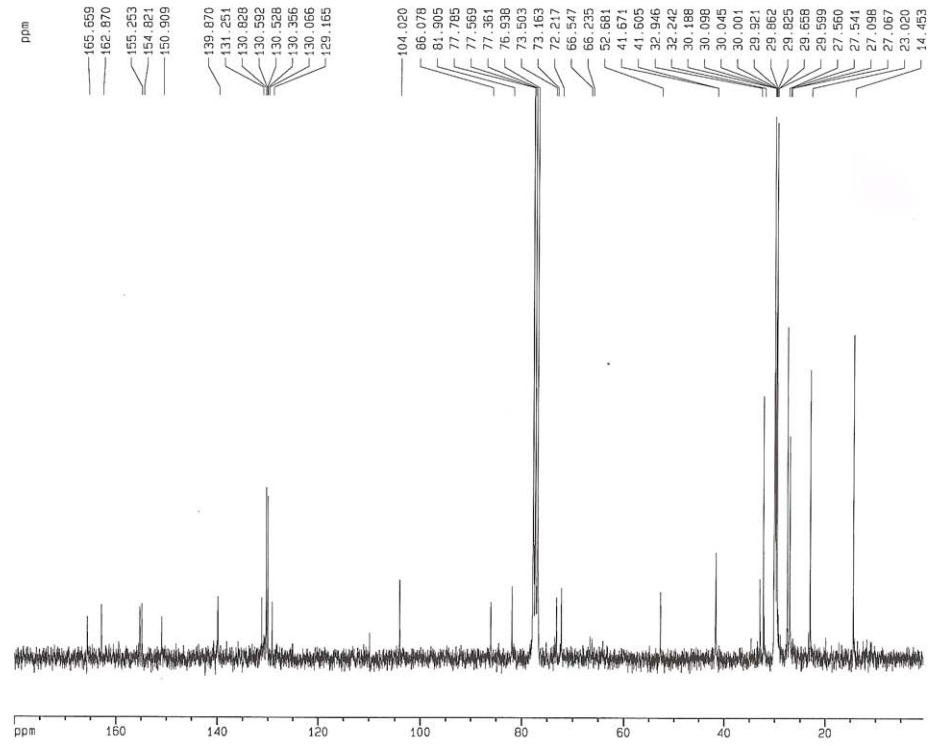


2',3'-Di-*O*-oleylcarbamyl-5'-azido-5'-deoxyuridine (4c)

^{13}H NMR

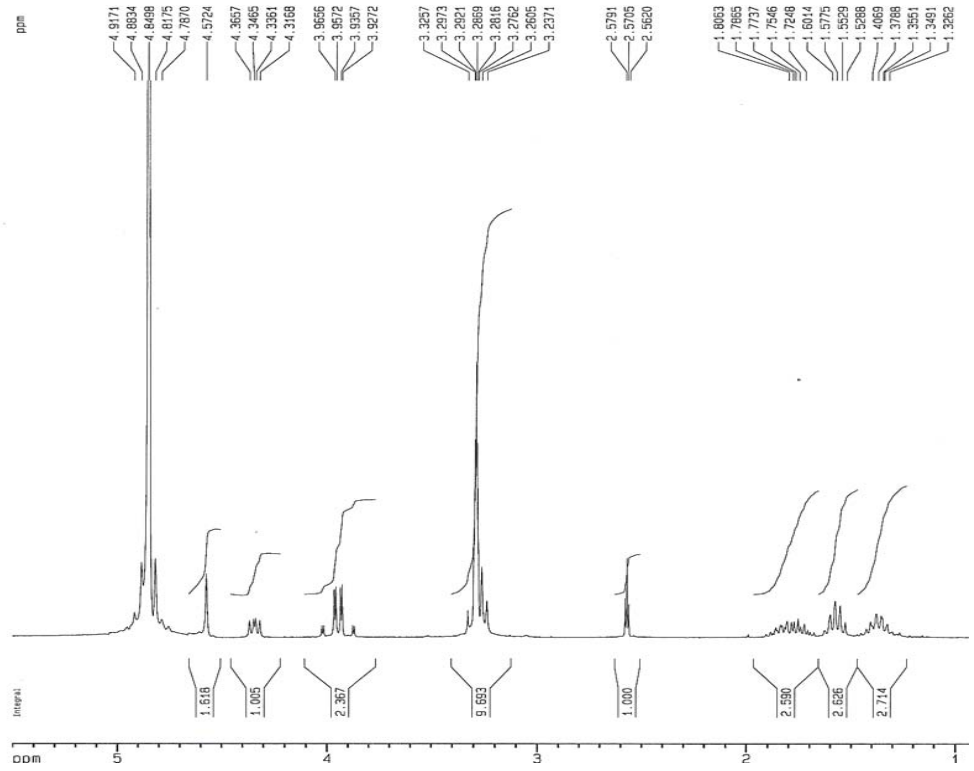


^{1}C NMR

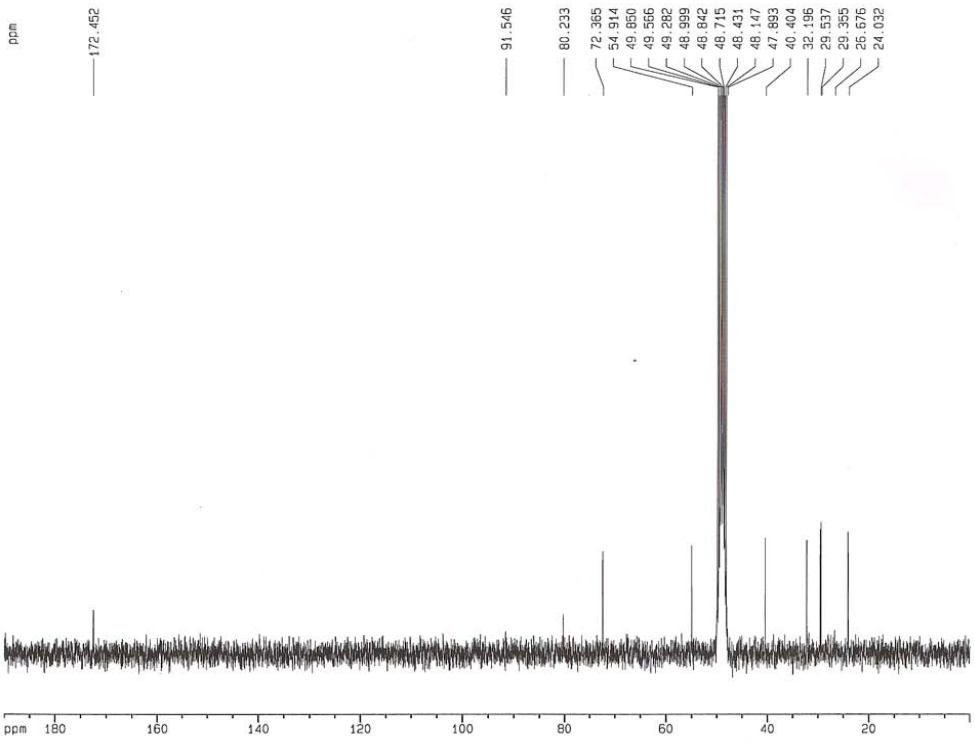


N_α, N_ε-bis(trifluoroacetyl)-L-lysine-*N*-propargylamide (5)

^{13}H NMR

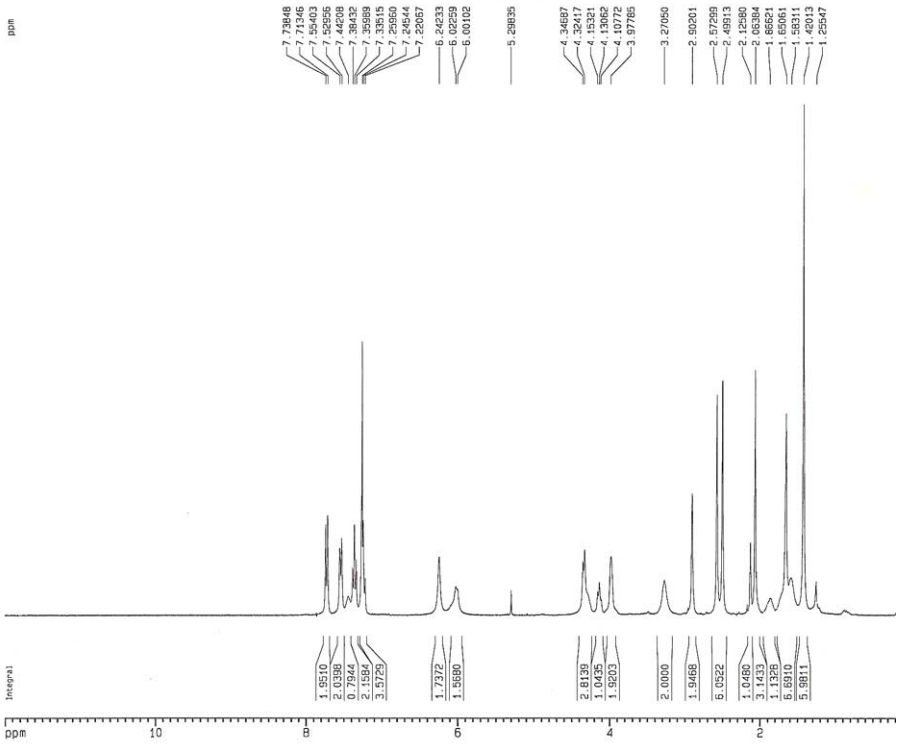


^{1}C NMR

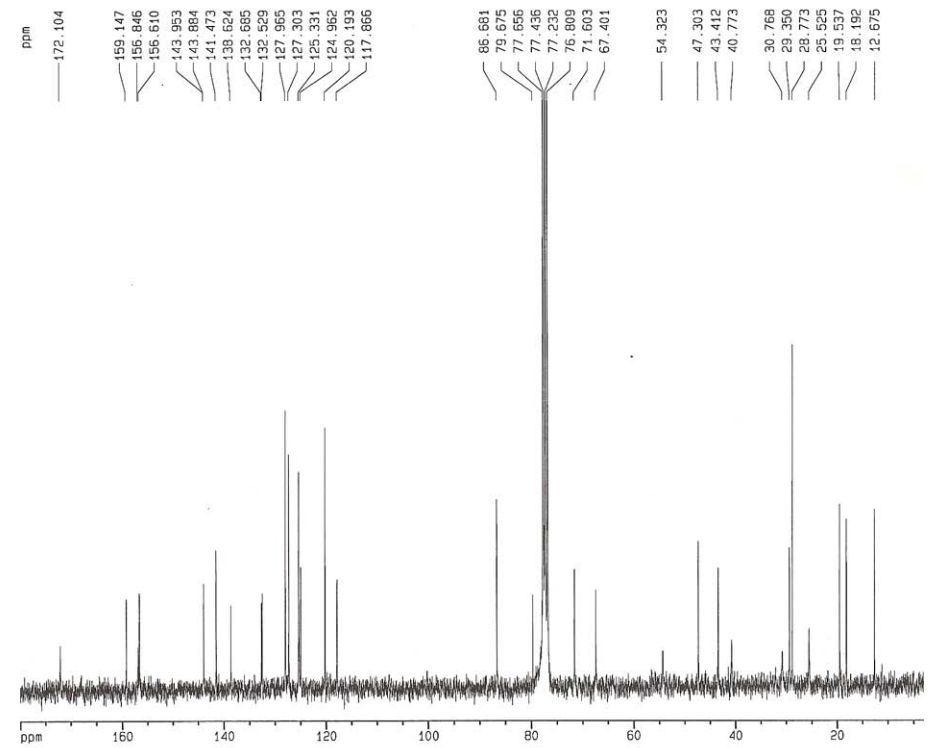


N_{α}-Fmoc-N_{ω}-Pbf-L-arginine-*N*-propargylamide (6)

^{13}H NMR

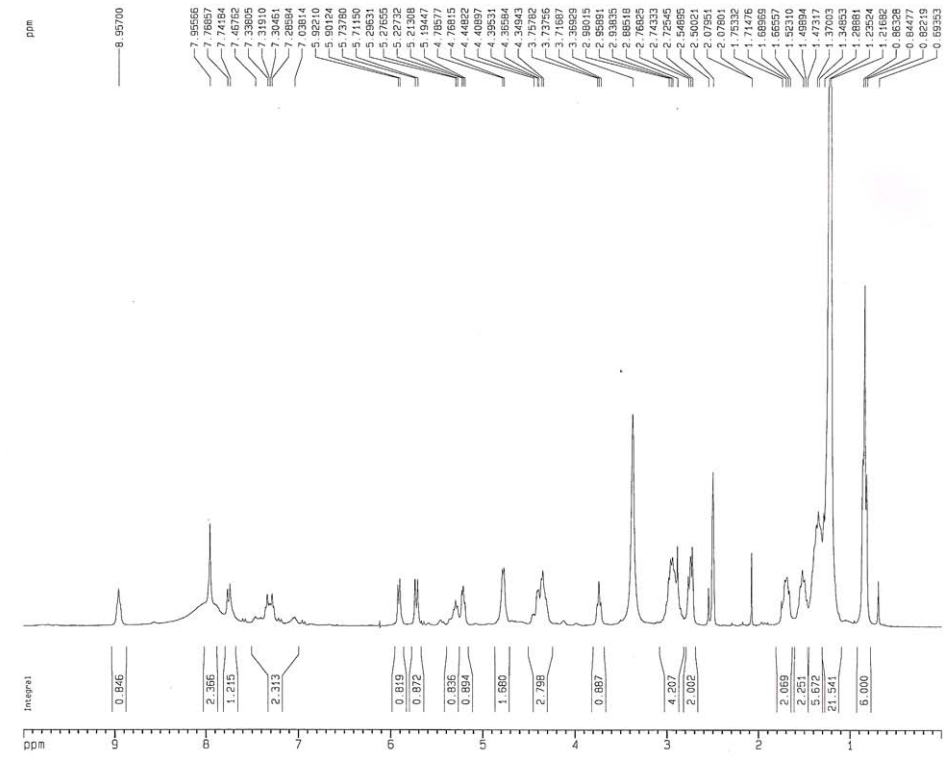


^{1}C NMR

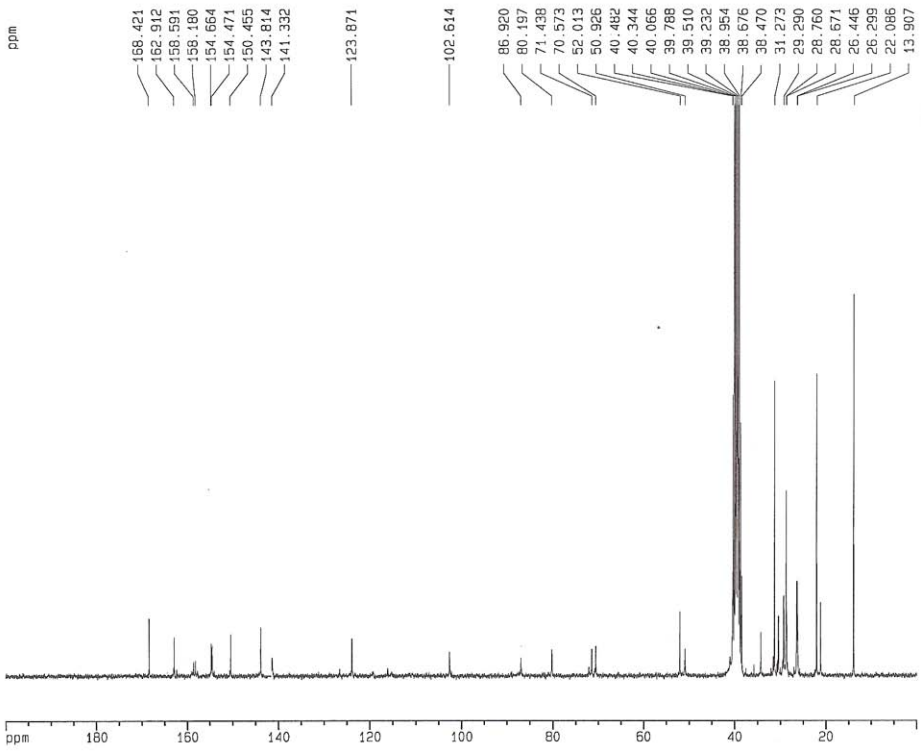


2',3'-Di-*O*-(octylcarbamyl)-5'-deoxy-5'-(4-lysylaminomethyl-*1H*-1,2,3-triazol-1-yl)uridine (7a)

^{13}H NMR

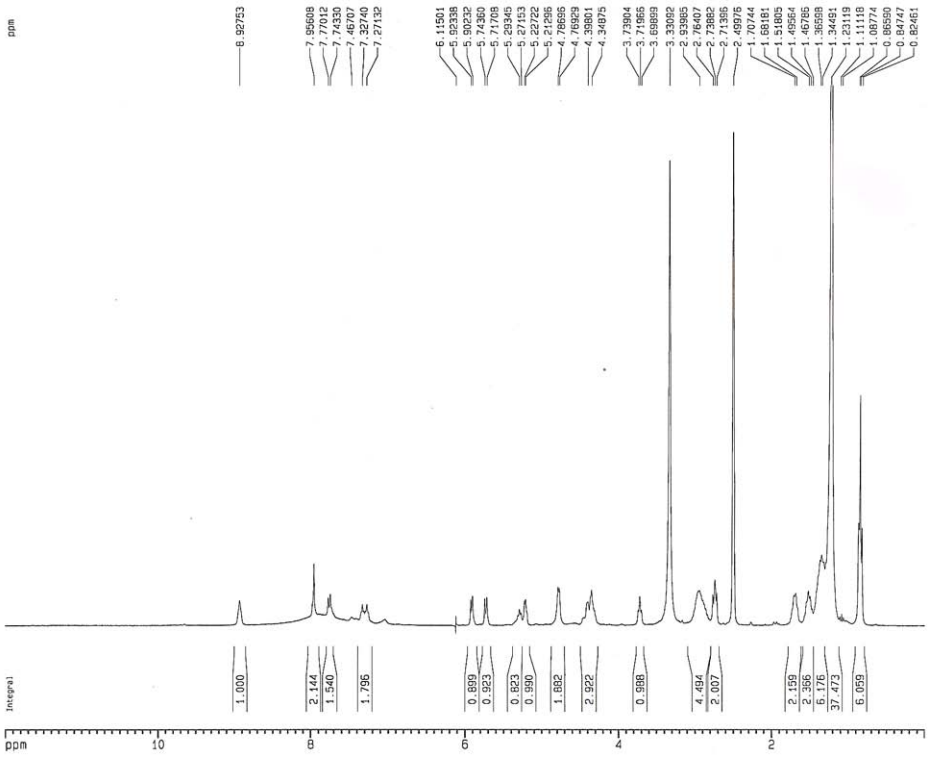


^{1}C NMR

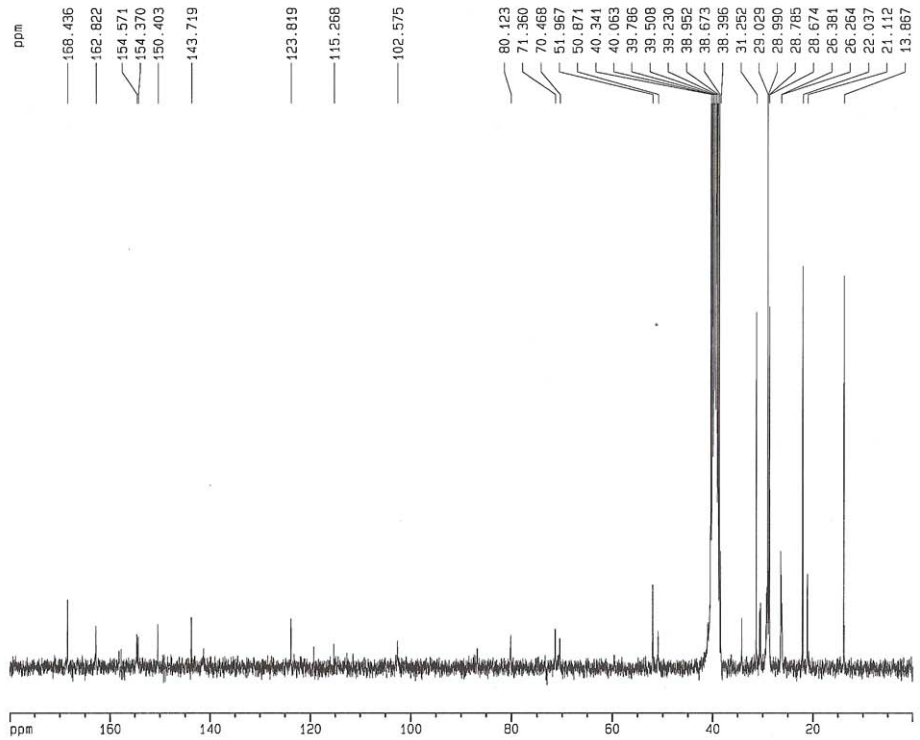


2',3'-Di-*O*-(dodecylcarbamyl)-5'-deoxy-5'-(4-lysylaminomethyl-*1H*-1,2,3-triazol-1-yl)uridine (7b)

^{13}H NMR

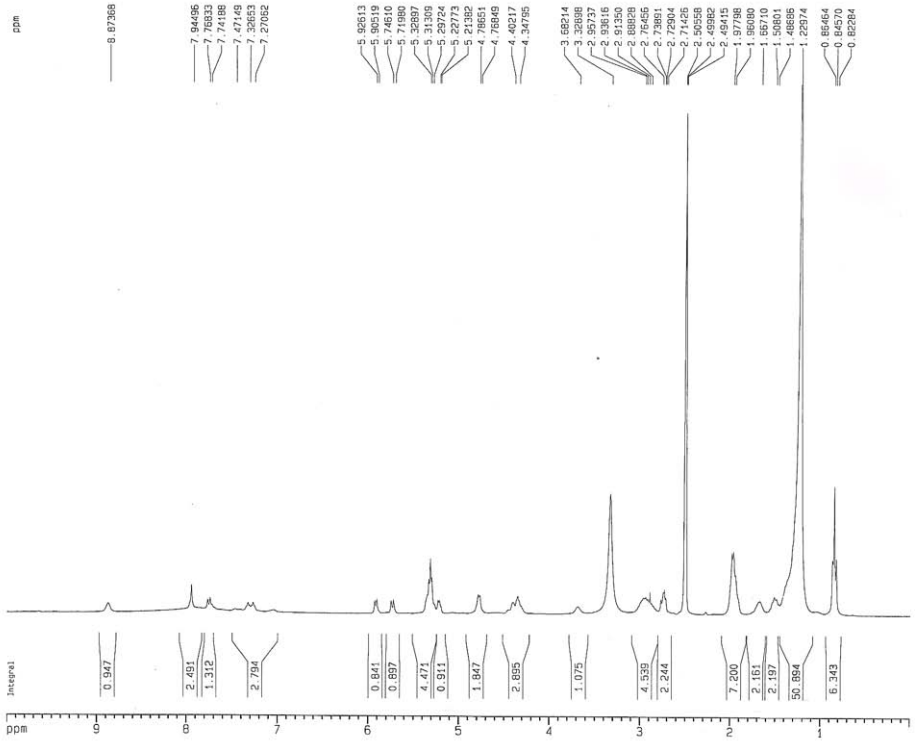


^{1}C NMR

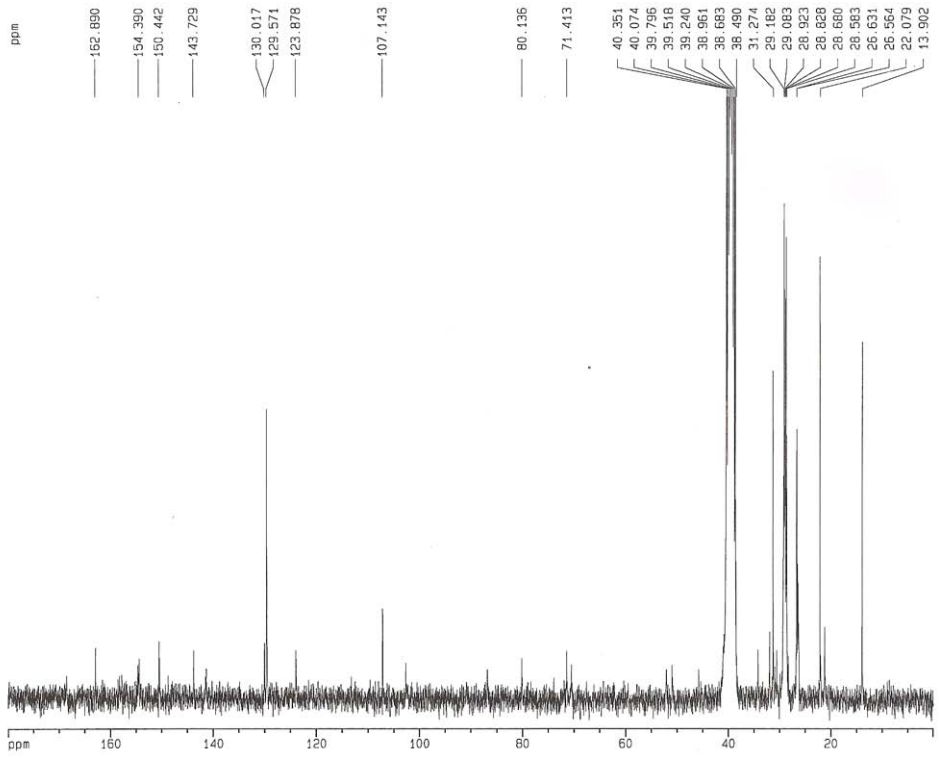


2',3'-Di-*O*-(oleylcarbamyl)-5'-deoxy-5'-(4-lysylaminomethyl-*1H*-1,2,3-triazol-1-yl)uridine (7c)

^{13}H NMR

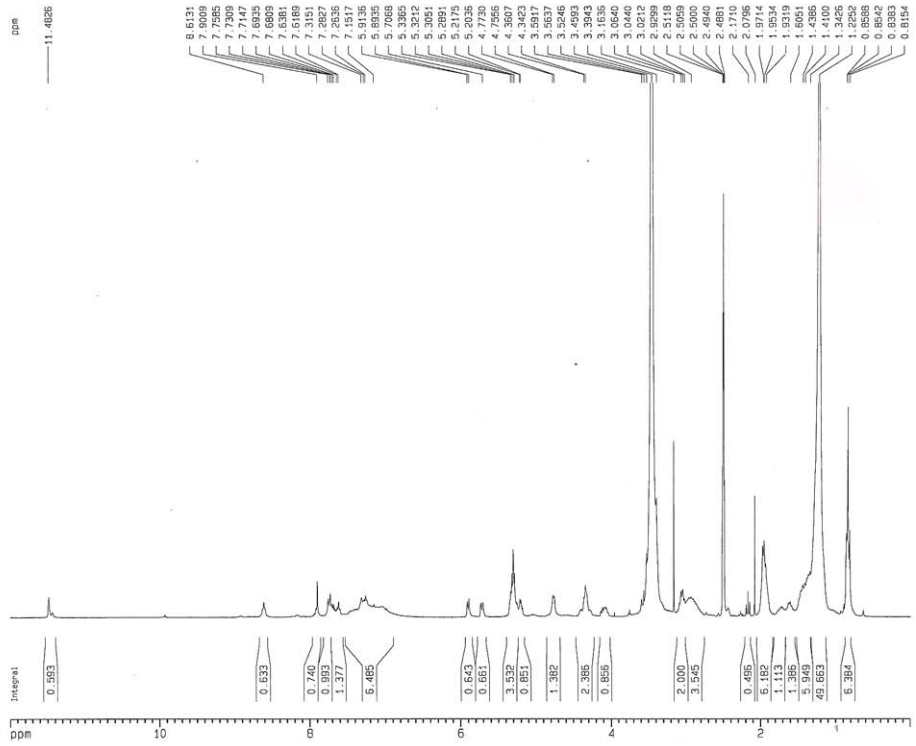


^{1}C NMR

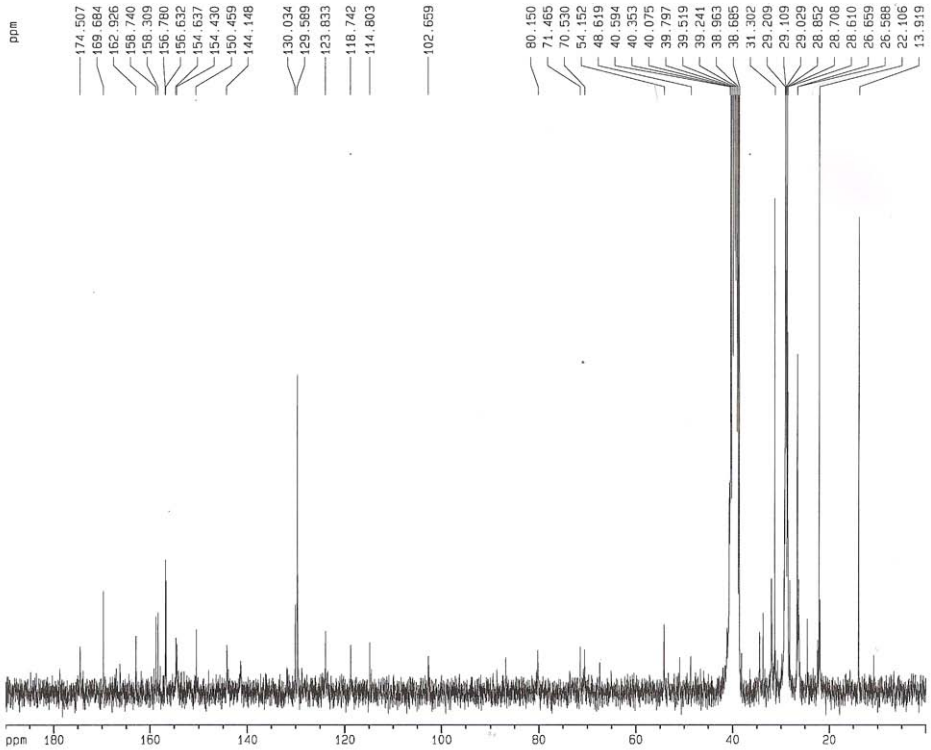


2',3'-Di-*O*-(oleylcarbamyl)-5'-deoxy-5'-(4-(*2S*-2,6-diguanidinohexanyl)aminomethyl-*1H*-1,2,3-triazol-1-yl)uridine (8)

^{13}H NMR

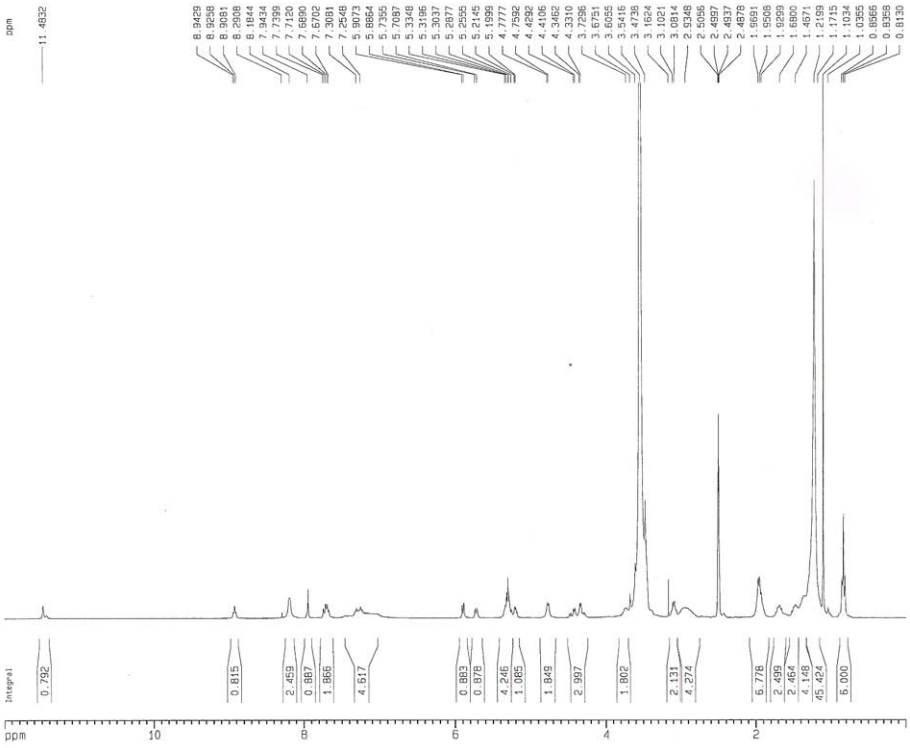


^{1}C NMR

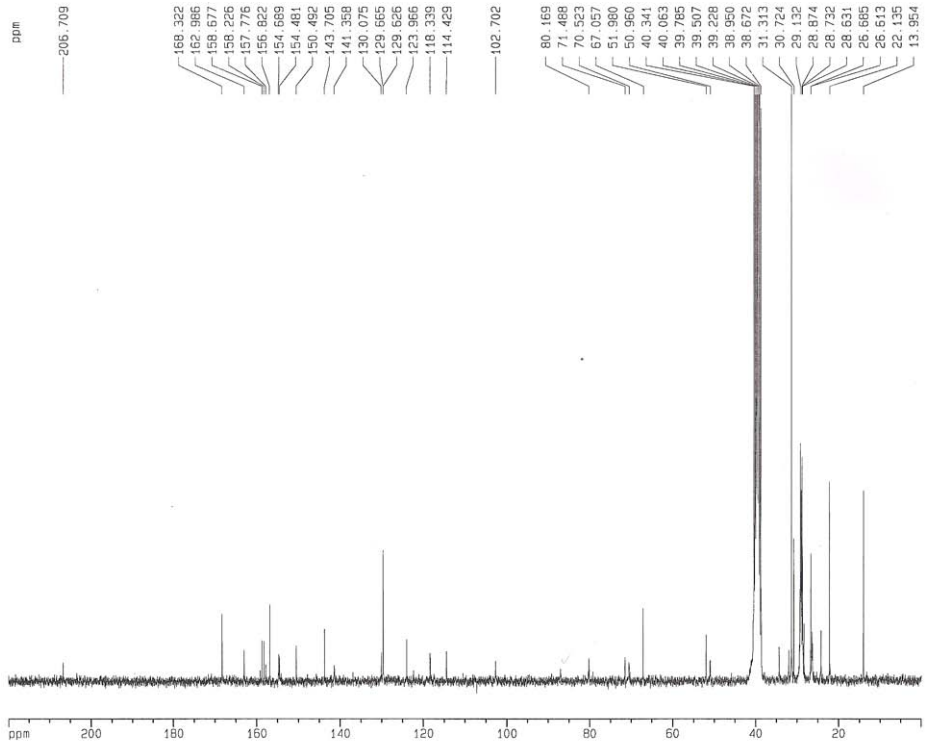


2',3'-Di-O-(oleylcarbamyl)-5'-deoxy-5'-(4-arginylaminomethyl-1H-1,2,3-triazol-1-yl)uridine (9)

^{13}H NMR



^{1}C NMR



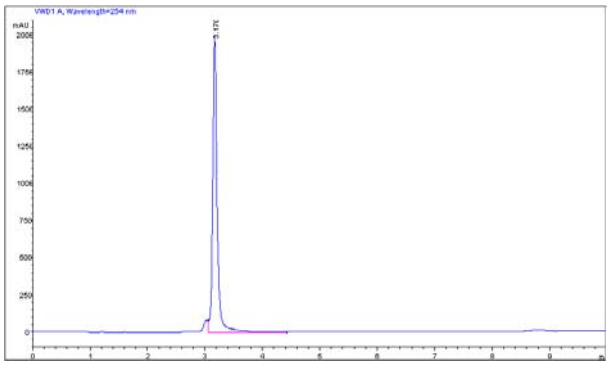
Synthesis of RNAs

The activator was a 0.25 M solution of 5-(2,4-bistrifluoromethylphenyl)-*1H*-tetrazole in anhydrous MeCN (Proligo, L8300212). The synthesized RNAs (1 μmol) were cleaved from the solid support, CPG, through treatment with a mixture of 41% aqueous methylamine and 33% ethanolic methylamine (3:1, v/v; 1.5 mL) for 4 h at room temperature. The resulting solution was filtered, lyophilized and then treated sequentially with 1-methyl-2-pyrrolidone (120 μL), anhydrous Et_3N (60 μL), and triethylamine trihydrofluoride (80 μL) for 2 h at 65 °C to desilylate the 2´-OTBDMS group. After quenching with TBE (Tris/borate/EDTA) buffer (600 μL), the oligonucleotide sample was directly applied to the HPLC (Vydac® C18 monomeric 120 Å, 10 x 250 mm). The gradient of the HPLC mobile phase was then increased linearly over 25 min from 5% MeCN /0.1 M triethylammonium acetate (TEAA, pH 7.2) buffer to 50% MeCN /0.1 M TEAA (pH 7.2) buffer at a flow rate of 3 mL/min. The fractions containing the purified RNA were treated with acidic NaOAc solution for 5 h at room temperature. The resulting solution was cooled at –20 °C overnight and then centrifuged at 3500 rpm for 1 h at 4 °C. The supernatant was discarded and the remaining RNA pellets were redissolved in distilled water (1 mL) and desalted using an NAP-10 column (GE Healthcare, 17-0854-02). The structures of the synthesized RNAs were confirmed using MALDI-TOF mass spectrometry. The confirmed sense and antisense strands were dissolved in the annealing buffer [100 mM KOAc, 30 mM HEPES-KOH, 2 mM $Mg(OAc)_2$; pH 7.4], heated at 90 °C for 5 min, slowly cooled to 37 °C, and then incubated at 37 °C for 1 h; **Antisense strand of VEGF-siRNAs** (5´-GAUCUCAUCAGGGUAC-UCCdTdT), 6603.9 (calcd. 6606.2); **sense strand of VEGF-N** (5´-GGAGUACCCUGAUGAGAUCdTdT), 6714.7 (calcd. 6709.2).

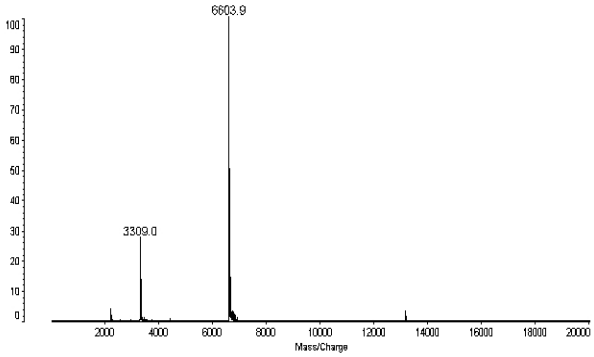
HPLC profiles and MALDI-MS spectra

Antisense strand

HPLC

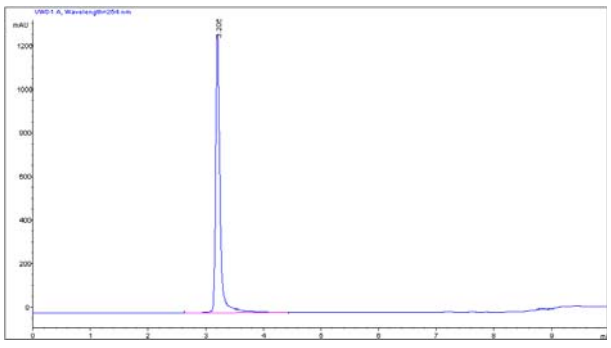


MALDI-MS

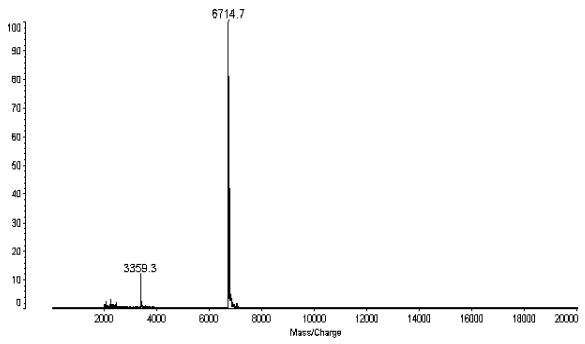


Sense strand

HPLC



MALDI-MS



The gradient of the HPLC (Agilent Ecilpse XDB-C8 5 μm, 4.6 x 150 mm) mobile phase was then increased linearly over 8 min from 5% MeCN /0.1 M triethylammonium acetate (TEAA, pH 7.2) buffer to 40% MeCN /0.1 M TEAA (pH 7.2) buffer at a flow rate of 1.5 mL/min.