Stereochemical Elucidation and Total Synthesis of Dihydropacidamycin D, a Semi-Synthetic Pacidamycin.

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Supporting information:

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commerial suppliers and used without further purification. The amino acid starting materials were purchased from Bachem, Aldrich, Sigma, or Novabiochem. The *N,N*-dimethylformamide (DMF) used was anhydrous (Aldrich). Ethyl acetate used in the formation of pentafluorophenyl esters was chromatography-grade solvent. Flash column chromatography was carried out using 'Baker' silica gel 40 μm flash chromatography packing. Sephadex G-10 was obtained from Sigma. Thin layer chromatography (TLC) analyses were performed with glass TLC plates coated with silica gel 60 F_{254}, manufactured by E. Merck and distributed by EM Science. ^{1}H NMR spectra were obtained using a 300 or 400 MHz Varian NMR spectrometer. Proton NMR data are presented in the format "chemical shift (multiplicity, integration, coupling constant)". Proton-decoupled ^{13}C NMR spectra were obtained on the same instruments at 75 or 100 MHz respectively, with a line broadening of 1.5 Hz. Chemical shifts are reported in ppm; coupling constants are reported in Hz. Unless otherwise noted, spectra were obtained in $CDCl_3$. Spectra obtained in $CDCl_3$ were referenced to the residual $CHCl_3$ (7.27 ppm); spectra obtained in d_6-DMSO were referenced to the residual d_5-DMSO (2.49 ppm); spectra obtained in CD_3OD were referenced to residual CHD_2OD (3.30 ppm). DCl in

D_2O was obtained from Aldrich. Chemical shifts taken in this solvent system are reported uncorrected to any reference. Elemental analyses were performed by Galbraith® Laboratories, Inc., Knoxville, TN. High-resolution mass spectrometry was performed at the University of Illinois Mass Spectrometry Laboratory, Urbana, IL. Infrared spectra were obtained using a Perkin Elmer 1600 series FTIR spectrometer, using samples prepared as either thin films on NaCl plates or anhydrous KBr windows (as indicated). Melting points were uncorrected.

Dihydropacidamycin D (2) from pacidamycin D. In 8 mL of DMF was dissolved pacidamycin D (25 mg, 0.035 mmol).[1,2] The mixture was purged with N_2 and 25 mg of 10% Pd/C was added. A supply of 1 atm of H_2 was provided by balloon, and the progress of the reaction monitored by mass spectral analysis of small filtered aliquots. When there was no longer any starting material present, the mixture was purged for 5 min with N_2 and the catalyst removed by filtration. The resulting residue was purified on semi-preparative C-18 HPLC column with UV detection at 260 nm (flow rate 1 mL/min, 0-100% acetonitrile in 0.1% aq TFA ramped over 20 min). Lyophilization of the product-containing fractions provided 1.8 mg white solid product as the TFA salt (0.0022 mmol, 6% yield). Low-resolution mass spectral analysis of the product indicated that it was contaminated with *ca.* 40% of tetrahydrogenated product. See compound **34** for complete characterization (note that since **2** also contained *ca.* 40% of tetrahydropacidamycin D, its ^1H NMR spectrum contained resonances in addition to those due to the dihydro compound; all resonances in the ^1H NMR spectrum of totally synthetic **34** could be correlated with a resonance in the ^1H NMR spectrum of **2**. HRMS (FAB$^+$) m/z: 714.3214 (MH^+ $C_{32}H_{44}N_9O_{10}$ requires 714.3211).

Degradation of pacidamycin 5 to provide a sample of 3-methylamino-2-aminobutyric acid. In a small screw-cap vial was placed 30 mg of pacidamycin 5.[1,2] To this was added 5 mL of a 50/50 v/v solution of 12 N HCl and glacial HOAc. The vial was sealed and the contents heated for 12 h at 110 °C. The resulting black suspension was filtered through a 0.2 μm nylon filter and concentrated to a black powder, which was lyophilized from water after coevaporation 3x from methanol/toluene mixture. The material was dissolved in a minimal amount of water and applied to the top of a column of Sephadex G-10. The column was eluted with the upper layer of a mixture of 4:1:5 *n*-BuOH/HOAc/H_2O, at a flow rate of 0.5 mL per min, changing fractions at intervals of 10 min. The fractions containing material that remained on TLC baseline (eluting with either 10:1 CH_2Cl_2/CH_3OH or 5:2:1 *n*-BuOH/HOAc/H_2O), was not UV active, but did stain with ninhydrin were chosen and combined. The resulting material was lyophilized to a white solid (5.0 mg). ^{1}H NMR (400 MHz, 2N DCl in D_2O; major isomer): δ 1.00 (d, 3H, *J* = 7.2), 2.35 (s, 3H), 3.50-3.54 (m, 1H), 4.08 (d, 1H, *J* = 4.4). ^{1}H NMR (400 MHz, 2N DCl in D_2O; minor isomer): δ 0.94 (d, 3H, *J* = 6.8), 2.36 (s, 3H), 3.31-3.34 (m, 1H), 4.16 (d, 1H, *J* = 2.8). ^{1}H NMR (400 MHz, unbuffered D_2O, major isomer): δ 1.54 (d, 3H, *J* = 6.8), 1.45 (d, 3H, *J* = 6.4), 2.90 (s, 3H), 3.77-3.81 (m, 1H), 4.04 (d, 1H, *J* = 8.4). ^{1}H NMR (400 MHz, unbuffered D_2O; minor isomer): δ 1.45 (d, 3H, *J* = 6.4), 2.92 (s, 3H), 3.72-3.78 (m, 1H), 4.25 (d, 1H, J = 2.8). Note: spectra measured in DCl/D_2O were uncorrected with regard to chemical shift.

Determination of the absolute stereochemistry of the 3-methylamino-2-aminobutyric acid. In 1 mL of 6 N aqueous HCl was suspended 2 mg of pacidamycin 4[1,2] in a screw-cap vial. The vial was sealed and the mixture was heated to 105 °C for 6

h. The resultant brown solution was cooled to rt and concentrated in vacuo. The solid hydrolysate was dissolved in 200 μL of H_2O to obtain a 10 mg/mL solution. Samples of 5 mg/mL, and 2.5 mg/mL were prepared by dilution with the appropriate amount of H_2O. Analytical HPLC analysis was performed on a 10 x 0.46 cm Hypersil ODS column (5 μm). The mobile phase contained a chiral additive: 4 mM *N*,*N*-di-n-propyl-L-alanine and 2 mM cupric acetate in water (pH = 5.5).[3] Injection of pacidamycin 4-hydrolysate demonstrated the presence of (2*S*,3*S*)-DABA by retention time (7.7 min) and by coinjection of authentic synthetic (2*S*,3*S*)-DABA (see below). The (2*R*,3*R*)-DABA standard eluted at 7.0 min.

Benzyl (2*S*,3*S*)-3-(benzyloxyamino)-2-(*tert*-butoxyformamido)butyric acid (8). To a solution of compound **7**, (3*S*,4*S*)-1-benzyloxyamino-3-*N*-*tert*-butyloxycarbonyl-4-methyl-2-azetidinone (5.635 g, 18.4 mmol), which was prepared using literature protocols,[4,5] in 35 mL 1:1 water/dioxane was added dropwise at ambient temperature, a 1 M solution of NaOH (18.4 mL, 18.4 mmol) over 1 hour. The mixture was stirred for 0.5 h after the addition was completed and 0.15 additional equivalents (2.8 mmol) of NaOH was added and the solution was stirred for an additional hour to ensure completion. The reaction mixture was then concentrated to 1/4 volume *in vacuo* and washed with EtOAc (2 x 25 mL). The aqueous fraction was then acidified to pH 1 with 1 N aqueous HCl, and the product extracted into EtOAc (2 x 30 mL). These two EtOAc layers were combined, washed with brine (2 x 20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to afford 5.94 g (100% yield) of (2*S*,3*S*)-3-(benzyloxyamino)-2-(*tert*-butoxyformamido)butyric acid **8** as a clear oil. IR (KBr, cm^{-1}) 3600-2400, 2305, 1715, 1500, 1368, 1265, 1163, 1023, 740. ^1H NMR (400 MHz,

CD_3OD): δ 0.98 (d, 3H, *J*= 6.8), 1.42 (s, 9H), 3.49 (dq, 1H, *J* = 6.8, 4.4), 4.57 (bs, 1H), 4.64 (d, 1H, *J* = 11.6), 4.68 (d, 1H, *J* = 11.6 Hz), 7.35 (m, 5H). ^{13}C NMR (100MHz, CD_3OD): δ 13.4, 28.6, 56.2, 57.8, 77.1, 80.8, 128.9, 129.4, 129.6, 139.0, 158.2, 174.8. Anal. Calcd for $C_{16}H_{24}N_2O_5$: C, 59.24%; H, 7.46%, N, 8.64%. Found: C, 58.93%; H, 7.62%; N, 9.11%.

(2*S*,3*S*)-3-((Benzyloxy)methylamino)-2-(*tert*-butoxyformamido)butyric acid (9). To a solution of (2*S*,3*S*)-3-(benzyloxyamino)-2-(*tert*-butoxyformamido)butyric acid (8.5 g, 26.2 mmol) in 70 mL methanol was added $NaCNBH_3$ (1.65 g, 26.2 mmol) over 15 minutes at 0 °C, followed by addition of formaldehyde 37 wt % aqueous solution (1.92 mL, 25.7 mmol) over 0.5 h. The reaction mixture was warmed to rt and stirred for 4 h before being concentrated *in vacuo*. The residue was dissolved in 200 mL water and the mixture was basified with K_2CO_3. The aqueous solution was washed with ether, then acidified to pH 2 with 1 M aqueous HCl, followed by extraction with EtOAc. The organic layers were then washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to afford 8.51 g (96% yield) of (2*S*,3*S*)-3-((benzyloxy)methylamino)-2-(*tert*-butoxyformamido)butyric acid (**9**) 96% yield. IR (KBr, cm^{-1}) 3500-2400, 2305, 1715, 1500, 1368, 1265, 1164, 1023, 747. ^1H NMR (400 MHz, CD_3OD): δ 1.08 (d, 3H, *J* = 6.8), 1.42 (s, 9H), 2.56 (s, 3H), 3.13 (dq, 1H, *J* = 6.8, 4.8), 4.50 (d, 1H, *J* = 4.8), 4.62 (s, 2H), 7.26 (m, 5H). ^{13}C NMR (100MHz, CD_3OD): δ 11.5, 28.9, 42.5, 57.5, 64.8, 75.5, 80.7, 129.0, 129.4, 130.0, 130.2, 138.5, 158.1, 175.1. Anal. Calcd for $C_{17}H_{26}N_2O_5$: C, 60.34%; H, 7.74%, N, 8.28%. Found: C, 59.92%; H, 7.87%; N, 8.47%.

(2*S*,3*S*)-3-Methylamino-2-(*tert*-butoxyformamido)butyric acid (10). A solution of **9** (2.4 g, 14.8 mmol) in 20 mL methanol was added under nitrogen to a suspension of 10% palladium on charcoal (0.6 g, 25 wt%) in 20 mL of methanol in a Parr hydrogenator flask. The mixture was placed on the Parr Hydrogenator and hydrogenated at 50 psi for 18 hours. The catalyst was filtered off and the filtrate evaporated *in vacuo*. The residue was then lyophilized, affording 1.55 g of (2*S*,3*S*)-3-methylamino-2-(*tert*-butoxyformamido)butyric acid (**10**) as a white solid (95% yield). The material was used in pacidamycin synthesis without any further purification. A 20 mg sample was recrystallized from iPr$_2$O/MeOH to provide fine white crystals (6.1 mg), mp 173-174.5 °C. IR (KBr, cm^{-1}) 3600-2500, 2344, 1702, 1676, 1524, 1203, 1056, 722. ^{1}H NMR (400 MHz, CD_3OD): δ 1.26 (d, 3H, J = 6.8), 1.48 (s, 9H), 2.76 (s, 3H), 3.72 (dq, 1H, J = 6.8, 4.0), 4.66 (bs, 1H). ^{13}C NMR (100MHz, CD_3OD): δ 12.3, 28.8, 31.8, 55.7, 58.0, 81.6, 158.7, 172.3. HRMS (FAB$^+$) m/z: 233.1502 (MH^+ $C_{10}H_{21}N_2O_4$ requires 233.1501).

1-(3'-Deoxy-2',5'-di-(*tert*-butyldimethylsilyloxy)-β-*D*-ribofuranosyl)uracil (13). To a solution of compound **12**,[6,7] 2',5'-di-*tert*-butyldimethylsilyoxyluridine (10.69 g, 22.6 mmol), in 80 mL of dichloroethane was added thiocarbonyldiimidazole (8.00 g, 45.2 mmol) in one portion. The resulting mixture was heated at reflux under nitrogen for 1.5 h before being cooled to rt and quenched by addition of 50 mL of water. The phases were separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with cold 1 M aqueous HCl, sat'd aqueous $NaHCO_3$, and brine. The organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was immediately carried on to the next reaction without further purification. To a solution of the thiocarbonyl compound in 100 mL of

degassed (3 freeze-pump-thaw cycles) xylene was added azacyclohexylcarbonitrile (3.0 g, 2.3 mmol) followed by *n*-Bu_3SnH (12 mL, 45 mmol). The reaction mixture was heated at reflux for 3 hours before being concentrated *in vacuo*. The residue was purified by silica gel chromatography eluting with 2:1 to 1:5 hexanes/chloroform, followed by 3:1 hexanes/EtOAc to afford 6.12 g of 1-(3'-deoxy-2',5'-di-(*tert*-butyldimethylsilyloxy)-β-*D*-ribofuranosyl)uracil **13** (59% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.12 (s, 12H), 0.91 (s, 18H), 1.71 (dd, 1H, *J* = 12.8, 4.0), 2.00-2.07 (m, 1H), 3.72 (d, 1H, *J* = 12.0), 4.17 (d, 1H, *J* = 12.0), 4.35 (d, 1H, *J* = 4.0), 4.47-4.50 (m, 1H), 5.63 (d, 1H, *J* = 8.0), 5.71 (s, 1H), 8.15 (d, 1H, *J* = 8.0), 9.57 (br s, 1H). This spectrum is consistent with that reported by Velazquez.[8]

1-(3'-Deoxy-2'-*tert*-butyldimethylsilyloxy-β-*D*-ribofuranosyl)uracil (14). A solution of 1-(3'-deoxy-2',5'-di-(*tert*-butyldimethylsilyloxy)-β-*D*-ribofuranosyl)uracil (**13**) (6.8 g, 15 mmol) in 200 mL 80% aqueous acetic was stirred at ambient temperature, under an N_2 atmosphere, for 24 h before being concentrated *in vacuo*. The residue was purified by silica gel chromatography eluting with 1:1 to 0:1 hexanes/EtOAc, to give 3.32 g of the desired 1-(3'-deoxy-2'-*tert*-butyldimethylsilyloxy-β-*D*-ribofuranosyl)uracil (yield 65%). A small amount was recrystallized from hexanes/ethyl acetate; plates separated (mp 149.5 - 150.0 °C). IR (thin film, cm^{-1}): 3390, 3161, 2921, 2856, 1725, 1687, 1474, 1382, 1262, 1120, 1000, 837. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.09 (s, 3H), 0.14 (s, 3H), 0.90 (s, 9H), 1.83 (dd, 1H, *J* = 12.8, 5.6), 2.05-2.11 (m, 1H), 3.75 (dd, 1H, *J* = 12.0, 4.0), 4.1 (dd, 1H, *J* = 12.0, 1.6), 4.47-4.50 (m, 1H), 4.51-4.54 (m, 1H), 5.64 (s, 1H), 5.68 (d, 1H, *J* = 8.0), 7.89 (d, 1H, *J* = 8.0), 8.99 (br s, 1H). ^{13}C ($CDCl_3$, 75 MHz): δ -5.26, -4.84, 17.8, 25.5, 33.2, 62.0, 76.6, 81.5, 93.4, 100.9, 141.3, 150.3, 164.6. Anal.

Calcd for $C_{15}H_{26}N_2O_5Si$: C, 52.61%; H, 7.65%, N, 8.18%. Found: C, 52.51%; H, 7.81%; N, 8.12%. Most of the remaining mass was recovered as 1-(3'-deoxy-β-*D*-ribofuranosyl)uracil (2.0 g, 30%).

1-(5'-Azido-3',5'-dideoxy-2'-*tert*-butyldimethylsilyloxy-β-*D*-ribofuranosyl)uracil (16). To a solution of 1-(3'-deoxy-2'-*tert*-butyldimethylsilyloxy-β-*D*-ribofuranosyl)uracil (**14**) (0.45 g, 1.3 mmol) in 7 mL of pyridine was added *p*-toluenesulfonyl chloride (0.75 g, 3.9 mmol) in one portion. The resulting mixture was stirred under N_2, slowly warming to rt overnight, before being quenched by addition of ice. The reaction mixture was then partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with dichloromethane and the organic extracts are washed with aqueous 1 N HCl and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude **15** was carried on to the next reaction without further purification. To a solution of crude tosylate **15** (0.69 g, 1.4 mmol) in 8 mL DMF was added LiN_3 (0.20 g, 4.1 mmol). (Note that NaN_3 has also been used successfully for this transformation. However, use of NaN_3 requires heating of the reaction mixture to 55-60 °C). The resulting mixture was stirred under nitrogen at 40 °C for 2 h, and at rt for 12 h. The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel chromatography eluting with 1:1 hexanes/EtOAc to give 0.40 g of 1-(5'-azido-3',5'-dideoxy-2'-*tert*-butyldimethylsilyloxy-β-*D*-ribofuranosyl)uracil **16** (yield 83%) as a clear oil. IR (thin film, cm^{-1}): 2929, 2360, 2102, 1687, 1461, 1261, 1101, 837, 780. ^1H NMR (CD_3OD, 400 MHz): δ 0.10 (s, 3H), 0.15 (s, 3H), 0.90 (s, 9H), 1.85-1.89 (m, 1H), 1.91-1.94 (m, 1H), 3.67 (dd, 1H, J = 13.6, 4.0), 3.83 (dd, 1H, J = 13.6,

3.2), 4.40-4.53 (m, 1H), 4.53-4.57 (m, 1H), 5.69 (s, 1H), 5.75 (d, 1H, J = 8.0), 7.66 (d, 1H, J = 8.0), 9.3 (br s, 1). ^{13}C NMR ($CDCl_3$, 75 MHz): δ -5.17, -4.77, 17.9, 25.6, 35.4, 53.0, 76.7, 78.7, 93.3, 101.8, 139.7, 150.0, 163.2. HRMS (FAB^+) m/z: 368.1755 (MH^+ $C_{15}H_{26}N_5O_4Si$ requires 368.1754).

1-(5'-Azido-3',5'-dideoxy-β-*D*-ribofuranosyl)uracil (17). To a solution of **16** (0.474 g, 1.29 mmol) in 10 ml of tetrahydrofuran was added tetrabutylammonium fluoride, 1 M in THF (1.5 mL, 1.5 mmol). The resulting mixture was stirred under nitrogen for 30 minutes before being concentrated *in vacuo*. The residue was purified by silica gel chromatography eluting with neat EtOAc to give 0.27 g of 1-(5'-azido-3',5'-dideoxy-β-*D*-ribofuranosyl)uracil **17** (yield 84%). ^{1}H NMR (CD_3OD, 400 MHz): δ 1.94-1.99 (m, 1H), 2.02-2.10 (m, 1H), 3.56 (dd, 1H, J = 13.6, 5.2), 3.69 (dd, 1H, J = 13.6, 3.6), 4.37-4.40 (m, 1H), 4.47-4.51 (m, 1H), 5.70 (d, 1H, J = 8.0), 5.75 (d, 1H, J = 2.0), 7.73 (d, 1H, J = 8.0). ^{13}C NMR (CD_3OD, 75 MHz): δ 36.2, 54.5, 76.5, 80.2, 94.5, 102.5, 142.1, 152.2, 166.2. HRMS (EI^+) m/z: 253.0825 (M^+ $C_9H_{11}N_5O_4$ requires 253.0811).

1-(5'-Amino-3',5'-dideoxy-β-*D*-ribofuranosyl)uracil (18). To a solution of **17** (0.37 g, 1.5 mmol) in 1.5 mL methanol was added 1,3-propanedithiol (1.5 mL, 0.15 mmol) in one portion. The resulting mixture was stirred at rt for 5 d. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in water and washed generously with dichloromethane. The aqueous layer was concentrated *in vacuo* to give 0.31 g of 1-(5'-amino-3',5'-dideoxy-β-*D*-ribofuranosyl)uracil (**18**) (yield 93%) as a white foam. ^{1}H NMR (CD_3OD, 300 MHz): δ 1.95-2.03 (m, 1H), 2.05-2.12 (m, 1H), 2.95-3.06 (m, 2H), 4.45-4.96 (m, 2H), 5.79 (d, 1H, J = 8.1), 5.82 (d, 1H, J = 1.8), 7.78 (d, 1H, J =

8.1). ^{13}C NMR (CD_3OD, 75 MHz): δ 37.2, 45.8, 76.6, 81.8, 95.3, 102.5, 142.7, 166.4. HRMS (EI^+) m/z: 228.0980 (MH^+ $C_9H_{14}N_3O_4$ requires 228.0984).

***tert*-Butyl (4*R*,5*R*)-4-(*tert*-butyldimethylsiloxy)-5-uracil-4,5-dihydro-2-furoate (20).** To a solution of *tert*-butyl 2',3'-*O*-isopropylideneuridine-5'-carboxylate (**19**)[9] (2.53 g, 7.47 mmol) in 50 mL of melted 4-Å molecular-sieve-dried 2-methyl-2-propanol was added with rapid stirring *t*-BuOK (1.68 g, 14.9 mmol). As soon as all of the *t*-BuOK dissolved, glacial acetic acid was added dropwide until the pH of the solution was 7.5-8. The resulting mixture was concentrated *in vacuo* to a yellow crude solid and maintained on a vacuum pump for 1 h. To the residue in 30 mL of DMF was added imidazole (2.54 g, 7.47 mmol) followed by *tert*-butyldimethylsilyl chloride. The reaction mixture was stirred at ambient temperature under N_2 for 1 h before being evaporated *in vacuo* with heating to no greater than 45 °C. The resulting residue was partitioned in a separatory funnel betwen 200 mL of 0.5 N aqueous HCl and 200 mL of 3.5:1 hexanes/ethyl acetate. The organic layer was washed with 1 N aqueous HCl, sat'd aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was recrystallized from boiling hexane/ethyl acetate to give 2.39 g (5.82 mmol, 78% yield) of *tert*-butyl (4*R*,5*R*)-4-(*tert*-butyldimethylsiloxy)-5-uracil-4,5-dihydro-2-furoate (**20**): mp 143.5-144 °C. IR (KBr, cm^{-1}): 2956, 2932, 1700, 1638, 1460, 1373, 1258, 1128, 1077, 841, 780. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.11 (s, 3H), 0.13 (s, 3H), 0.91 (s, 9H), 1.55 (s, 9H), 5.08 (app t, 1H, J = 2.9), 5.77 (dd, 1H, J = 8.1, 2.4), 5.93 (d, 1H, J = 2.7), 6.27 (d, 1H, J = 3.0), 7.04 (d, 1H, J = 8.1), 8.34 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ -4.6, 18.1, 25.7, 28.1, 79.8, 83.5, 94.4, 103.4, 111.2, 139.5,

149.4, 151.0, 157.9, 162.8. Anal. Calcd for $C_{19}H_{30}N_2O_6Si$: C, 55.59%; H, 7.37%; N, 6.82%. Found: C, 55.59%; H, 7.46%; N, 6.73%.

***tert*-Butyl (2*R*,4*R*,5*R*)-4-(*tert*-butyldimethylsiloxy)-5-uracil-tetrahydro-2-furoate (21).** To a solution of **20** (2.10 g, 5.12 mmol) in 100 mL methanol purged for 10 minutes with a stream of N_2 by cannula needle dropped to the bottom of the flask was added 10% Pd/C (0.40 g). The resulting suspension was purged with N_2 for an additional 10 minutes and then purged with H_2 (1 atm) for 10 min. The resulting mixture was stirred under H_2 (1 atm) for an additonal 30 minutes before being purged with N_2 for 10 minutes. The Pd/C was removed by filtration though a pad of Celite and the filtrate was concentrated *in vacuo* to a crude white solid composed of a 9/1 mixture of the desired product to its C(4') diastereomer. The residue was recrystallized from boiling EtOAc/hexanes (hexanes added to turbidity) to give 1.80 g of pure *tert*-butyl (2*R*,4*R*,5*R*)-4-(*tert*-butyldimethylsiloxy)-5-uracil-tetrahydro-2-furoate **21** (4.36 mmol, 85% yield): mp 162.5-163.0 °C. IR (KBr, cm^{-1}): 3149, 3046, 2931, 2860, 1750, 1687, 1459, 1377, 1255, 1166, 1111, 1079, 838, 810, 776. ^1H NMR ($CDCl_3$, 400 MHz): δ 0.09 (s, 3H), 0.11 (s, 3H), 0.89 (s, 9H), 1.50 (s, 9H), 2.21-2.27 (m, 1H), 2.39-2.46 (m, 1H), 4.55 (m, 1H), 4.78 (dd, 1H, J = 8.8, 4.4), 5.71 (dd, 1H, J = 8.0, 2.4), 5.75, (d,1H, J = 2.0), 7.21 (d, 1H, J = 8.0), 8.53 (b s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ -4.95, -4.72, 18.0, 25.7, 28.1, 36.9, 75.2, 78.4, 82.1, 95.9, 102.0, 140.1, 149.7, 163.3, 169.6. Anal. Calcd for $C_{19}H_{32}N_2O_6Si$: C, 55.32%; H, 7.82%, N, 6.79%. Found: C, 55.03%; H, 7.97%; N, 6.88%.

1-(3-Deoxy-2-*tert*-butyldimethylsiloxy-α-*L*-arabinofuranosyl)uracil (22). A solution of **21** (1.80 g, 4.36 mmol) in 10 mL of 9:1 TFA/CH_2Cl_2 was stirred at ambient

temperature for no longer than 5-10 minutes before being concentrated *in vacuo* to a clear oil. The residue was co-evaporated from 1:1 dichloromethane/toluene and then from a mixture of 1:1 methanol/toluene. The product was carried on to the next step without further purification. To a solution of the resulting free carboxylic acid (47.0 mg, 0.131 mmol) in 5 mL of THF was added at 0 °C EtOCOCl (49.7 mg, 43.8 μL, 0.459 mmol) followed by Et_3N (21.4 mg, 29.4 μL, 0.211 mmol). The resulting mixture was stirred at ambient temperature for 2 h and then recooled to 0 °C. In one portion, $NaBH_4$ (35 mg, 0.917 mmol) was added with rapid stirring followed by dropwise addition of H_2O over 0.5 hour to dissolve the $NaBH_4$. The resulting mixture was stirred at 0 °C for 1 h, the reaction mixture was concentrated *in vacuo*, and the residue was partitioned between EtOAc and water. The layers were separated and the organic layer was washed with 1 N aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography eluting with 1:1 to 3:1 EtOAc/hexanxes to give 44.8 mg of 1-(3-deoxy-2-*tert*-butyldimethylsiloxy-α-*L*-arabinofuranosyl)uracil **22** (yield 100%): mp 136-137 °C. IR (KBr, cm^{-1}): 3515, 3156, 2955, 2858, 1689 (s), 1466, 1383, 1262, 1114, 1079, 843, 777. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.13 (s, 3H). 0.18 (s, 3H), 0.91 (s, 9H), 1.89 (ddd, 1H, J = 14.0, 3.6, 2.4), 2.19 (ddd, 1H, J = 14.1, 8.8, 5.4), 3.77-3.78 (m, 2H), 4.56-4.58 (m, 1H), 4.62-4.67 (m, 1H), 5.69 (s, 1H), 5.73 (d, 1H, J = 8.1), 7.27 (d, 1H, J = 8.1), 9.33 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ -9.4, -9.0, 13.6, 21.4, 29.6, 60.7, 71.7, 78.6, 90.2, 97.6, 134.9, 145.6, 159.1. Anal. Calcd for $C_{15}H_{26}N_2O_5Si$: C, 52.61%; H, 7.65%, N, 8.18%. Found: C, 52.67%; H, 7.91%; N, 8.29%.

1-(5'-(4-Toluenesulfonyl)-3'-deoxy-2'-*tert*-butyldimethylsilyloxy-α-*L*-arabinofuranosyl)uracil (23). To a solution of **22** (42.1 mg, 0.123 mmol) in 2 mL of pyridine was added at ambient temperature 4-toluenesulfonyl chloride (141 mg, 0.738 mmol). The resulting mixture was stirred at rt for 25 h under N_2 before being partitioned between 50 mL of EtOAc and 50 mL of H_2O. The layers were separated and the organic layer was washed with 1 N aqueous HCl, sat'd aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatograpy eluting with EtOAc/hexanes, 1:2 to 2:1, to give 54.8 mg of **23** (yield 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.07 (s, 3H), 0.11 (s, 3H), 0.84 (s, 9H), 1.85 (m, 1H), 2.11-2.18 (m, 1H), 2.46 (s, 3H), 4.12 (dd, 1H, *J* = 10.4, 4.8), 4.23 (dd, 1H, *J* = 10.4, 6.8), 4.48-4.49 (m, 1H), 4.68-4.74 (m, 1H), 5.55 (s, 1H) 5.70 (d, 1H, *J* = 7.6), 7.16 (d, 1H, *J* = 8.0), 7.36 (d, 2H, *J* = 8.0), 7.82 (d, 2H, *J* = 7.6), 8.18 (b s, 1H). This compound was used without further characterization.

1-(5'-Azido-3',5'-dideoxy-2'-*tert*-butyldimethylsilyloxy-α-*L*-arabinofuranosyl)uracil (24). To a solution of 5'-(4-toluensulfonyl)-3'-deoxy-2'-(O-*tert*-butyldimethylsilyloxy)-α-arabinofuranosyl)uracil **23** (46.8 mg, 0.0942 mmol) in 3 mL of DMF was added NaN_3 (75 mg, 1.2 mmol) in one portion. The resulting mixture was heated at 90 °C for 5 min and then stirred at 50 °C for 5 h before being evaporated *in vacuo*. The residue was partioned between water and ethyl acetate. The layers were separated and the organic layer was washed with sat'd aqueous $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography eluting with 1:1 hexanes/EtOAc to give 29.5 mg of 1-(5'-azido-3',5'-dideoxy-2'-*tert*-butyldimethylsilyloxy-α-*L*-arabinofuranosyl)uracil (**24**) (yield 85%). An

aliquot was recrystallized from hexanes/EtOAc, providing a sample for melting point: mp 102-103 °C. IR (KBr, cm^{-1}): 3161, 3099, 3055, 2955, 2859, 2105, 1697, 1668, 1468, 1387, 1262, 1121, 1058, 862, 836, 785. ^1H NMR (400 MHz, CD_3OD): δ 0.12 (s, 3H), 0.17 (s, 3H), 0.91 (s, 9H), 1.87 (ddd, 1H, *J* = 13.6, 3.6, 3.2), 2.16-2.23 (m, 1H), 3.38 (dd, 1H, *J* = 12.8, 4.4 Hz), 3.64 (dd, 1H, *J* = 12.8, 7.2), 4.56-4.58 (m, 1H), 4.59-4.65 (m, 1H), 5.68 (s, 1H), 5.73 (d, 1H, *J* = 8.0), 7.23 (d, 1H, *J* = 8.0), 8.78 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ -5.19, -4.80, 17.9, 25.6, 35.4, 55.2, 76.0, 81.0, 95.1, 102.0, 139.5, 149.7, 162.9. HRMS (FAB^+) m/z: 368.1755 (MH^+ $C_{15}H_{26}N_5O_4Si$ requires 368.1754).

1-(5'-Azido-3',5'-dideoxy-α-*L*-arabinofuranosyl)uracil (25). To a solution of **24** (29.5 mg, 0.080 mmol) in 5 mL of THF was added tetrabutylammonium fluoride, 1 M in THF (0.0964 mmol, 96 μL). The resulting mixture was stirred at ambient temperature for 1 h before being evaporated *in vacuo*. The residue was purified by silica gel chromatography, eluting with neat EtOAc, to give 16.9 mg of 1-(5'-azido-3',5'-dideoxy-α-*L*-arabinofuranosyl)uracil **25** (yield 83%). IR (KBr, cm^{-1}): 3422, 2104, 1686, 1459, 1265, 1101, 813. ^1H NMR (400 MHz, CD_3OD): δ 1.88 (ddd, *J* = 13.2, 6.2, 5.0 Hz, 1H), 2.30-2.37 (m, 1H), 3.40 (dd, *J* = 13.2, 4.0 Hz, 1H), 3.51 (dd, *J* = 13.2, 6.8 Hz, 1H), 4.48-4.51 (m, 1H), 4.62-4.65 (m, 1H), 5.68 (d, *J* = 8.0 Hz, 1H), 5.76 (d, *J* = 3.2 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (CD_3OD, 101 MHz): δ 36.1, 56.0, 76.3, 81.6, 95.1, 102.6, 142.5, 152.3, 166.3. HRMS (FAB^+) m/z: 276.0708 (MNa^+ $C_9H_{11}N_5O_4Na$ requires 276.0709).

1-(5'-amino-3',5'-dideoxy-α-*L*-arabinofuranosyl)uracil 26. To a solution of 1-(5'-azido-3',5'-dideoxy-α-*L*-arabinofuranosyl)uracil **25** (16.9 mg, 0.0667 mmol) in 50 μL of methanol was added 1 mL of 1,3-propanedithiol. The resulting mixture was stirred at

ambient temperature for 4 d before being concentrated *in vacuo*. The residue was partitioned between water and CH_2Cl_2. The layers were separated and the aqueous layer was washed with CH_2Cl_2 repeatedly. The aqueous layer was lyophilized to give 9.0 mg of 1-(5'-amino-3',5'-dideoxy-α-*L*-arabinofuranosyl)uracil **26** (yield 60%): mp 166 °C (begin dec). IR (KBr, cm^{-1}): 3174, 1688, 1630, 1503, 1458, 1265, 1100, 815. ^1H NMR (400 MHz, CD_3OD): δ 1.8 (ddd, 1H, *J*= 13.8, 4.8, 3.8 Hz), 2.33 (ddd, 1H, *J*= 13.8, 8.4, 6.2 Hz), 2.81 (dd, 1H, *J*= 13.2, 6.0 Hz), 2.90 (dd, 1H, *J*= 13.2, 3.6 Hz), 4.41 (ddd, 1H, *J*= 6.0, 3.6, 2.0 Hz), 4.54-4.59 (m, 1H), 5.68 (d, 1H, *J*= 8 Hz), 5.74 (d, 1H, *J*= 2.0 Hz), 7.55 (d, 1H, *J*= 8.4 Hz). ^{13}C NMR (CD_3OD, 101 MHz): δ 36.2, 46.3, 76.5, 83.8, 95.7, 102.4, 142.2, 152.4, 166.6. Anal. Calcd for $C_9H_{13}N_3O_4$: C, 47.58%; H, 5.77%, N, 18.49%. Found: C, 47.52%; H, 5.85%; N, 18.42%.

General procedure for the synthesis of compounds of structural type **2**: The synthesis shown employs reagents that provide the diastereomeric product equivalent to semi-synthetic **2**.

***N*-[[(1S)-1-[(Benzyloxy)carbonyl]ethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester (27).** A solution of *L*-alanyl benzyl ester 4-toluenesulfonic acid salt (2.0 g, 5.89 mmol) and *N,N*-diisopropylethylamine (1.12 mL, 6.45 mmol) in 20 mL of CH_2Cl_2 was added dropwise, at rt to a solution of triphosgene (0.582 g, 1.96 mmol) in 100 mL of CH_2Cl_2. The resulting mixture as stirred at rt for 5 minutes before addition of a solution of *L*-tryptophan *tert*-butyl ester hydrochloride (1.75 g, 5.89 mmol) and *N,N*-diisopropylethylamine (1.12 mL, 6.45 mmol) in 10 mL of CH_2Cl_2. The resulting mixture was stirred at rt for 30 min before being washed sequentially with 1 M aqueous HCl, 10% aqueous $NaHCO_3$, and brine. The combined organic layers were dried over anhydrous

Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by flash-chromatography on silica gel eluting with hexanes/EtOAc 8:2 to 6:4) to afford 2.41 g of *N*-[[(1*S*)-1-[(benzyloxy)carbonyl]ethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester (**27**) (yield 88%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (d, 3H, *J* = 5.4), 1.37 (s, 9H), 3.10-3.3 (m, 2H), 4.63 (quintet, 1H, *J* = 5.4), 4.7-4.8 (m, 2H), 5.05 (d, 1H, *J* = 9.3), 5.70 (d, 1H, *J* = 5.7), 5.83 (d, 1H, *J* = 6.3), 6.92 (d, 1H, *J* = 1.5), 7.0-7.4 (m, 9H), 7.58 (d, 1H, *J* = 6.0), 8.69 (s, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 18.8, 27.9, 48.5, 53.8, 67.1, 81.8, 109.7, 111.2, 118.8, 119.0, 121.7, 123.4, 127.6, 128.0, 128.2, 128.4, 128.5, 135.3, 135.85, 156.9, 172.1, 175.0. HRMS (FAB$^+$) m/z: 466.2344 (MH^+ $C_{26}H_{32}N_3O_5$ requires 466.2342).

***N*-[[(1*S*)-1-Carboxyethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester (28).** A solution of *N*-[[(1*S*)-1-[(benzyloxy)carbonyl]ethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester (**27**) (2.4 g, 5.15 mmol) in 10 mL of methanol (+10 mL of methanol to rinse) was added under nitrogen to a suspension of 10% palladium on charcoal (0.24 g, 20 wt. %) in 20 mL of MeOH. The flask was then flushed with H_2 (1 atm) and the mixture was vigorously stirred at rt, under H_2 (1 atm) for 5 h. The catalyst was filtered off and rinsed with MeOH. The filtrate was evaporated *in vacuo* and the residue was purified by silica gel chromatography eluting with hexanes/EtOAc/HOAc 1:1:0.01 to 1:2:0.01) to afford 1.5 g of *N*-[[(1*S*)-1-carboxyethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester **28** (yield 78%). IR (KBr, cm^{-1}) 3396, 2981, 1723, 1639, 1561, 1458, 1369, 1231, 1155, 743. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.20 (3, d, *J* = 7.2), 1.38 (9, s), 3.16 (2, m), 4.30 (1, br t, *J* = 6), 4.67 (1, br d, *J* = 6.4), 5.71 (1, br s, 1H), 5.87 (1, d, *J* = 7.6), 6.89 (1, d, *J* = 2.0), 7.05 (1, t, *J* = 7.2), 7.11 (1, t, *J* = 7.2), 7.30-7.15 (3, m), 7.53 (1, d), 8.70 (1, s). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 17.9, 28.1, 49.2, 54.4, 82.8, 109.6, 111.6, 118.8, 119.3, 122.0,

123.8, 125.5, 127.7, 128.4, 129.2, 136.3, 158.2, 173.2, 177.1. HRMS (FAB^+) m/z: 376.1872 (MH^+ $C_{19}H_{26}N_3O_5$ requires 376.1872).

***N*-[[(1*S*)-1-[(Pentafluorophenoxy)carbonyl]ethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester (29).** At 0°C, a solution of pentafluorophenol (0.4 g, 2.17 mmol) in 2 mL of EtOAc was added to a solution of *N*-[[(1*S*)-1-carboxyethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester (**28**) (0.75 g, 2 mmol) in 20 mL of EtOAc, followed by *N,N'*-dicyclohexylcarbodiimide (0.45 g, 2.18 mmol). The resulting mixture was stirred at 0 °C for 1 h and 0.5 h at rt. The reaction mixture was recooled to 0 °C and filtered through a cotton plug (rinsed 1 x 2 mL of cold EtOAc). The filtrate was then evaporated *in vacuo* and to afford 0.85 g (yield 92%) of *N*-[[(1*S*)-1-[(pentafluorophenoxy)carbonyl]ethyl]carbamoyl]-*L*-tryptophan, *tert*-butyl ester (**29**). This compound was used immediately without further purification or characterization.

(2*S*,3*S*)-N^2-[(*tert*-Butoxy)carbonyl]-N^3-[[*N*-(fluoren-9-ylmethoxy)carbonyl]-*L*-alanyl]-N^3-methyl-diaminobutyramide (30). A solution of 9-fluorenylmethyloxycarbonyl-*L*-alanine pentafluorophenyl ester (0.48 g, 1.03 mmol) and **10** (0.2 g, 0.861 mmol) in 10 mL of DMF was stirred at 50-55 °C for 14 h. The reaction mixture was then evaporated *in vacuo* and the residue purified by silica gel chromatography, eluting with EtOAc/CH_2Cl_2/MeOH/HOAc 1:0:0:0 to 1:1:0:0 to 6:4:1:0.1 to afford 0.344 g of (2*S*,3*S*)-N^2-[(*tert*-butoxy)carbonyl]-N^3-[[*N*-(fluoren-9-ylmethoxy)carbonyl]-*L*-alanyl]-N^3-methyl-diaminobutyramide (**30**) (yield 77%). IR (KBr, cm^{-1}) 3600-2500, 1716, 1502, 1422, 1265, 1162, 896, 748. ^1H NMR (300 MHz, CD_3OD): δ 1.31 (d, 3H, *J* = 6.9), 1.39 (d, 3H, *J* = 6.9 Hz), 1.55 (s, 9H), 3.12 (s, 3H), 4.0-4.70 (m, 5H), 5.02 (m, 1H), 7.3-7.6 (m, 4H), 7.75 (m, 2H), 7.89 (m, 2H). ^{13}C NMR

(100MHz, CD_3OD): δ 14.0, 17.6, 21.6, 28.8, 30.5, 51.8, 57.4, 68.1, 80.9, 121.0, 126.4, 128.3, 128.9, 129.3, 130.1, 139.0, 142.7, 145.3, 145.4, 158.0, 158.2, 174.0, 175.5. HRMS (FAB^+) m/z: 526.2555 (MH^+ $C_{28}H_{36}N_3O_7$ requires 526.2553).

1-[5-(2*S*,3*S*)-N^2-[(*tert*-Butoxy)carbonyl]-N^3-[[*N*-(fluoren-9-ylmethoxy)carbonyl]-*L*-alanyl]-N^3-methyl-diaminobutyramido]-3,5,dideoxy-α-*L*-arabinofuranosyl]uracil (31). At 0 °C, a solution of pentafluorophenol (0.0087 g, 0.0472 mmol) in 0.25 mL of EtOAc was added to a solution of (2*S*,3*S*)-N^2-[(tert-butoxy)carbonyl]-N^3-[[*N*-(fluoren-9-ylmethoxy)carbonyl]-*L*-alanyl]-N^3-methyl-diaminobutyramide (**30**) (0.025 g, 0.0476 mmol) in 0.25 mL of EtOAc, followed by *N*,*N*-dicyclohexylcarbodiimide (0.0098 g, 0.0475 mmol). The resulting mixture was stirred at 0 °C for 1 h and 0.5 h at rt. The reaction mixture was recooled to 0 °C and filtered through a cotton plug (rinsed once with 2 mL of cold EtOAc). The filtrate was then evaporated *in vacuo* and the residue was dissolved in 0.25 mL of DMF to which 1-(5'-amino-3',5'-dideoxy-α-*L*-arabinofuranosyl)uracil (**26**) (0.009 g, 0.0396 mmol) was added. The resulting mixture was stirred at 50-55 °C for 16 h. The reaction mixture was evaporated and the residue purified by flash-chromatography on silica gel eluting with CH_2Cl_2/EtOAc/MeOH (1:0:0 to 6:4:0 to 6:4:0.1 to 6:4:0.2 to 6:4:0.3 to 6:4:0.4 to 6:4:0.5 to 6:4:0.6 to 6:4:0.7) to afford 0.0132 g of **31** (yield 45%). IR (KBr, cm^{-1}) 3500-2600, 1711, 1682, 1625, 1421, 1265, 1165, 896, 741. ^1H NMR (400 MHz, CD_3OD, tabulated data represent complex mixture of rotamers in the spectrum): δ 1.28 (d, J = 6.3), 1.3-1.5 (m, 12H), 2.02 (m, 1H), 2.25 (m, 1H), 2.82 (s, 3H), 3.3-3.4 (m, 1H), 4.0-4.7 (m, 4H), 5.6-5.75 (m, 2H), 7.2-7.8 (m, 8H). ^{13}C NMR (100MHz, CD_3OD, tabulated data represent complex mixture of rotamers in the spectrum), δ 14.0, 15.5, 17.8, 19.2, 28.8, 35.9, 36.4,

45.1, 45.7, 54.9, 58.5, 68.0, 76.5, 76.6, 81.0, 95.0, 95.3, 102.5, 102.6, 121.0, 126.3, 128.3, 128.9, 142.4, 142.7, 145.3, 145.4, 145.5, 152.3, 157.5, 158.1, 166.4, 166.5, 172.3, 172.8, 174.8, 175.4. HRMS (FAB^+) m/z: 735.3353 (MH^+ $C_{37}H_{47}N_6O_{10}$ requires 735.3354).

1-(5-[(2*S*,3*S*)-N^2-[*N*-[(1*S*)-1-(*tert*-Butoxycarbonyl)-2-indol-3-ylethyl]carbamoyl]-*L*-alanyl]-N^3-[[*N*-[(fluoren-9-ylmethoxy)carbonyl]-*L*-alanyl]-N^3-[methyl-2,3-diaminobutyramido]-3,5-dideoxy-α-*L*-arabinofuranosyl)uracil (32). A solution of compound **31** (0.0132 g, 0.018 mmol) in 1 mL of trifluoroacetic acid/water (9:1) was stirred at rt for 1 h. The reaction mixture was diluted with 2 mL of methanol and 2 mL of toluene and was evaporated *in vacuo* and then reevaporated once from 2 mL of toluene. The residue was dissolved in 0.25 mL of a diisopropylethylamine solution in dimethylformamide prepared by diluting 28 μL diisopropylethylamine in 2 mL of dimethylformamide. Compound **29** (0.020 g, 0.0369 mmol) was added and the resulting mixture was stirred at 50-55 °C for 14 h. The reaction mixture was evaporated *in vacuo* and the residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/EtOAc/MeOH/HOAc (1:0:0:0 to 6:4:0:0 to 6:4:0.5:0.1 to 6:4:0.75:0.1 to 6:4:1:0.1) to afford 0.0175 g of **32** (yield > 95%). IR (KBr, cm^{-1}) 3600-2900, 1676, 1685, 1544, 1456, 1261, 1206, 1153, 1102, 743. ^1H NMR (300 MHz, CD_3OD, most resonances very broad, complex mixture of rotamers, diagnostic resonances shown): δ 1.06 (s), 2.56 (s), 2.58 (s), 3.8-4.4 (m), 5.2-5.5 (m), 6.6-8 (m). ^{13}C NMR (100MHz, CD_3OD, tabulated data represent complex mixture of rotamers in the spectrum): δ 14.1, 15.6, 17.7, 18.5, 18.6, 19.2, 27.9, 28.3, 28.6, 28.8, 29.3, 29.5, 31.5, 35.8, 36.2, 44.9, 45.6, 51.1, 54.9, 55.8, 57.0, 57.1, 67.9, 76.4, 80.8, 81.7, 82.6, 94.7, 94.8, 102.5, 110.6, 110.7, 112.2, 119.5, 119.7, 120.8, 122.3, 122.4, 124.5, 126.1, 128.0, 128.7, 128.7, 137.8, 142.0,

142.2, 142.4, 145.0, 145.1, 145.2, 152.0, 157.87, 159.1, 159.5, 166.1, 171.3, 171.8, 173.6, 175.2, 175.3, 175.7, 175.9.

1-(5-[(2*S*,3*S*)-N^2-[*N*-[[(1*S*)-1-Carboxy-2-indol-3ylethyl]carbomoyl]-*L*-alanyl]-N^3-*L*-alanyl-N^3-methyl-2,3-diaminobutyramido]-3,5-dideoxy-α-*L*-arabinofuranosyl)uracil (34). A solution of compound **32** (0.0175 g, 0.018 mmol) in 1 mL of trifluoroacetic acid/water/ethanedithiol (9:1:0.1) was stirred at rt for 2.5 h. The reaction mixture, diluted with 2 mL of methanol and 2 mL of toluene, was evaporated *in vacuo* and reevaporated once from 2 mL of toluene. The residue was used directly in the next step without any further purification. A solution of the residue (0.018 mmol, theoretical) in 2 mL of $Et_2NH/CH_3CN/CH_2Cl_2/H_2O$ (50:23:23:4) was stirred at rt for 2 h. The precipitate was removed by filtration and rinsed once with 2 mL of CH_2Cl_2. The solid was redissolved in 5 mL of methanol/water (1:1) and the solution was evaporated *in vacuo*. The residue was dissolved in 1 mL of water and was loaded onto a C-18 Analtech Spice Sample Preparation Cartridge. The cartridge was rinsed with 2 x 1 mL of H_2O and 2 x 1 mL of acetonitrile/water (1:1). The filtrate was evaporated *in vacuo* and the residue dissolved in 1 mL of water and was filtered through a nylon filter. The filter was rinsed with 2 x 2 mL of H_2O. Purification was performed by HPLC on semi-preparative C-18 column (eluant CH_3CN/0.1% TFA acid in H_2O 0:100 to 70:30 over 20 minutes, 10 mL/minute flow rate, detection 260 nm, injections of 0.5 mL of the filtrate). The combined pure fractions were evaporated *in vacuo* and the residue was dissolved in 1 mL of H_2O and filtered through a Rainin Nylon Filter unit (0.3 U/13 mm). The filter was rinsed with 2 x 1 mL of water. Finally, the combined aqueous solution was lyophilized to afford 0.0046 g of 1-(5-((2*S*,3*S*)-N^2-(2-indol-3ylethyl)carbomoyl)-*L*-alanyl)-N^3-(L-

alanyl)-N^3-methyl-2,3-diaminobutyramido)-3,5-dideoxy-α-*L*-arabinofuranosyl)uracil (**34**) as its trifluoroacetic acid salt (yield 36% over 4 steps). IR (KBr pellet, cm^{-1}) 3600-2500, 2363, 1685, 1655, 1560, 1544, 1204. ^1H NMR (400 MHz, CD_3OD, mixture of rotamers, diagnostic resonances and integration tabulated): δ 1.15 (d, J = 7.6), 1.6-1.27 (m), 1.33 (d, J = 6.8), 1.38 (d, J = 6.8), 1.65-1.80 (m, doubled signals, 1H), 2.1-2.3 (m, doubled signals, 1H), 2.80 (s, 1.5H), 2.83 (s, 1.5H), 3.1-3.5 (m), 4.0-4.2 (m), 4.35-4.45 (m), 4.5-4.65 (m), 5.55-5.75 (m), 6.85-7.15 (m), 7.30 (t, 1H, J = 7.6), 7.45 (d, 0.5 H, J = 8), 7.55 (t, 1H, J = 6.4), 7.61 (d, 0.5H, J = 8.0). ^{13}C NMR (100MHz, CD_3OD, tabulated data represent a complex mixture of rotamers): δ 13.6, 15.2, 16.5, 17.2, 18.2, 18.3, 28.7, 29.2, 30.9, 36.0, 36.4, 44.9, 45.2, 51.1, 51.3, 52.9, 54.7, 55.2, 56.6, 57.3, 76.5, 80.9, 81.4, 95.0, 95.4, 102.5, 102.7, 110.8, 110.9, 112.4, 119.6, 120.0, 122.5, 124.8, 124.9, 129.0, 138.2, 142.5, 142.9, 152.4, 159.9, 160.1, 166.4, 166.6, 171.3, 171.9, 176.1, 176.4, 176.5. HRMS (FAB^+) m/z: 714.3214 (MH^+ $C_{32}H_{44}N_9O_{10}$ requires 714.3211).

Analytical HPLC Analysis of Semi-synthetic 2 and Totally Synthetic 34. Both purified compounds were subjected to analytical HPLC analysis on a Phenomenex C-8 reversed-phase analytical HPLC column (1 mL/min flow rate, 0-100% CH_3CN in H_2O with 0.1% TFA, ramped over 20 min; UV detection at 254 nm). For totally synthetic **34**, t_R = 12.2 min; for semi-synthetic **2**, t_R = 12.3 min.

References and Notes

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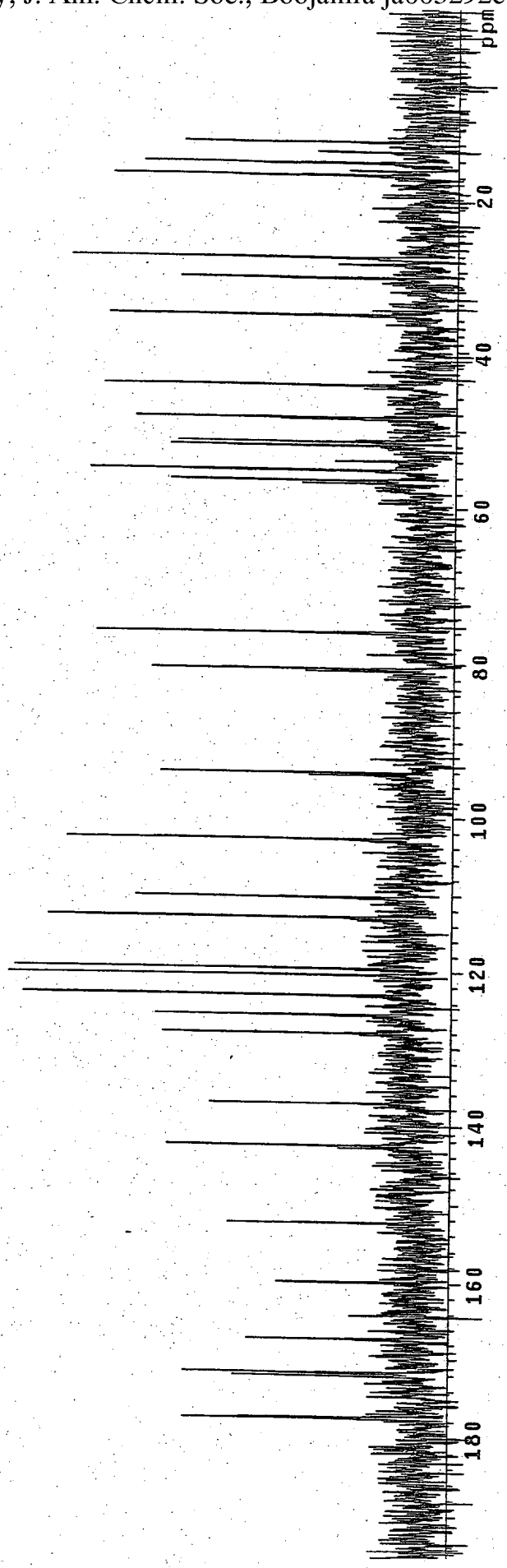
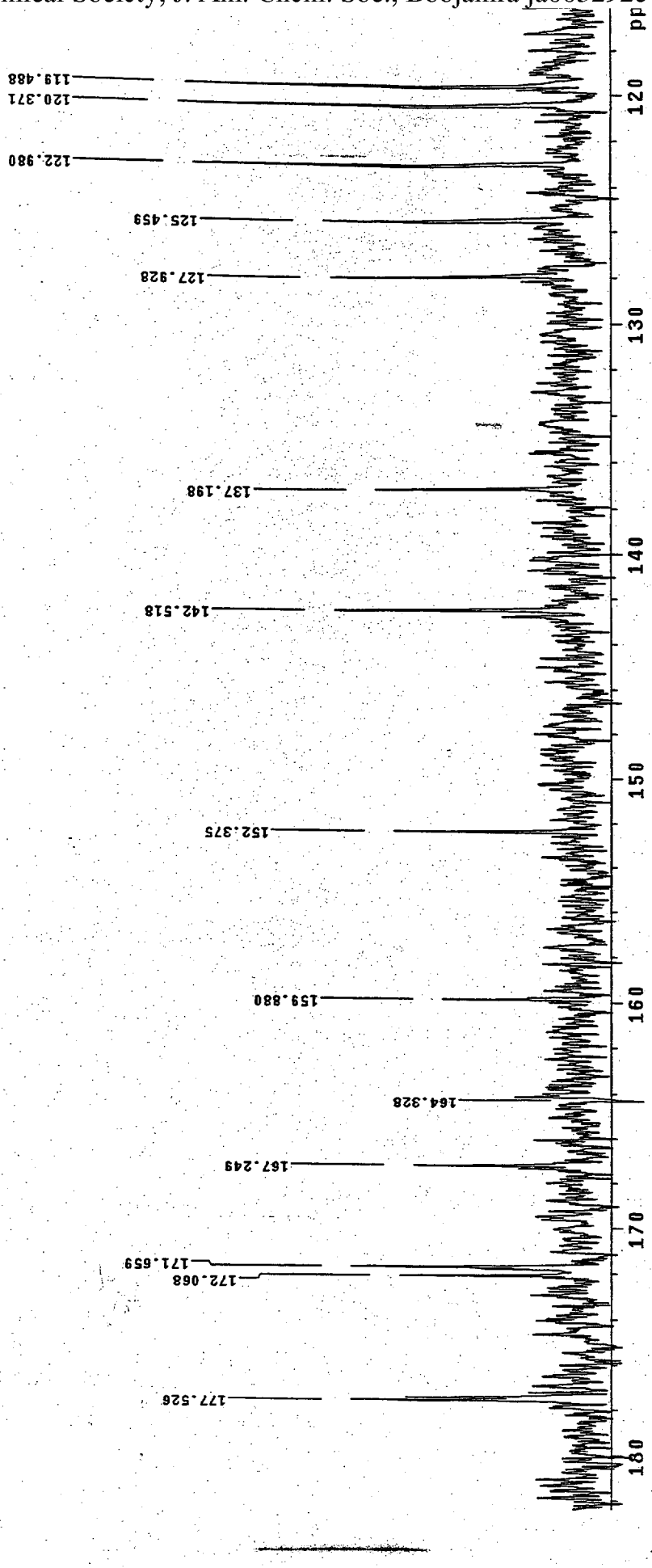
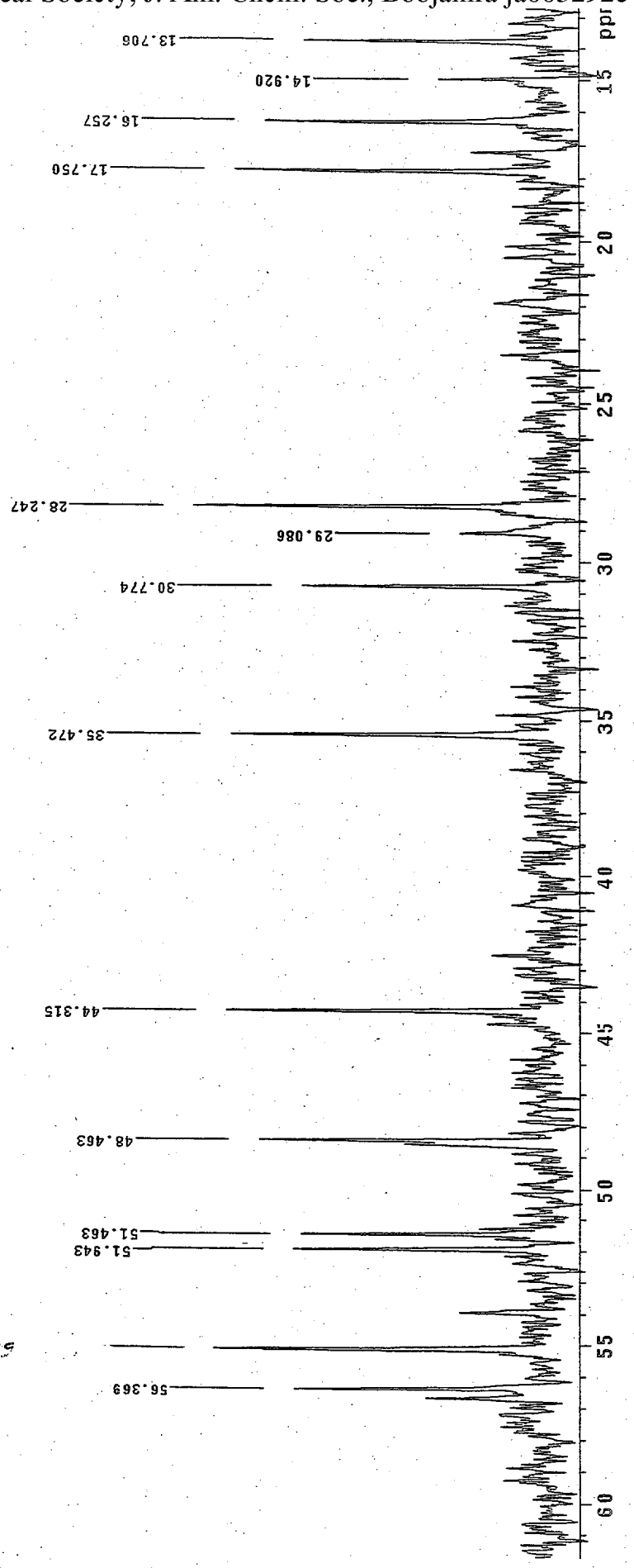
^{13}C NMR spectrum page 1 of 4. compound 34 (totally synthetic dihydropacidamycin D)
D_2O externally referenced to acetone
100 MHz (line broadening 4 Hz)

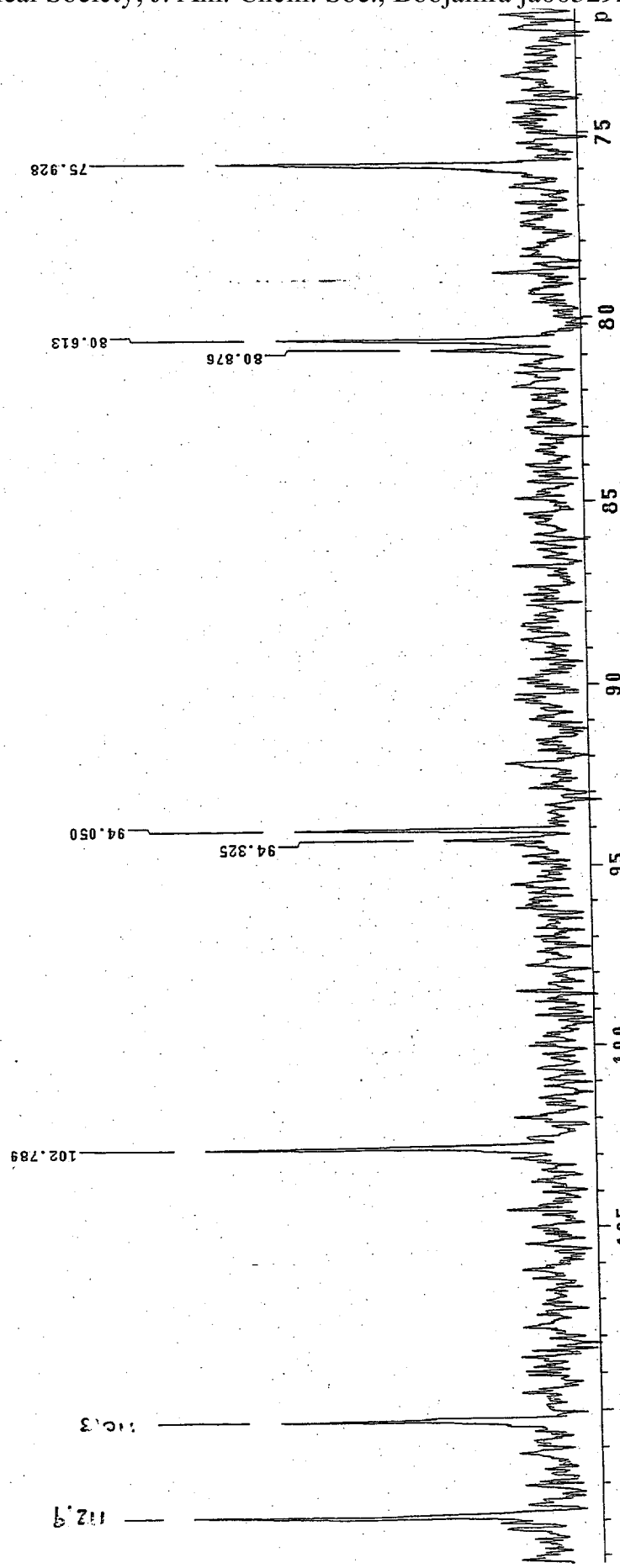


^{13}C NMR spectrum page 2 of 4, compound 34 (totally synthetic dihydropacidamycin D)
D_2O externally referenced to acetone
100 MHZ (line broadening 4 Hz)





^{13}C NMR page 3 of 4, compound 34



^{13}C NMR page 4 of 4, compound 34

^{1}H NMR of compound 34: totally synthetic dihydropacidamycin D

CD_3OD 400 MHz