Supplementary Material for

Synthesis and Solution Conformation Studies of 3-Substituted Uridine and Pseudouridine Derivatives

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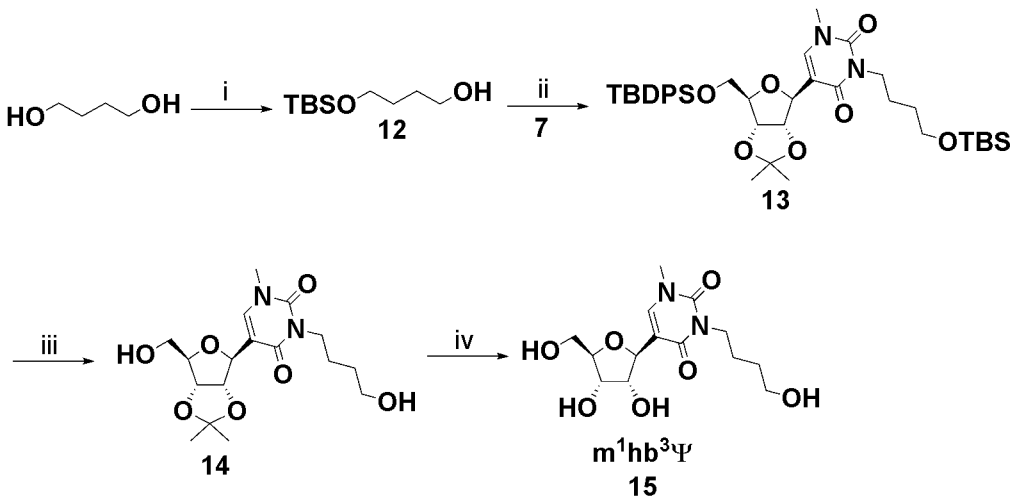
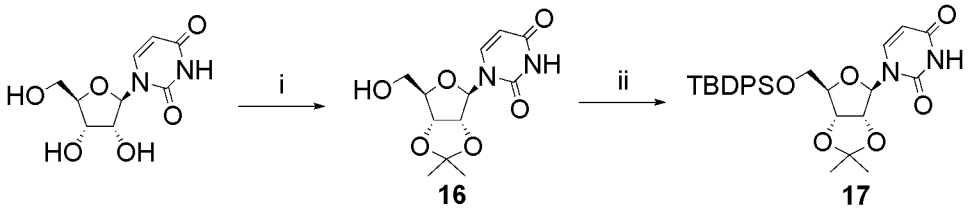
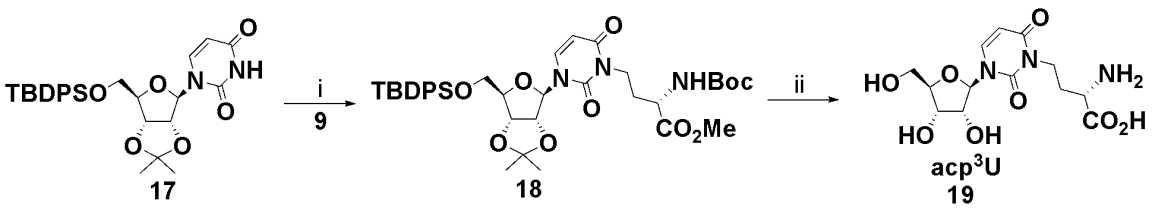
Schemes



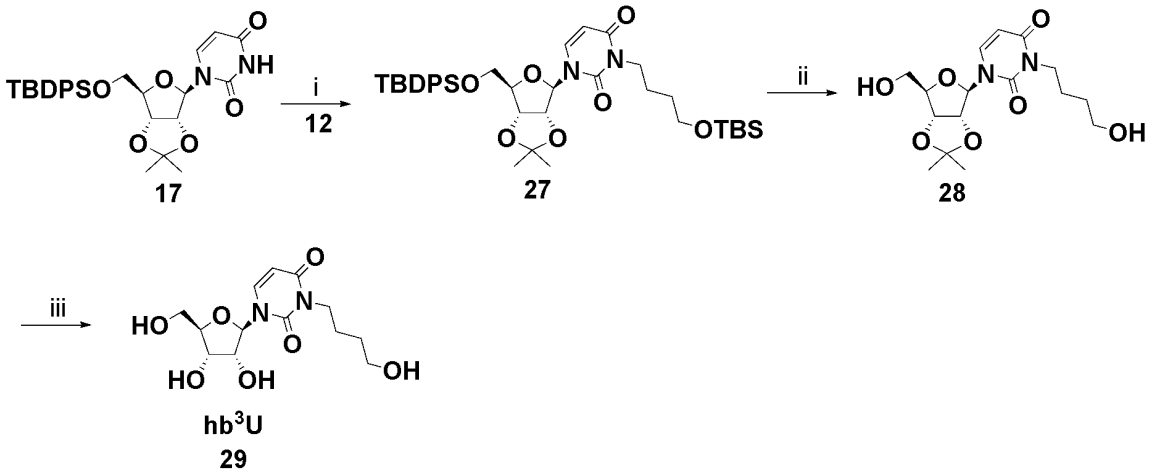
Scheme S1. *Reagents and conditions*: (i) *tert*-butyldimethylsilyl chloride, imidazole, DMF, rt, 2 h, 68%; (ii) **7**, DIAD, PPh_3, THF, rt, 1 h, 77%; (iii) tetrabutylammonium fluoride, THF, rt, 2.5 h, 89%; (iv) TFA, H_2O/acetone (9/1, v/v), rt, 2 h, 99%.



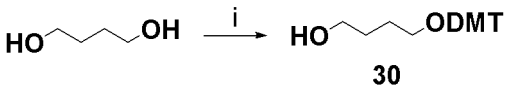
Scheme S2. *Reagents and conditions*: (i) *conc*. H_2SO_4, acetone, rt, 2.5 h, 95% yield; (ii) *tert*-butyldiphenylsilyl chloride, imidazole, DMF, rt, 18 h, 95% yield.



Scheme S3. *Reagents and conditions*: (i) **9**, DIAD, PPh_3, THF, rt, 1 h, 92%; (iii) a) 0.67 N $NaOH_{(aq)}$, dioxane, rt, 25 min; b) TFA/H_2O (9/1), 1 h, rt, 92% in two steps.



Scheme S4. *Reagents and conditions*: (i) **12**, DIAD, PPh_3, THF, rt, 1 h, 92%; (ii) TBAF, THF, rt, 2.5 h, 96%; (iii) TFA/H_2O (9/1), 1 h, rt, 79%.



Scheme S5. *Reagents and conditions*: (i) Dimethoxytrityl chloride, pyridine, rt, 20 h, 87%.

Experimental Section

General Procedure for Deprotection Using TFA/H_2O (9/1) Solution. TFA/H_2O (9/1, 10 mL) solution was added to the protected modified nucleoside. Stirring was continued for 1 h. TFA and water were evaporated under reduced pressure in a hot water bath. The product was washed with chloroform several times to give the corresponding deprotected compound.

1-*O*-(*tert*-Butyldimethylsilyl)-butane-4-ol (12). To a solution of 1,4-butanediol (10.0 g, 111 mmol) and imidazole (5.29 g, 77.7 mmol) in DMF (30 mL) was added *tert*-butyldimethylsilyl chloride (4.18 g, 27.7 mmol) and stirred for 2 h. The solution was diluted with H_2O (150 mL) and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with H_2O and dried over Na_2SO_4. The solvent was evaporated to yield crude product and purified with column chromatography using ethyl acetate/hexane (15–35%) to give **12** (3.85 g, 68%) as a colorless oil: R_f 0.58 (EtOAc/hexane 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.64 (m, 4 H), 2.64 (br s, 1 H), 1.63 (m, 4 H), 0.89 (s, 9 H), 0.06 (s, 6 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 63.6, 62.9, 30.4, 30.0, 18.5, -5.2; ESI-MS (ES^+) m/z calcd for $C_{10}H_{24}O_2Si$ 204.15, found 205.31 ($M+H^+$); HRMS calcd for $C_6H_{15}O_2Si$ ($M^+–C_4H_9$) 147.0841, found 147.0841.

1-Methyl-3-[4-*O*-(*tert*-butyldimethylsilyl)-butyl]-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*O*-(isopropylidene)pseudouridine (13). The procedure was the same as for **10** using alcohol **12.** The crude product was purified with column chromatography using EtOAc/hexane (15–40%) to give **13** (0.25 g, 77%) as a colorless oil: R_f 0.27 (EtOAc/hexane 3:7); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (m, 4 H), 7.40 (m, 6 H), 7.27 (d, J = 7.5 Hz, 1 H), 4.92 (d, J = 3.0 Hz, 1 H), 4.75 (m, 1 H), 4.65 (dd, J = 6.5, 3.5 Hz, 1 H), 4.14 (m, 1 H), 3.96 (m, 3 H), 3.85 (dd, J = 12.0, 4.0 Hz, 1 H), 3.61 (t, J = 6.5 Hz, 2 H), 3.08 (s, 3 H), 1.66 (m, 2 H), 1.59 (s, 3 H), 1.56 (m, 2 H), 1.36 (s, 3 H), 1.06 (s, 9 H), 0.88 (s, 9 H), 0.04 (s, 6 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 162.1, 151.6,

140.1, 135.8, 135.6, 133.7, 133.2, 130.2, 130.1, 128.1, 128.0, 114.4, 112.4, 85.7, 85.0, 81.3, 81.2, 64.2, 63.2, 41.4, 36.9, 30.6, 27.8, 27.1, 26.2, 25.8, 24.4, 19.6, 18.6, -5.05; ESI-MS (ES^+) m/z calcd for $C_{39}H_{58}N_2O_7Si_2$ 722.28, found 745.19 ($M+Na^+$), 761.13 ($M+K^+$).

3-[(*S*)-3-*N*-(*tert*-Butoxycarbonyl)-amino-3-methyl-carboxypropyl]-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*O*-(isopropylidene)uridine (18). The procedure was the same as for **10** using compounds **17** and **9**. The crude product was purified by column chromatography using EtOAc/hexane (40–60%) to give **18** (1.79 g, 91%) as a white foam: R_f 0.37 (EtOAc/hexane 1:1); mp 51–54 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 7.60 (m, 5 H), 7.40 (m, 6 H), 5.92 (s, 1 H), 5.47 (m, 2 H), 4.76 (m, 2 H), 4.37 (dd, *J* = 14.0, 6.5 Hz, 1 H), 4.30 (m, 1 H), 3.99 (m, 3 H), 3.80 (dd, *J* = 11.5, 3.5 Hz, 1 H), 3.64 (s, 3H), 2.08 (m, 2 H), 1.57 (s, 3 H), 1.44 (s, 9 H), 1.35 (s, 3 H), 1.05 (s, 9 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 172.8, 162.7, 155.8, 150.9, 138.6, 135.8, 135.6, 132.9, 132.5, 130.4, 130.3, 128.2, 128.2, 114.4, 101.8, 93.2, 87.0, 85.6, 80.4, 80.1, 64.2, 52.5, 51.6, 37.6, 29.6, 28.6, 27.5, 27.1, 25.6, 22.2, 19.5; ESI-MS (ES^+) m/z calcd for $C_{38}H_{51}N_3O_{10}Si$ 737.33, found 776.29 ($M+K^+$).

3-(3-Amino-3-carboxypropyl)uridine (19, acp^{3}U). The same procedure for compound **11** was employed with compound **18** (430 mg, 0.58 mmol) to give **19** (201 mg, 92%) as a white solid: mp 159–161 °C; ^1H NMR (500 MHz, D_2O) δ 7.73 (d, *J* = 8.0 Hz, 1 H), 5.82 (d, *J* = 8.5 Hz, 1 H), 5.79 (d, *J* = 4.0 Hz, 1 H), 4.22 (m, 1 H), 4.09 (m, 1 H), 3.96 (m, 3 H), 3.79 (dd, *J* = 13.0, 2.5 Hz, 1 H), 3.68 (dd, *J* = 12.5, 4.5 Hz, 1 H), 3.58 (m, 1 H), 2.10 (m, 2 H); ^{13}C NMR (500 MHz, D_2O) δ 174.1, 165.4, 152.0, 140.1, 101.8, 90.6, 84.2, 79.9, 69.4, 60.8, 52.6, 37.6, 28.2; ESI-MS (ES^+) m/z calcd for $C_{13}H_{19}N_3O_8$ 345.12, found 346.15 ($M+H^+$), 368.12 ($M+Na^+$); HRMS calcd for $C_{12}H_{15}N_3O_7$ (M^+-CH_4O) 313.0910, found 313.0901.

1-*O*-Benzoyl-*(S)*-2-*N*-(*tert*-butoxycarbonyl)-pentan-5-ol (22). To a solution of compound **21** (0.61 g, 1.09 mmol) in dry THF (20 mL) was added tetrabutylammonium fluoride (1.0 M solution in THF, 1.20 mL, 1.20 mmol) and stirred at rt for 3 h. The solvent was evaporated under reduced pressure to give the crude product. The residue was purified by column chromatography using ethyl acetate/hexane (45–70%) to give **22** (0.30 g, 84%) as a white solid: R_f 0.38 (EtOAc/hexane 7:3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (m, 2 H), 7.56 (m, 1 H), 7.43 (t, J = 7.5 Hz, 1 H), 4.73 (br s, 1 H), 4.31 (d, J = 4.5 Hz, 1 H), 4.04 (br s, 1 H), 3.69 (m, 2 H), 1.90 (br s, 1 H), 1.63 (m, 4 H), 1.41 (s, 9 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.7, 155.9, 133.4, 130.1, 129.9, 128.6, 79.8, 67.1, 62.6, 49.8, 29.0, 28.8, 28.6; ESI-MS (ES^+) m/z calcd for $C_{17}H_{25}NO_5$ 323.17, found 346.28 ($M+Na^+$), 362.25 ($M+K^+$); HRMS calcd for $C_{13}H_{16}NO_4$ (M^+-C_4H_9O) 250.1079, found 250.1084.

3-[(*S*)-4-*N*-(*tert*-Butoxycarbonyl)-amino-5-*O*-benzoyl-pentyl]-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*O*-(isopropylidene)uridine (23). The procedure was the same as for **10** using **17** and alcohol **22**. The crude product was purified with column chromatography using EtOAc/hexane (25–45%) to give **23** (140 mg, 96%) as a white foam: R_f 0.44 (EtOAc/hexane 1:1); mp 58–61 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (m, 2 H), 7.62 (m, 4 H), 7.56 (m, 2 H), 7.41 (m, 8 H), 5.93 (d, J = 2.5 Hz, 1 H), 5.50 (d, J = 8.0 Hz, 1 H), 4.75 (m, 2 H), 4.64 (d, J = 9.0 Hz, 1 H), 4.30 (m, 3 H), 3.94 (m, 4 H), 3.81 (dd, J = 11.5, 4.0 Hz, 1 H), 1.75 (m, 3 H), 1.65 (s, 3 H), 1.53 (m, 1 H), 1.40 (s, 9 H), 1.35 (s, 3 H), 1.06 (s, 9 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.7, 162.8, 155.7, 151.0, 138.4, 135.8, 135.6, 133.3, 132.9, 132.5, 130.4, 130.3, 130.2, 130.0, 128.6, 128.2, 128.2, 114.4, 102.0, 93.1, 86.9, 85.7, 80.5, 79.7, 67.2, 64.2, 49.8, 41.0, 29.5, 28.6, 27.5, 27.2, 25.6, 24.4, 19.5; ESI-MS (ES^+) m/z calcd for $C_{45}H_{57}N_3O_{10}Si$ 827.4, found 850.2 ($M+Na^+$), 866.2 ($M+K^+$).

3-(4-Amino-4-carboxybutyl)uridine, TFA Salt (26, acb^{3}U). Compound **25** was reacted with TFA/H_2O to give **26** (160 mg, 94%) as an off-white solid: mp 158–160 °C; ^{1}H NMR (500 MHz, D_2O) δ 7.72 (d, *J* = 8.0 Hz, 1 H), 5.81 (d, *J* = 8.5 Hz, 1 H), 5.79 (d, *J* = 4.5 Hz, 1 H), 4.20 (t, *J* = 5.0 Hz, 1 H), 4.08 (t, *J* = 5.0 Hz, 1 H), 4.00 (m, 1 H), 3.79 (m, 4 H), 3.67 (dd, *J* = 12.5, 4.5 Hz, 1 H), 1.80 (m, 2 H), 1.62 (m, 2 H); ^{13}C NMR (500 MHz, D_2O) δ 173.6, 165.4, 152.0, 140.0, 101.9, 90.4, 84.2, 73.9, 69.5, 60.8, 53.9, 40.8, 27.7, 22.7; ESI-MS (ES^+) m/z calcd for $C_{14}H_{21}N_3O_8$ 359.1, found 360.2 ($M+H^+$), 398.1 ($M+K^+$); Anal. Calcd for $C_{16}H_{22}F_3N_3O_{10}$: C, 40.60; H, 4.68; N, 8.88; O, 33.80. Found: C, 41.99; H, 5.58; N, 9.59; O, 36.89.

3-[4-*O*-(*tert*-Butyldimethylsilyl)butyl]-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*O*-(isopropylidene)uridine (27). The procedure was the same as for **10** using **17** and alcohol **12**. The crude product was purified with column chromatography using EtOAc/hexane (15–35%) to give **27** (1.09 g, 92%) as a colorless oil: R_f 0.26 (EtOAc/hexane 1:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (m, 4 H), 7.55 (d, *J* = 8.0 Hz, 1 H), 7.41 (m, 6 H), 5.93 (d, *J* = 2.5 Hz, 1 H), 5.51 (d, *J* = 8.5 Hz, 1 H), 4.75 (m, 2 H), 4.31 (dd, *J* = 7.0, 3.0 Hz, 1 H), 3.97 (dd, *J* = 11.5, 3.0 Hz, 1 H), 3.90 (m, 2 H), 3.81 (dd, *J* = 11.5, 4.0 Hz, 1 H), 3.61 (t, *J* = 6.5 Hz, 2 H), 1.65 (m, 2 H), 1.58 (s, 3 H), 1.54 (m, 2 H), 1.35 (s, 3 H), 1.06 (s, 9 H), 0.88 (s, 9 H), 0.38 (s, 6 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 162.8, 151.0, 138.3, 135.8, 135.6, 132.9, 132.6, 130.4, 130.3, 128.2, 128.2, 114.4, 102.1, 93.2, 86.9, 85.7, 80.6, 64.2, 63.1, 41.2, 30.5, 27.5, 27.1, 26.2, 25.6, 24.3, 19.5, 18.6, -5.1; ESI-MS (ES^+) m/z calcd for $C_{38}H_{56}N_2O_7Si_2$ 708.36, found 709.24 ($M+H^+$); HRMS calcd for $C_{38}H_{56}N_2O_7Si_2Na^+$ 731.3518, found 731.3501.

3-(4-Hydroxybutyl)-2',3'-*O*-(isopropylidene)uridine (28). The crude product was purified by column chromatography using methanol/ethyl acetate/hexane (0.02/1/1 to 0.30/1/1) to give **28** (0.15 g, 96%) as a colorless oil: R_f 0.14 (MeOH/EtOAc/hexane 0.2:1:1); ^{1}H NMR

(500 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.0 Hz, 1 H), 5.75 (d, *J* = 7.5 Hz, 1 H), 5.56 (d, *J* = 2.5 Hz, 1 H), 5.02 (dd, *J* = 6.5, 3.0 Hz, 1 H), 4.96 (dd, *J* = 6.5, 3.0 Hz, 1 H), 4.30 (dd, *J* = 6.5, 3.5 Hz, 1 H), 3.93 (m, 3 H), 3.80 (dd, *J* = 12.0, 3.5 Hz, 1 H), 3.66 (t, *J* = 6.5 Hz, 2 H), 1.70 (m, 2 H), 1.59 (m, 5 H), 1.36 (s, 3 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 162.8, 151.2, 140.8, 114.5, 102.3, 97.1, 87.2, 84.2, 80.6, 62.9, 62.5, 41.0, 29.9, 27.5, 25.5, 24.1; ESI-MS (ES^+) m/z calcd for $C_{16}H_{24}N_2O_7$ 356.16, found 379.23 ($M+Na^+$), 395.20 ($M+K^+$), HRMS calcd for $C_{16}H_{24}N_2O_7$ (M^+) 356.1584, found 356.1595.

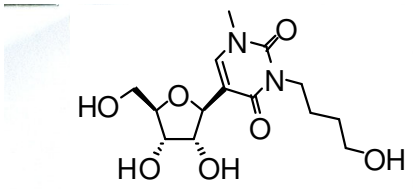
3-(4-Hydroxybutyl)uridine (29, hb^{3}U). The crude product was purified by column chromatography using MeOH/methylene chloride (10–30%) to give **29** (85 mg, 79%) as a white foam: R_f 0.36 (MeOH/CH_2Cl_2 1:4); mp 55−58 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (d, *J* = 8.0 Hz, 1 H), 5.92 (d, *J* = 4.0 Hz, 1 H), 5.76 (d, *J* = 8.4 Hz, 1 H), 4.17 (m, 2 H), 4.02 (m, 1 H), 3.93 (m, 2 H), 3.84 (dd, *J* = 12.4, 2.4 Hz, 1 H), 3.73 (dd, *J* = 12.0, 2.4 Hz, 1 H), 3.57 (t, *J* = 6.4 Hz, 2 H), 1.67 (m, 2 H), 1.55 (m, 2 H); ^{13}C NMR (400 MHz, CD_3OD) δ 163.8, 151.4, 139.6, 100.9, 90.4, 85.1, 74.7, 70.0, 61.4, 61.0, 40.8, 29.7, 24.0; ESI-MS (ES^+) m/z calcd for $C_{13}H_{20}N_2O_7$ 316.1, found 339.3 ($M+Na^+$); HRMS calcd for $C_{13}H_{20}N_2O_7$ (M^+) 316.1271, found 316.1276.

1-*O*-(Dimethoxytrityl)-butane-4-ol (30). To a solution of 1,4-butanediol (5.0 g, 55.5 mmol) in pyridine (10 mL) was added dimethoxytrityl chloride (1.87 g, 5.55 mmol) and stirred for 20 h. The solvent was evaporated to yield crude product, which was purified by column chromatography using ethyl acetate/hexane (30–50%) to give **30** (1.89 g, 87%) as a colorless oil: R_f 0.36 (EtOAc/hexane 1:1); ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (m, 2 H), 7.19 (m, 6 H), 7.09 (m, 1 H), 6.73 (m, 4 H), 3.66 (s, 6 H), 3.45 (t, *J* = 6.8 Hz, 2 H), 3.00 (t, *J* = 6.4 Hz, 2 H), 1.56 (m, 4 H); ^{13}C NMR (400 MHz, CD_3OD) δ 158.8, 145.7, 136.6, 130.0, 128.1, 127.5, 126.5, 112.8,

86.0, 63.1, 61.7, 54.5, 29.5, 26.4; ESI-MS (ES^+) m/z calcd for $C_{25}H_{28}O_4$ 392.2, found 431.3 ($M+K^+$); HRMS calcd for $C_{25}H_{28}O_4$ (M^+) 392.1988, found 392.2003.

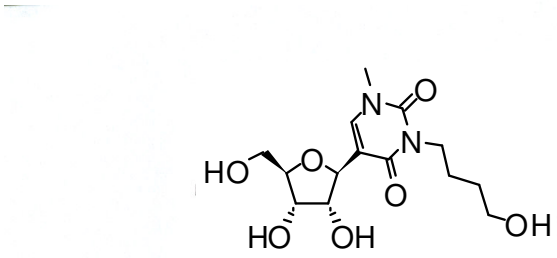
3-(3-Carboxypropyl)-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*O*-(isopropylidene)uridine (32). The procedure is the same as for compound **25**. The crude product was purified by column chromatography using MeOH/ CH_2Cl_2 (10–20%) to give **32** (350 mg, 80%) as a colorless oil: R_f 0.09 (MeOH/ CH_2Cl_2 1:9); ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (m, 4 H), 7.58 (d, J = 8.4 Hz, 1 H), 7.41 (m, 6 H), 5.93 (d, J = 1.6 Hz, 1 H), 5.51 (d, J = 8.0 Hz, 1 H), 4.75 (m, 2 H), 4.31 (dd, J = 6.0, 2.8 Hz, 1 H), 3.97 (m, 3 H), 3.81 (dd, J = 12.0, 4.0 Hz, 1 H), 2.39 (t, J = 7.2 Hz, 2 H), 1.96 (m, 2 H), 1.58 (s, 3 H), 1.35 (s, 3 H), 1.06 (s, 9 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.9, 163.0, 151.0, 138.6, 135.8, 135.6, 132.9, 132.5, 130.4, 130.3, 128.2, 128.2, 114.4, 102.0, 93.2, 86.9, 85.6, 80.6, 64.2, 40.4, 31.5, 27.5, 27.2, 25.6, 23.0, 19.5; ESI-MS (ES^+) m/z calcd for $C_{32}H_{40}N_2O_8Si$ 608.3, found 631.1 ($M+Na^+$); HRMS calcd for $C_{31}H_{37}N_2O_8Si$ ($M^+–CH_3$) 593.2319, found 593.2328.

3-(3-Carboxypropyl)uridine (33, cp^{3}U). Compound **32** was treated with TFA/H_2O solution to give compound **33** as a yellow oil. The residue was purified by column chromatography using MeOH/CH_2Cl_2 (15–30%) to give **33** (96 mg, 90%) as a colorless oil: R_f 0.08 (MeOH/ CH_2Cl_2 1:4); ^1H NMR (500 MHz, CD_3OD) δ 8.03 (d, J = 8.0 Hz, 1 H), 5.91 (d, J = 4.0 Hz, 1 H), 5.76 (d, J = 8.0 Hz, 1 H), 4.16 (m, 2 H), 4.00 (m, 3 H), 3.85 (dd, J = 12.5, 2.5 Hz, 1 H), 3.74 (dd, J = 12.0, 3.0 Hz, 1 H), 2.34 (t, J = 7.0 Hz, 2 H), 1.92 (m, 2 H); ^{13}C NMR (500 MHz, CD_3OD) δ 175.5, 163.9, 151.4, 139.7, 100.8, 90.5, 85.1, 74.7, 69.9, 60.9, 40.3, 31.1, 22.8; ESI-MS (ES^+) m/z calcd for $C_{13}H_{18}N_2O_8$ 330.1, found 353.1 ($M+Na^+$).



$m^1hb^3\Psi$

15



$m^1hb^3\Psi$

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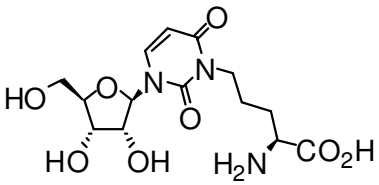

acb^{3}U
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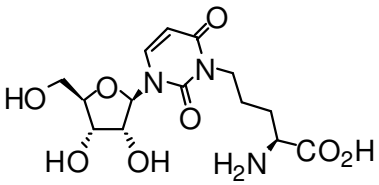

acb^{3}U
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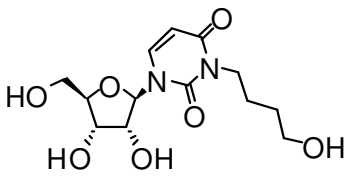

hb^{3}U
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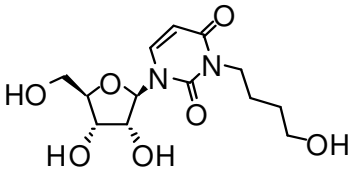
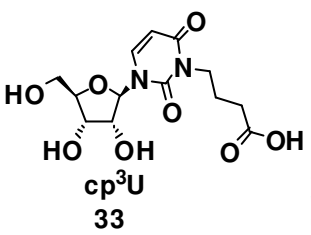
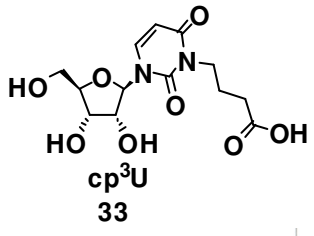


hb^{3}U
29



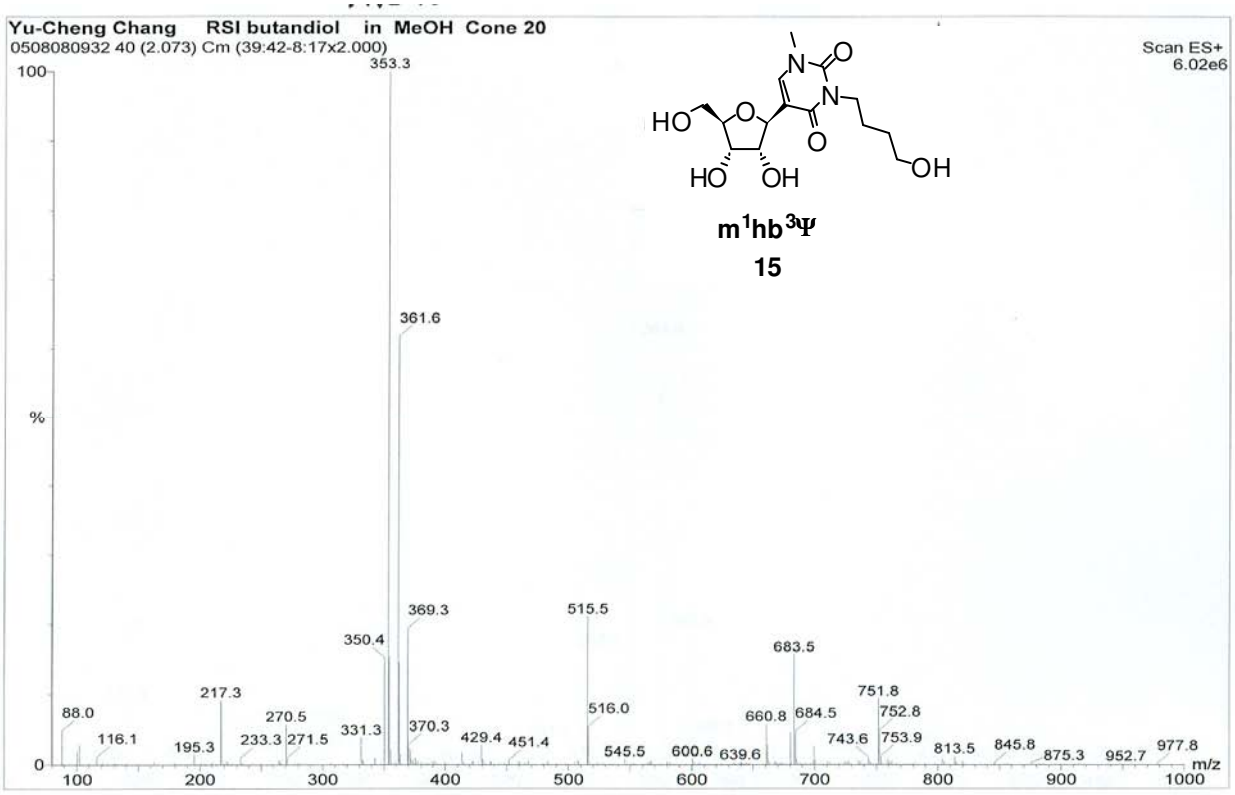
cp^{3}U

33



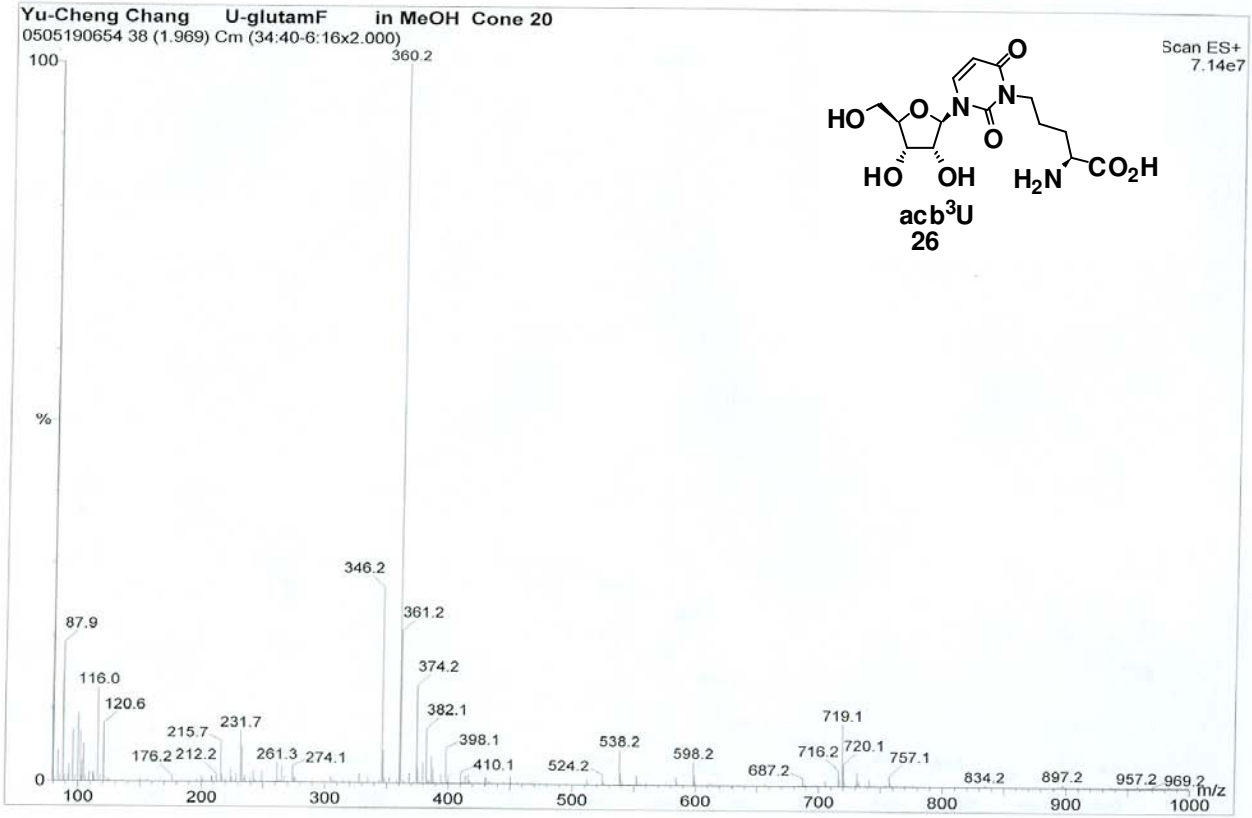
cp^{3}U

33



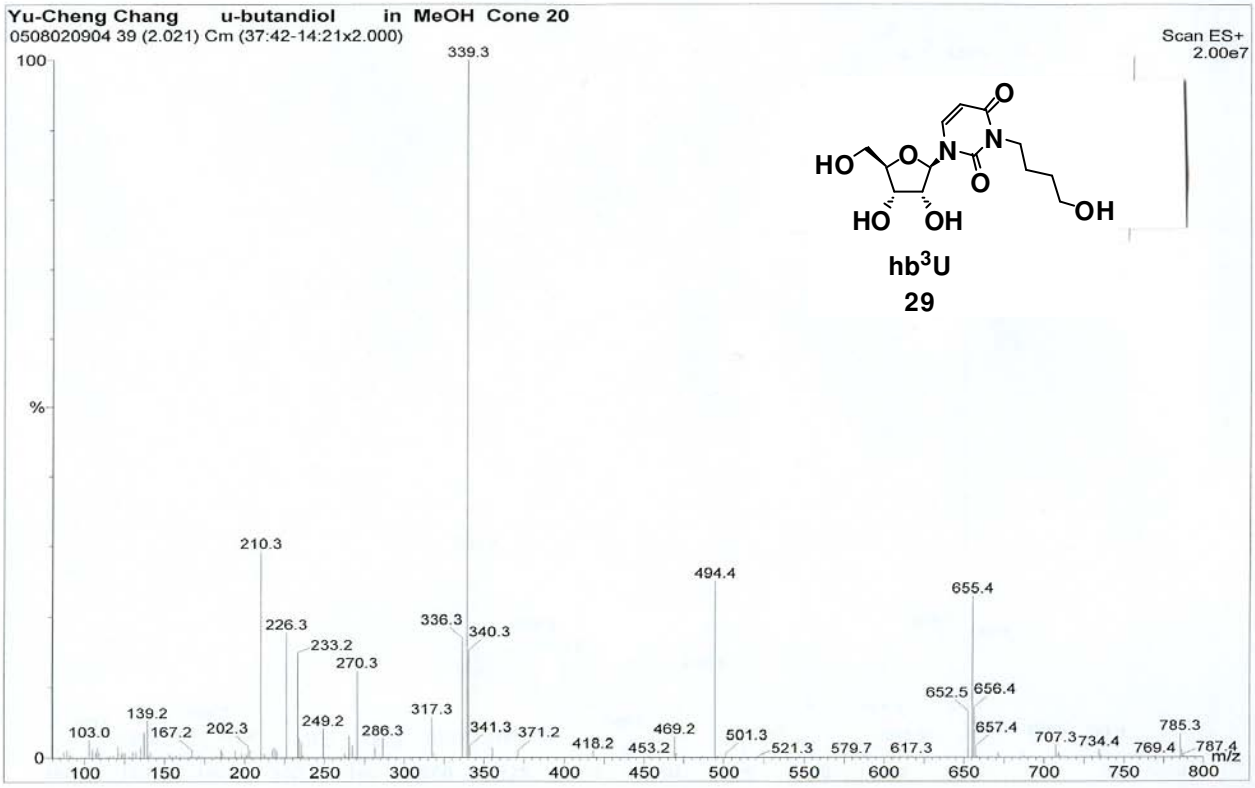
$m^1hb^{3}\Psi$

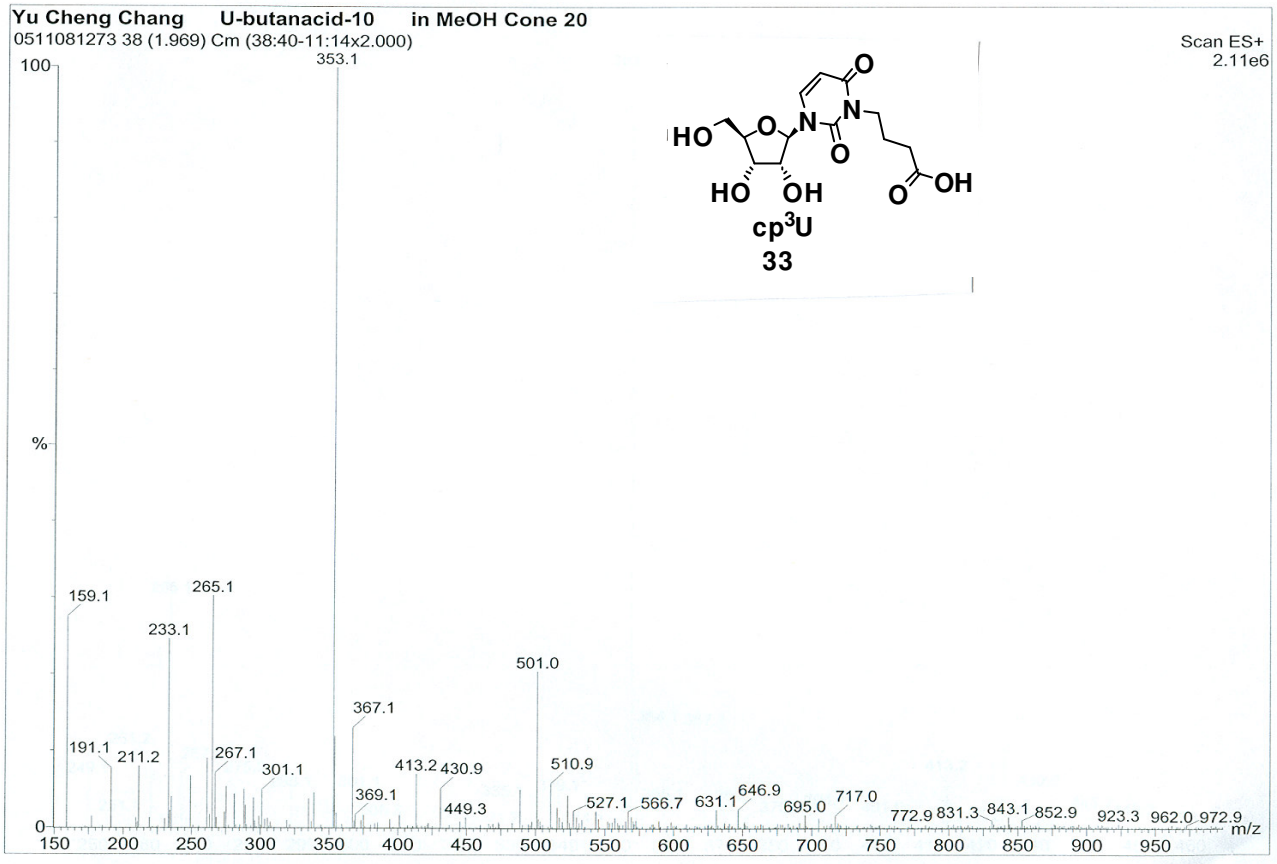
15



$acb^{3}U$

26


Yu-Cheng Chang u-butandiol in MeOH Cone 20
0508020904 39 (2.021) Cm (37:42-14:21x2.000)
Scan ES+
2.00e7
100
%
0
339.3
210.3
494.4
655.4
226.3
336.3
340.3
233.2
270.3
652.5
656.4
139.2
317.3
103.0
167.2
202.3
249.2
286.3
341.3
371.2
418.2
469.2
453.2
501.3
521.3
579.7
617.3
657.4
707.3
734.4
785.3
769.4
787.4
m/z
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800
HO
O
N
O
HO
OH
OH
hb3U
29


Yu Cheng Chang U-butanacid-10 in MeOH Cone 20
0511081273 38 (1.969) Cm (38:40-11:14x2.000)
Scan ES+
2.11e6
100
%
0
353.1
159.1
265.1
233.1
501.0
367.1
191.1
211.2
267.1
301.1
413.2
430.9
510.9
369.1
449.3
527.1
566.7
631.1
646.9
695.0
717.0
772.9
831.3
843.1
852.9
923.3
962.0
972.9
m/z
150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950
HO
O
N
O
HO
OH
O
OH
cp3U
33