Contents

Supplementary Figures

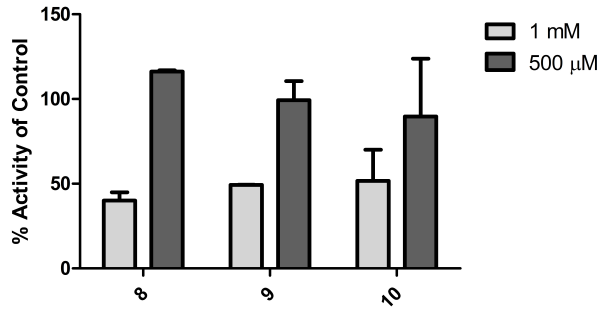


Figure S1. Biological evaluation of compounds **8**-**10** at 1 mM (light grey) and 500 μM (dark grey) in the PglC activity assay, using the radioactivity-based extraction assay. Data represented are obtained in duplicate. Error bars indicate mean ± SD.

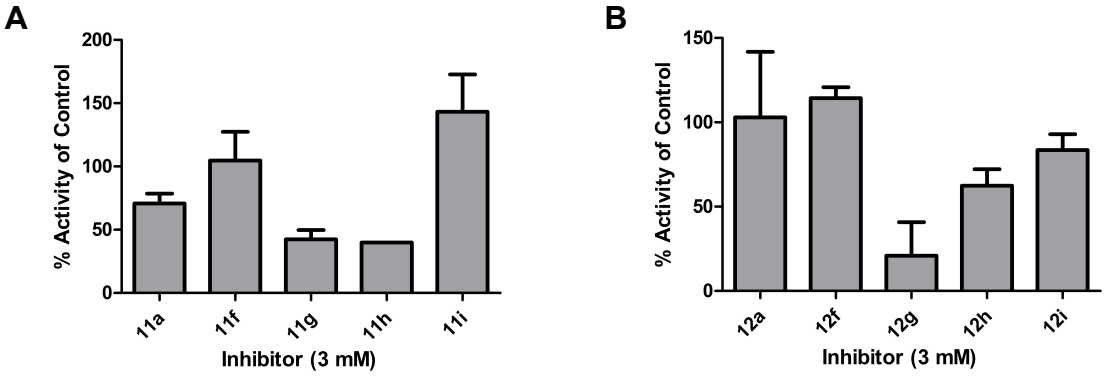
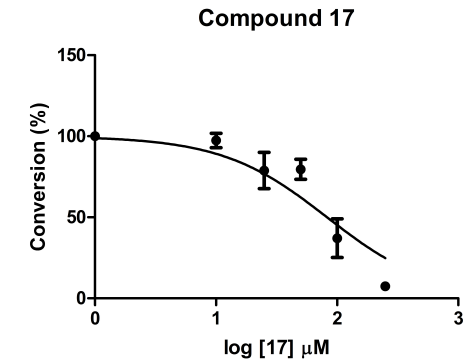
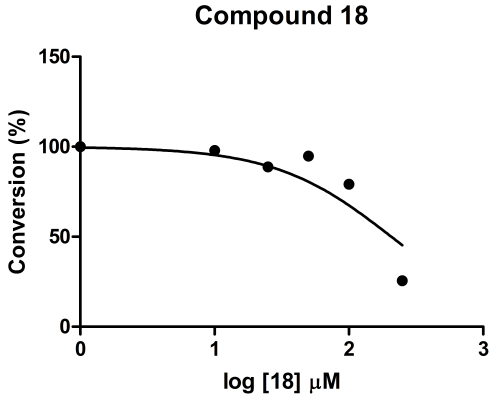
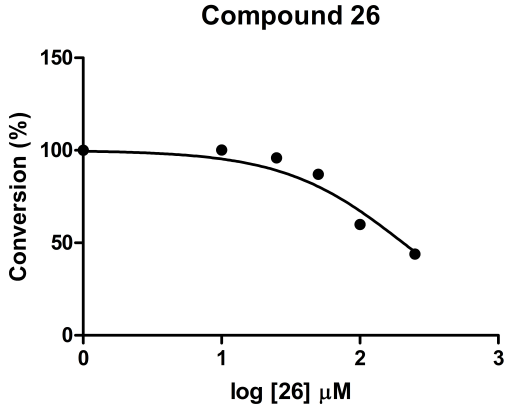


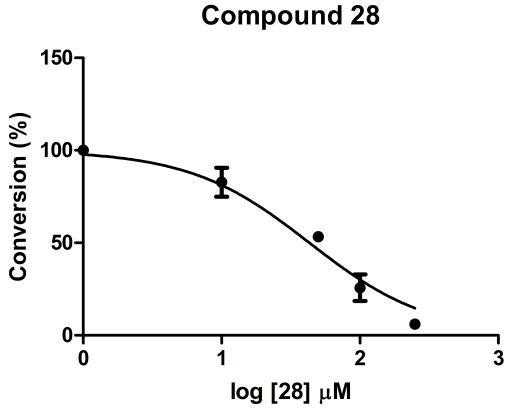
Figure S2. Biological evaluation of clicked products based on compounds **11** (A) and **12** (B) in a PglC activity assay using the radioactivity-based extraction assay. Data represented are obtained in duplicate. Error bars indicate mean ± SD.

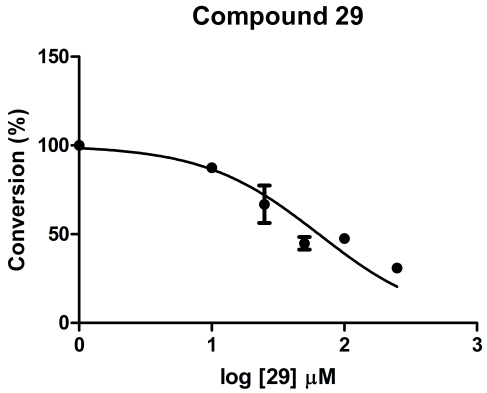
IC_{50} curves (generated using GraphPad Prism)

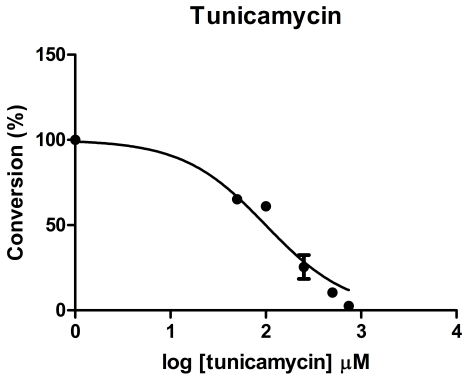




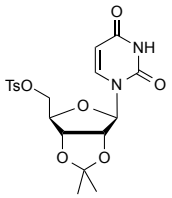




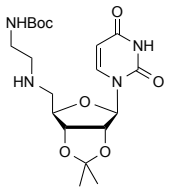




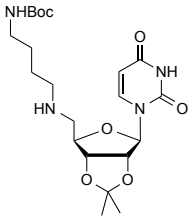
Experimental details



2',3'-*O*-isopropylidene-5'-*O*-(*p*-toluenesulfonyl)uridine (1). 2',3'-*O*-isopropylidene uridine[1] (1.0 g, 3.52 mmol) was dissolved in DCM/pyridine (20 mL, 3/1, v/v) under a nitrogen atmosphere. *p*-Toluenesulfonyl chloride (1.34 g, 7.04 mmol) and 4-dimethylaminopyridine (43 mg, 0.35 mmol) were added, and the resulting solution was stirred at RT for 24 h. The reaction was quenched by the addition of methanol (2 mL), diluted with DCM and washed with sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated and the title compound was isolated after flash column chromatography (silica gel, 33% hexane in EtOAc) as a colorless foam (Yield: 1.37 g, 3.12 mmol, 89%). The analytical data were in accord with those previously reported.[2]

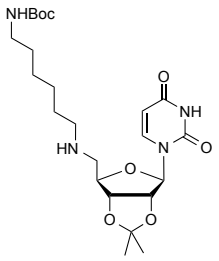


5'-*N*-(*tert*-butyl *N*-[2-aminoethyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (2). Tosyl uridine **1** (200 mg, 0.46 mmol) was dissolved in dry THF (2 mL) and treated with *N*-Boc-ethylenediamine (0.14 mL, 0.91 mmol) and K_2CO_3 (0.13 g, 0.91 mmol). The resulting suspension was stirred for 7 days, after which time the mixture was diluted with DCM and washed with H_2O (2x). The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 10% MeOH in DCM) to afford the title compound as a colorless foam (Yield: 151 mg, 0.35 mmol, 78%). TLC: R_f = 0.38 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.21 (d, 1H, *J* = 7.4 Hz, H-6) 7.05 (s, 1H, NH), 5.72 (d, 1H, *J* = 7.6 Hz, H-5), 5.56 (s, 1H, NH), 5.19 (d, 1H, *J* = 3.9 Hz, H-1'), 4.95 (dd, 1H, *J* = 1.8, 6.5 Hz, H-3'), 4.90 (dd, 1H, *J* = 4.6, 6.4 Hz, H-2'), 4.28 (d, 1H, *J* = 1.3 Hz, H-4'), 3.90 (dd, 1H, *J* = 1.8, 12.2 Hz, H-5'), 3.85 (d, 1H, *J* = 11.4 Hz, H-5'), 3.42 – 3.55 (m, 2H, CH_2), 3.20 – 3.33 (m, 2H, CH_2), 1.52 (s, 3H, CH_3 iPr), 1.32 (s, 9H, CH_3 tBu), 1.29 (s, 3H, CH_3 iPr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.9 (C=O Boc), 156.7 (C-4), 153.2 (C-2), 141.1 (C-6), 114.9 (C_q iPr), 106.0 (C-5), 99.0 (C-1'), 85.6 (C-4'), 81.9, 80.7 (C-2', C-3'), 79.3 (C_q tBu), 61.0 (C-5'), 41.1, 39.9 (CH_2), 28.4 (CH_3 tBu), 27.3, 25.2 (CH_3 iPr); LC: R_t = 6.36 min; ESI-MS: m/z = 427.13 ($M+H^+$).

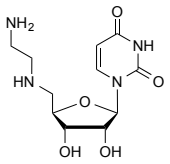


5'-*N*-(*tert*-butyl *N*-[4-aminobutyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (3). Tosyl uridine **1** (110 mg, 0.25 mmol) was dissolved in dry THF (2 mL) and treated with *N*-Boc-1,4-butanediamine (94 mg, 0.50 mmol) and K_2CO_3 (138 mg, 1.0 mmol). The resulting suspension was stirred for 3 days, after which time the mixture was diluted with DCM and washed with H_2O (2x). The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 8% MeOH in DCM) to afford the title compound as a colorless oil (Yield: 103 mg, 0.23 mmol, 91%). TLC: R_f = 0.37 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.18 (d, 1H, *J* = 7.7 Hz, H-6), 7.12 (t, 1H, *J* = 4.6 Hz, NH), 6.31 (bs, 1H, NH), 5.75 (d, 1H, *J* = 7.6 Hz, H-5), 5.30 (t, 1H, *J* = 5.0 Hz, NH), 5.17 (d, 1H, *J* = 4.8 Hz, H-1'), 4.97 (dd, 1H, *J* = 1.9, 6.6 Hz, H-3'), 4.92 (dd, 1H, *J* = 5.0, 6.4 Hz, H-2'), 4.30 (s, 1H, H-4'), 3.92 (d, 1H, *J* = 11.7 Hz, H-5'), 3.88 (dd, 1H, *J* = 1.3, 11.8 Hz, H-5'), 3.31 – 3.38 (m, 2H, CH_2), 2.98 – 3.07 (m, 2H, CH_2), 1.53 – 1.62 (m, 2H, CH_2), 1.54 (s, 3H, CH_3 iPr), 1.38 – 1.47 (m, 2H, CH_2), 1.38 (s, 9H, CH_3 tBu), 1.30 (s, 3H, CH_3 iPr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.0 (C=O Boc), 156.5 (C-4), 152.9 (C-2), 141.3 (C-6), 114.9 (C_q iPr), 105.9 (C-5), 99.5 (C-1'), 85.2 (C-4'), 81.2, 80.7 (C-2', C-3'), 79.2 (C_q tBu), 60.9 (C-5'), 40.8, 40.0 (CH_2), 28.4 (CH_3 tBu), 27.3 (CH_3 iPr), 27.2, 25.8 (CH_2), 25.1 (CH_3 iPr); LC: R_t = 6.65 min; ESI-MS: m/z = 455.13 ($M+H^+$).

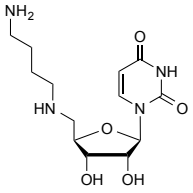
[1] A. M. Bello, E. Poduch, M. Fujihashi, M. Amani, Y. Li, I. Crandall, R. Hui, P. I. Lee, K. C. Kain, E. F. Pai, L. P. Kotra, *J. Med. Chem.* **2007**, *50*, 915-921.
[2] H. J. Korhonen, H. L. Bolt, D. R. W. Hodgson, *Beilstein J. Org. Chem.* **2015**, *11*, 469-472.



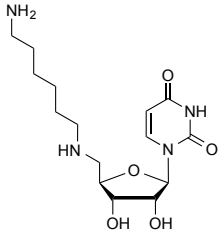
5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (4). Tosyl uridine **1** (200 mg, 0.46 mmol) was dissolved in dry THF (2 mL) and treated with *N*-Boc-1,6-hexanediamine (230 mg, 0.91 mmol) and K_2CO_3 (378 mg, 2.74 mmol). The resulting suspension was stirred for 7 days, after which time the mixture was diluted with DCM and washed with H_2O (2x). The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 8% MeOH in DCM) to afford the title compound as a colorless oil (Yield: 210 mg, 0.44 mmol, 96%). TLC: R_f = 0.46 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.27 (d, 1H, *J* = 7.8 Hz, H-6), 6.76 (t, 1H, *J* = 4.8 Hz, NH), 6.81 (t, 1H, *J* = 4.6 Hz, NH), 6.33 (bs, 1H, NH), 5.69 (d, 1H, *J* = 7.6 Hz, H-5), 5.18 (d, 1H, *J* = 4.8 Hz, H-1'), 4.97 (bs, 1H, NH), 4.95 (dd, 1H, *J* = 1.8, 6.4 Hz, H-3'), 4.85 (dd, 1H, *J* = 5.1, 6.0 Hz, H-2'), 4.31 (d, 1H, *J* = 1.3 Hz, H-4'), 3.87 (s, 2H, H-5'), 3.22 – 3.38 (m, 2H, CH_2), 2.94 – 3.02 (m, 2H, CH_2), 1.52 (s, 3H, CH_3 iPr), 1.46 – 1.54 (m, 2H, CH_2), 1.35 (s, 9H, CH_3 tBu), 1.32 – 1.40 (m, 2H, CH_2), 1.28 (s, 3H, CH_3 iPr), 1.18 – 1.25 (m, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.1 (C=O Boc), 156.2 (C-4), 152.9 (C-2), 140.6 (C-6), 114.7 (C_q iPr), 105.6 (C-5), 98.7 (C-1'), 85.6 (C-4'), 81.9, 81.1 (C-2', C-3'), 79.0 (C_q tBu), 61.1 (C-5'), 41.5, 40.4, 29.7, 28.5 (CH_2), 28.4 (CH_3 tBu), 27.3 (CH_3 iPr), 26.4, 26.2 (CH_2), 25.2 (CH_3 iPr); LC: R_t = 7.48 min; ESI-MS: m/z = 483.20 ($M+H^+$).



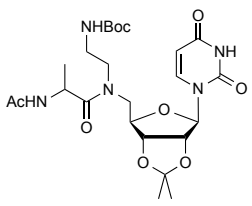
5'-*N*-(2-aminoethyl)-5'-amino-5'-deoxy-uridine (5). The title compound was obtained from compound **2** (43 mg, 0.10 mmol) using general procedure D (Yield: 28 mg, 0.10 mmol, quant.). ^{1}H NMR (D_2O, 400 MHz) δ 8.11 (d, 1H, *J* = 7.7 Hz, H-6), 6.28 (d, 1H, *J* = 7.7 Hz, H-5), 5.64 (d, 1H, *J* = 6.0 Hz, H-1'), 4.44 (dd, 1H, *J* = 6.1, 5.0 Hz, H-2'), 4.15 – 4.24 (m, 2H, H-3', H-4'), 3.65 – 3.84 (m, 4H, H-5', CH_2), 3.20 (t, 2H, *J* = 5.9 Hz, CH_2); ^{13}C NMR (D_2O, 100 MHz): δ 170.7 (C-4), 153.9 (C-2), 144.6 (C-6), 102.1 (C-5), 93.1 (C-1'), 86.7 (C4'), 73.1 (C-2'), 70.0 (C-3'), 60.5 (C-5'), 39.3, 38.4 (CH_2).



5'-*N*-(4-aminobutyl)-5'-amino-5'-deoxy-uridine (6). The title compound was obtained from compound **3** (53 mg, 0.12 mmol) using general procedure D (Yield: 36 mg, 0.12 mmol, quant.). ^{1}H NMR (D_2O, 400 MHz) δ 7.91 (d, 1H, *J* = 8.1 Hz, H-6), 6.08 (d, 1H, *J* = 8.0 Hz, H-5), 5.59 (d, 1H, *J* = 6.4 Hz, H-1'), 4.42 (dd, 1H, *J* = 6.4, 5.3 Hz, H-2'), 4.17 – 4.25 (m, 2H, H-3', H-4'), 3.78 (ddd, *J* = 2.8, 12.5 Hz, H-5'), 3.72 (dd, 1H, *J* = 3.1, 12.7 Hz, H-5'), 3.42 – 3.48 (m, 2H, CH_2), 2.90 – 2.96 (m, 2H, CH_2), 1.60 – 1.70 (m, 4H, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 164.2 (C-4), 151.4 (C-2), 143.1 (C-6), 103.8 (C-5), 93.5 (C-1'), 87.0 (C-4'), 72.9 (C-2'), 70.2 (C-3'), 60.5 (C-5'), 41.9, 38.9, 24.5, 23.9 (CH_2).

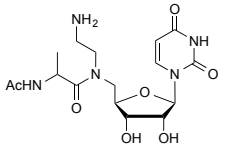


5'-*N*-(6-aminohexyl)-5'-amino-5'-deoxy-uridine (7). The title compound was obtained from compound **4** (63 mg, 0.13 mmol) using general procedure D (Yield: 44 mg, 0.13 mmol, quant.). ^{1}H NMR (D_2O, 400 MHz) δ 7.86 (dd, 1H, *J* = 3.9, 8.2 Hz, H-6), 6.02 (dd, 1H, *J* = 4.3, 8.1 Hz, H-5), 5.55 (dd, 1H, *J* = 4.2, 6.5 Hz, H-1'), 4.35 – 4.44 (m, 1H, H-2'), 4.13 – 4.23 (m, 2H, H-3', H-4'), 3.68 – 3.79 (m, 2H, H-5'), 3.32 – 3.42 (m, 2H, CH_2), 2.82 – 2.89 (m, 2H, CH_2), 1.48 – 1.60 (m, 4H, CH_2), 1.23 – 1.32 (m, 4H, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 163.1 (C-4), 150.9 (C-2), 142.9 (C-6), 103.9 (C-5), 93.7 (C-1'), 87.1 (C-4'), 72.8 (C-2'), 70.3 (C-3'), 60.5 (C-5'), 42.8, 39.3, 27.1, 26.8, 25.3, 25.1 (CH_2).

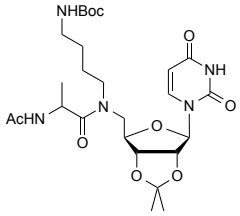


5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(*tert*-butyl *N*-[2-aminoethyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (30). Ac-L-Ala-OH (81 mg, 0.62 mmol) in DMF (300 µL) was pre-activated with EDC·HCl (119 mg, 0.62 mmol) and HOBt (95 mg, 0.62 mmol) for 15 min at RT, followed by addition of the mixture to compound **2** (88 mg, 0.21 mmol) in DMF (200 µL). The resulting mixture was stirred for 6 days, after which time the mixture was diluted with DCM, washed with H_2O (4x), dried over

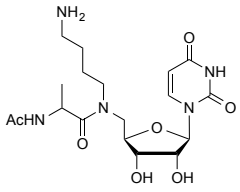
Na_2SO_4 and concentrated *in vacuo*. Flash column purification (silica gel, 15% MeOH in DCM) yielded the title compound as a colorless oil (Yield: 60 mg, 0.11 mmol, 54%). Due to epimerization and rotamerization, the ^1H and ^{13}C spectra were difficult to interpret. Based on the purity by LC-MS, compound **30** was used in the next step, and fully characterized thereafter. TLC: R_f = 0.40 (DCM/MeOH, 9/1, v/v); LC: R_t = 6.78 min; ESI-MS: m/z = 540.27 ($M+H^+$).



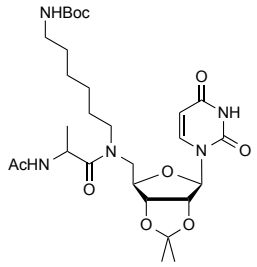
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(2-aminoethyl)-5'-amino-5'-deoxy-uridine (8). Compound **30** (23 mg, 43 μmol) was deprotected using general procedure D to give the product as white solids (Yield: 16.7 mg, 42 μmol, quant., A : B = 0.3 : 1). ^1H NMR (D_2O, 600 MHz) δ 8.02 (d, 0.3H, J = 7.7 Hz, H-6_A), 7.94 (dd, 1H, J = 4.8, 7.7 Hz, H-6_B), 6.14 – 6.22 (m, 1.3H, H-5_A, H-5_B), 5.61 (dd, 1H, J = 1.9, 5.8 Hz, H-1'$_B$), 5.53 (d, 0.3H, J = 6.3 Hz, H-1'$_A$), 4.20 – 4.36 (m, 4.3H, H-2'$_A$, H-2'$_B$, H-4'$_B$, H-5'$_B$), 4.05 – 4.18 (m, 2.9H, H-3'$_A$, H-3'$_B$, H-4'$_A$, CH-Ala_A, CH-Ala_B), 3.62 – 3.68 (m, 3.2H, H-5'$_A$, CH_2), 3.09 – 3.13 (m, 2.6H, CH_2), 1.83 (s, 0.9H, CH_3 NHAc-$_A$), 1.80 (s, 3H, CH_3 NHAc-$_B$), 1.19 – 1.24 (m, 3H, CH_3-Ala_B), 1.13 – 1.18 (m, 0.9H, CH_3-Ala_A); ^{13}C NMR (D_2O, 150 MHz) δ 174.2, 174.1, 173.9, 173.8 (C=O), 171.1 (C-4_B), 170.9 (C-4_A), 154.2 (C-2_B), 154.0 (C-2_A), 144.4 (C-6_A), 142.5 (C-6_B), 102.7 (C-5_B), 102.3 (C-5_A), 93.0 (C-1'$_A$), 91.1 (C-1'$_B$), 86.6 (C-4'$_A$), 83.7 (C-4'$_B$), 74.3 (C-2'$_B$), 73.0 (C-2'$_A$), 70.2 (C-3'$_B$), 70.0 (C-3'$_A$), 64.3 (C-5'$_B$), 60.5 (C-$_5$'A), 48.8 (CH-Ala_B), 48.6 (CH-Ala_A), 39.3, 39.2, 38.5 (CH_2), 21.4, 21.3 (CH_3 NHAc), 16.0, 15.7 (CH_3 Ala); LC: R_t = 1.96 min; ESI-MS: m/z = 400.27 ($M+H^+$).



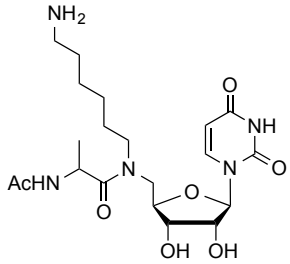
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(*tert*-butyl *N*-[4-aminobutyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (31). Ac-L-Ala-OH (74 mg, 0.56 mmol) in DMF (300 μL) was pre-activated with EDC·HCl (109 mg, 0.56 mmol) and HOBt (87 mg, 0.56 mmol) for 15 min at RT, followed by addition of the mixture to compound **3** (86 mg, 0.19 mmol) in DMF (200 μL). The resulting mixture was stirred for 6 days, after which time the mixture was diluted with DCM, washed with H_2O (4x), dried over Na_2SO_4 and concentrated *in vacuo*. Flash column purification (silica gel, 8% MeOH in DCM) yielded the title compound as a colorless oil (40 mg, 70.5 μmol, 37%). Due to epimerization and rotamerization, the ^1H and ^{13}C spectra were difficult to interpret. Based on the purity by LC-MS, compound **31** was used in the next step, and fully characterized thereafter. TLC: R_f = 0.34 (DCM/MeOH, 9/1, v/v); LC: R_t = 6.71, 6.78 min; ESI-MS: m/z = 590.70 ($M+Na^+$).



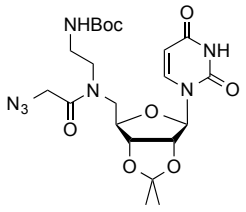
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(4-aminobutyl)-5'-amino-5'-deoxy-uridine (9). Compound **31** (14 mg, 24 μmol) was deprotected using general procedure D to give the product as white solids (Yield: 9.7 mg, 23 μmol, quant., A : B = 1 : 0.14). ^1H NMR (D_2O, 600 MHz) major species: δ 7.87 (dd, 1H, J = 8.1, 9.8 Hz, H-6), 6.11 (d, 1H, J = 8.1 Hz, H-5), 5.66 (dd, 1H, J = 1.9, 5.9 Hz, H-1'), 4.38 – 4.42 (m, 2H, H-2', H-3'), 4.36 (q, 1H, J = 4.36 Hz, CH Ala), 4.26 – 4.32 (m, 1H, H-4'), 4.18 – 4.24 (m, 2H, H-5'), 3.41 – 3.48 (m, 2H, CH_2), 2.89 (m, 2.94 (m, 2H, CH_2), 1.87 (s, 3H, CH_3 NHAc), 1.60 – 1.69 (m, 4H, CH_2), 1.26 (t, 3H, J = 7.4 Hz, CH_3 Ala); ^{13}C NMR (D_2O, 150 MHz) major species: δ 174.2, 173.9 (C=O), 164.4 (C-4), 151.8 (C-2), 141.0 (C-6), 104.0 (C-5), 91.3 (C-1'), 84.2 (C-4'), 74.4 (C-2'), 70.3 (C-3'), 64.4 (C-5'), 48.8 (CH Ala), 42.0, 38.9, 24.5, 23.9 (CH_2), 21.3 (CH_3 NHAc), 15.7 (CH_3 Ala); LC: R_t = 1.65 min; ESI-MS: m/z = 450.33 ($M+Na^+$).



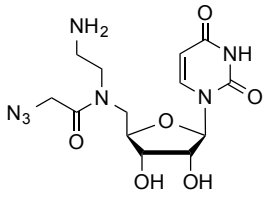
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (32). Ac-L-Ala-OH (72 mg, 0.55 mmol) in DMF (300 µL) was pre-activated with EDC·HCl (86 mg, 0.55 mmol) and HOBt (69 mg, 0.55 mmol) for 15 min at RT, followed by addition of the mixture to compound **4** (72 mg, 0.15 mmol) in DMF (200 µL). The resulting mixture was stirred for 6 days, after which time the mixture was diluted with DCM, washed with H_2O (4x), dried over Na_2SO_4 and concentrated *in vacuo*. Flash column purification (silica gel, 10% MeOH in DCM) yielded the title compound as a colorless oil (Yield: 66 mg, 0.11 mmol, 74%). Due to epimerization and rotamerization, the ^1H and ^{13}C spectra were difficult to interpret. Based on the purity by LC-MS, compound **32** was used in the next step, and fully characterized thereafter. TLC: R_f = 0.34 (DCM/MeOH, 9/1, v/v); LC: R_t = 7.36, 7.43 min; ESI-MS: m/z = 618.33 ($M+Na^+$).



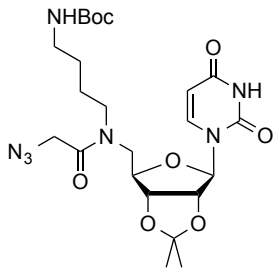
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(6-aminohexyl)-5'-amino-5'-deoxy- uridine (10). Compound **32** (16 mg, 27 µmol) was deprotected using general procedure D to give the product as white solids (Yield: 12.3 mg, 27 µmol, quant., A : B = 1 : 0.13). ^1H NMR (D_2O, 600 MHz) major species: δ 7.84 (dd, 1H, *J* = 8.2, 9.6 Hz, H-6), 6.07 (d, 1H, *J* = 8.1 Hz, H-5), 5.65 (dd, 1H, *J* = 2.2, 5.8 Hz, H-1'), 4.37 – 4.44 (m, 2H, H-2', H-3'), 4.34 – 4.37 (m, 1H, CH Ala), 4.26 – 4.31 (m, 1H, H-5'), 4.18 – 4.24 (m, 3H, H-4', H-5'), 3.37 – 4.43 (m, 2H, CH_2), 2.84 – 2.89 (m, 2H, CH_2), 1.87 (s, 3H, CH_3 NHAc), 1.52 – 1.63 (m, 4H, CH_2), 1.23 – 1.34 (m, 7H, CH_2, CH_3 Ala); ^{13}C NMR (D_2O, 150 MHz) major species: δ 174.2, 173.9 (C=O), 163.3 (C-4), 151.3 (C-2), 140.7 (C-6), 104.3 (C-5), 91.4 (C-1'), 84.3 (C-4'), 74.4 (C-2'), 70.4 (C-3'), 64.4 (C-5'), 48.8 (CH Ala), 42.7, 39.3, 27.1, 26.5, 25.2, 25.2 (CH_2), 21.3 (CH_3 NHAc), 15.7 (CH_3 Ala); LC: R_t = 1.80 min; ESI-MS: m/z = 456.40 ($M+H^+$).



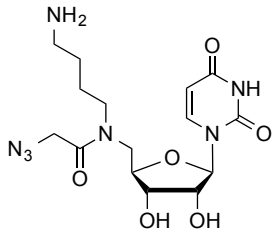
5'-*N*-azidoacetyl-5'-*N*-(*tert*-butyl *N*-[2-aminoethyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (33). Compound **2** (40 mg, 94 µmol) was dissolved in dry DMF (800 µL) and treated with chloroacetic anhydride (32 mg, 187 µmol) and pyridine (15 µL, 187 µmol) for 20 min at RT. The mixture was diluted with DCM and quenched by the addition of aq. $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The intermediate was re-dissolved in DMF (800 µL) and treated with NaN_3 overnight at RT. The mixture was diluted with DCM and washed with sat. aq. NaCl. The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and the product was purified using flash column chromatography (silica gel, 5% MeOH in DCM) (Yield: 48 mg, 93 µmol, quant.). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37 (d, 1H, *J* = 7.8 Hz, H-6), 5.91 (d, 1H, *J* = 7.5 Hz, H-5), 5.59 (d, 1H, *J* = 3.9 Hz, H-1'), 5.52 (bs, 1H, NH), 4.89 (dd, 1H, *J* = 4.0, 6.7 Hz, H-3'), 4.81 (dd, *J* = 6.7, 2.7 Hz, H-2'), 4.48 – 455 (m, 3H, H-4', H-5'), 3.99 (s, 2H, CH_2-N_3), 3.45 – 3.62 (m, 2H, CH_2), 3.25 – 3.44 (m, 2H, CH_2), 1.65 (s, 3H, CH_3 iPr), 1.43 (s, 9H, CH_3 tBu), 1.40 (s, 3H, CH_3 iPr); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.9 (C=O), 93.8 (C-1'), 83.7 (C-4'), 82.5, 80.4 (C-2', C-3'), 79.5 (C_q tBu), 64.4 (C-5'), 50.3 (CH_2-N_3), 28.4 (CH_3 tBu), 27.0, 25.3 (CH_3 iPr); LC: R_t = 8.11 min; ESI-MS: m/z = 510.20 ($M+H^+$).



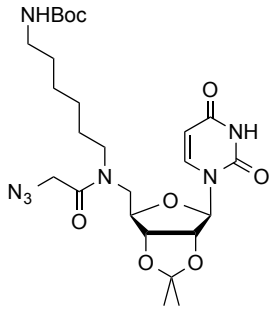
5'-*N*-azidoacetyl-5'-*N*-(2-aminoethyl)-5'-amino-5'-deoxy-uridine (11). Compound **33** (48 mg, 93 µmol) was deprotected using general procedure D to give the product as a colorless oil (Yield: 34 mg, 93 µmol, quant.). ^1H NMR (DMSO-d_6, 500 MHz): δ 8.02 (d, 1H, *J* = 7.7 Hz, H-6), 5.92 (d, 1H, *J* = 7.8 Hz, H-5), 5.58 (d, 1H, *J* = 5.8 Hz, H-1'), 4.68 (d, 1H, *J* = 5.3 Hz, H-2'), 4.15 – 4.20 (m, 2H, H-3', H-4'), 3.95 – 4.00 (m, 2H, H-5'), 3.97 (s, 2H, CH_2-N_3), 3.47 – 3.52 (m, 2H, CH_2), 2.94 – 3.05 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 171.2 (C=O), 169.7 (C-4), 155.9 (C-2), 142.5 (C-6), 105.2 (C-5), 95.6 (C-1'), 87.4 (C-4'), 74.7 (C-2'), 70.9 (C-3'), 61.6 (C-5'), 50.6 (CH_2-N_3), 39.5, 39.4 (CH_2); LC: R_t = 3.09 min; ESI-MS: m/z = 370.13 ($M+H^+$).



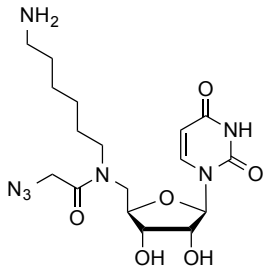
5'-*N*-azidoacetyl-5'-*N*-(*tert*-butyl *N*-[4-aminobutyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (34). Compound **3** (87 mg, 192 μmol) was dissolved in dry DMF (800 μL) and treated with chloroacetic anhydride (65 mg, 383 μmol) and pyridine (31μL, 383 μmol) for 20 min at RT. The mixture was diluted with DCM and quenched by the addition of aq. $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The intermediate was re-dissolved in DMF (800 μL) and treated with NaN_3 (37 mg, 569 μmol) overnight at RT. The mixture was diluted with DCM and washed with sat. aq. NaCl. The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and the product was purified using flash column chromatography (silica gel, 5% MeOH in DCM) (Yield: 97 mg, 181 μmol, 94%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35 (d, 1H, *J* = 3.9 Hz, H-6), 5.55 – 5.59 (m, 2H, H-5, NH), 5.53 (d, 1H, *J* = 3.9 Hz, H-1'), 4.55 – 4.58 (m, 3H, H-2', H-3', NH), 4.48 – 4.52 (m, 3H, H-4', H-5'), 3.94 (s, 2H, CH_2-N_3), 3.40 – 3.48 (m, 2H, CH_2), 3.05 – 3.15 (m, 2H, CH_2), 1.56 – 1.63 (m, 5H, CH_2, CH_3 iPr), 1.50 – 1.56 (m, 2H, CH_2), 1.41 (s, 9H, CH_3 tBu), 1.38 (s, 3H, CH_3 iPr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.8, 156.1 (C=O), 136.2 (C-6), 116.2 (C_q iPr), 106.3 (C-5), 93.6 (C-1'), 84.0 (C-4'), 82.5, 80.8 (C'-2, C-3'), 79.0 (C_q tBu), 64.5 (C-5'), 50.3 (CH_2-N_3), 41.8, 40.1 (CH_2), 28.4 (CH_3 tBu), 27.5 (CH_2), 27.0 (CH_3 iPr), 26.1 (CH_2), 25.2 (CH_3 iPr); LC: R_t = 7.93 min; ESI-MS: m/z = 538.13 ($M+H^+$).



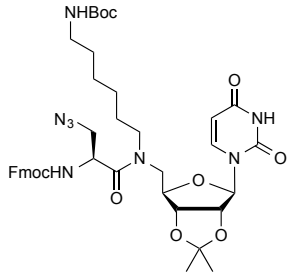
5'-*N*-azidoacetyl-5'-*N*-(4-aminobutyl)-5'-amino-5'-deoxy-uridine (12). Compound **34** (79 mg, 148 μmol) was deprotected using general procedure D to give the product as a colorless oil (Yield: 57 mg, 146 μmol, quant.). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.06 (d, 1H, *J* = 7.8 Hz, H-6), 6.02 (d, 1H, *J* = 7.7 Hz, H-5), 5.57 (d, 1H, *J* = 6.4 Hz, H-1'), 4.68 (d, 1H, *J* = 5.3 Hz, H-2'), 4.16 – 4.20 (m, 1H, H-3'), 4.01 – 4.04 (m, 1H, H-4'), 3.97 (s, 2H, CH_2-N_3), 3.62 – 3.66 (m, 2H, H-5'), 3.27 – 3.33 (m, 2H, CH_2), 2.75 – 2.85 (m, 2H, CH_2), 1.50 – 1.58 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 171.2 (C=O), 169.6 (C-4), 153.6 (C-2), 144.4 (C-6), 104.1 (C-5), 94.0 (C-1'), 87.9 (C-4'), 73.9 (C-2'), 71.1 (C-3'), 61.7 (C-5'), 50.6 (CH_2-N_3), 41.4, 39.6, 26.4, 25.4 (CH_2); LC: R_t = 2.32 min; ESI-MS: m/z = 398.20 ($M+H^+$).



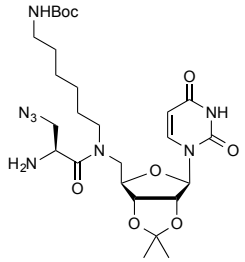
5'-*N*-azidoacetyl-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (35). Compound **4** (112 mg, 232 μmol) was dissolved in dry DMF (1 mL) and treated with chloroacetic anhydride (80 mg, 464 μmol) and pyridine (37 μL, 464 μmol) for 20 min at RT. The mixture was diluted with DCM and quenched by the addition of aq. $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The intermediate was re-dissolved in DMF (1 mL) and treated with NaN_3 (45 mg, 692 μmol) overnight at RT. The mixture was diluted with DCM and washed with sat. aq. NaCl. The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and the product was purified using flash column chromatography (silica gel, 5% MeOH in DCM) (Yield: 129 mg, 230 μmol, quant.). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.23 – 7.30 (m, 1H, H-6), 5.74 (d, *J* = 7.7 Hz, H-5), 5.66 (bs, 1H, NH), 5.43 (d, 1H, *J* = 4.0 Hz, H-1'), 4.83 (bs, 1H, NH), 4.72 – 4.77 (m, 1H, H-3'), 4.66 – 4.72 (m, 1H, H-2'), 4.45 – 4.50 (m, 1H, H-4'), 4.32 – 4.44 (m, 2H, H-5'), 3.87 (s, 2H, CH_2-N_3), 3.29 – 3.38 (m, 2H, CH_2), 2.93 – 3.05 (m, 2H, CH_2), 1.44 – 1.55 (m, 5H, CH_2, CH_3 iPr), 1.20 – 1.40 (m, 18H, CH_2, CH_3 iPr, CH_3 tBu); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.8, 156.0 (C=O), 135.9 (C-6), 115.8 (C_q iPr), 106.4 (C-5), 93.4 (C-1'), 84.0 (C-4'), 82.4, 80.8 (C-2', C-3'), 78.7 (C_q tBu), 64.5 (C-5'), 50.2 (CH_2-N_3), 41.8, 40.2, 29.9, 28.7 (CH_2), 28.3 (CH_3 tBu), 27.0 (CH_3 iPr), 26.3, 26.2 (CH_2), 25.1 (CH_3 iPr); LC: R_t = 9.49 min; ESI-MS: m/z = 565.33 ($M+H^+$).



5'-*N*-azidoacetyl-5'-*N*-(6-aminohexyl)-5'-amino-5'-deoxy-uridine (13). Compound **35** (119 mg, 211 μmol) was deprotected using general procedure D to give the product as a colorless oil (Yield: 89 mg, 208 μmol, quant.). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.15 (d, 1H, J = 7.7 Hz, H-6), 6.11 (d, 1H, J = 7.6 Hz, H-5), 5.62 (d, 1H, J = 6.3 Hz, H-1'), 4.20 – 4.23 (m, 1H, H-2'), 4.02 – 4.05 (m, 2H, H-3', H-4'), 3.97 (s, 2H, CH_2-N_3), 3.64 (d, 2H, J = 2.4 Hz, H-5'), 3.30 – 3.40 (m, 2H, CH_2), 2.70 – 2.80 (m, 2H, CH_2), 1.47 – 1.55 (m, 4H, CH_2), 1.24 – 1.35 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 171.2 (C=O), 169.5 (C-4), 153.5 (C-2), 145.2 (C-6), 103.3 (C-5), 94.3 (C-1'), 88.2 (C-4'), 74.0 (C-2'), 71.1 (C-3'), 61.6 (C-5'), 50.6 (CH_2-N_3), 42.6, 39.9, 29.0, 28.0, 26.7, 26.5 (CH_2); LC: R_t = 1.64 min; ESI-MS: m/z = 425.47 ($M+H^+$).

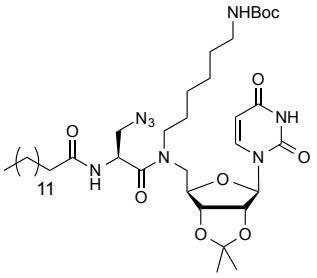


5'-*N*-(*N*-[9-fluorenylmethoxycarbonyl]-3-azido-L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (14). Fmoc-L-Aza-OH (119 mg, 338 μmol) was dissolved in DMF (250 μL) and pre-activated by the addition of EDC·HCl (69 mg, 360 μmol) and HOBt (55 mg, 360 μmol) and the solution was stirred for 10 min at RT. The mixture was subsequently added to a solution of compound **4** in DMF (200 μL), and the resulting solution was stirred overnight. The mixture was diluted with DCM and washed with H_2O (4x), dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (silica gel, 4% MeOH in DCM) afforded the pure title compound as a colorless oil (Yield: 82 mg, 101 μmol, 45%). TLC: R_f = 0.53 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67 (d, 2H, J = 7.6 Hz, CH_{arom}), 7.51 (d, 2H, J = 7.4 Hz, CH_{arom}), 7.31 (t, 3H, J = 7.9 Hz, CH_{arom}, H-6), 7.18 – 7.25 (m, 2H, CH_{arom}), 6.28 (d, 1H, J = 6.0 Hz, NH), 5.79 (d, 1H, J = 7.3 Hz, H-5), 5.35 (bs, 1H, NH), 5.24 (d, 1H, J = 3.5 Hz, H-1'), 4.64 – 4.75 (m, 2H, H-2', H-3'), 4.34 – 4.49 (m, 4H, H-4', CH_2 Fmoc, C*H*H Aza), 4.25 – 4.32 (m, 2H, CH Aza, CH*H* Aza), 4.12 (t, 1H, J = 6.9 Hz, CH Fmoc), 3.64 – 3.75 (m, 2H, H-5'), 3.25 – 3.37 (m, 2H, CH_2), 2.94 – 3.03 (m, 2H, CH_2), 1.52 (s, 3H, CH_3 iPr), 1.42 – 1.50 (m, 2H, CH_2), 1.35 (s, 9H, CH_3 tBu), 1.30 – 1.40 (m, 2H, CH_2), 1.28 (s, 3H, CH_3 iPr), 1.16 – 1.28 (m, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1 (C=O), 169.5 (C-4), 156.0, 155.9 (C=O), 152.5 (C-2), 143.8, 143.5 (C_q, C-6), 141.3 (C_q), 135.7, 127.8, 127.2, 125.1, 120.0 (CH_{arom}), 116.2 (C_q iPr), 106.8 (C-5), 93.3 (C-1'), 83.9 (C-2'), 82.3 (C-4'), 80.9 (C_q tBu), 79.0 (C-3'), 67.4 (CH_2 Fmoc), 64.5 (C-5'), 54.0, 52.0 (CH Aza, CH_2 Aza), 47.0 (CH Fmoc), 41.9, 40.3, 30.0, 28.8 (CH_2), 28.4 (CH_3 tBu), 27.1 (CH_3 iPr), 26.5, 26.3 (CH_2), 25.2 (CH_3 iPr); LC: R_t = 10.19 min; ESI-MS: m/z = 817.33 ($M+H^+$).

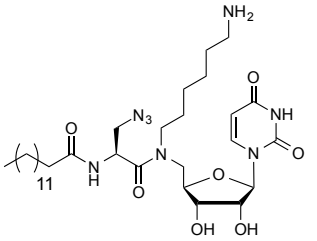


5'-*N*-(3-azido-L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (15). Compound **14** (35 mg, 43 μmol) was dissolved in DCM (3 mL) and treated with immobilized piperazine (430 mg, 430-860 μmol[3]) for 24 h, after which time the solution was filtered through a syringe filter and concentrated at RT. The resulting amine was directly used in the next step without further purification. TLC: R_f = 0.29 (DCM/MeOH, 9/1, v/v + 1% Et_3N); LC: R_t = 7.32 min; ESI-MS: m/z = 595.27 ($M+H^+$).

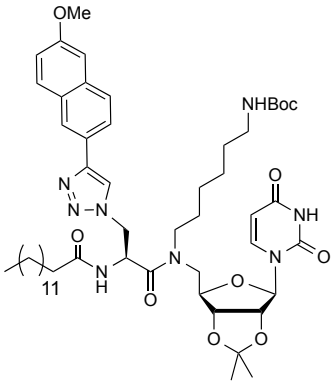
[3] The extent of labeling of commercially available immobilized piperazine is reported to range between 1-2 mmol/g.



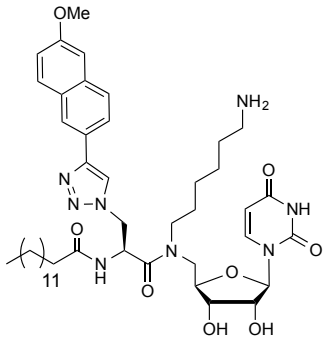
5'-*N*-(*N*-myristoyl-3-azido-L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (16). Myristic acid (20 mg, 86 μmol) was dissolved in DMF (250 μL) and stirred with EDC·HCl (16.4 mg, 86 μmol) and HOBt (13 mg, 86 μmol) at RT for 15 min. This solution was added to the crude amine **15** (~43 μmol) in DMF (350 μL) and the resulting solution was stirred until complete consumption of the starting material was observed using LC-MS (2 h). The mixture was diluted with DCM, washed with H_2O (4x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 10% MeOH in DCM) afforded the title compound as a colorless oil (Yield: 16.7 mg, 21 μmol, 48% over two steps). TLC: R_f = 0.50 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.32 (d, 1H, *J* = 7.7 Hz, H-6), 6.86 (bs, 1H, NH), 5.84 (d, 1H, *J* = 6.7 Hz, H-5), 5.45 (bs, 1H, NH), 5.31 (s, 1H, H-1'), 4.68 – 4.75 (m, 2H, H-2', H-3'), 4.65 (dt, 1H, *J* = 4.3, 7.1 Hz, CH Aza), 4.56 (bs, 1H, NH), 4.43 – 4.51 (m, 2H, H-4', H-5'), 4.35 – 4.42 (m, 1H, H-5'), 3.68 (d, 2H, *J* = 4.3 Hz, CH_2 Aza), 3.30 – 3.43 (m, 2H, CH_2), 2.97 – 3.09 (m, 2H, CH_2), 2.10 – 2.25 (m, 2H, CH_2), 1.57 (s, 3H, CH_3 iPr), 1.48 – 1.57 (m, 4H, CH_2), 1.37 (s, 9H, CH_3 tBu), 1.33 (s, 3H, CH_3 iPr), 1.27 – 1.43 (m, 6H, CH_2), 1.12 – 1.25 (m, 20H, CH_2), 0.81 (t, 3H, *J* = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.6, 171.2 (C=O), 169.7 (C-4), 156.1 (C-2), 136.1 (C-6), 116.2 (C_q iPr), 106.5 (C-5), 93.6 (C-1'), 83.9 (C-2' or C-3'), 82.5 (C-4'), 81.0 (C-2' or C-3'), 79.1 (C_q tBu), 64.4 (C-5'), 52.3, 51.9 (CH Aza, CH_2 Aza), 42.0 (CH_2), 40.3 (CH_2), 36.2, 31.9, 30.0, 29.6, 29.5, 29.4, 29.3, 28.4, 27.1, 26.5, 26.3, 25.5, 25.2 (CH_3, CH_3 tBu, CH_3 iPr), 22.7 (CH_2), 14.1 (CH_3); LC: R_t = 11.50 min; ESI-MS: m/z = 805.40 ($M+H^+$).



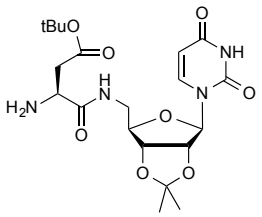
5'-*N*-(*N*-myristoyl-3-azido-L-alanine)-5'-*N*-(6-aminohexyl)-5'-amino-5'-deoxy uridine (17). Compound **16** (4.5 mg, 5.6 μmol) was deprotected according to general procedure D to give the title compound as a white solid (Yield: 4.2 mg, 5.35 μmol, 96%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.54 (d, 1H, *J* = 7.8 Hz, NH), 7.73 (d, 1H, *J* = 7.8 Hz, H-6), 5.94 (d, 1H, *J* = 7.6 Hz, H-5), 5.65 (d, 1H, *J* = 5.8 Hz, H-1'), 4.52 (dt, 1H, *J* = 7.1, 7.1 Hz, CH Aza), 4.30 (d, 2H, *J* = 3.7 Hz, H-5'), 4.16 – 4.20 (m, 1H, H-4'), 4.14 (t, 1H, *J* = 5.5 Hz, H-2'), 4.00 (dd, 1H, *J* = 4.0, 4.8 Hz, H-3'), 3.55 – 3.70 (m, 2H, CH_2 Aza), 3.30 – 3.36 (m, 2H, CH_2), 2.75 – 2.83 (m, 2H, CH_2), 2.12 – 2.17 (m, 2H, CH_2), 1.51 – 1.59 (m, 4H, CH_2), 1.44 – 1.51 (m, 2H, CH_2), 1.28 – 1.37 (m, 4H, CH_2), 1.18 – 1.27 (m, 20H, CH_2), 0.86 (t, 3H, *J* = 6.7 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 173.3, 170.1 (C=O), 169.1 (C-4), 153.3 (C-2), 140.3 (C-6), 105.0 (C-5), 90.0 (C-1'), 83.0 (C-4'), 74.2 (C-2'), 70.0 (C-3'), 65.0 (C-5'), 52.3 (CH Aza), 51.8 (CH_2 Aza), 41.5, 39.3, 35.4, 31.8, 29.5, 29.4, 29.3, 29.2, 29.0, 28.3, 27.3, 26.0, 25.7, 25.5, 22.6 (CH_2), 14.4 (CH_3); LC: R_t = 9.84 min; ESI-MS: m/z = 665.40 ($M+H^+$).



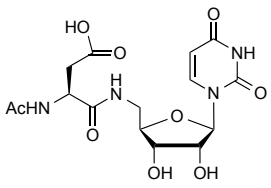
5'-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl]carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (36). Compound **16** (9.6 mg, 11.9 μmol) was converted to the title compound using general procedure C (Yield: 9.1 mg, 9.2 μmol, 77%). TLC: R_f = 0.46 (DCM/MeOH, 9/1, v/v); LC: R_t = 11.90 min; ESI-MS: m/z = 987.60 ($M+H^+$). Due to rotamerization, the ^{1}H and ^{13}C spectra were difficult to interpret. Based on the purity by LC-MS, compound **36** was used in the next step, and fully characterized thereafter.



5'-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alanine)-5'-*N*-(6-aminohexyl)-5'-amino-5'-deoxy uridine (18). Compound **36** (~9.2 μmol) was fully deprotected according to general procedure D to give the title compound as a slightly colored solid (Yield: 6.9 mg, 7.8 μmol, 85%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.54 (s, 1H, $CH_{triazole}$), 8.50 (d, 1H, J = 7.8 Hz, NH), 8.29 (s, 1H, CH_{arom}), 7.85 – 7.92 (m, 3H, CH_{arom}), 7.73 – 7.82 (m, 2H, NH), 7.52 (d, 1H, J = 7.8 Hz, H-6), 7.34 (d, 1H, J = 2.5 Hz, CH_{arom}), 7.15 – 7.20 (m, 2H, CH_{arom}, NH), 5.70 (d, 1H, J = 7.8 Hz, H-5), 5.52 – 5.62 (m, 3H, H-1', 2'-OH, 3'-OH), 4.88 – 4.93 (m, 1H, CH Aza), 4.86 (dd, 1H, J = 4.7, 14.0 Hz, C*H*H Aza), 4.75 (dd, 1H, J = 9.1, 13.9 Hz, CH*H* Aza), 4.29 – 4.36 (m, 2H, H-5'), 4.13 – 4.16 (m, 1H, H-4'), 4.08 – 4.12 (m, 1H, H-2'), 3.99 – 4.03 (m, 1H, H-3'), 3.89 (s, 3H, CH_3 OMe), 3.21 – 3.27 (m, 2H, CH_2), 2.75 – 2.83 (m, 2H, CH_2), 2.05 (t, 2H, J = 7.3 Hz, CH_2), 1.49 – 1.60 (m, 4H, CH_2), 1.28 – 1.40 (m, 4H, CH_2), 1.00 – 1.25 (m, 22H, CH_2), 0.86 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 173.2, 170.0 (C=O), 169.7 (C-4), 157.9 (C_q), 153.6 (C-2), 146.9 (C_q), 138.1 (C-6), 134.4 (C_q), 130.0 (CH_{arom}), 129.0 (C_q), 127.8 (CH_{arom}), 126.3 (C_q), 124.5, 123.8 (CH_{arom}), 122.5 ($CH_{triazole}$), 119.6 (CH_{arom}), 106.8 (C-5), 106.4 (CH_{arom}), 89.3 (C-1'), 82.5 (C-4'), 73.8 (C-2'), 69.9 (C-3'), 65.3 (C-5'), 55.7 (CH_3 OMe), 52.6 (CH Aza), 49.9 (CH_2 Aza), 40.5, 39.3, 31.8, 29.6, 29.5, 29.3, 29.2, 28.9, 28.4, 27.1, 25.9, 25.6, 25.4, 22.6 (CH_2), 14.4 (CH_3); LC: R_t = 10.35 min; ESI-MS: m/z = 847.53 ($M+H^+$).

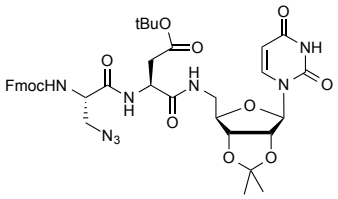


5'-*N*-(*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (20). Starting from 5'-amino-5-deoxy-2',3'-*O*-isopropylidene uridine **19**,[4] compound **20** was obtained using general procedure A (Yield: 82 mg, 180 μmol, 62%). TLC: R_f = 0.24 (DCM/MeOH, 9/1, v/v + 1% Et_3N); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.65 (t, 1H, J = 5.6 Hz, NH), 7.22 (m, 1H, H-6), 5.67 (d, 1H, J = 8.0 Hz, H-5), 5.46 (d, 1H, J = 2.2 Hz, H-1'), 4.98 (dd, 1H, J = 2.2, 6.6 Hz, H-2'), 4.71 (dd, 1H, J = 4.7, 6.4 Hz, H-3'), 4.29 (bs, 2H, NH_2), 4.11 (dd, 1H, J = 4.7, 9.5 Hz, H-4'), 3.68 (dd, 1H, J = 4.4, 7.6 Hz, CH Asp), 3.61 (dt, 1H, J = 6, 14.2 Hz, H-5'), 3.48 (dt, 1H, J = 4.8, 14.2 Hz, H-5'), 2.78 (dd, 1H, J = 4.3, 16.6 Hz, C*H*H Asp), 2.51 (dd, 1H, J = 7.9, 16.6 Hz, CH*H* Asp), 1.47 (s, 3H, CH_3 iPr), 1.37 (s, 9H, CH_3 tBu), 1.26 (s, 3H, CH_3 iPr);); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.7, 171.2 (C=O), 163.6 (C-4), 150.3 (C-2), 143.1 (C-6), 114.7 (C_q iPr), 102.8 (C-5), 95.1 (C-1'), 85.5 (C-4'), 83.9 (C-2'), 81.3, 81.1 (C-3', C_q tBu), 52.1 (CH Asp), 40.6, 40.1 (C-5', CH_2 Asp), 28.1 (CH_3 tBu), 27.2, 25.3 (CH_3 iPr); LC: R_t = 5.77 min; ESI-MS: m/z = 454.93 ($M+H^+$).

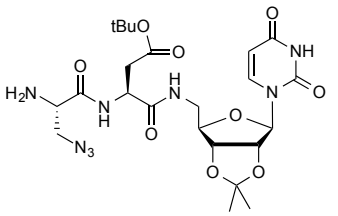


5'-*N*-(*N*-acetyl-L-aspartate)-5'-amino-5'-deoxy uridine (21). Amine **20** (10 mg, 22 μmol) was dissolved in MeOH (300 μL) and treated with Ac_2O (10 μL, 110 μmol) and Et_3N (15 μL, 110 μmol) for 2 h, after which time the mixture was diluted with toluene and concentrated *in vacuo*. Flash column chromatography (silica gel, 5% MeOH in DCM) gave the acetylated intermediate (LC: R_t = 5.91 min; ESI-MS: m/z = 496.93 ($M+H^+$), which was subsequently treated with TFA/H_2O (500 μL, 1/1, v/v) overnight. LC-MS confirmed full consumption of the acetylated intermediate, and the reaction mixture was lyophilized to give the title compound as a colorless oil (Yield: 7.8 mg, 19 μmol, 86%). ^{1}H NMR (D_2O, 600 MHz): δ 7.54 (d, 1H, J = 8.1 Hz, H-6), 5.77 (d, 1H, J = 8.1 Hz, H-5), 5.68 (d, 1H, J = 4.6 Hz, H-1'), 4.59 (dd, 1H, J = 5.7, 7.4 Hz, CH Asp), 4.23 (t, 1H, J = 4.7 Hz, H-2'), 3.98 – 4.02 (m, 2H, H-3', H-4'), 3.45 – 3.51 (m, 2H, H-5'), 2.80 (dd, 1H, J = 5.7, 17.1 Hz, C*H*H Asp), 2.73 (dd, 1H, J = 7.4, 17.0 Hz, CH*H* Asp), 1.92 (s, 3H, CH_3 NHAc); ^{13}C NMR (D_2O, 150 MHz): δ 174.1, 174.0, 172.9 (C=O), 166.1 (C-4), 151.5 (C-2), 142.2 (C-6), 102.2 (C-5), 90.2 (C-1'), 81.9 (C-4'), 73.0 (C-2'), 70.3 (C-3'), 50.2 (CH Asp), 40.6 (C-5'), 35.5 (CH_2 Asp), 21.8 (CH_3 NHAc); LC: R_t = 1.68 min; ESI-MS: m/z = 400.93 ($M+H^+$).

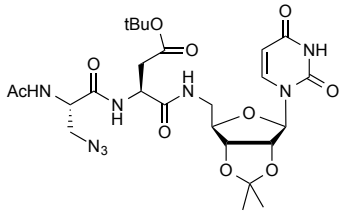
[4] A. Babič, Gobec, S.; C. Gravier-Pelletier, Y. Le Merrer, S. Pečar, *Tetrahedron* **2008**, *64*, 9093-9100.



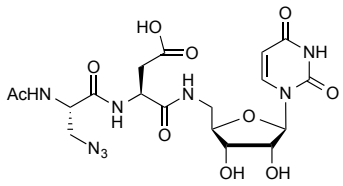
5'-*N*-(*N*-[9-fluorenylmethoxycarbonyl]-3-azido-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (22). Fmoc-L-Aza-OH (70 mg, 198 μmol) was dissolved in DMF (400 μL) and treated with EDC·HCl (41 mg, 216 μmol) and HOBt (33 mg, 216 μmol) for 10 min at RT. This mixture was added to compound **20** (82 mg, 180 mmol) and the resulting solution was stirred until complete consumption of compound **20** was observed using LC-MS (~1 h). The mixture was diluted with DCM, washed with H_2O (4x), dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (silica gel, 5% MeOH in DCM) afforded the title compound as a colorless oil (Yield: 101 mg, 129 μmol, 71%). TLC: R_f = 0.47 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.83 (bs, 1H, NH), 7.68 (d, 2H, *J* = 7.5 Hz, CH_{arom}), 7.48 – 7.59 (m, 2H, CH_{arom}), 7.32 (t, 2H, *J* = 7.5 Hz, CH_{arom}), 7.23 (t, 2H, *J* = 7.5 Hz, CH_{arom}), 7.10 (d, 1H, *J* = 8.0 Hz, H-6), 5.76 (bs, 1H, NH), 5.67 (dd, 1H, *J* = 2.0, 8.0 Hz, H-5), 5.24 (s, 1H, H-1'), 5.00 (d, 1H, *J* = 6.1 Hz, H-2'), 4.68 – 4.77 (m, 2H, H-3', CH Asp), 4.50 – 4.58 (m, 1H, CH Aza), 4.35 – 4.50 (m, 2H, CH_2 Fmoc), 4.16 (t, 1H, *J* = 6.7 Hz, CH Fmoc), 4.10 (s, 1H, H-4'), 3.78 (dd, 1H, *J* = 3.6, 11.8 Hz, C*H*H Aza), 3.62 – 3.71 (m, 1H, H-5'), 3.52 (dd, 1H, *J* = 5.2, 11.9 Hz, CH*H* Aza), 3.37 – 3.45 (m, 1H, H-5'), 2.82 – 2.90 (m, 1H, C*H*H Asp), 2.57 (dd, 1H, *J* = 5.3, 16.8 Hz, CH*H* Asp), 1.45 (s, 3H, CH_3 iPr), 1.30 (s, 9H, CH_3 tBu), 1.24 (s, 3H, CH_3 iPr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.9, 170.5 (C=O), 169.3 (C-4), 162.7, 156.5 (C=O), 150.7 (C-2), 143.6, 143.5 (C_q, C-6), 141.3 (C_q), 127.8, 127.2, 125.0, 120.0 (CH_{arom}), 114.8 (C_q iPr), 102.9 (C-5), 96.2 (C-1'), 84.7 (C-4'), 83.5 (C-2'), 81.8 (C_q tBu), 80.3 (C-3'), 67.7 (CH_2 Fmoc), 54.3, 52.0, 50.0 (CH Aza, CH Asp, CH_2 Aza), 47.1 (CH Fmoc), 40.8 (C-5'), 36.9 (CH_2 Asp), 28.0 (CH_3 tBu), 27.3, 25.3 (CH_3 iPr); LC: R_t = 9.51 min; ESI-MS: m/z = 789.07 ($M+H^+$).



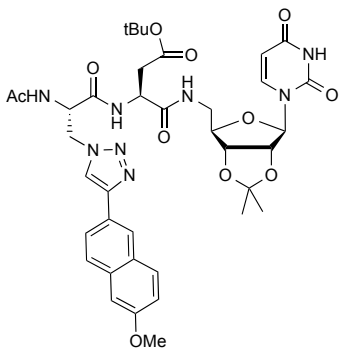
5'-*N*-(3-azido-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (23). Compound **22** (35 mg, 44 μmol) was dissolved in DCM (2.5 mL) and treated with immobilized piperazine (440 mg, 440-880 μmol[3]) for 24 h, after which time the solution was filtered through a syringe filter and concentrated at RT. The resulting amine was directly used in the next step without further purification. LC: R_t = 5.95 min; ESI-MS: m/z = 567.07 ($M+H^+$).



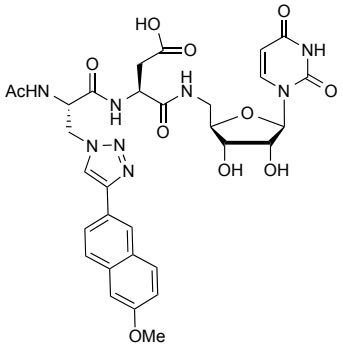
5'-*N*-(*N*-acetyl-3-azido-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (24). The crude amine **23** (9 mg, 16 μmol) was dissolved in MeOH (400 μL) and treated with Ac_2O (7.7 μL, 82 μmol) and Et_3N (11.4 μL, 82 μmol) for 2 h, after which time the mixture was diluted with toluene and concentrated *in vacuo*. Flash column chromatography (silica gel, 5% MeOH in DCM) gave the title compound as a white solid (Yield: 9 mg, 14.6 μmol, 89%). TLC: R_f = 0.44 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.92 (s, 1H, NH), 7.47 (d, 1H, *J* = 8.9 Hz, NH), 7.23 (dd, 1H, *J* = 4.3, 5.6 Hz, NH), 7.14 (d, 1H, *J* = 8.1 Hz, H-6), 6.49 (d, 1H, *J* = 8.4 Hz, NH), 5.70 (dd, 1H, *J* = 2.1, 8.0 Hz, H-5), 5.27 (d, 1H, *J* = 2.4 Hz, H-1'), 5.05 (dd, 1H, *J* = 2.3, 6.6 Hz, H-2'), 4.82 (ddd, 1H, *J* = 4.6, 5.7, 8.4 Hz, CH Aza), 4.72 – 4.77 (m, 2H, H-3', CH Asp), 4.13 (dt, 1H, *J* = 3.3, 4.9 Hz, H-4'), 3.77 (dd, 1H, *J* = 4.6, 12.4 Hz, C*H*H Aza), 3.67 (ddd, 1H, *J* = 3.3, 6.3, 14.6 Hz, H-5'), 3.53 (dd, 1H, *J* = 5.7, 12.4 Hz, CH*H* Aza), 3.47 (dt, 1H, *J* = 3.7, 14.7 Hz, H-5'), 2.85 (dd, 1H, *J* = 5.1, 16.7 Hz, C*H*H Asp), 2.58 (dd, 1H, *J* = 5.3, 16.7 Hz, CH*H* Asp), 2.03 (s, 3H, CH_3 NHAc), 1.48 (s, 3H, CH_3 iPr), 1.34 (s, 9H, CH_3 tBu), 1.27 (s, 3H, CH_3 iPr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.0, 170.5 (C=O), 169.2 (C-4), 163.0 (C=O), 150.9 (C-2), 143.5 (C-6), 114.9 (C_q iPr), 103.0 (C-5), 96.7 (C-1'), 84.8 (C-4'), 83.6 (C-2'), 81.9 (C_q tBu), 80.1 (C-3'), 52.3, 51.7, 50.0 (CH Aza, CH Asp, CH_2 Aza), 40.8 (C-5'), 37.0 (CH_2 Asp), 28.0 (CH_3 tBu), 27.3, 25.3 (CH_3 iPr), 23.2 (CH_3 NHAc); LC: R_t = 6.55 min; ESI-MS: m/z = 608.93 ($M+H^+$).



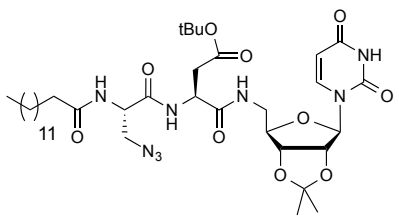
5'-*N*-(*N*-acetyl-3-azido-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (25). Compound **24** was fully deprotected using general procedure D to afford the title compound as a white solid (Yield: 1.75 mg, 3.42 μmol, 83%). ^{1}H NMR (D_2O, 600 MHz): δ 7.57 (d, 1H, *J* = 8.1 Hz, H-6), 5.81 (d, 1H, *J* = 8.1 Hz, H-5), 5.69 (d, 1H, *J* = 4.4 H-1'), 4.60 – 4.75 (m, 1H, CH Asp), 4.43 (t, 1H, *J* = 5.5 Hz, CH Aza), 4.26 (t, 1H, *J* = 4.7 Hz, H-2'), 4.97 – 4.05 (m, 2H, H-3', H-4'), 3.66 (dd, 1H, *J* = 6.0, 12.9 Hz, C*H*H Aza), 3.59 (dd, 1H, *J* = 5.2, 12.9 Hz, CH*H* Aza), 3.48 – 3.53 (m, 2H, H-5'), 2.87 (dd, 1H, *J* = 5.9, 16.9 Hz, C*H*H Asp), 2.76 (dd, 1H, *J* = 7.4, 16.9 Hz, CH*H* Asp), 1.95 (s, 3H, CH_3 NHAc); ^{13}C NMR (D_2O, 150 MHz): δ 174.6, 174.0, 172.2, 171.2 (C=O), 166.2 (C-4), 151.5 (C-2), 142.4 (C-6), 102.3 (C-5), 90.5 (C-1'), 81.8 (C-4'), 73.0 (C-2'), 70.2 (C-3'), 53.2 (CH Aza), 50.9 (CH_2 Aza), 50.1 (CH Asp), 40.6 (C-5'), 35.2 (CH_2 Asp), 21.6 (CH_3 NHAc); LC: R_t = 1.61 min; ESI-MS: m/z = 513.07 ($M+H^+$).



5'-*N*-(*N*-acetyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-*O-tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (37). Compound **24** was converted to the title compound using general procedure C. TLC: R_f = 0.41 (DCM/MeOH, 9/1, v/v); LC: R_t = 8.26 min; ESI-MS: m/z = 791.20 ($M+H^+$). In spite of extensive purification, the NMR spectra were difficult to interpret. Purity and identify of compound **37** were assessed through LC-MS, and the compound was extensively characterized after the subsequent reaction step.

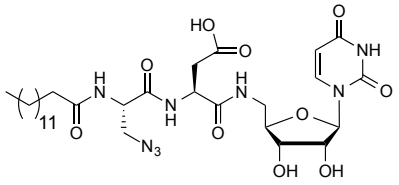


5'-*N*-(*N*-acetyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (26). The title compound was obtained from compound **37** using general procedure D (Yield: 2.1 mg, 3.0 mmol, 61% over two steps). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.36 (s, 1H, NH), 8.59 (d, 1H, *J* = 7.7 Hz, NH), 8.54 (s, 1H, $CH_{triazole}$), 8.32 (s, 1H, CH_{arom}), 8.29 (d, 1H, *J* = 7.9 Hz, NH), 8.11 (t, 1H, *J* = 5.8 Hz, NH), 7.88 – 7.94 (m, 3H, CH_{arom}), 7.68 (d, 1H, *J* = 8.0 Hz, H-6), 7.35 (d, 1H, *J* = 2.0 Hz, CH_{arom}), 7.19 (dd, 1H, *J* = 2.4, 8.9 Hz, CH_{arom}), 5.76 (d, 1H, *J* = 6.1 Hz, H-1'), 5.62 (dd, 1H, *J* = 1.9, 8.0 Hz, H-5), 5.42 (d, 1H, *J* = 5.8 Hz, 2'-OH), 5.19 (d, 1H, *J* = 4.8 Hz, 3'-OH), 4.81 – 4.86 (m, 1H, CH Aza), 4.79 (dd, 1H, *J* = 4.6, 14.0 Hz, C*H*H Aza), 4.64 (dd, 1H, *J* = 7.4, 14.2 Hz, CH*H* Aza), 4.57 – 4.61 (m, 1H, CH Asp), 4.05 – 4.09 (m, 1H, H-2'), 3.86 – 3.93 (m, 5H, H-3', H-4', CH_3 OMe), 3.30 – 3.45 (m, 2H, H-5'), 2.73 (dd, 1H, *J* = 5.2, 16.7 Hz, C*H*H Asp), 2.57 (dd, 1H, *J* = 8.0, 16.7 Hz, CH*H* Asp), 1.85 (s, 3H, CH_3 NHAc); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 172.2, 171.1, 170.3 (C=O), 169.0 (C-4), 163.5, 157.9 (C_q), 151.2 (C-2), 146.8 (C_q), 141.7 (C-6), 134.3 (C_q), 132.0, 131.9, 130.0, 129.3, 129.2, 129.0, 127.9 (CH_{arom}), 126.4 (C_q), 124.6, 123.9 (CH_{arom}), 122.6 ($CH_{triazole}$), 119.6, 106.5 (CH_{arom}), 102.4 (C-5), 88.3 (C-1'), 83.0 (C-4'), 72.9 (C-2'), 71.2 (C-3'), 55.7 (CH_3 OMe), 53.2 (CH Aza), 51.1 (CH_2 Aza), 50.3 (CH Asp), 41.6 (C-5'), 36.5 (CH_2 Asp), 22.9 (CH_3 NHAc); LC: R_t = 6.43 min; ESI-MS: m/z = 695.20 ($M+H^+$).

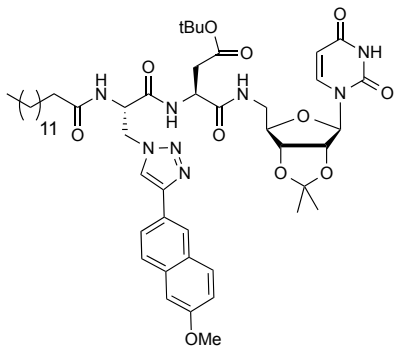


5'-*N*-(*N*-myristoyl-3-azido-L-alaninyl-*O-tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (27). Myristic acid (19 mg, 82 μmol) was dissolved in DMF (200 μL) and stirred with EDC·HCl (16 mg, 82 μmol) and HOBt (13 mg, 82 μmol) at RT for 15 min. This solution was added to the crude amine **23** (~41 μmol) in DMF (200 μL) and the resulting solution was stirred until complete consumption of the starting

material was observed using LC-MS (2 h). The mixture was diluted with DCM, washed with H_2O (4x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 5% MeOH in DCM) afforded the title compound as a colorless oil (Yield: 20 mg, 26 μmol, 63% over two steps). TLC: R_f = 0.41 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.04 (s, 1H, NH), 7.54 (d, 1H, *J* = 8.9 Hz, NH), 7.24 (dd, 1H, *J* = 4.0, 6.2 Hz, NH), 7.15 (d, 1H, *J* = 8.1 Hz, H-6), 6.55 (d, 1H, *J* = 6.9 Hz, NH), 5.69 (d, 1H, *J* = 8.0 Hz, H-5), 5.29 (d, 1H, *J* = 2.3 Hz, H-1'), 5.03 (dd, 1H, *J* = 2.2, 6.6 Hz, H-2'), 4.82 (dt, 1H, *J* = 5.2, 8.0 Hz, CH Aza), 4.72 – 4.78 (m, 2H, H-3', CH Asp), 4.10 – 4.15 (m, 1H, H-4'), 3.75 (dd, 1H, *J* = 4.7, 12.5 Hz, C*H*H Aza), 3.65 (ddd, 1H, *J* = 3.3, 6.2, 14.7 Hz, H-5'), 3.53 (dd, 1H, *J* = 5.7, 12.4 Hz, CH*H* Aza), 3.47 (dt, 1H, *J* = 3.6, 14.6 Hz, H-5'), 2.85 (app dd, 1H, *J* = 5.0, 16.8 Hz, C*H*H Asp), 2.56 (dd, 1H, *J* = 5.3 Hz, 16.8 Hz, CH*H* Asp), 2.18 – 2.25 (m, 2H, CH_2), 1.52-1.63 (m, 2H, CH_2), 1.48 (s, 3H, CH_3 iPr), 1.33 (s, 9H, CH_3 tBu), 1.27 (s, 3H, CH_3 iPr), 1.15 – 1.26 (m, 20H, CH_2), 0.81 (t, 3H, *J* = 6.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.9, 170.6 (C=O), 169.4 (C-4), 163.1, 162.6 (C=O), 150.9 (C-2), 143.4 (C-6), 114.8 (C_q iPr), 103.0 (C-5), 96.4 (C-1'), 84.7 (C-4'), 83.6 (C-2'), 81.8 (C_q tBu), 80.1 (C-3'), 52.2 (CH Aza), 51.7 (CH_2 Aza), 50.0 (CH Asp), 40.8 (C-5'), 36.9 (CH_2 Asp), 36.5, 31.9, 29.7, 29.6, 29.5, 29.3, 29.3, 29.1 (CH_2), 28.0 (CH_3 tBu), 27.3 (CH_3 iPr), 25.5, 25.3 (CH_3 iPr, CH_2), 22.7 (CH_2), 14.1 (CH_3); LC: R_t = 11.41 min; ESI-MS: m/z = 777.07 ($M+H^+$).

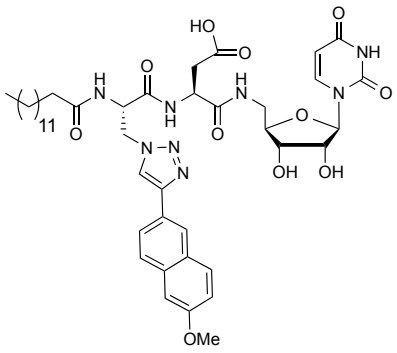


5'-*N*-(*N*-myristoyl-3-azido-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (28). Compound **27** was fully deprotected using general procedure D to afford the title compound as an off-white solid (Yield: 3.0 mg, 4.4 μmol, quant.). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.33 (d, 1H, *J* = 7.3 Hz, NH), 8.26 (d, 1H, *J* = 8.0 Hz, NH), 7.92 (t, 1H, *J* = 5.7 Hz, NH), 7.66 (d, 1H, *J* = 8.1 Hz, H-6), 5.73 (d, 1H, *J* = 5.8 Hz, H-1'), 5.65 (d, 1H, *J* = 8.0 Hz, H-5), 5.39 (d, 1H, *J* = 5.6 Hz, 2'-OH), 4.50 – 4.57 (m, 2H, CH Asp, CH Aza), 3.98 – 4.03 (m, 1H, H-2'), 4.83 – 4.87 (m, 1H, H-3'), 3.77 – 3.82 (m, 1H, H-4'), 3.53 (dd, 1H, *J* = 4.5, 12.6 Hz, C*H*H Aza), 3.40 – 3.46 (m, 1H, CH*H* Aza), 3.28 – 3.39 (m, 2H, H-5'), 2.66 (dd, 1H, *J* = 5.6, 16.6 Hz, C*H*H Asp), 2.53 – 2.56 (m, 1H, CH*H* Asp), 2.12 – 2.17 (m, 2H, CH_2), 1.45 – 1.53 (m, 2H, CH_2), 1.20 – 1.30 (m, 20H, CH_2), 0.86 (t, 3H, *J* = 6.7 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 173.3, 172.2, 171.0 (C=O), 169.6 (C-4), 163.5 (C=O), 151.2 (C-2), 141.7 (C-6), 102.4 (C-5), 88.4 (C-1'), 83.0 (C-4'), 72.9 (C-2'), 71.0 (C-3'), 52.7, 52.0 (CH Aza, CH_2 Aza), 50.1 (CH Asp), 41.5 (C-5'), 36.6 (CH_2 Asp), 35.6, 31.8, 29.5, 29.4, 29.3, 29.2, 29.1, 25.5, 22.6 (CH_2), 14.4 (CH_3); LC: R_t = 9.84 min; ESI-MS: m/z = 681.20 ($M+H^+$).



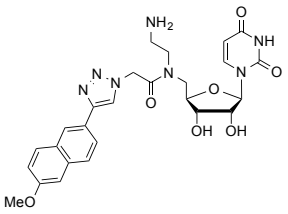
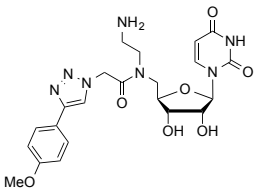
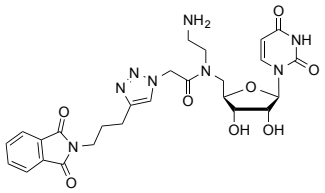
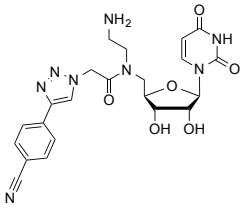
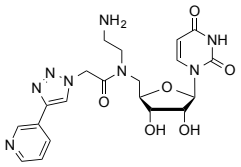
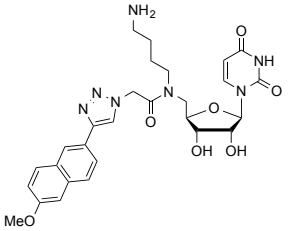
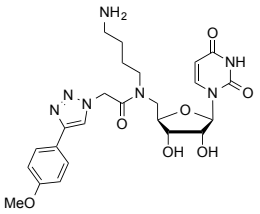
5'-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (38). The title compound was obtained from compound **27** according to general procedure C (8.4 mg, 8.8 μmol, 93%). TLC: R_f = 0.60 (DCM/MeOH, 9/1, v/v); ^{1}H NMR ($CDCl_3$/MeOH-d_4, 400 MHz): δ 8.17 (s, 1H, CH_{arom}), 7.97 (s, 1H, $CH_{triazole}$), 7.81 (dd, 1H, *J* = 1.6, 8.5 Hz, CH_{arom}), 7.72 – 7.77 (m, 2H, CH_{arom}), 7.25 (d, 1H, *J* = 8.0 Hz, H-6), 7.09 – 7.15 (m, 2H, CH_{arom}), 5.64 (d, 1H, *J* = 8.0 Hz, H-5), 5.40 (d, 1H, *J* = 2.5 Hz, H-1'), 4.98 – 5.03 (m, 2H, H-2', CH Aza), 4.86 (dd, 1H, *J* = 5.6, 14.2 Hz, C*H*H Aza), 4.70 – 4.81 (m, 3H, H-3', CH Asp, CH*H* Aza), 4.15 – 4.20 (m, 1H, H-4'), 3.90 (s, 3H, CH_3 OMe), 3.64 (dd, 1H, *J* = 3.8, 14.4 Hz, H-5'), 3.53 (dd, 1H, *J* = 4.1, 14.4 Hz, H-5'), 2.70 (dd, 1H, *J* = 6.0, 16.7 Hz, C*H*H Asp), 2.63 (dd, 1H, *J* = 5.7, 16.7 Hz, CH*H* Asp), 2.21 (t, 2H, *J* = 7.5 Hz, CH_2), 1.51 – 1.60 (m, 2H, CH_2), 1.48 (s, 3H, CH_3 iPr), 1.34 (s, 9H, CH_3 tBu), 1.27 (s, 3H, CH_3 iPr), 1.12 – 1.24 (m, 20H, CH_2), 0.83 (t, 3H, *J* = 6.7 Hz, CH_3); ^{13}C NMR ($CDCl_3$/MeOH-d_4, 100 MHz): δ 174.8, 170.7 (C=O), 168.7 (C-4), 163.7 (C=O), 158.0 (C_q), 150.8 (C-2), 148.0 (C_q), 143.2 (C-6), 134.5 (C_q), 129.7 (CH_{arom}), 128.9 (C_q), 127.5 (CH_{arom}), 125.0 (C_q), 124.4, 124.1 (CH_{arom}), 121.4 ($CH_{triazole}$), 119.3 (CH_{arom}), 114.7 (C_q iPr), 105.8 (CH_{arom}), 102.7 (C-5), 95.5 (C-1'), 84.5 (C-4'), 83.5 (C-2'), 81.8 (C_q tBu), 80.5 (C-3'), 55.3 (OMe), 52.8 (CH Aza), 50.4 (CH_2 Aza), 49.8 (CH Asp), 40.5 (C-5'), 36.8

(CH_2 Asp), 36.2 (CH_2), 31.8, 29.6, 29.5, 29.4, 29.3, 29.2 (CH_2), 27.8 (CH_3 tBu), 27.1 (CH_3 iPr), 25.5 (CH_2), 25.2 (CH_3 iPr), 22.6, 14.0 (CH_2); LC: R_t = 12.01 min; ESI-MS: m/z = 959.40 ($M+H^+$).

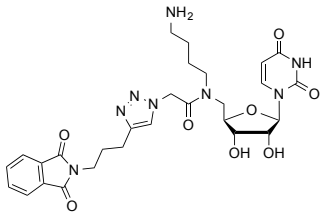


5'-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (29). Compound **38** was fully deprotected using general procedure D to afford the title compound as a slightly colored solid (Yield: 5.1 mg, 5.9 µmol, 66%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.4 (s, 1H, NH), 8.53 (d, 1H, *J* = 7.7 Hz, NH), 8.48 (s, 1H, $CH_{triazole}$), 8.28 (s, 1H, CH_{arom}), 8.21 (d, 1H, *J* = 8.2 Hz, NH), 8.11 (t, 1H, *J* = 5.6 Hz, NH), 7.86 – 7.92 (m, 3H, CH_{arom}), 7.68 (d, 1H, *J* = 8.0 Hz, H-6), 7.33 (d, 1H, *J* = 1.9 Hz, CH_{arom}), 7.18 (dd, 1H, *J* = 2.3, 8.9 Hz, CH_{arom}), 5.75 (d, 1H, *J* = 5.9 Hz, H-1'), 5.64 (dd, 1H, *J* = 1.3, 7.8 Hz, H-5), 5.41 (d, 1H, *J* = 5.7 Hz, 2'-OH), 5.18 (d, 1H, *J* = 4.4 Hz, 3'-OH), 4.87 – 4.92 (m, 1H, CH Aza), 4.82 (dd, 1H, *J* = 4.1, 14.0 Hz, C*H*H Aza), 4.57 – 4.62 (m, 2H, CH Asp, CH*H* Aza), 4.04 – 4.08 (m, 1H, H-2'), 3.85 – 3.92 (m, 5H, H-3', H-4', CH_3 OMe), 3.40 – 3.45 (m, 2H, H-5'), 2.71 (dd, 1H, *J* = 4.5, 16.8 Hz, C*H*H Asp), 2.57 – 2.61 (m, 1H, CH*H* Asp), 2.02 – 2.13 (m, 2H, CH_2), 1.29 – 1.38 (m, 2H, CH_2), 0.95 – 1.26 (m, 20H, CH_2), 0.86 (t, 3H, *J* = 7.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 173.1, 171.1 (C=O), 169.2 (C-4), 163.5 (C=O), 157.9 (C_q), 151.2 (C-2), 146.7 (C_q), 141.7 (C-6), 134.3 (C_q), 130.0, 129.0, 127.8 (CH_{arom}), 126.4 (C_q) 124.5, 123.8 (CH_{arom}), 122.5 ($CH_{triazole}$), 119.5, 106.4 (CH_{arom}), 102.4 (C-5), 88.4 (C-1'), 83.0 (C-4'), 73.0 (C-2'), 71.2 (C-3'), 55.7 (CH_3 OMe), 52.9 (CH Aza), 50.9 (CH_2 Aza), 50.3 (CH Asp), 41.6 (C-5'), 35.7 (CH_2 Asp), 31.8, 29.5, 29.4, 29.3, 29.2, 29.0, 25.6, 22.6 (CH_2),14.4 (CH_3); LC: R_t = 10.45 min; ESI-MS: m/z = 863.3 ($M+H^+$).

Overview of the click library based on azides 11, 12 and 13 (synthesized using general procedure B)

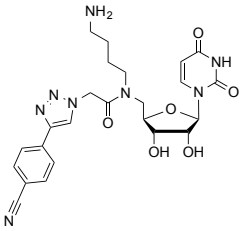
Compound	Structure	LC-MS data
11a		R_t = 7.35 min m/z = 552.20 ($M+H^+$)
11f		R_t = 6.15 min m/z = 502.13 ($M+H^+$)
11g		R_t = 6.61 min m/z = 582.20 ($M+H^+$)
11h		R_t = 6.07 min m/z = 496.20 ($M+H^+$)
11i		R_t = 2.00 min m/z = 473.07 ($M+H^+$)
12a		R_t = 8.04 min m/z = 580.27 ($M+H^+$)
12f		R_t = 6.13 min m/z = 530.27 ($M+H^+$)

12g



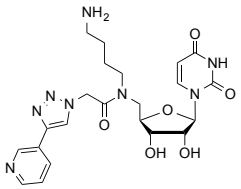
R_t = 6.52 min
m/z = 611.27 ($M+H^+$)

12h



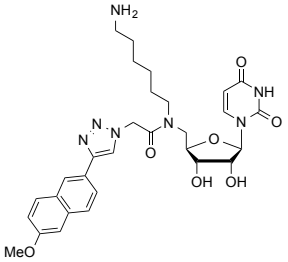
R_t = 5.89 min
m/z = 525.27 ($M+H^+$)

12i



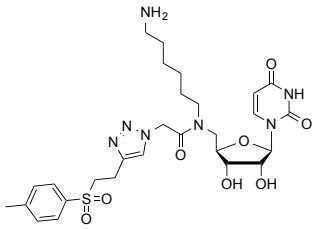
R_t = 2.03 min
m/z = 501.20 ($M+H^+$)

13a



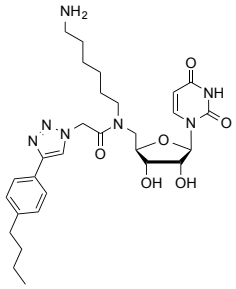
R_t = 6.98 min
m/z = 608.27 ($M+H^+$)

13b



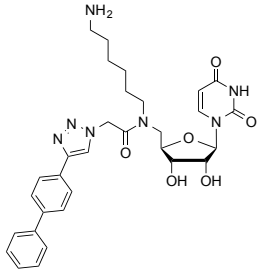
R_t = 6.93 min
m/z = 650.33 ($M+16+H^+$)

13c

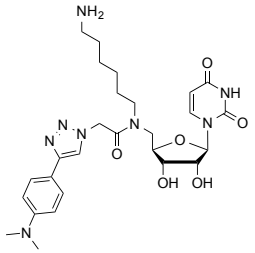
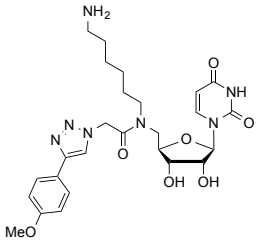
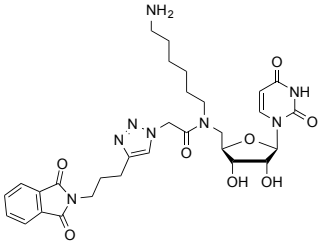
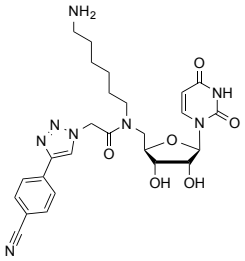
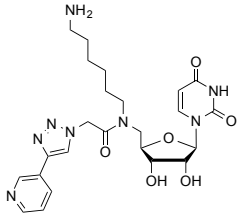


R_t = 8.18 min
m/z = 584.33 ($M+H^+$)

13d

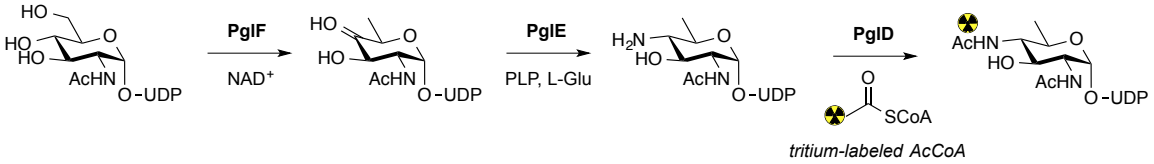


R_t = 7.77 min
m/z = 604.33 ($M+H^+$)

13e		R_t = 4.95 min m/z = 571.33 ($M+H^+$)
13f		R_t = 6.10 min m/z = 558.33 ($M+H^+$)
13g		R_t = 6.70 min m/z = 639.33 ($M+H^+$)
13h		R_t = 6.16 min m/z = 553.27 ($M+H^+$)
13i		R_t = 1.94 min m/z = 529.27 ($M+H^+$)

Chemo-enzymatic synthesis of radiolabeled UDP-diNAcBac

[^{3}H]-UDP-diNAcBac was prepared by previously described chemoenzymatic methods, with a modification at the final step to incorporate the tritium radiolabel.[5] UDP-GlcNAc was converted to UDP-4-amino-NAcBac using PglF and PglE. After purification, 5 mM UDP-4-amino-NAcBac was incubated with [^{3}H]-Acetyl coenzyme A (20 Ci/mmol, American Radiolabeled Chemicals), 50 mM HEPES pH 7.5, 50 mM NaCl, and 260 μM PglD, followed by a chase with 5 mM unlabeled acetyl coenzyme A for 2 hours. The radiolabeled product was purified on a Phenomenex Synergi C18 reverse-phase HPLC column, to yield [^{3}H]-UDP-diNAcBac (5.4 mCi/mmol).



Purification of PglC

PglC was cloned into the pET-SUMO vector (Life Technologies) using the BsaI and XhoI restriction sites and the following primers:

PglC Forward Primer	5′-CGCCGGTCTCCAGGTATGTATGAAAAA-3′
PglC Reverse Primer	5′-ATCGCTCGAGTTATGCCGTCCCGGTCTT-3′

The pET-SUMO-PglC plasmid was transformed into BL21-RIL cells (Agilent) for overexpression, using kanamycin and chloramphenicol for selection. Overexpression was performed using the Studier method.[19] In this method, 1 mL of an overnight cell culture was added to expression media containing 30 μg/mL kanamycin and 30 μg/mL chloramphenicol in 1 L of autoinduction media (0.1% (w/v) tryptone, 0.05% (w/v) yeast extract, 2 mM $MgSO_4$, 0.05% (v/v) glycerol, 0.005% (w/v) glucose, 0.02% (w/v) α-lactose, 2.5 mM Na_2HPO_4, 2.5 mM KH_2PO_4, 5 mM NH_4Cl, 0.5 mM Na_2SO_4). Cells were allowed to grow with shaking for 3 h at 37 °C. After 3 h, the temperature was decreased to 16 °C, and the cells were incubated for 16 hours. Cells were harvested by centrifuging at 9000 x g, and cells were stored at -80 °C. Cell pellets were thawed in 10% of the original culture volume in 50 mM Tris pH 8.0, 150 mM NaCl, 40 μL protease inhibitor cocktail (Calbiochem). The cells were lysed by two rounds of sonication for 90 seconds each, at an amplitude of 50% with one-second on/off pulses. The cells were incubated on ice for ten minutes between rounds of sonication. Cellular debris was removed by centrifugation at 9000 x *g* for 45 minutes. The resulting supernatant was transferred to a clean centrifuge tube and subjected to centrifugation at 142,000 x g for 65 minutes to pellet the CEF. If the CEF was to be used for activity assays, it was homogenized into 1% of the original culture volume in 50 mM HEPES pH 7.5, 100 mM NaCl and stored at -80 °C. If protein was to be purified from the CEF, it was isolated and homogenized into 10% of the original culture volume in 50 mM HEPES pH 7.5, 100 mM NaCl, 1% n-dodecyl beta-D-maltoside (DDM), using a glass homogenizer. 20 μL protease inhibitor cocktail was added to prevent proteolysis. This sample was incubated at 4 °C with gentle rocking for 16 hours, after which it was centrifuged (145,000 x g) to remove insoluble material. The resulting supernatant was incubated with 1 mL Ni-NTA resin for 1-2 hours. The resin was washed with 30 ml Wash 1 buffer (50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% DDM, 20 mM imidazole), followed by a wash with 30 ml Wash 2 buffer (50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% DDM, 45 mM imidazole). PglC was eluted in 4 x 1 mL fractions of elution buffer (50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% DDM, 300 mM imidazole). Gel filtration analysis was performed using a Superdex S200 10/300 column (GE Healthcare) equilibrated with 50 mM HEPES pH 7.5, 100 mM NaCl, and 0.03% DDM.

[5] N. B. Olivier, M. M. Chen, J. R. Behr, B. Imperiali, *Biochemistry* **2006**, *45*, 13659-13669.

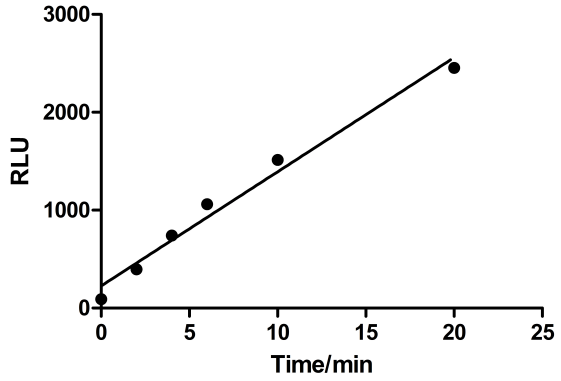
Radioactivity-Based Activity Assays with PglC.

Assays contained 20 μM Und-P, 10% DMSO, 0.1% Triton X-100, 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 20 μM [^{3}H]-UDP-diNAcBac, and 1 nM PglC in a final volume of 60 μL. Inhibitors were added in DMSO, in a volume such that the total concentration of DMSO in the reaction was equal to 10% (v/v). PglC was pre-incubated in the reaction mixture lacking [^{3}H]-UDP-diNAcBac for five minutes at RT. After initiation of the reaction with [^{3}H]-UDP-diNAcBac, aliquots (20 μL) were taken at twenty minute time points and quenched in 1 mL $CHCl_3$:MeOH. The organic layer was washed three times with 400 μL PSUP (Pure Solvent Upper Phase, composed of 15 mL $CHCl_3$, 240 mL MeOH, 1.83 g KCl in 235 mL H_2O). The resulting aqueous layers were combined with 5 mL EcoLite (MP Biomedicals) liquid scintillation cocktail. Organic layers were combined with 5 mL OptiFluor (PerkinElmer). Both layers were analyzed using scintillation counting. Conversion was calculating by dividing the radioactive counts in the organic layer over the total number of counts. The data was plotted as percentage remaining activity compared to the positive control (no inhibitor), and the IC_{50} values were obtained by plotting residual activity versus concentration (GraphPad Prism).

Luminescence assay using UMP/CMP-Glo.

The quenching solution was prepared as described by Promega. Assays contained 20 μM Und-P, 10% DMSO, 0.1% Triton X-100, 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 20 μM UDP-diNAcBac, and 1 nM PglC in a final volume of 25 μL. Inhibitors were added in DMSO, in a volume such that the total concentration of DMSO in the reaction was equal to 10% (v/v). PglC was pre-incubated in the reaction mixture lacking UDP-diNAcBac for five minutes at RT. After initiation of the reaction with UDP-diNAcBac, the reaction was halted after 20 min by the addition of 25 μL quenching buffer. The mixture was transferred to a 96-well plate (white, half area, Corning) and placed in the plate reader. The plate was shaken at low speed for 16 min, and incubated at RT for 44, after which time the luminescence was read. Background inhibition of the UMP/CMP-Glo assay was established for each inhibitor,[6] and the luminescent reads were adjusted accordingly. Conversion was calculating by dividing the luminescence units (RLU) in the sample with inhibitor over the RLU obtained for the positive control (no inhibitor). The data was plotted as percentage remaining activity compared to the positive control (no inhibitor), and the IC_{50} values were obtained by plotting residual activity versus concentration (GraphPad Prism). In our experiments, using the conditions described in the protocol, generally 2000-2500 RLU (relative luminescence units) is generated. We note that this value is subject to small day-to-day variations and can vary between plate readers.

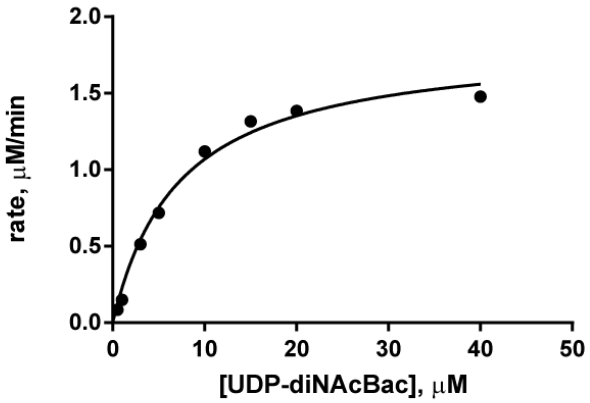
Progress curve of an inhibited reaction using the UMP/CMP-Glo assay

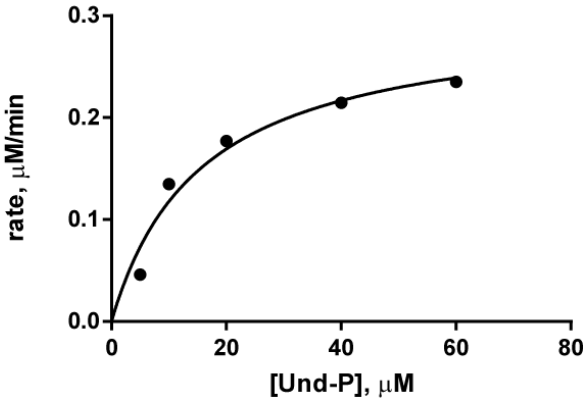


[6] Some inhibitors, especially the first-and second-generation scaffolds, inhibited the UMP/CMP-Glo assay itself. Fortunately, the more elaborate compounds and end products were not inhibiting the assay.

Michaelis Menten plots for UDP-diNAcBac and Und-P

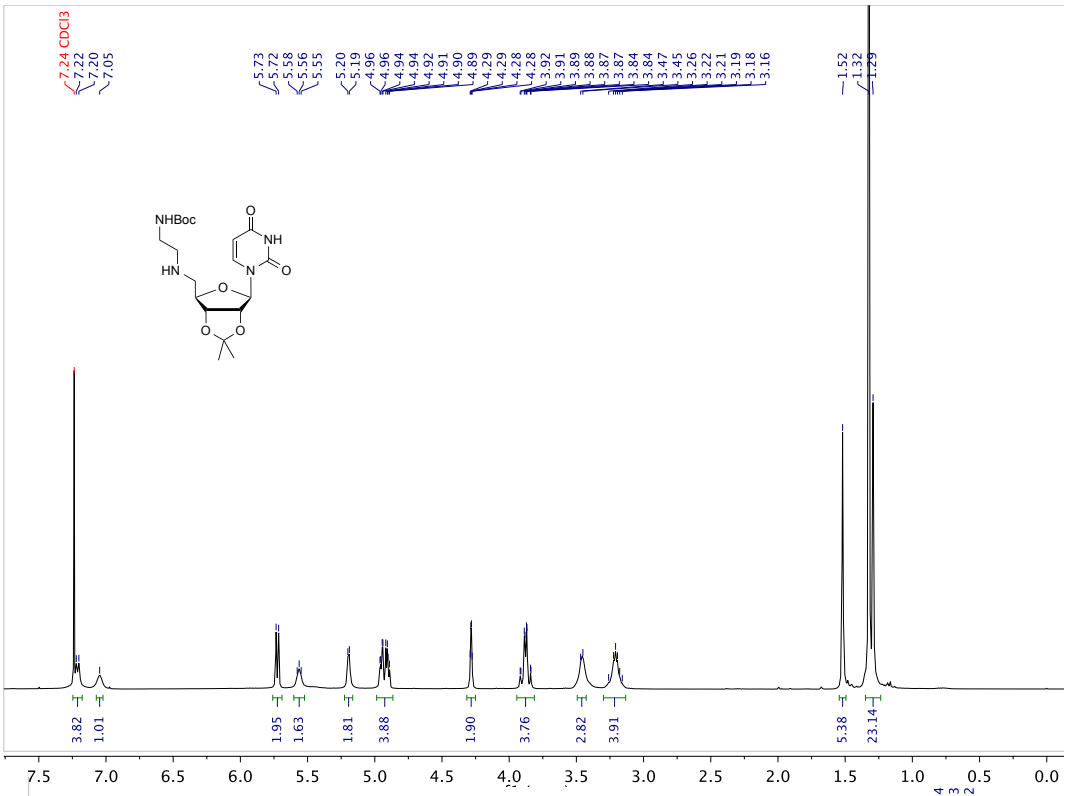
Kinetic parameters were determined for each of the substrates of the PglC reaction. The radioactivity-based assay was used to determine the K_M of UDP-diNAcBac, at 20 μM Und-P, while the UMP/CMP-Glo assay was used to determine the K_M of Und-P, at 20 μM UDP-diNAcBac. An important caveat for the determination of kinetic parameters for Und-P is that it is difficult to predict how this substrate is distributed among detergent micelles that contain PglC, and it is not apparent what the effective concentration of Und-P is in the microenvironment of the micelles. Thus, the reported parameters for Und-P are apparent values. The data was fit to a Michaelis Menten plot using non-linear regression.

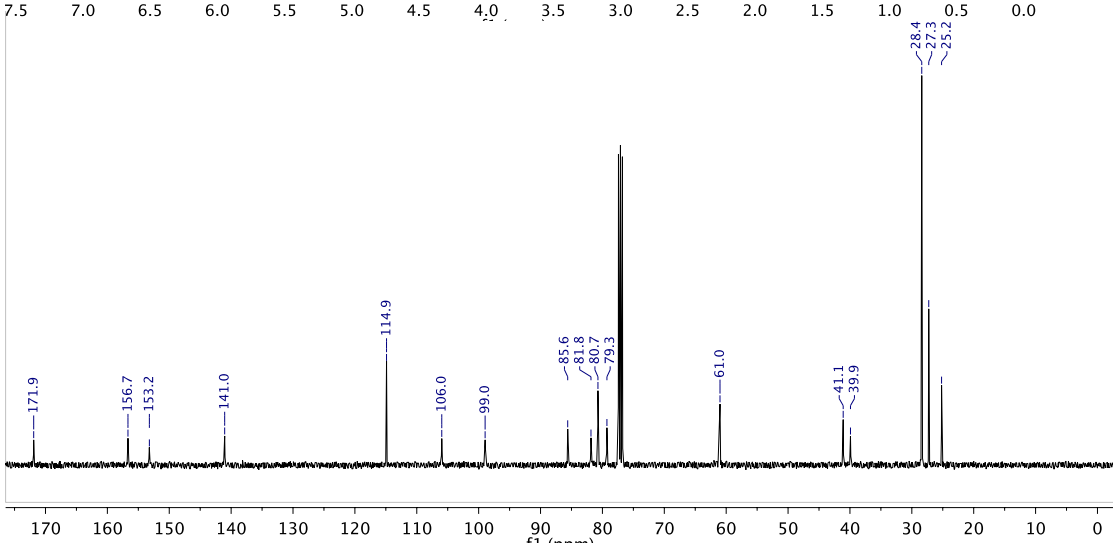


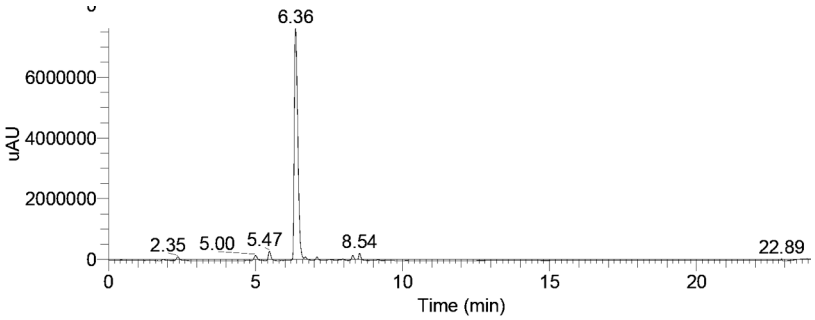


	$K_{M\ app}$	$V_{max\ app}$	k_{cat}
UDP-diNAcBac	7.2 ± 1.1 μM	1.84 ± 0.09 μM/min	303 ± 90 min^{-1}
Und-P	15.6 ± 5.1 μM	0.3 ± 0.04 μM/min	460 ± 10 min^{-1}

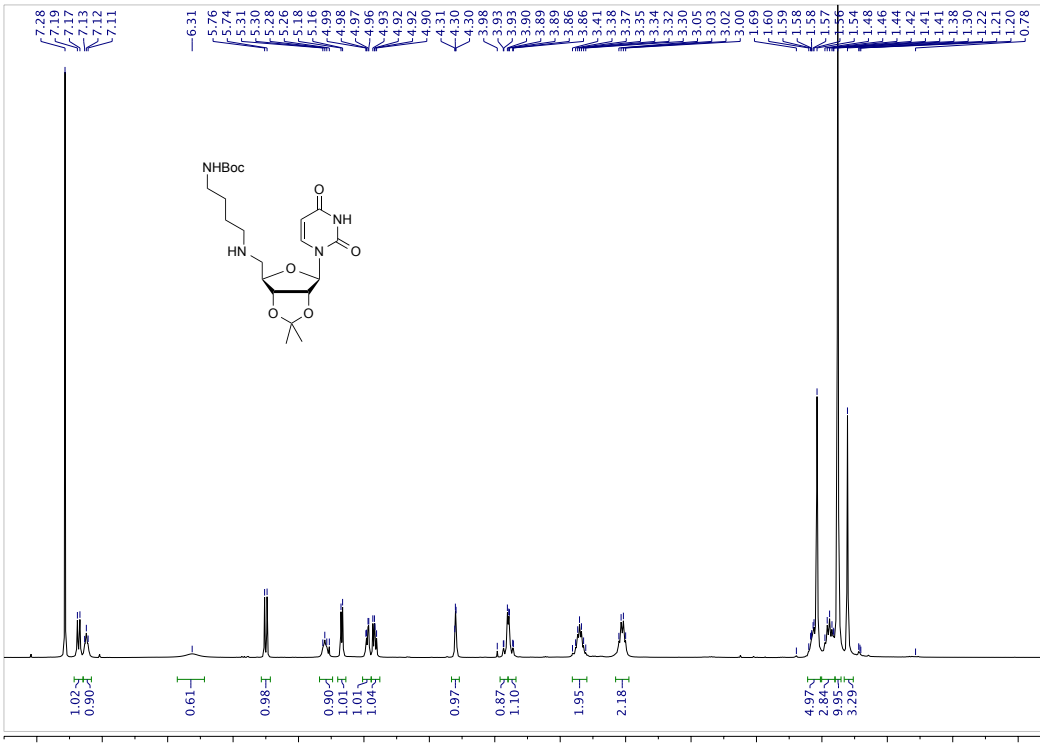
5'-*N*-(*tert*-butyl *N*-[2-aminoethyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (2).

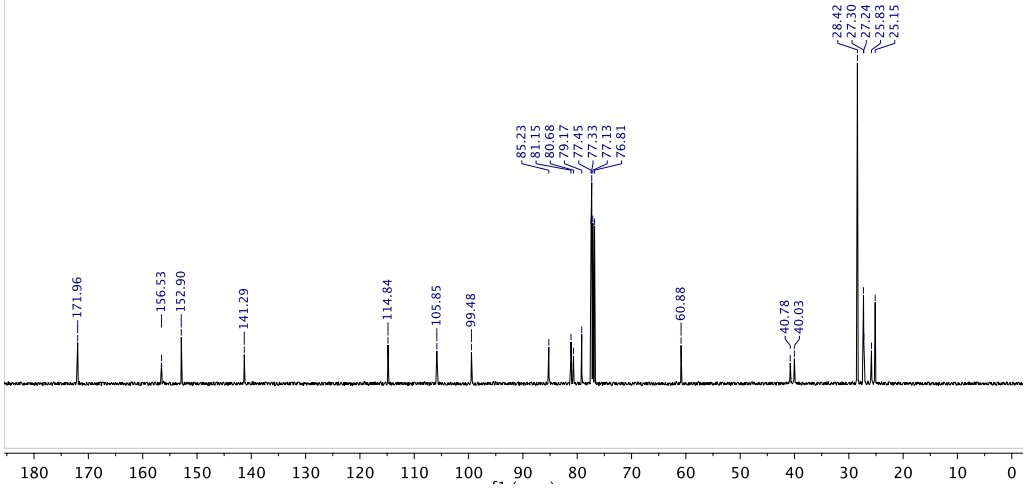


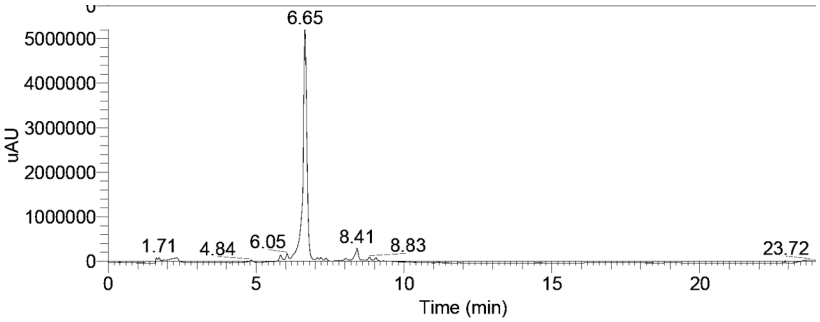




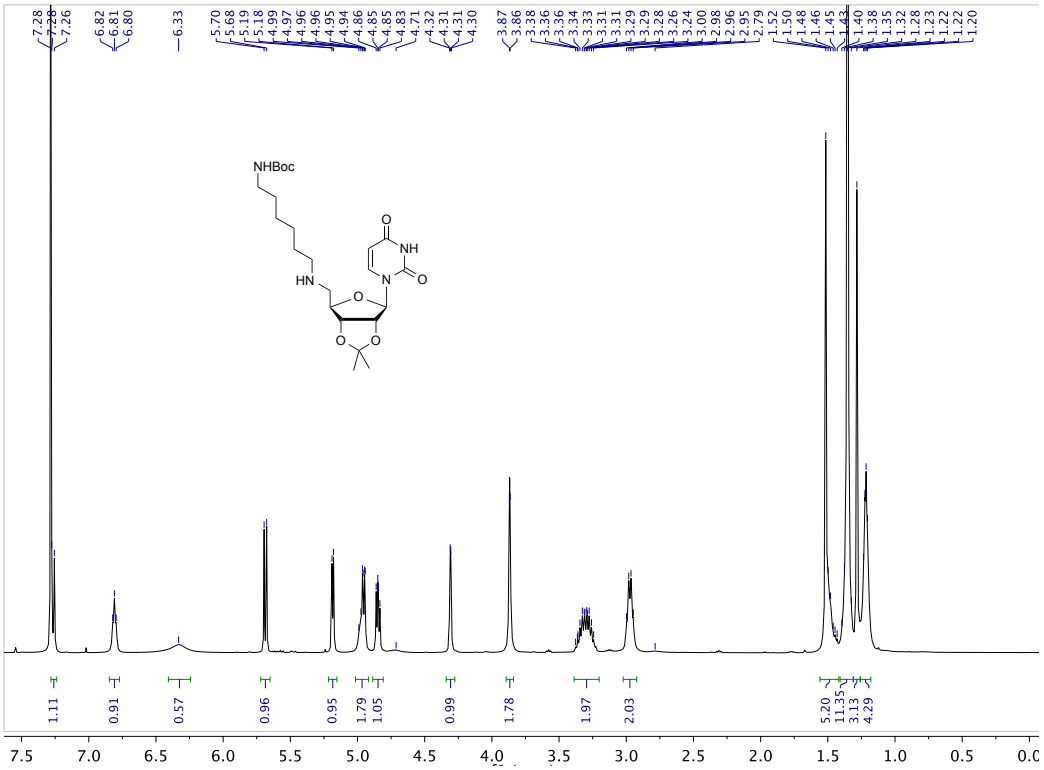
5'-*N*-(*tert*-butyl *N*-[4-aminobutyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (3).

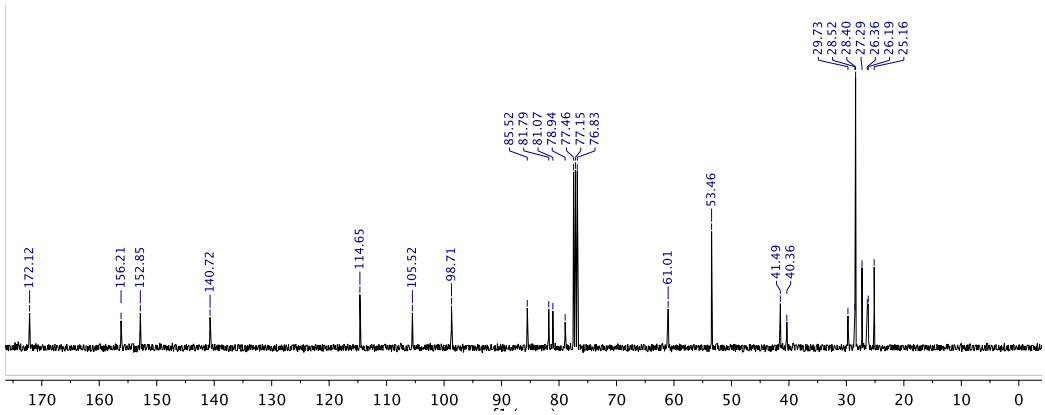


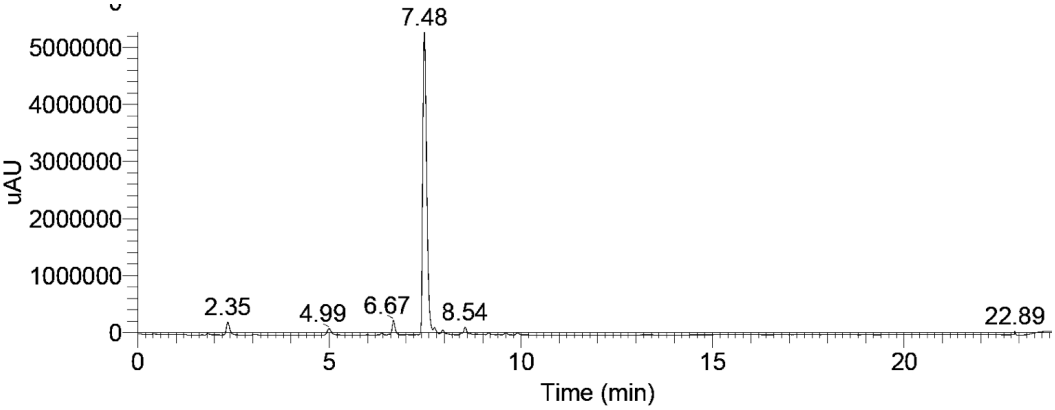




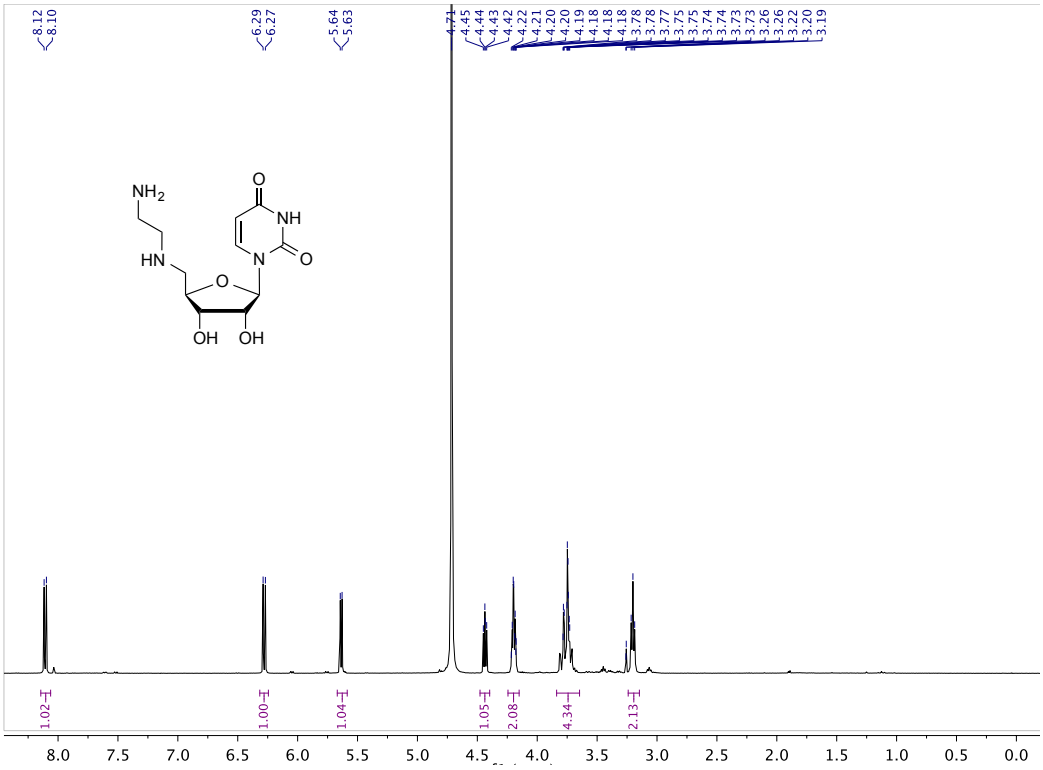
5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (4).

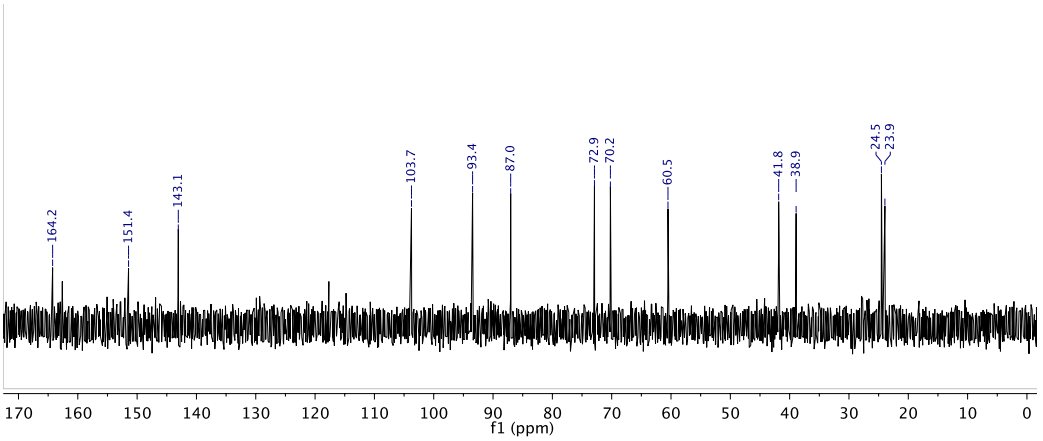




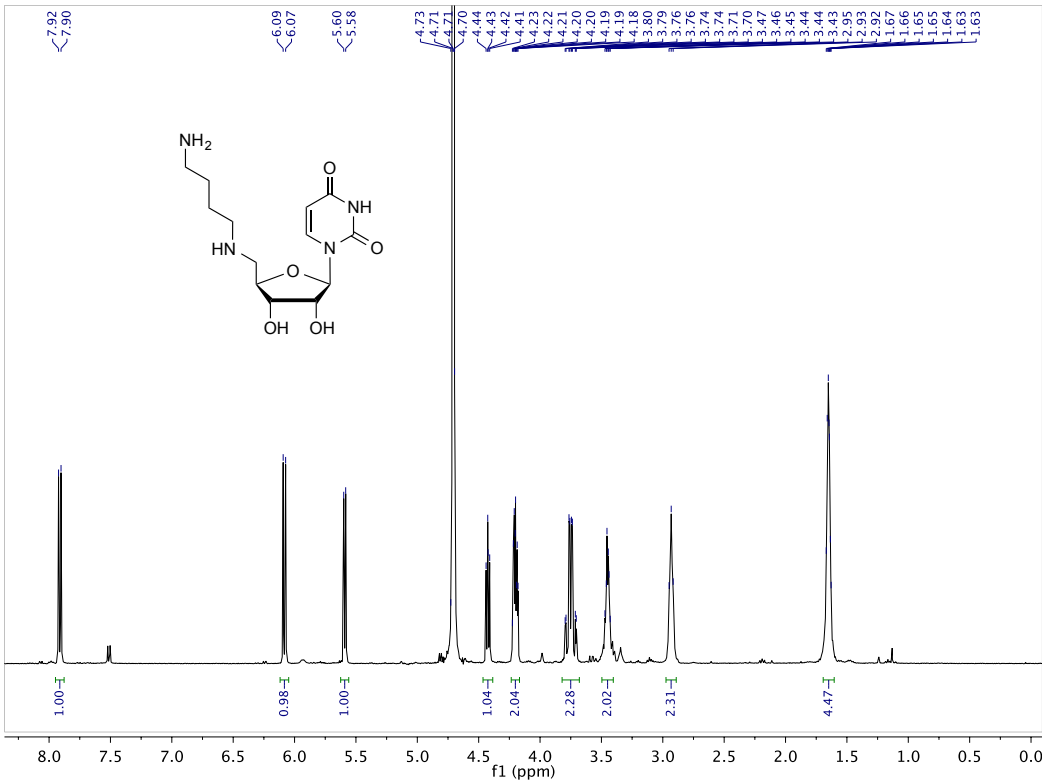


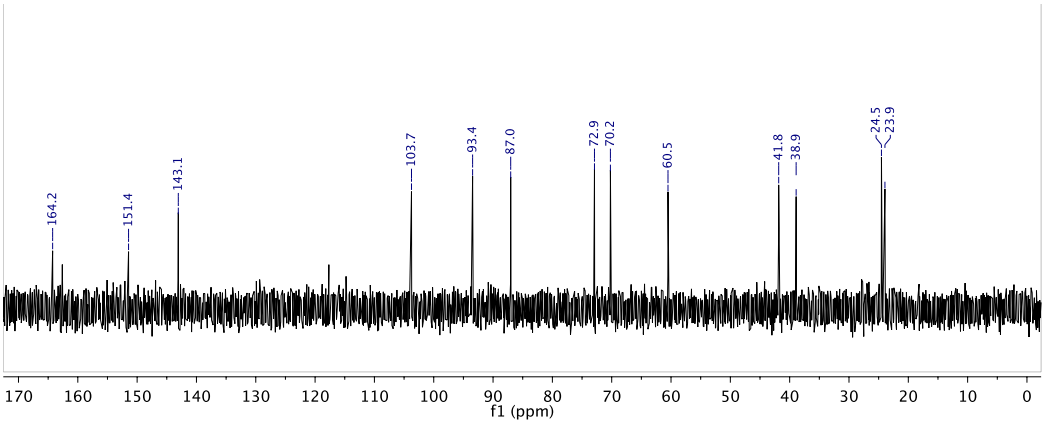
5’-*N*-(2-aminoethyl)-5’-amino-5’-deoxy-uridine (5).



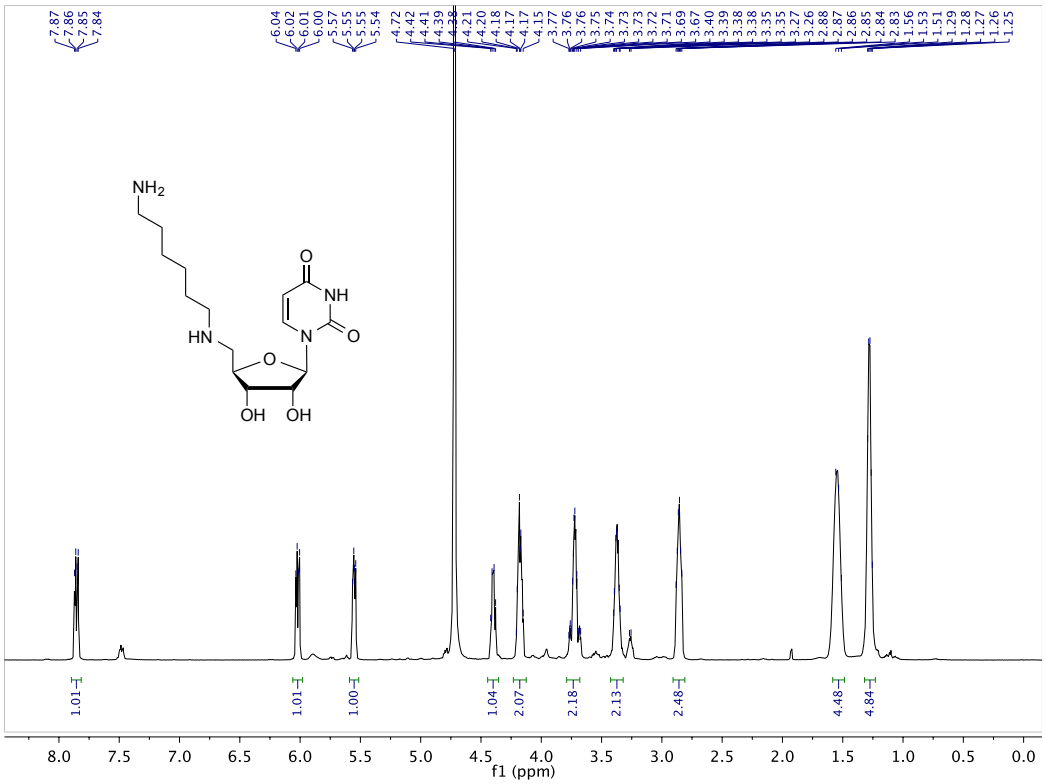


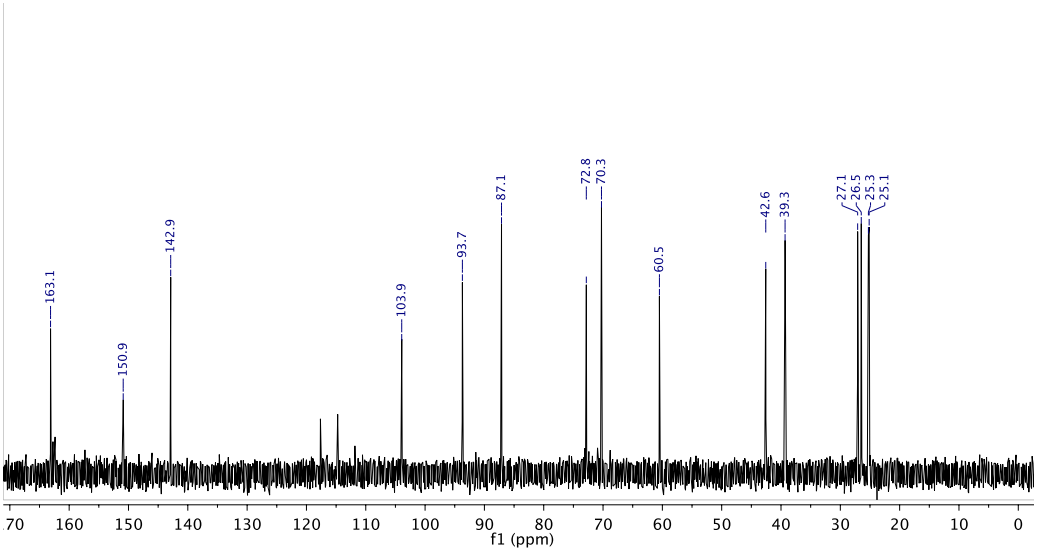
5’-*N*-(4-aminobutyl)-5’-amino-5’-deoxy-uridine (6).



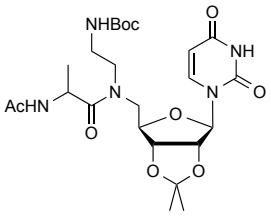


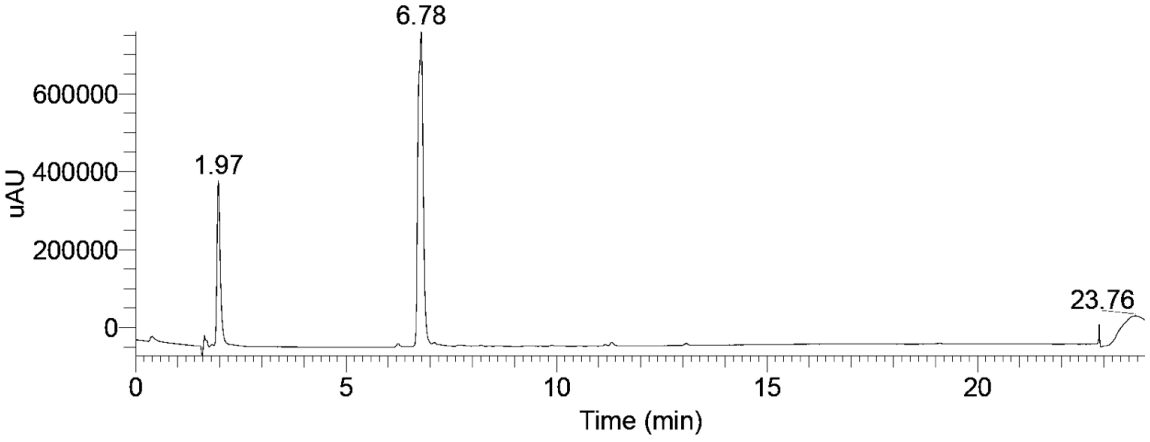
5’-*N*-(6-aminohexyl)-5’-amino-5’-deoxy-uridine (7).



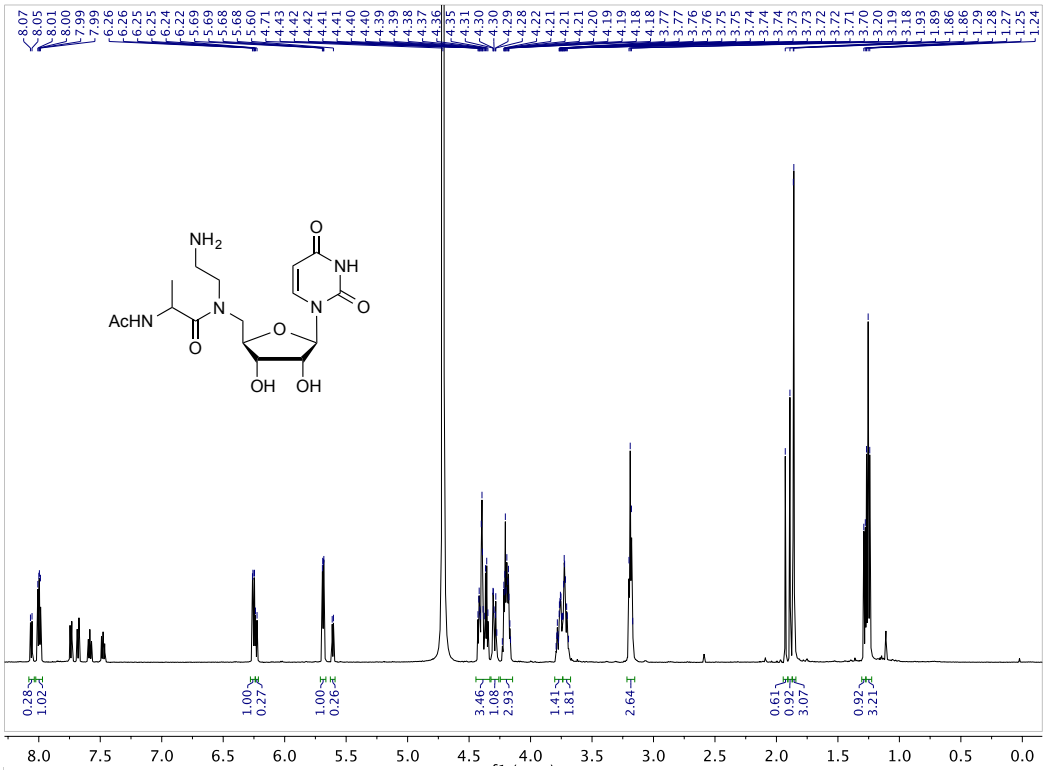


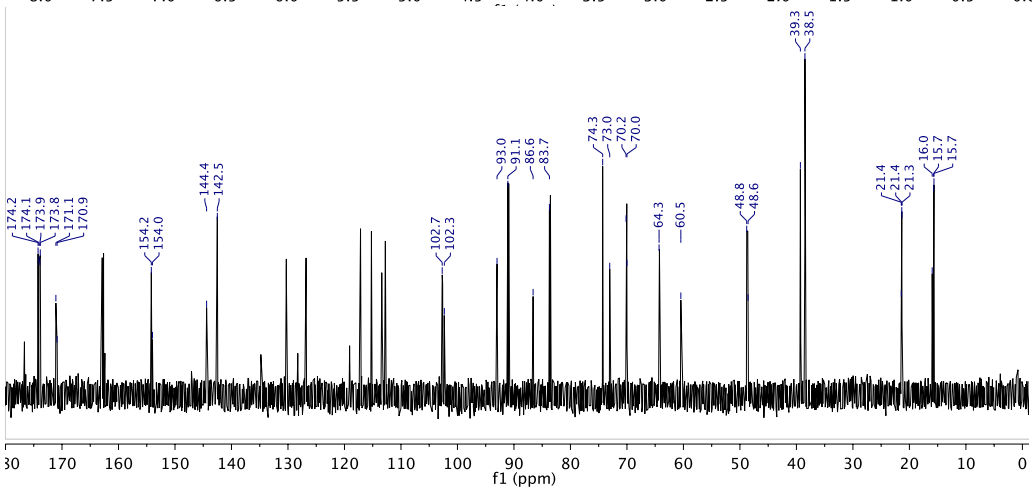
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(*tert*-butyl *N*-[2-aminoethyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (30).

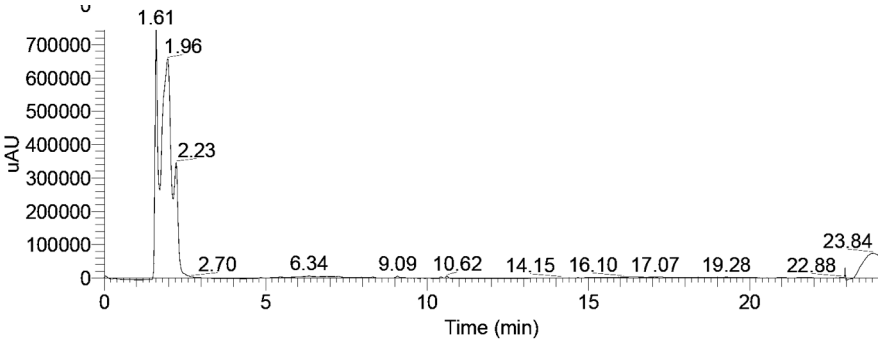




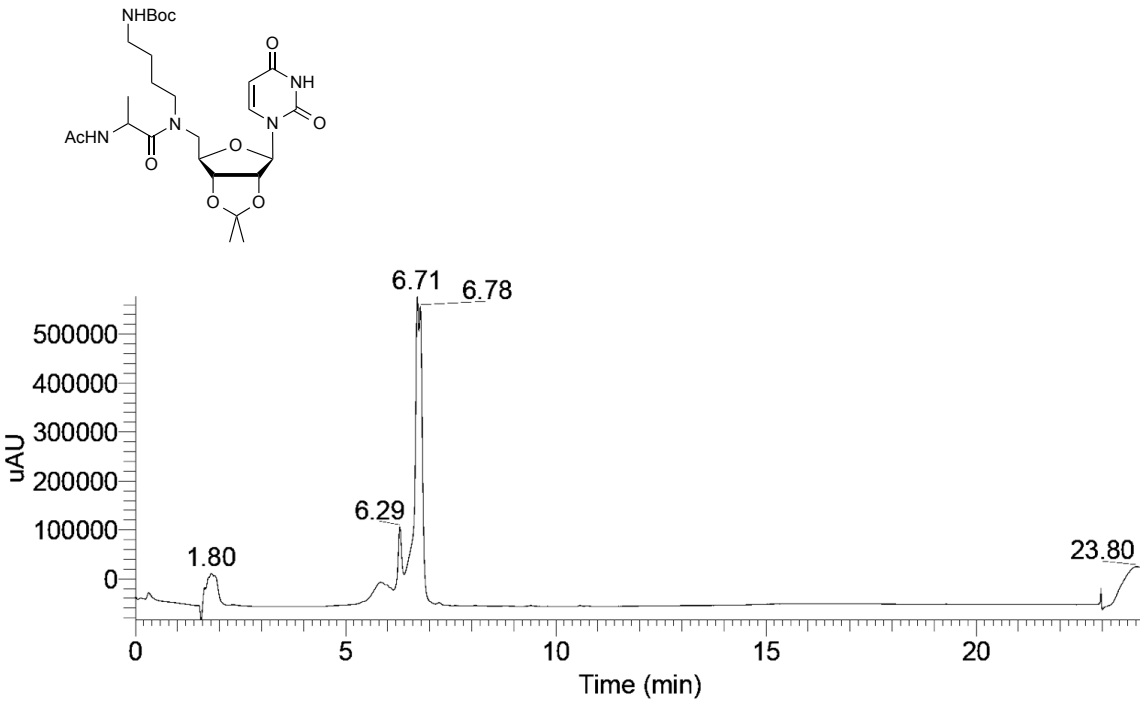
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(2-aminoethyl)-5'-amino-5'-deoxy-uridine (8).



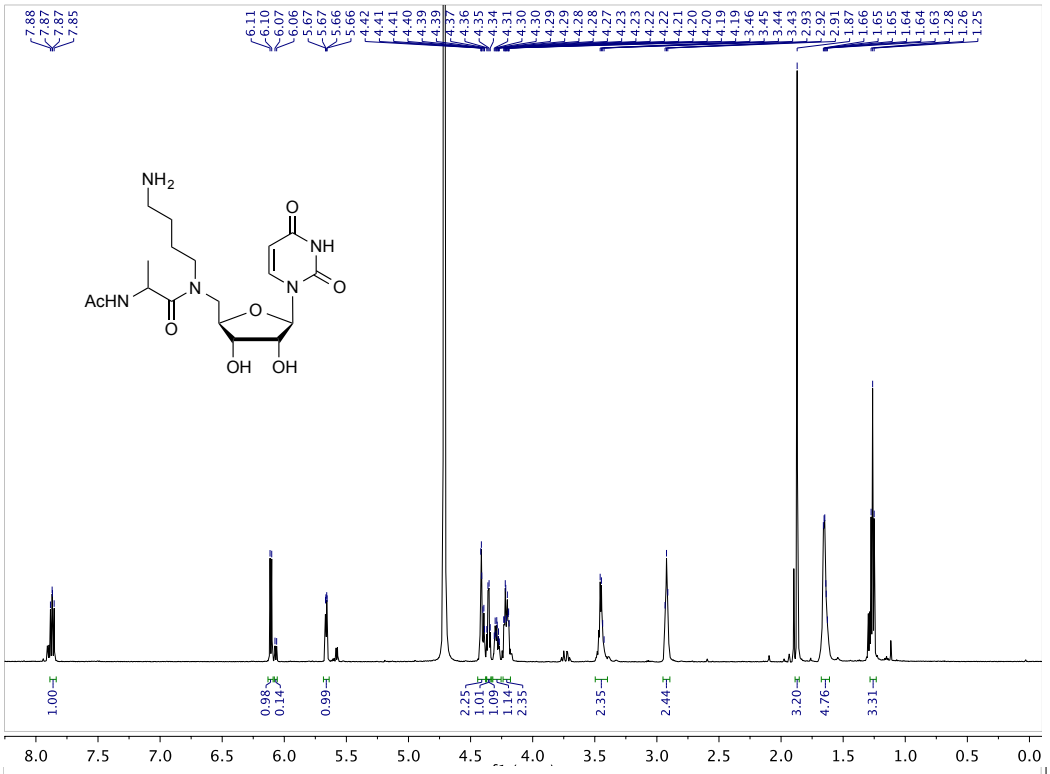


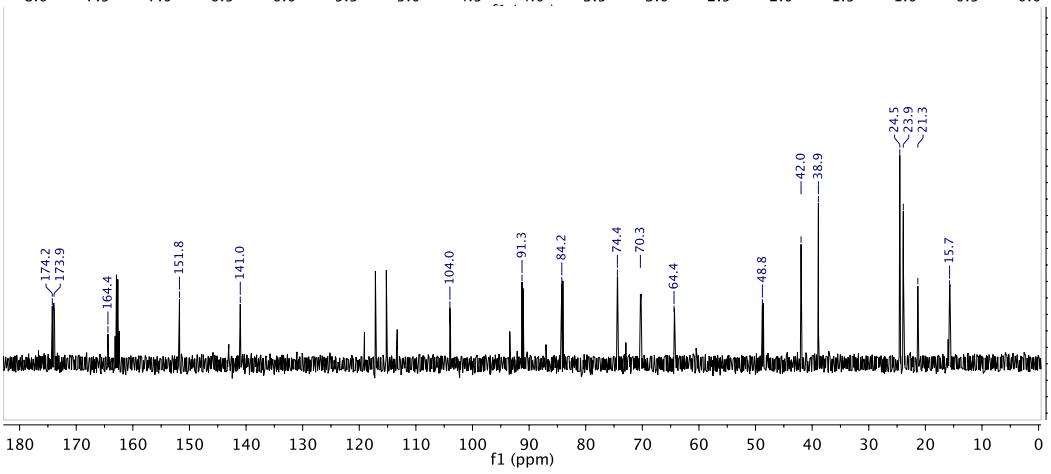


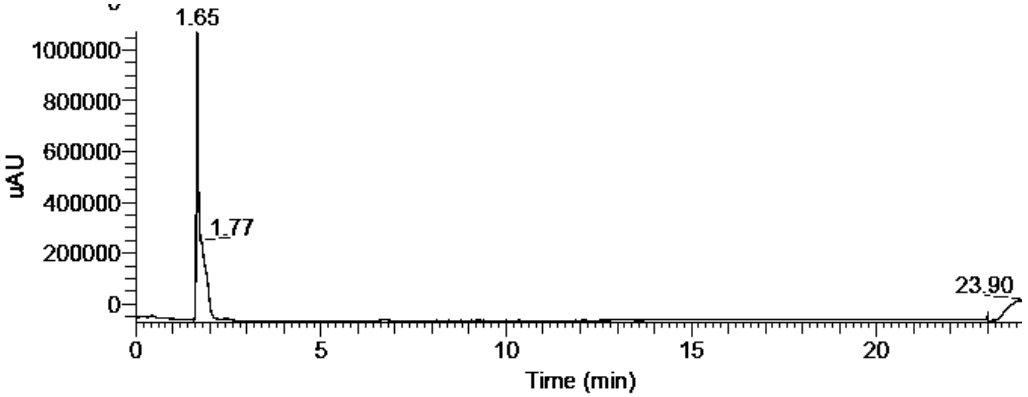
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(*tert*-butyl *N*-[4-aminobutyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (31).



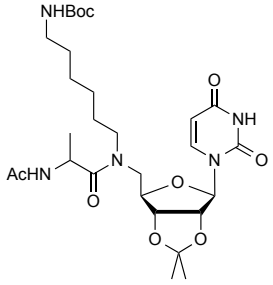
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(4-aminobutyl)-5'-amino-5'-deoxy-uridine (9).

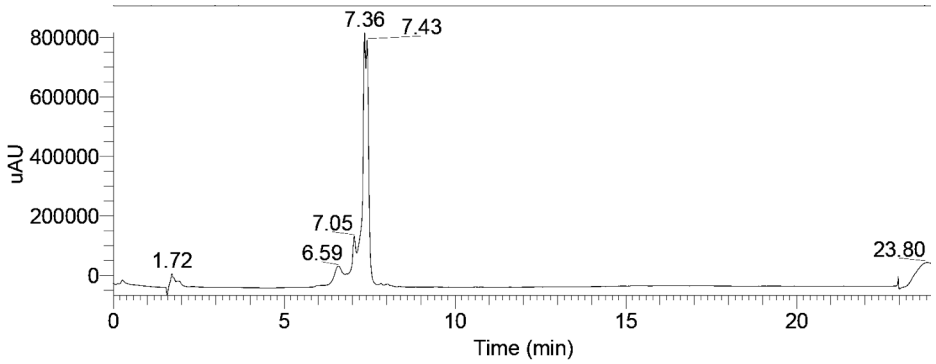




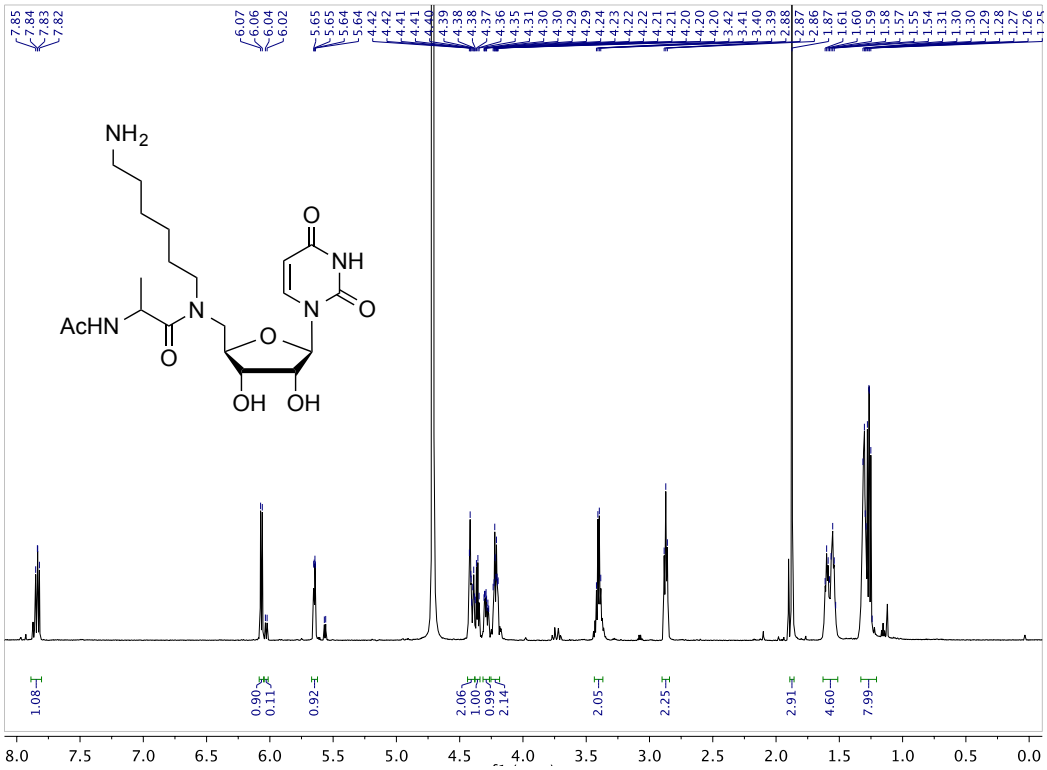


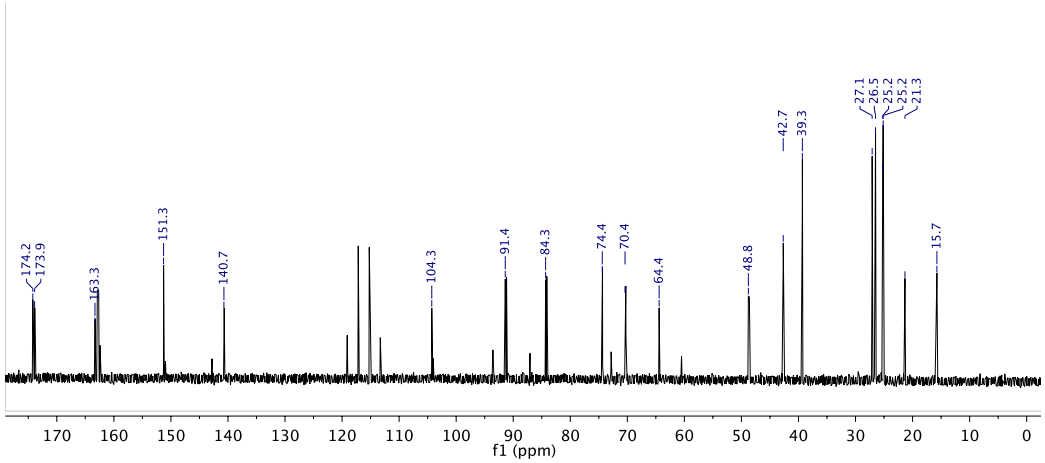
5'-*N*-(*N*-acetyl-D/L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (32).

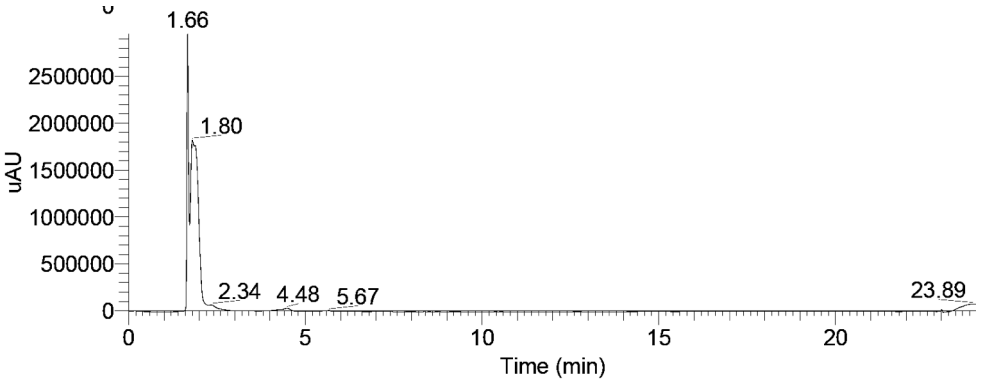




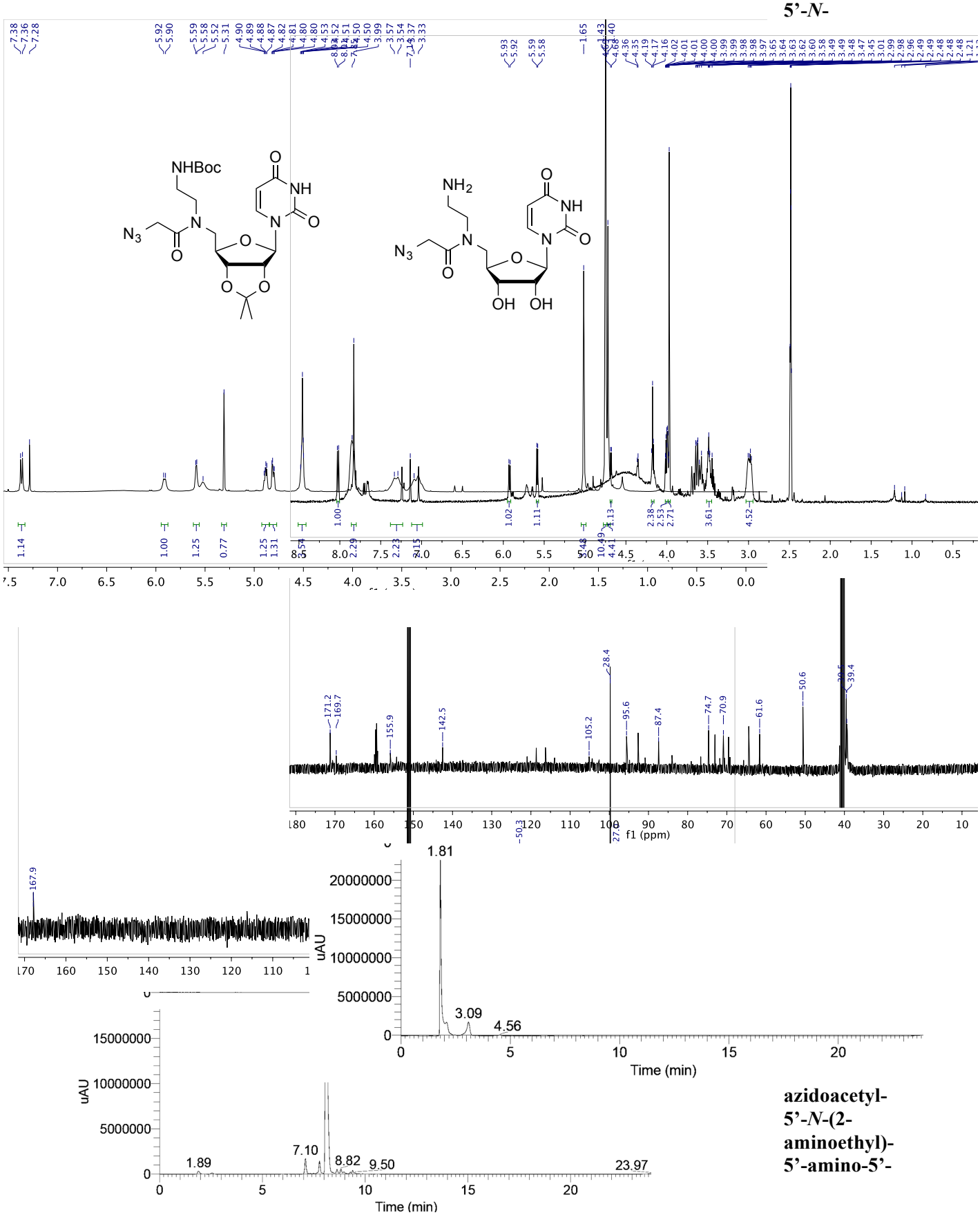
5’-*N*-(*N*-acetyl-D/L-alanine)-5’-*N*-(6-aminohexyl)-5’-amino-5’-deoxy- uridine (10).







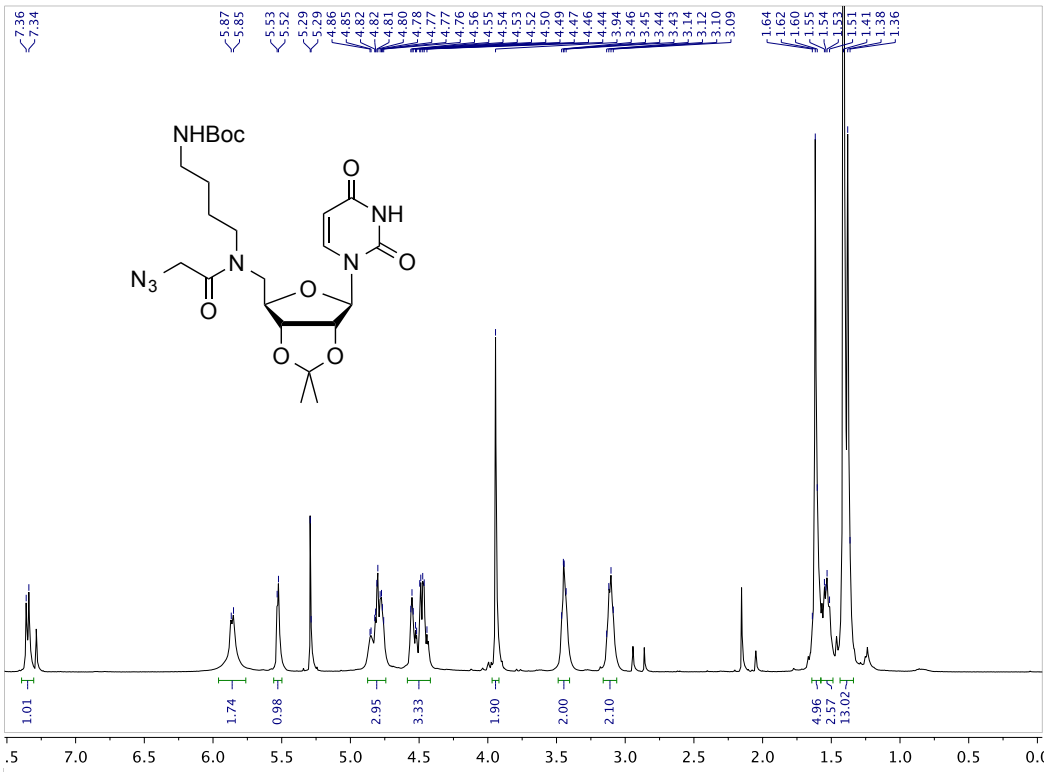
5’-*N*-azidoacetyl-5’-*N*-(*tert*-butyl *N*-[2-aminoethyl] carbamate)-5’-amino-5’-deoxy-2’,3’-*O*-isopropylidene uridine (33).

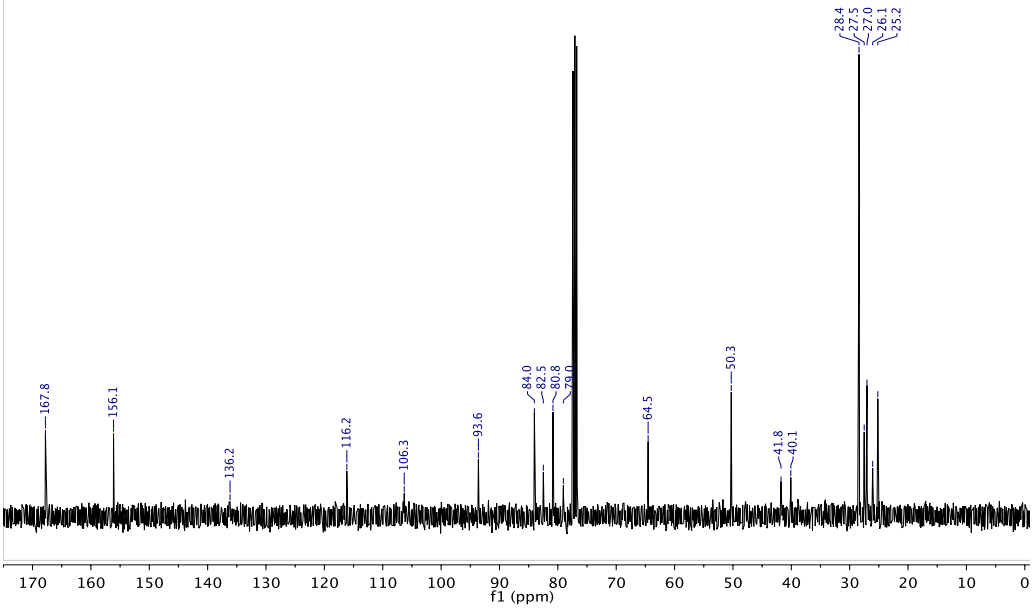


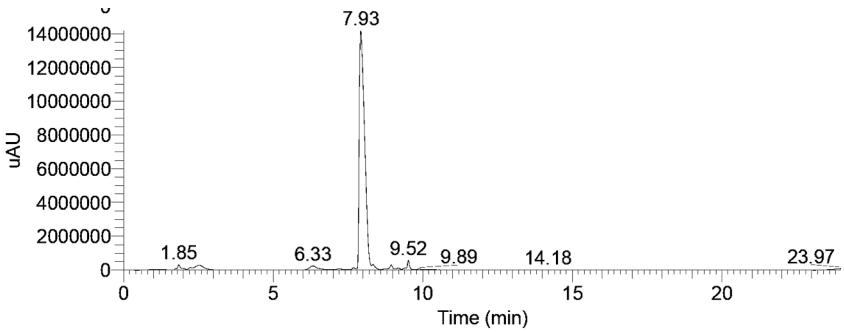
5’-*N*-azidoacetyl-5’-*N*-(2-aminoethyl)-5’-amino-5’-

deoxy-uridine (11).

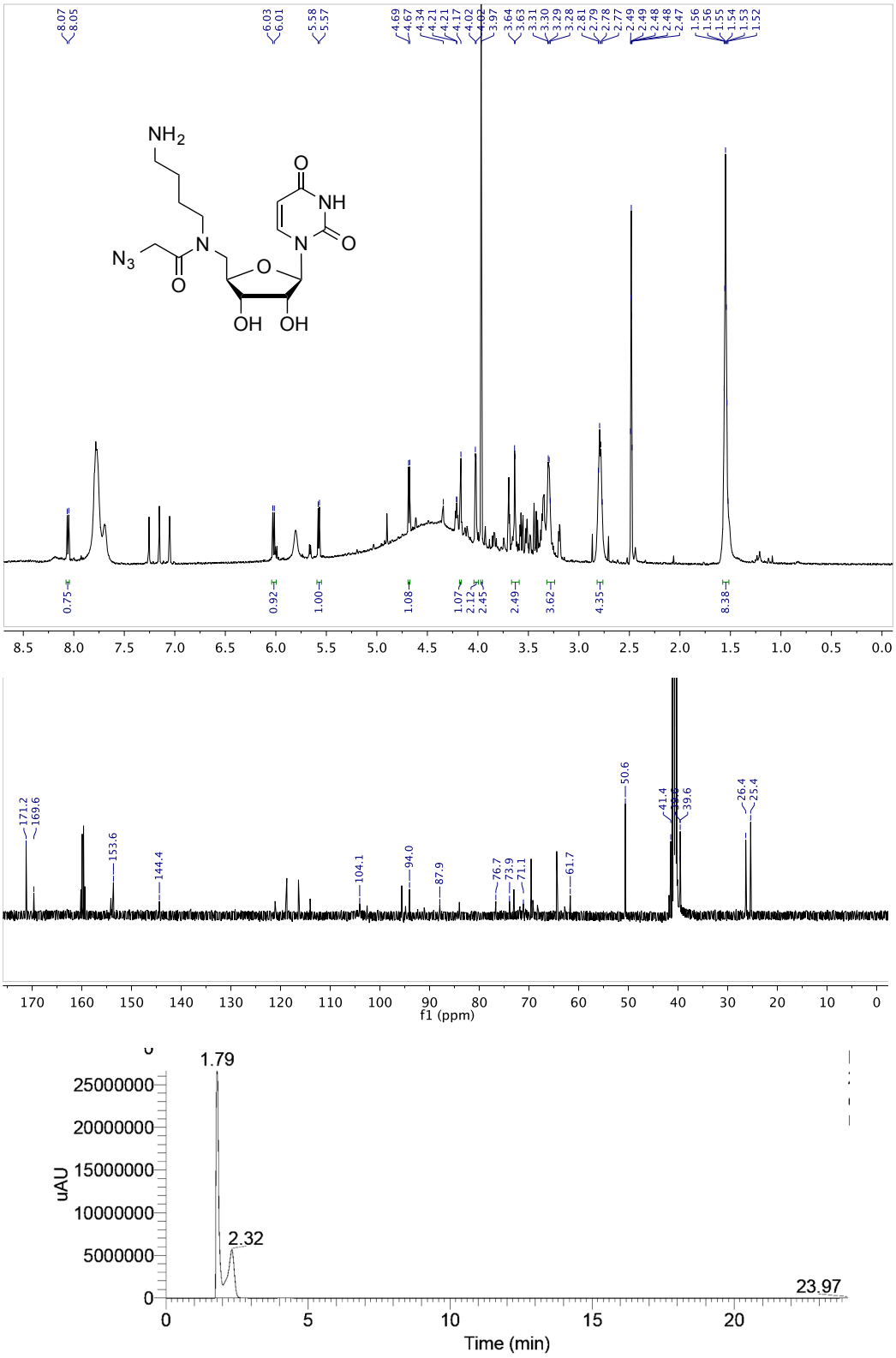
5'-*N*-azidoacetyl-5'-*N*-(*tert*-butyl *N*-[4-aminobutyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (34).



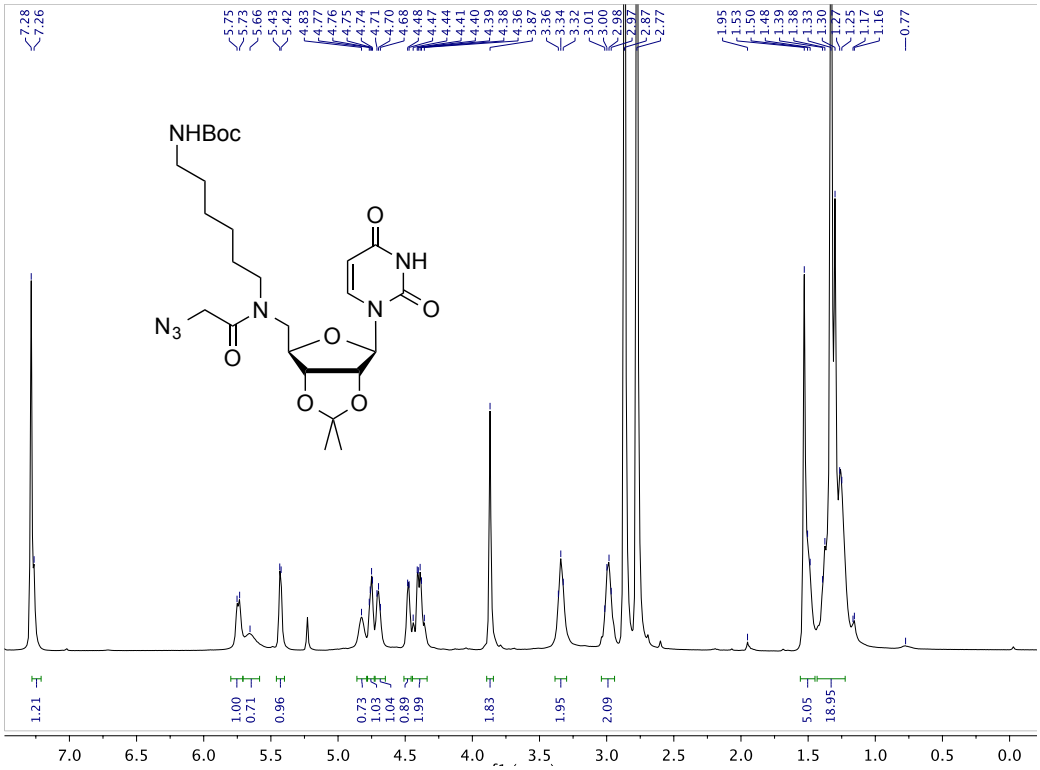


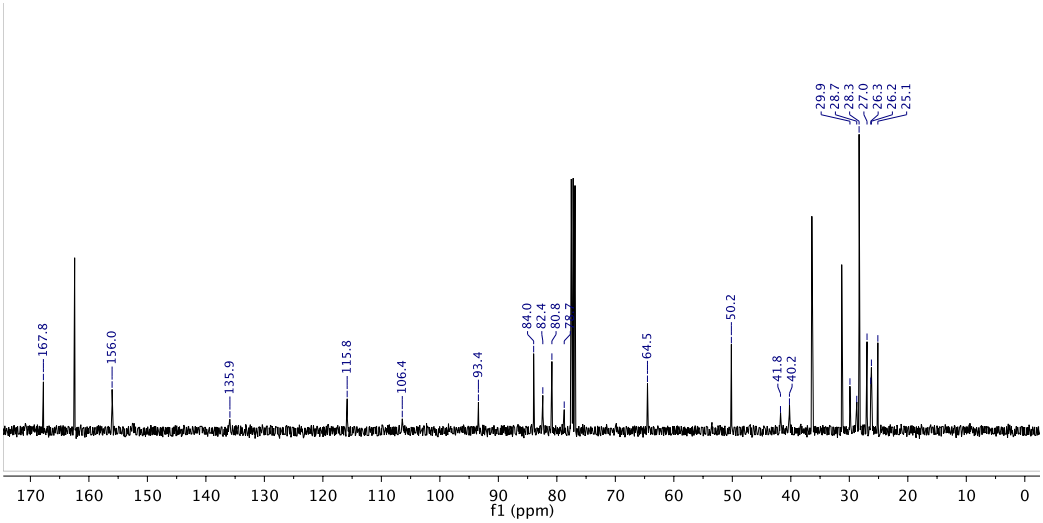


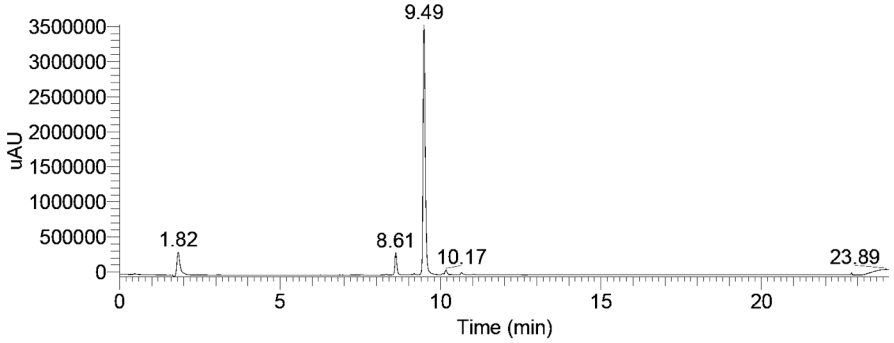
5'-*N*-azidoacetyl-5'-*N*-(4-aminobutyl)-5'-amino-5'-deoxy-uridine (12).



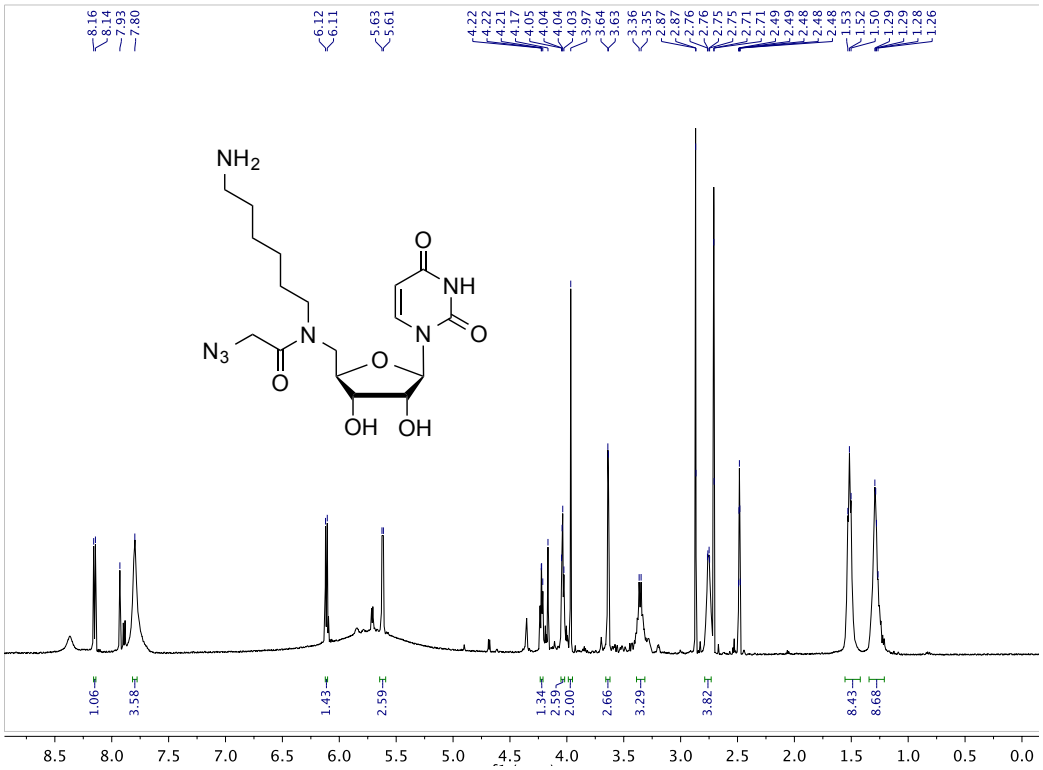
5'-*N*-azidoacetyl-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (35).

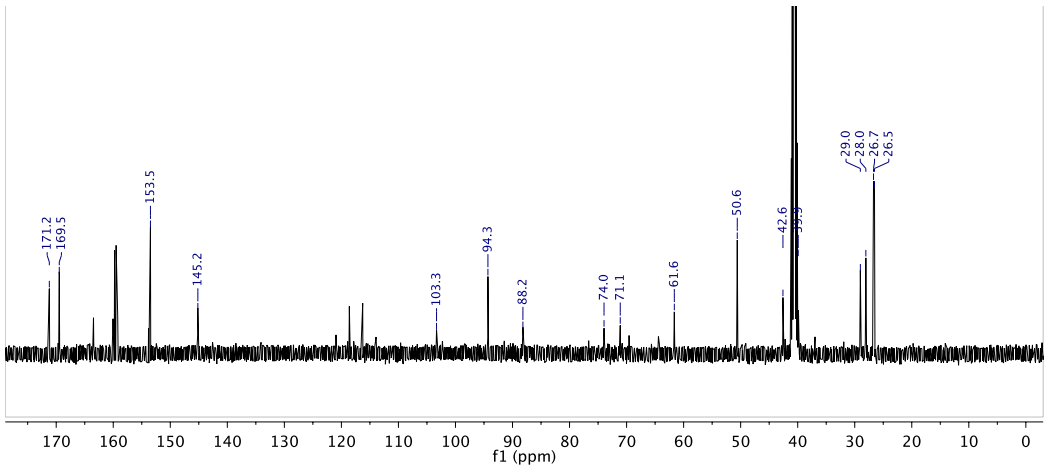


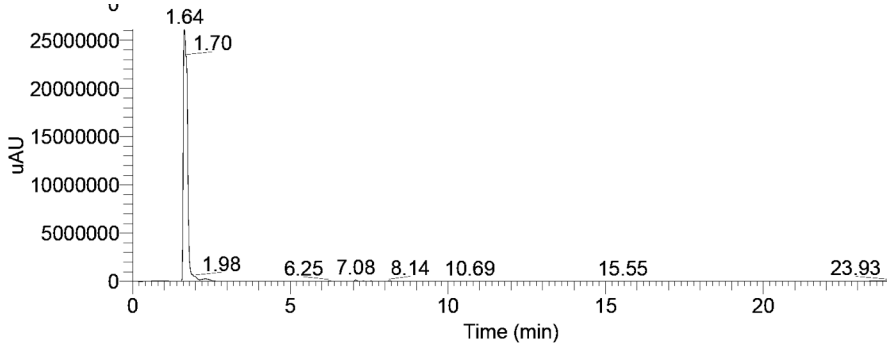




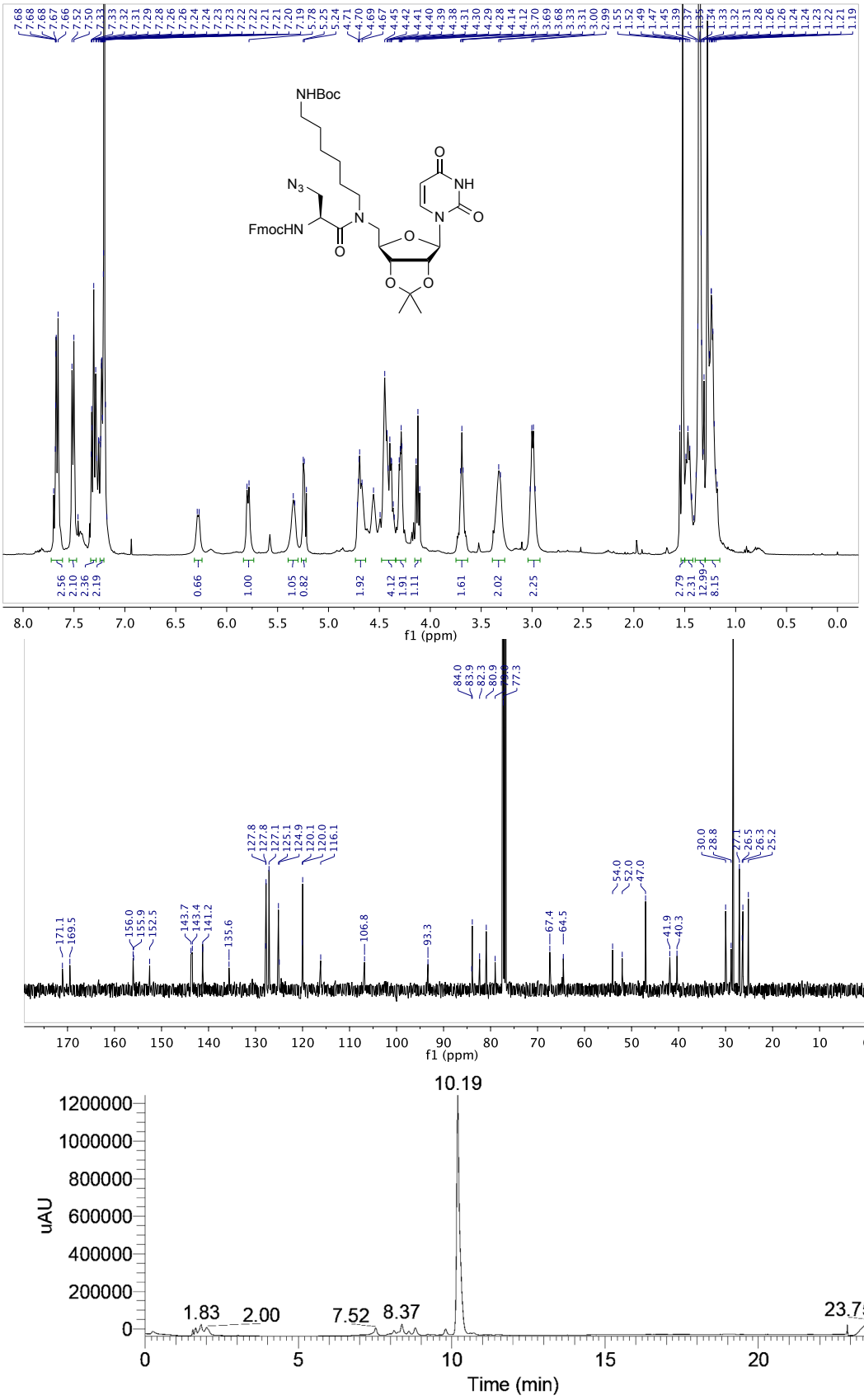
5'-*N*-azidoacetyl-5'-*N*-(6-aminohexyl)-5'-amino-5'-deoxy-uridine (13).



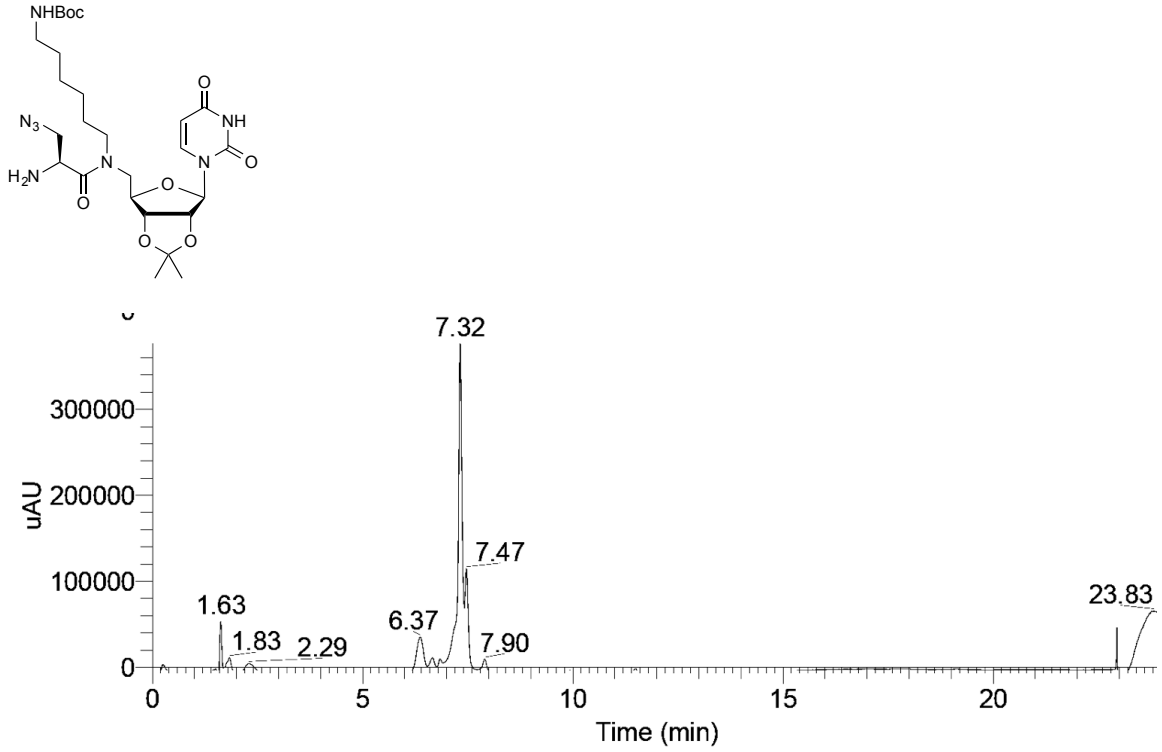




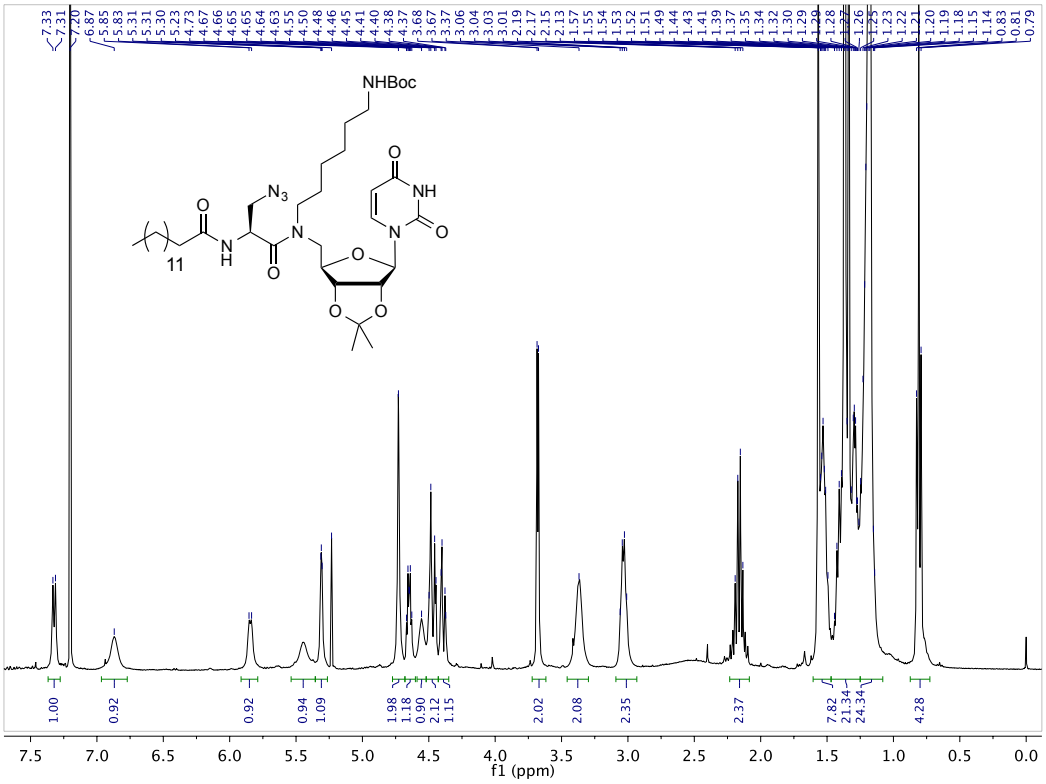
5'-*N*-(*N*-[9-fluorenylmethoxycarbonyl]-3-azido-L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (14).

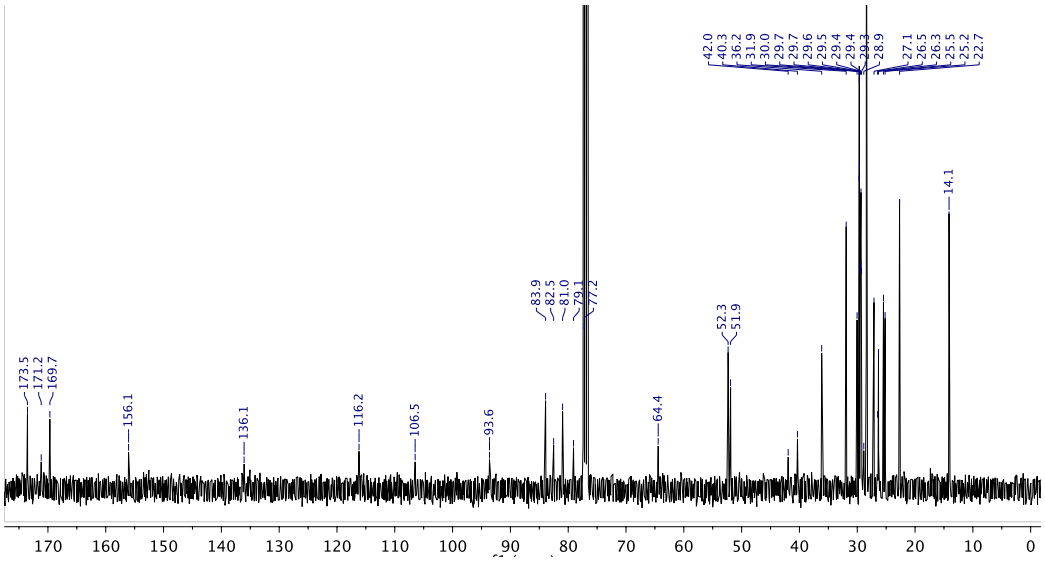


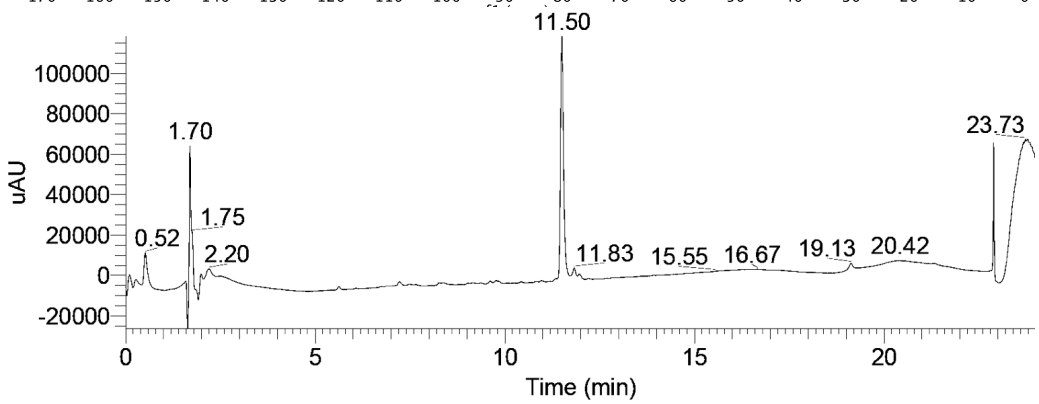
5'-*N*-(3-azido-L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (15).



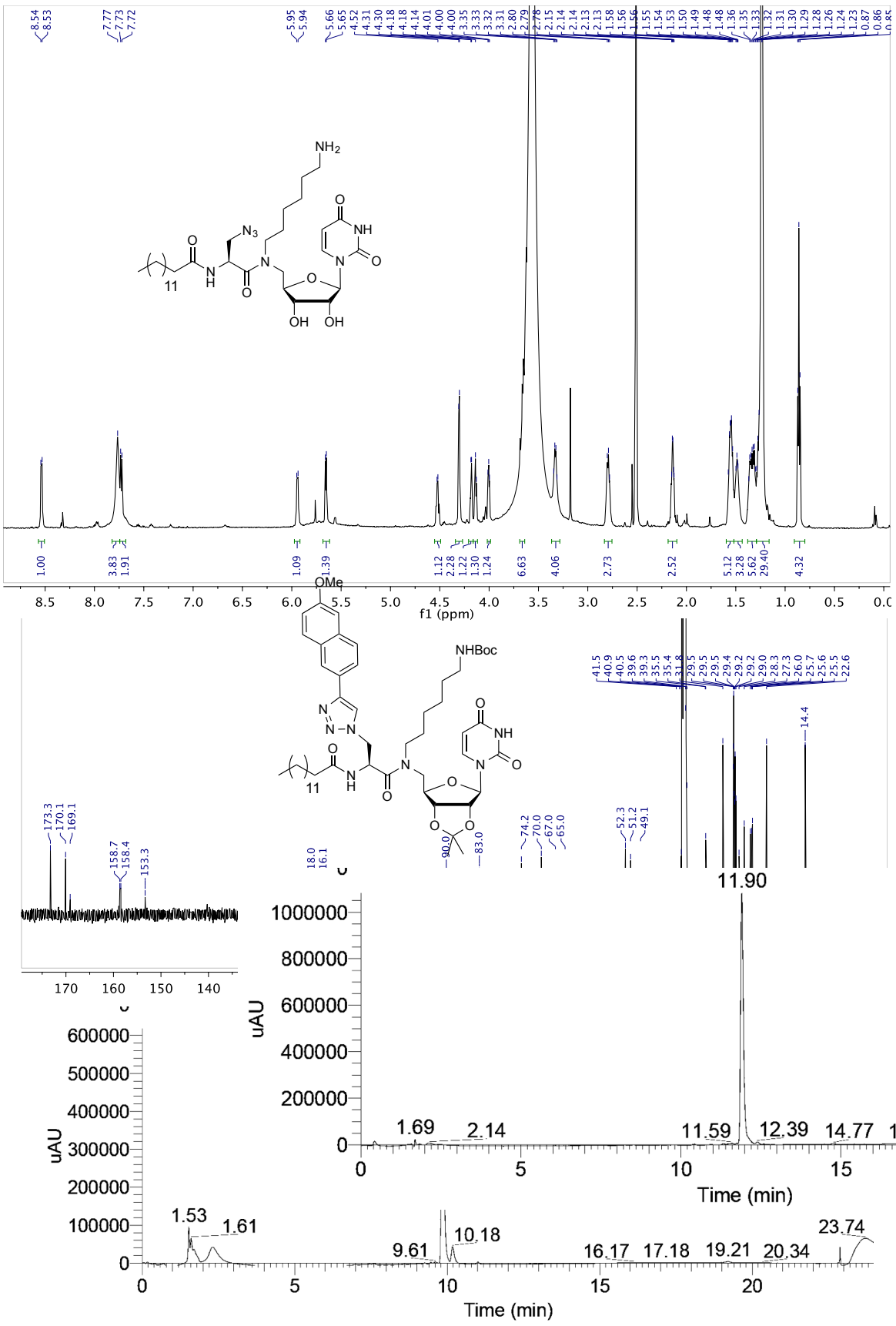
5'-*N*-(*N*-myristoyl-3-azido-L-alanine)-5'-*N*-(*tert*-butyl *N*-[6-aminohexyl] carbamate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (16).







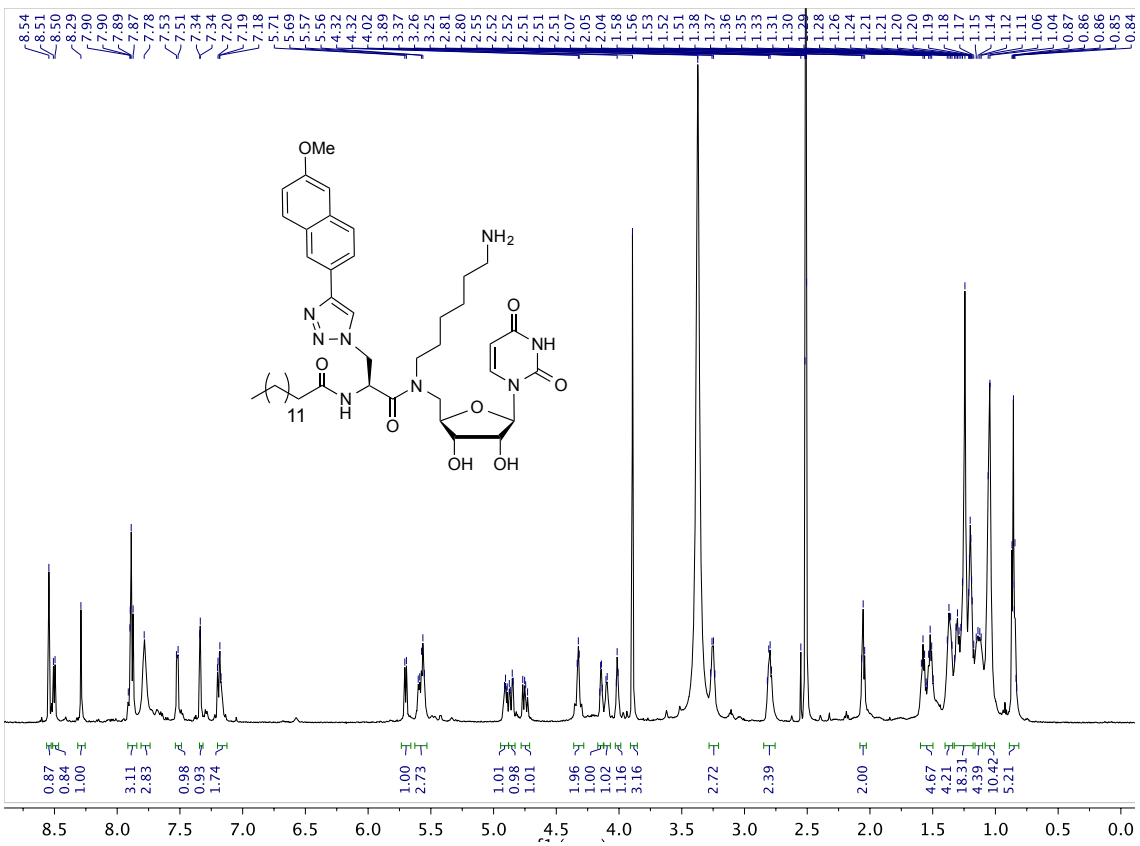
5’-*N*-(*N*-myristoyl-3-azido-L-alanine)-5’-*N*-(6-aminohexyl)-5’-amino-5’-deoxy uridine (17).

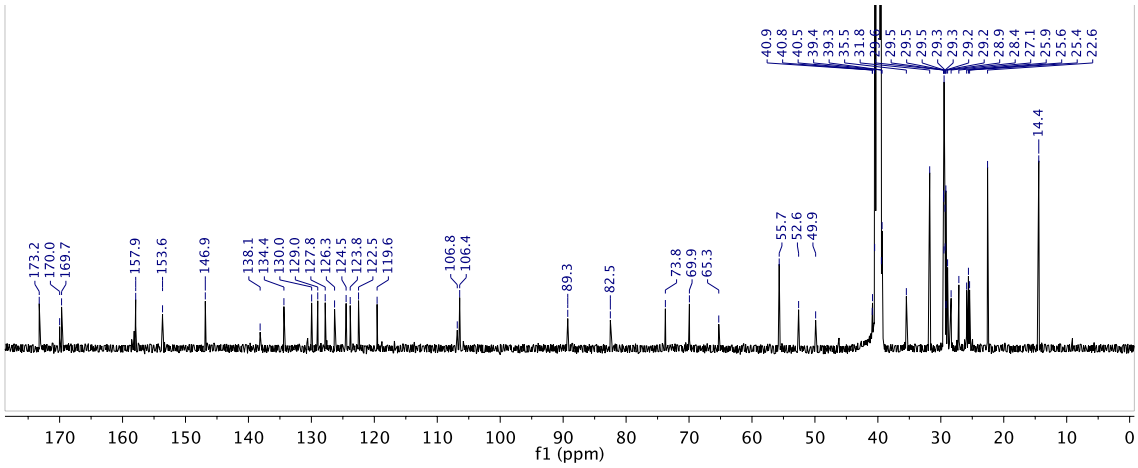


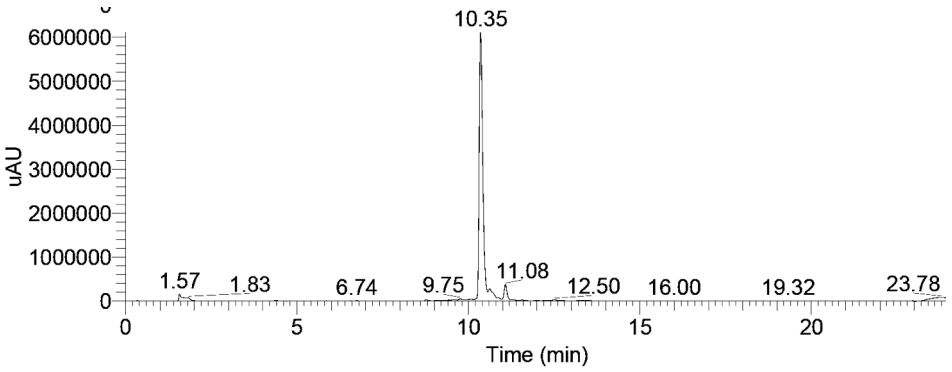
5’-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alanine)-5’-*N*-(*tert*-butyl *N*-[6-aminohexyl]carbamate)-5’-amino-5’-deoxy-2’,3’-*O*-isopropylidene uridine (36).

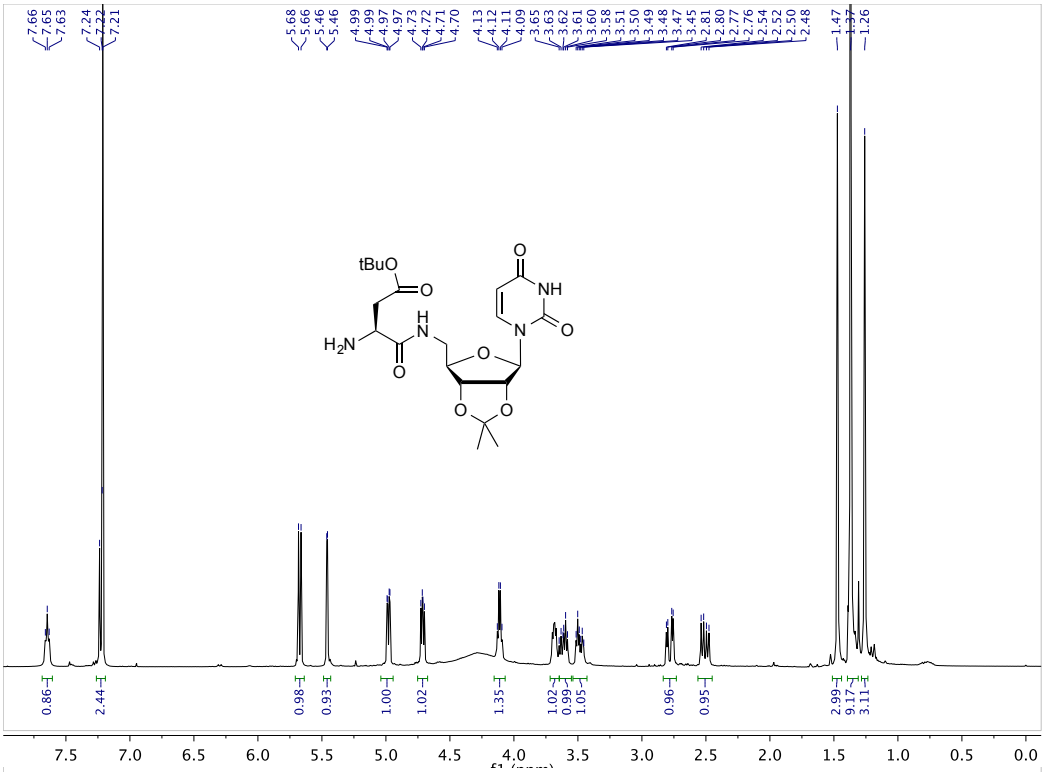
5’-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alanine)-5’-*N*-(6-aminohexyl)-5’-amino-5’-deoxy uridine (18).

5’-*N*-(*O*-*tert*-butyl-L-aspartate)-5’-amino-5’-deoxy-2’,3’-*O*-isopropylidene uridine (20).








7.66
7.65
7.63
7.24
7.22
7.21
5.68
5.66
5.46
5.46
4.99
4.99
4.97
4.97
4.73
4.72
4.71
4.70
4.13
4.12
4.11
4.09
3.65
3.63
3.62
3.61
3.60
3.58
3.51
3.50
3.49
3.48
3.47
3.45
2.81
2.80
2.77
2.76
2.54
2.52
2.50
2.48
1.47
1.37
1.26
tBuO
O
NH
H
N
H2N
O
O
O
O
O
0.86
2.44
0.98
0.93
1.00
1.02
1.35
1.02
0.99
1.05
0.96
0.95
2.99
9.17
3.11
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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3.0
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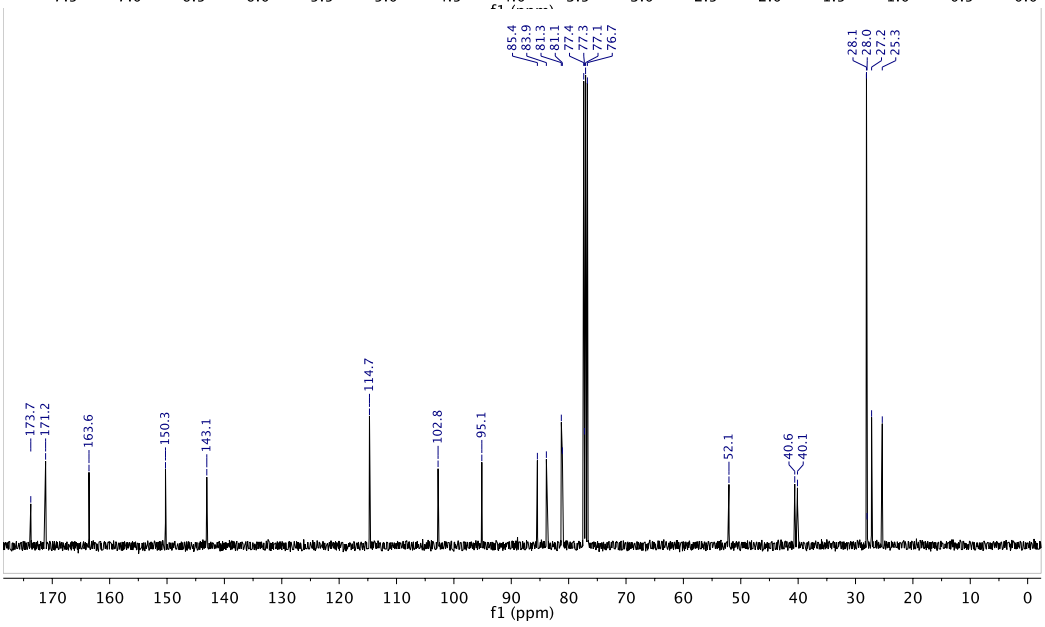
2.0
1.5
1.0
0.5
0.0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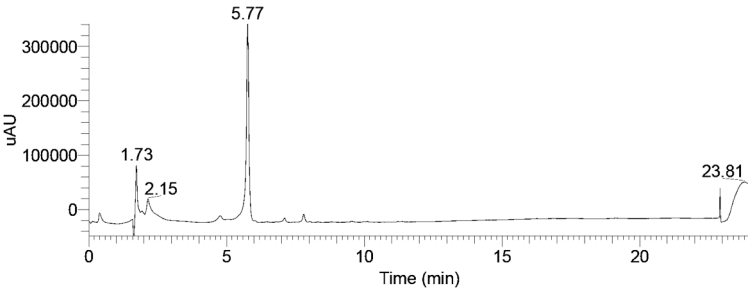
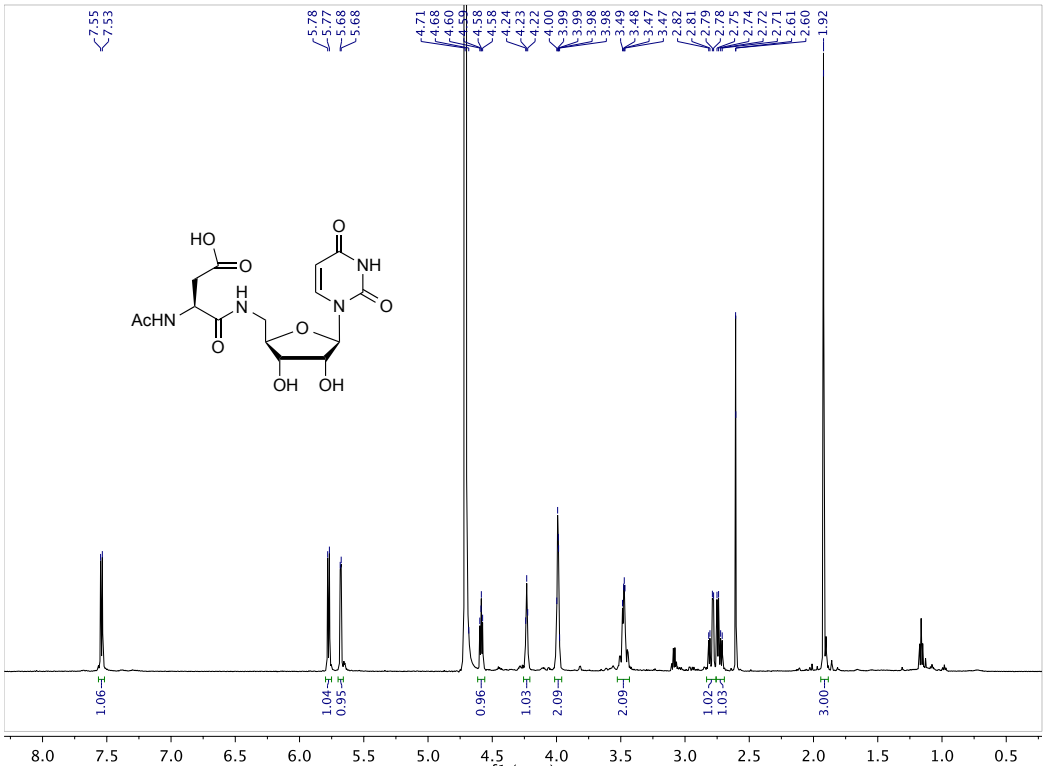
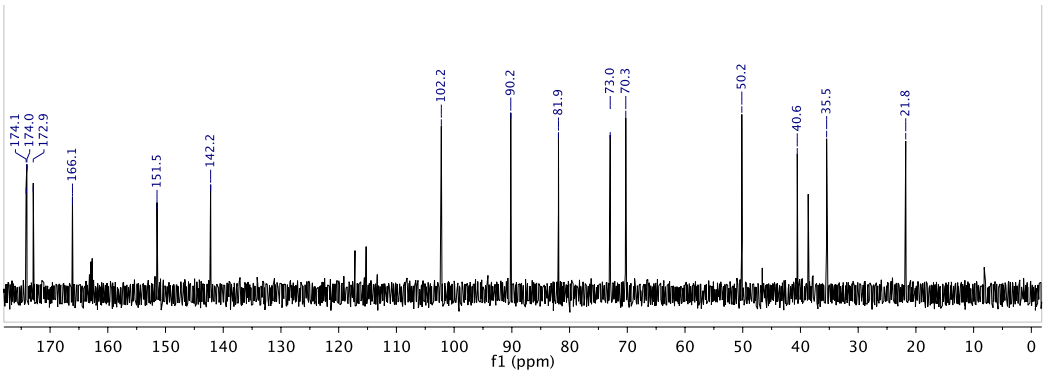
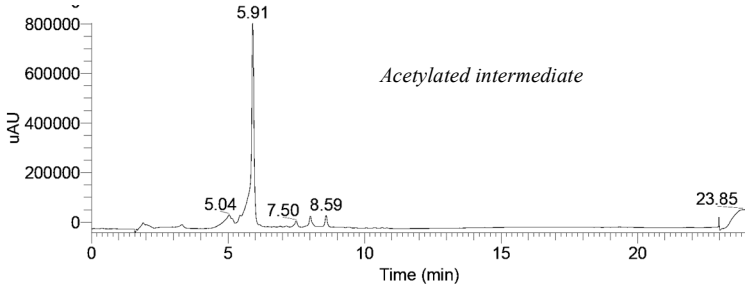
83.9
81.3
81.1
77.4
77.3
77.1
76.7
28.1
28.0
27.2
25.3
173.7
171.2
163.6
150.3
143.1
114.7
102.8
95.1
52.1
40.6
40.1
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


5.77
300000
200000
100000
0
uAU
1.73
2.15
23.81
0
5
10
15
20
Time (min)

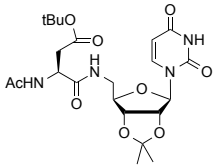
5'-*N*-(*N*-acetyl-L-aspartate)-5'-amino-5'-deoxy uridine (21).

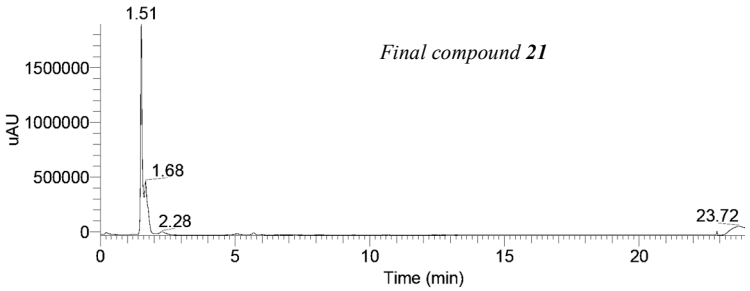






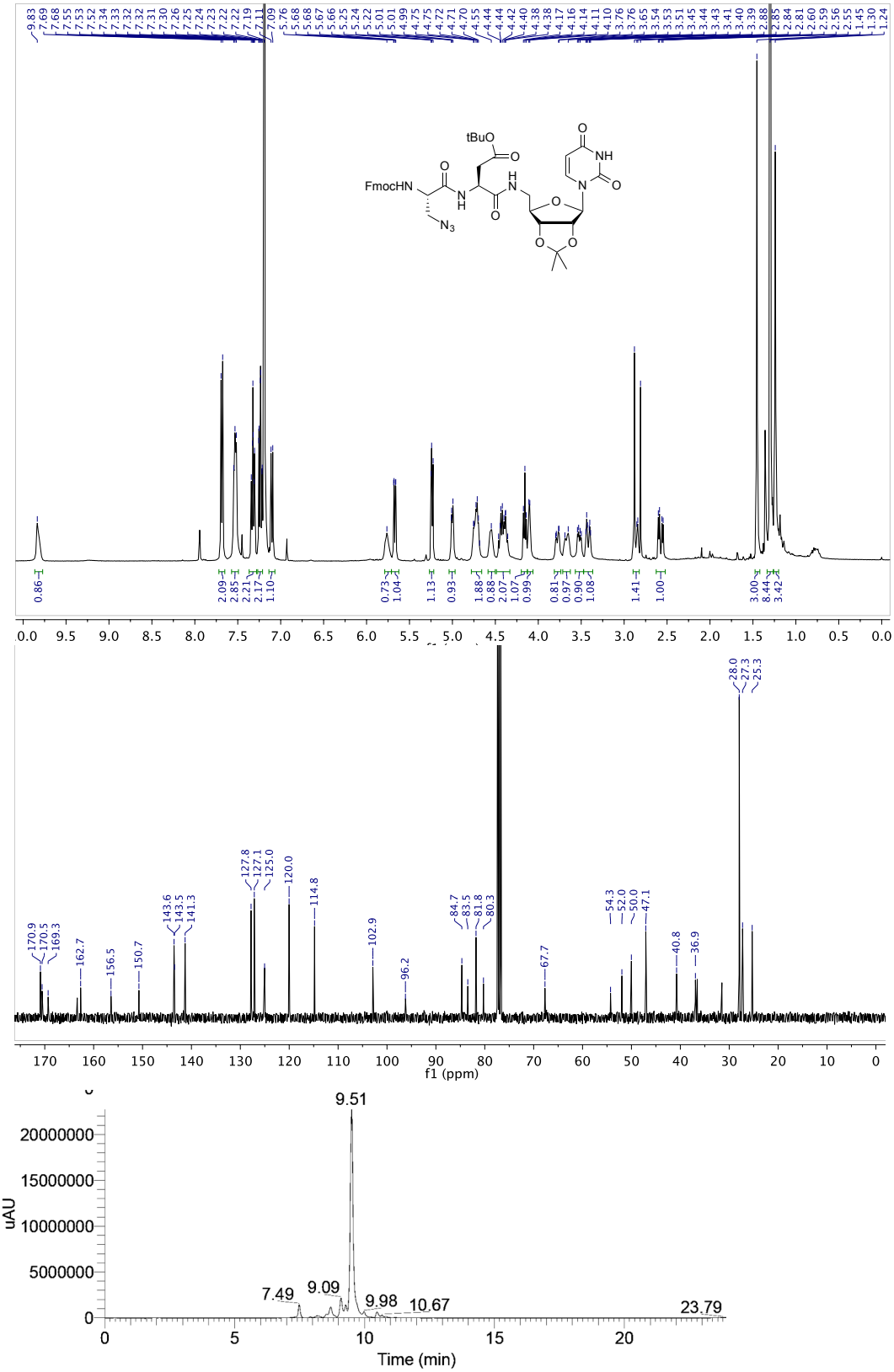
Acetylated intermediate



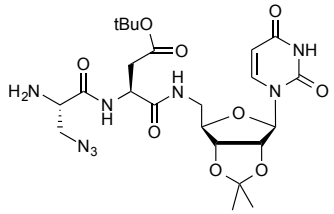


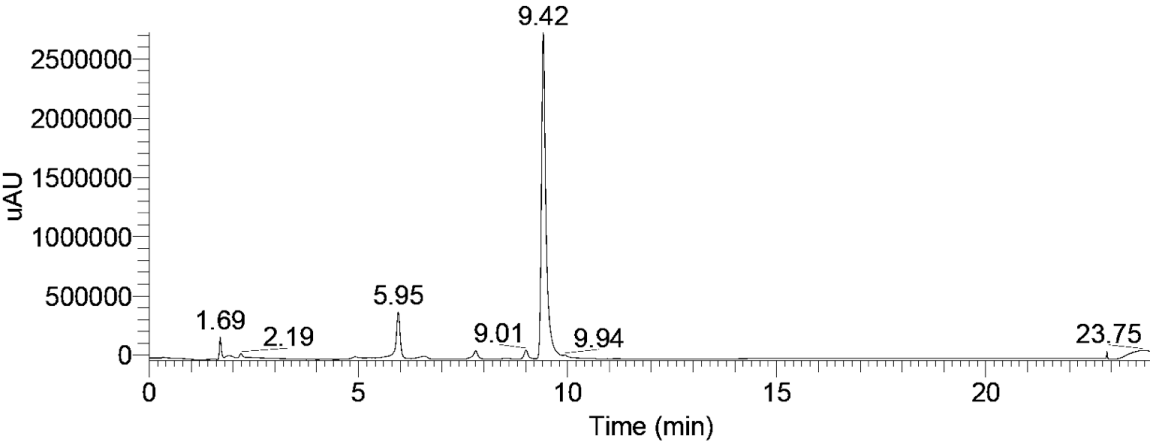
*Final compound **21***

5'-*N*-(*N*-[9-fluorenylmethoxycarbonyl]-3-azido-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (22).

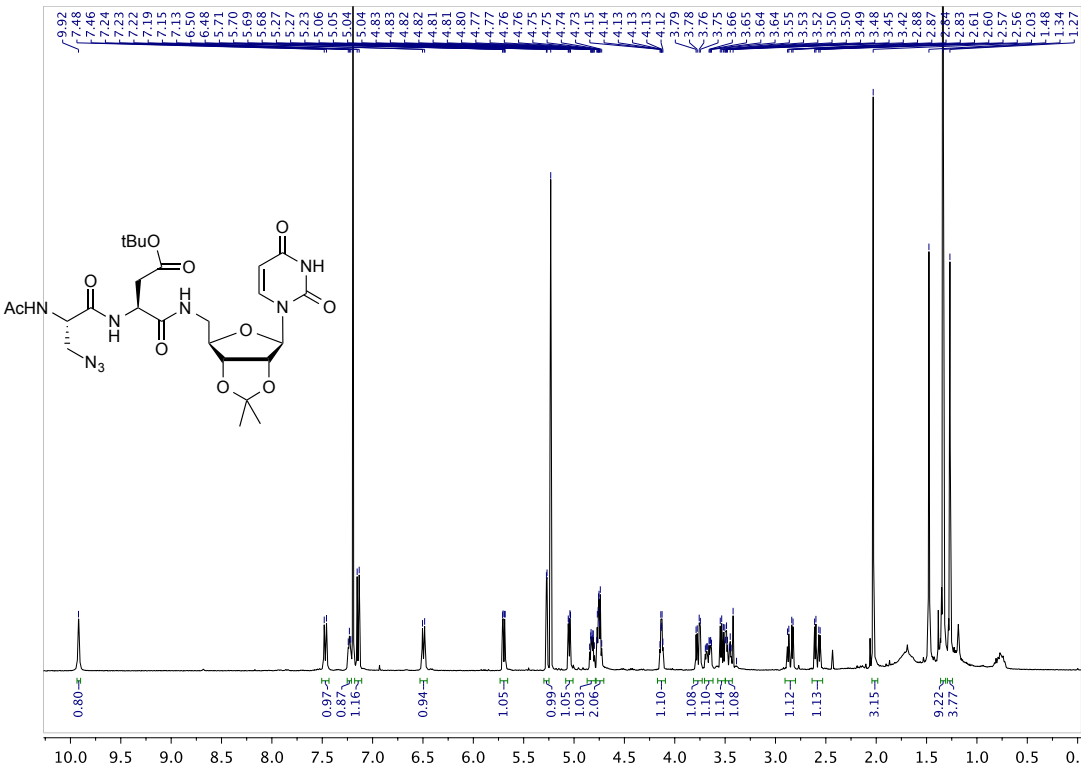


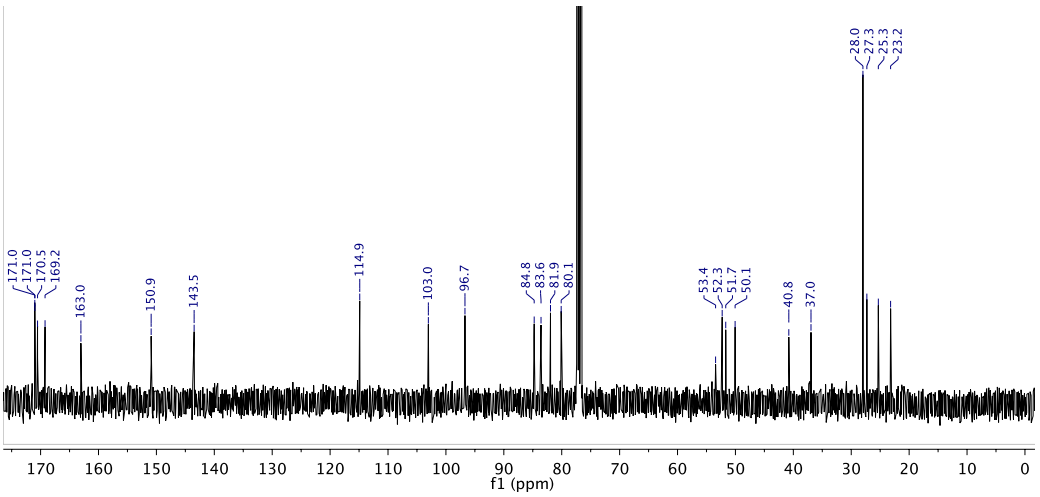
5'-*N*-(3-azido-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (23).

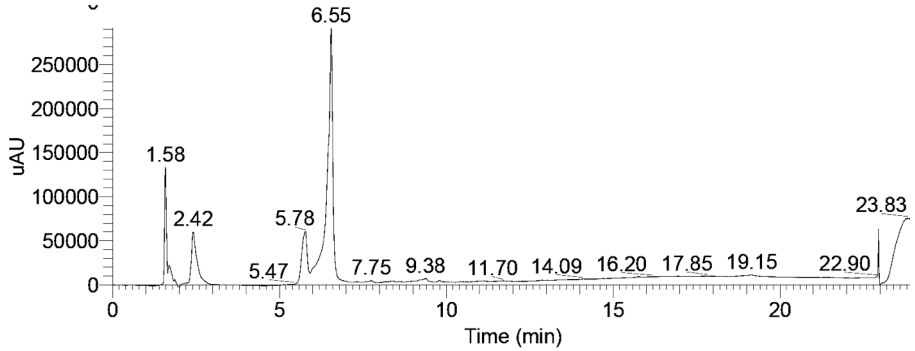




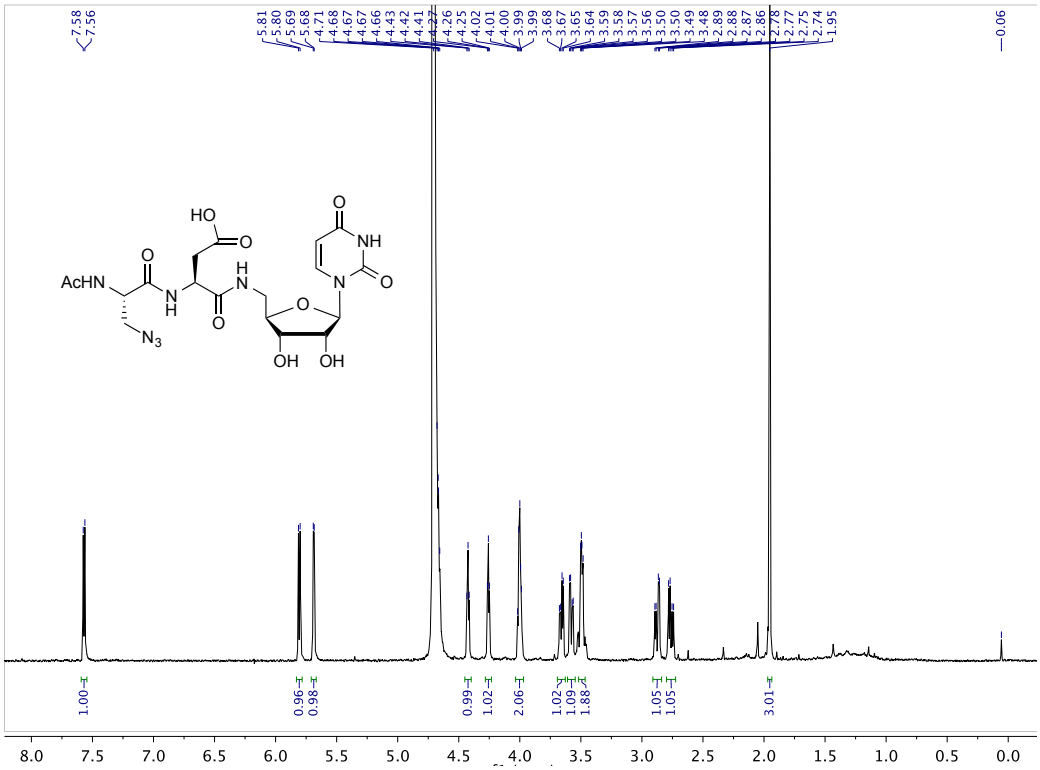
5'-*N*-(*N*-acetyl-3-azido-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (24).

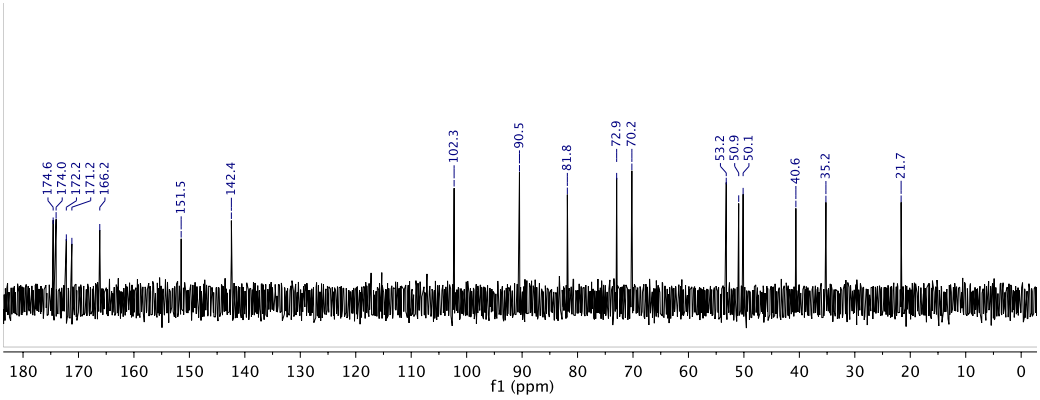


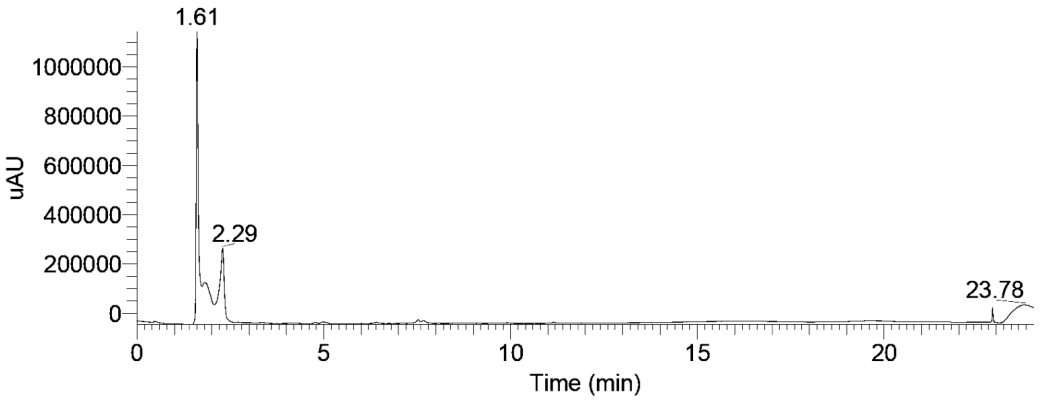




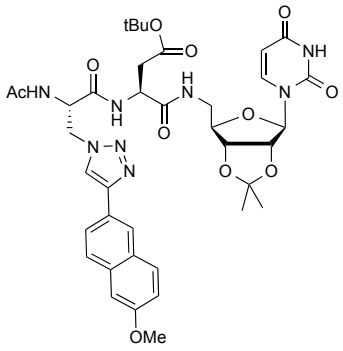
5'-*N*-(*N*-acetyl-3-azido-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (25).

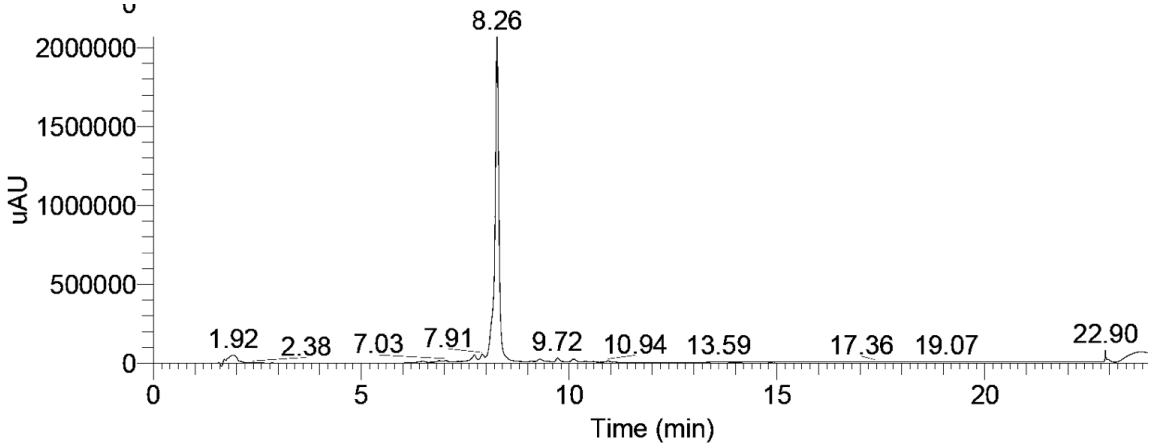




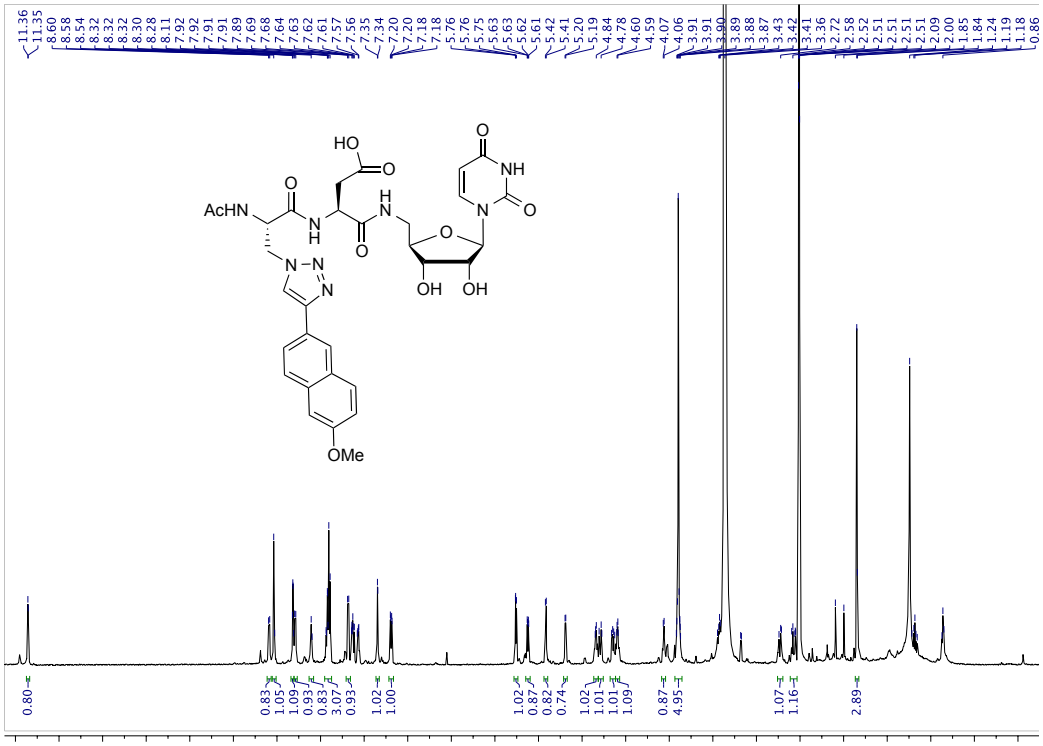


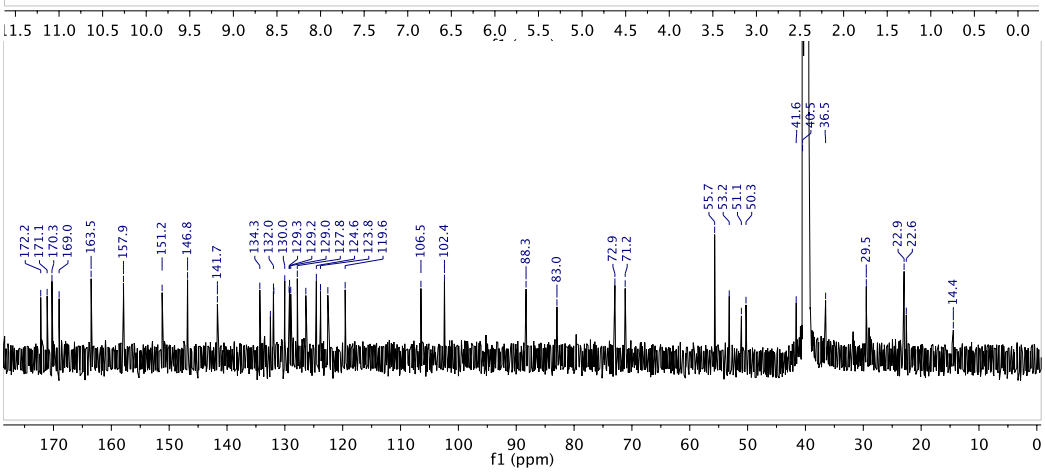
5'-*N*-(*N*-acetyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (37).

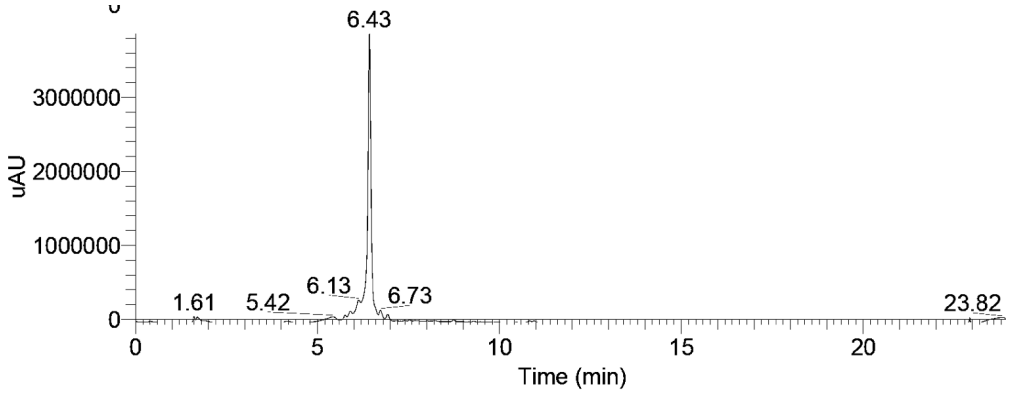




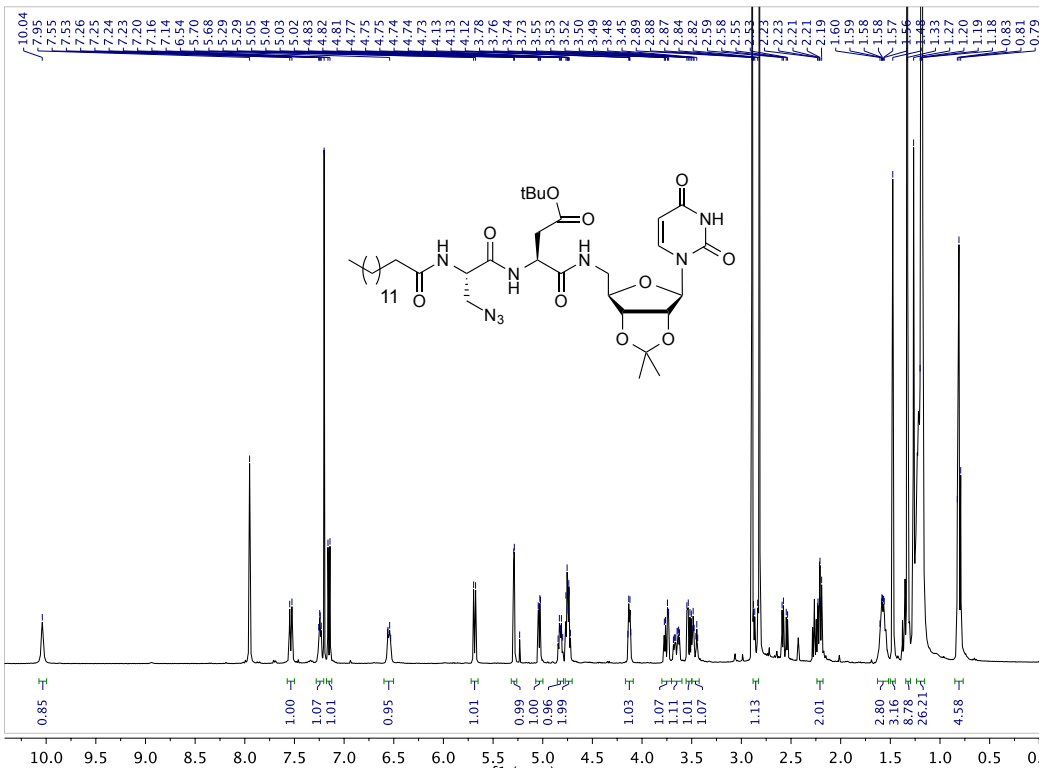
5'-*N*-(*N*-acetyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (26).

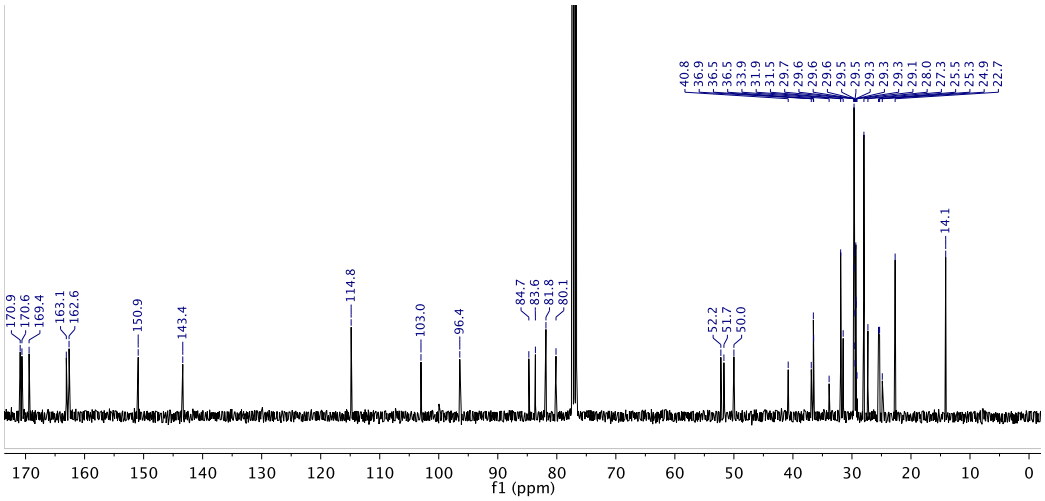


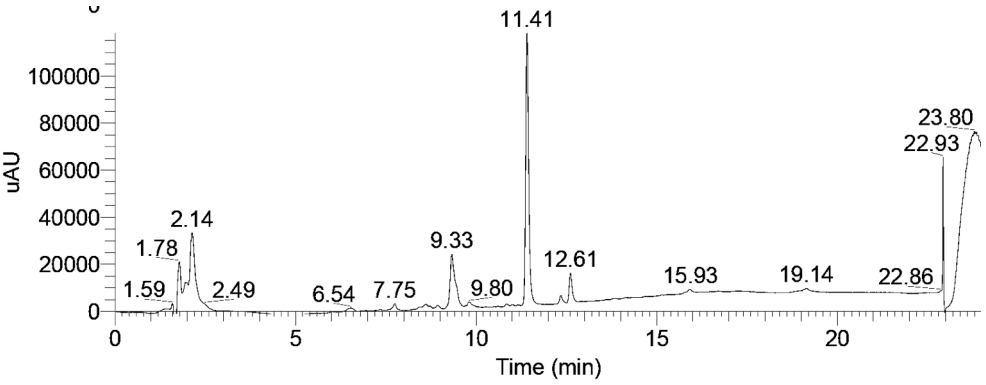




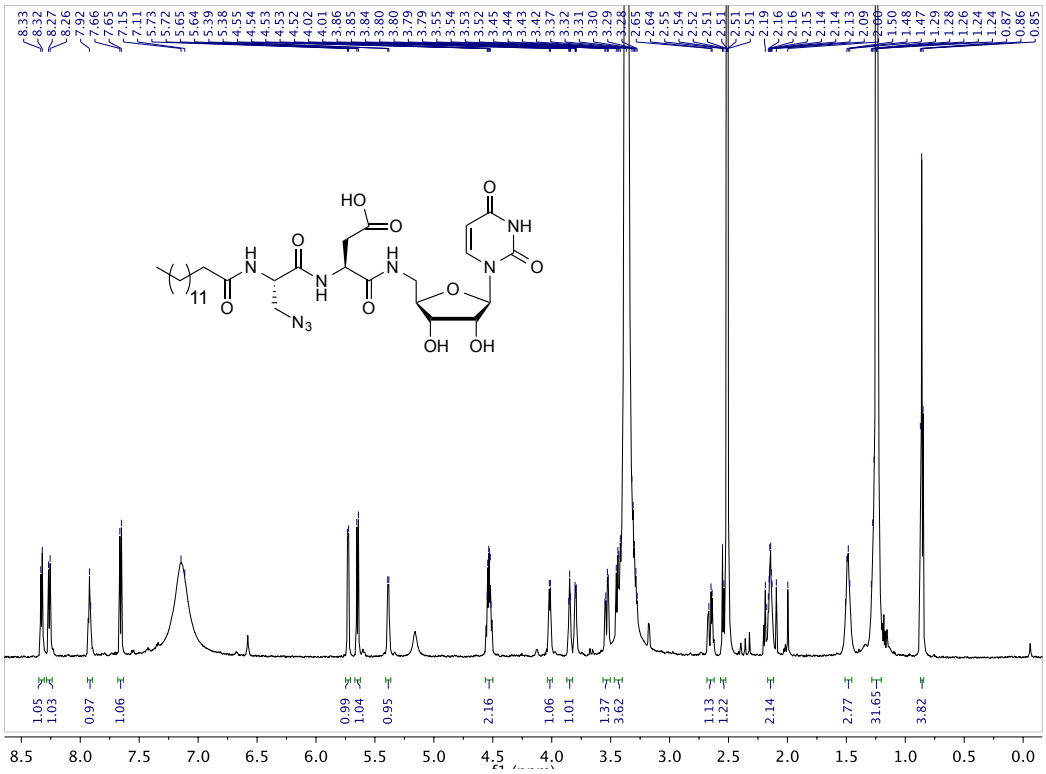
5'-*N*-(*N*-myristoyl-3-azido-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (27).

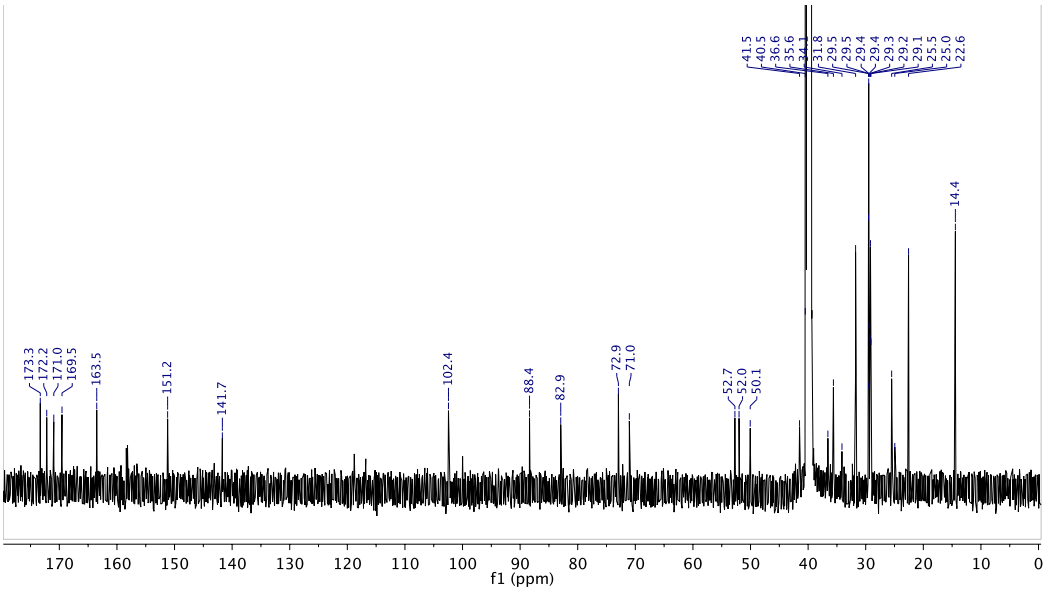


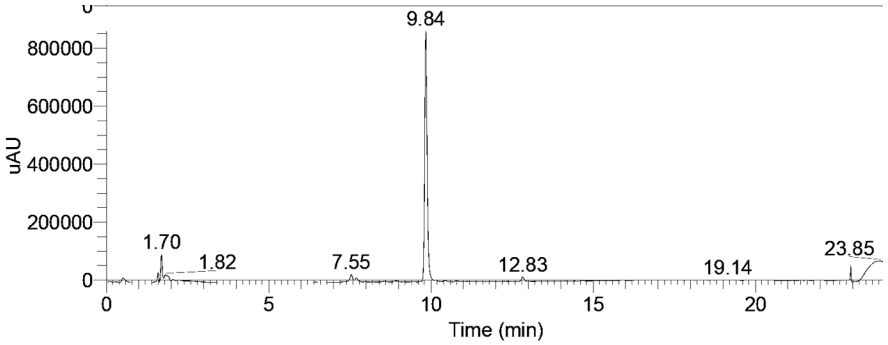




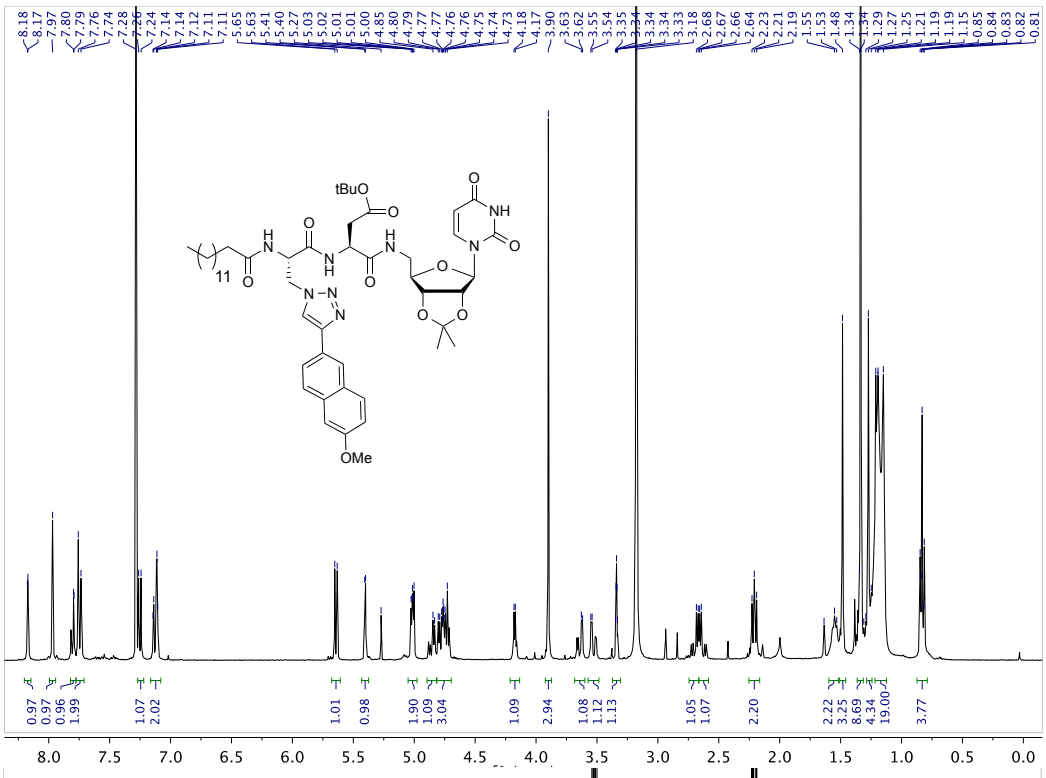
5'-*N*-(*N*-myristoyl-3-azido-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (28).

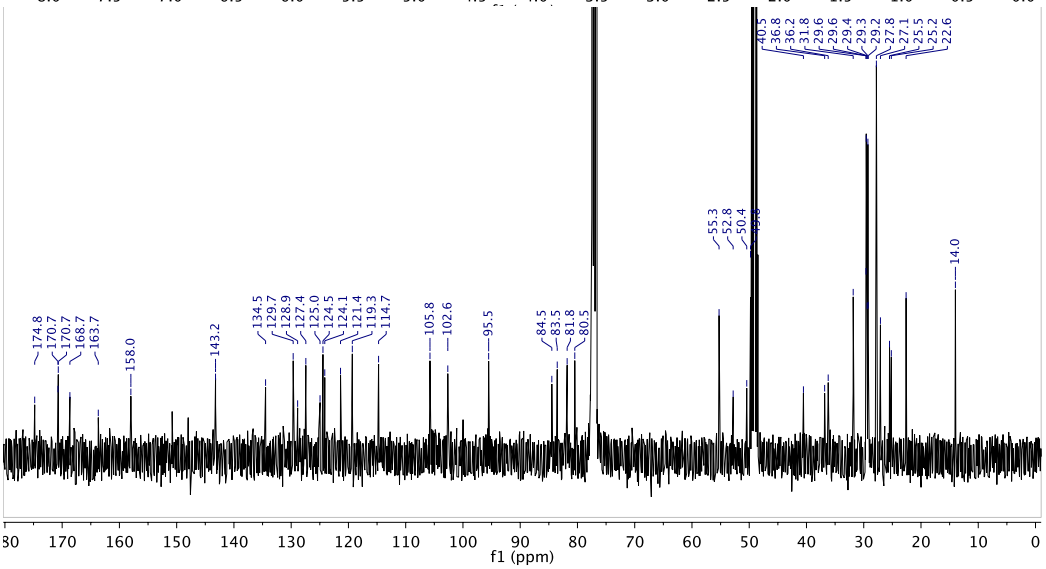


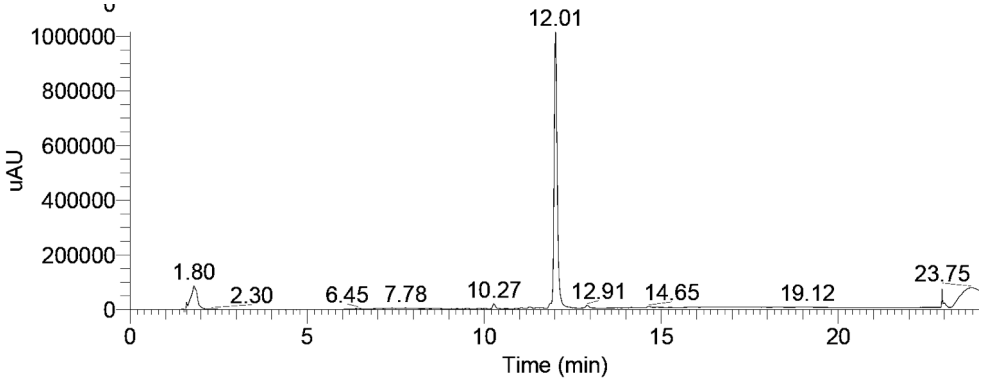




5'-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-*O*-*tert*-butyl-L-aspartate)-5'-amino-5'-deoxy-2',3'-*O*-isopropylidene uridine (38).







5'-*N*-(*N*-myristoyl-3-[4-(6-methoxynaphthyl)-1H-1,2,3-triazol-1-yl]-L-alaninyl-L-aspartate)-5'-amino-5'-deoxy uridine (29).