Supporting Information
Total Syntheses of (–)-Pyrimidoblamic Acid and P-3A

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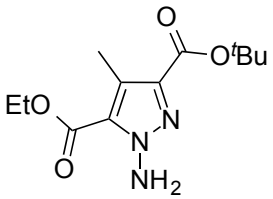
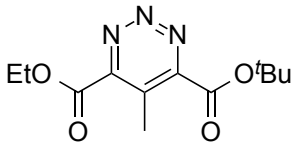
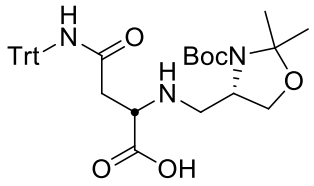


3-(*tert*-Butyl) 5-Ethyl 1-Amino-4-methyl-1*H*-pyrazole-3,5-dicarboxylate (9a). A solution of potassium *t*-butoxide (1 M in NMP, 17 mL) was added to a solution of **8a** (15.3 mmol, 3.9 g) in NMP (20 mL) at 20 °C. After 30 min, a solution of *O*-4-nitrobenzoylhydroxylamine (17.6 mmol, 3.2 g) in NMP (15 mL) was added at < 25 °C. The mixture was stirred at 20 °C for 45 min, at which time LC/MS analysis showed complete conversion of **8a**. The reaction mixture was quenched with addition of 7% aqueous NaCl and the aqueous layer was extracted with EtOAc. The organic layers were combined, washed with saturated aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl before being dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 20% EtOAc–hexanes) to yield **9a** as a ~2:1 mixture of N-amination regioisomers (3.01 g, 73%). Isomer A: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.68 (s, 2H), 4.38 (q, *J* = 7.1 Hz, 2H), 2.50 (s, 3H), 1.61 (s, 9H), 1.39 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4, 160.3, 135.2, 126.6, 125.0, 83.9, 60.9, 28.4 (3C), 14.5, 10.5. Isomer B: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.58 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 2.50 (s, 3H), 1.60 (s, 9H), 1.42 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.7, 161.1, 136.8, 125.9, 124.8, 81.9, 61.7, 28.4 (3C), 14.3, 10.6; IR for mixture (film) ν_{max} 3334, 3235, 2979, 2934, 1709, 1582, 1446, 1369, 1298, 1243, 1168, 1131, 1092, 1017, 842, 786 cm^{-1}; HRESI-TOF *m/z* 270.1445 ($C_{12}H_{19}N_3O_4$ + H^+ requires 270.1448).

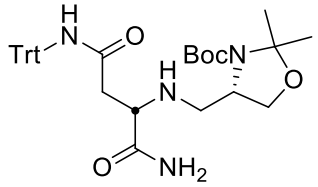


4-(*tert*-Butyl) 6-Ethyl 5-Methyl-1,2,3-triazine-4,6-dicarboxylate (5a). A 20% aqueous solution of $KHCO_3$ (5 mL) was added dropwise at 25 °C to a solution of **9a** (0.74 mmol, 200 mg) in CH_2Cl_2 (3.5 mL). A solution of iodine (1.11 mmol, 283 mg) in CH_2Cl_2 (10 mL) was added to the emulsion at 25 °C. After complete addition, the reaction mixture was allowed to stir at ambient temperature and monitored by TLC. After 5 h, the reaction was quenched with addition of 10% aqueous sodium thiosulfate. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, 15% EtOAc–hexanes) to yield **5a** as an orange oil (149 mg, 75%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.55 (q, *J* = 7.1 Hz, 2H), 2.62 (s,

3H), 1.67 (s, 9H), 1.47 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.3, 162.4, 153.9, 152.0, 126.7, 85.9, 63.4, 28.2 (3C), 14.2, 14.1; IR (film) ν_{max} 2981, 2937, 1732, 1459, 1371, 1296, 1248, 1178, 1148, 1101, 1050, 1015, 841, 815, 744, 716 cm^{-1}; HRESI-TOF *m/z* 268.1298 ($C_{12}H_{17}N_3O_4$ + H^+ requires 268.1292).

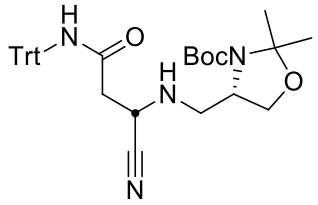


(*S*)-2-((((*S*)-3-(*tert*-Butyloxycarbonyl)-2,2-dimethyloxazolidin-4-yl)methyl)amino)-4-oxo-4-(tritylamino)butanoic Acid (10). A solution of (*R*)-(+)-3-Boc-2,2-dimethyloxazolidine-4-carboxaldehyde (**7**, 6.06 mmol, 1.4 g) in anhydrous CH_2Cl_2 (20 mL) at 25 °C was treated with N'-trityl-L-asparagine (**6**, 3.03 mmol, 1.1 g) and powdered 4Å molecular sieves. The suspension was stirred for 15 min before $NaBH(OAc)_3$ (7.58 mmol, 1.6 g) was added and the reaction mixture was stirred for 12 h under argon. The reaction was quenched with the slow addition of saturated aqueous $NaHCO_3$ until gas evolution ceased. The mixture was poured into H_2O and the product was extracted with CH_2Cl_2. The organic phases were combined, filtered through Celite, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and purified by flash chromatography (SiO_2) using a gradient elution of 5% MeOH–CH_2Cl_2 to elute the less polar by-products and then 10% MeOH–CH_2Cl_2 to elute **10** (1.6 g, 84%) as an amorphous white solid: $[\alpha]^{23}_D$ +8.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz, 55 °C) δ 7.26 (m, 15H), 4.10 (m, 1H), 3.98 (t, J = 8.0 Hz, 1H), 3.80 (m, 1H), 3.67 (m, 1H), 3.11 (m, 3H), 2.85 (dd, J = 8.4, 16.7 Hz, 1H), 1.48 (s, 3H), 1.45 (s, 3H), 1.44 (s, 9H); IR (film) ν_{max} 3056, 2979, 2934, 2882, 1679, 1529, 1492, 1447, 1388, 1377, 1366, 1251, 1170, 766, 700 cm^{-1}; HRESI-TOF *m/z* 588.3072 ($C_{34}H_{41}N_3O_6$ + H^+ requires 588.3068).

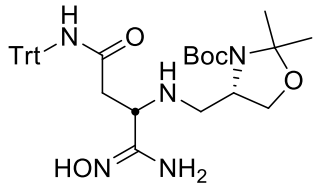


(*S*)-*tert*-Butyl-4-((((*S*)-1-Amino-1,4-dioxo-4-(tritylamino)butan-2-yl)amino)methyl)-2,2-dimethyloxazolidine-3-carboxylate (11). A suspension of **10** (0.73 mmol, 0.43 g) and HOBt (0.88 mmol, 0.12 g) in anhydrous CH_2Cl_2 (7.3 mL) under argon at 0 °C was treated with EDCI•HCl (0.88 mmol, 0.17 g). The reaction mixture was warmed to 25 °C and stirred for 30 min. The reaction mixture was cooled to 0 °C and a 0.5 M solution of NH_3 in THF (2.92 mmol, 5.8 mL) was added quickly by syringe. Upon complete addition, the reaction mixture was allowed to warm to 25 °C and stir for 30 min at which time the reaction was judged complete by TLC. The reaction mixture was concentrated in vacuo, redissolved in CH_2Cl_2 and purified by flash chromatography (SiO_2) using a gradient elution of 70% EtOAc–hexanes to elute the less polar by-products and then 5% MeOH–CH_2Cl_2 to elute **11** (0.35 g, 81%) as an amorphous white solid: $[\alpha]^{23}_D$ +28 (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.22 (s, 1H), 7.28 (m, 15H), 6.69 (bs, 1H), 5.76 (bs, 1H), 3.80 (m, 2H), 3.70 (m, 1H), 3.39 (m, 1H), 2.75 (m, 2H), 2.57

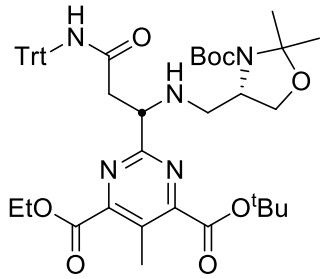
(m, 3H), 1.50 (s, 3H), 1.46 (s, 9H), 1.44 (s, 3H); IR (film) ν_{max} 3301, 1670, 1525, 1492, 1448, 1390, 1367, 1257, 1173, 1085, 700 cm^{-1}; HRESI-TOF *m/z* 587.3238 ($C_{34}H_{42}N_4O_5$ + H^+ requires 587.3228).



(*S*)-*tert*-Butyl-4-((((*S*)-1-Cyano-3-oxo-3-(tritylamino)propyl)amino)methyl)-2,2-dimethyloxazolidine-3-carboxylate (12). A solution of **11** (1.5 mmol, 0.88 g) in anhydrous THF (15 mL) at 0 °C was treated with *i*-Pr_2NEt (6.0 mmol, 1 mL). This mixture was treated with a 50% wt. solution of T3P in EtOAc (6.0 mmol, 3.6 mL). Upon complete addition, the reaction mixture was warmed at 50 °C and allowed to stir until complete conversion was noted by TLC (~2 h). The reaction was quenched with addition of water and the product was extracted with CH_2Cl_2. The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was redissolved in CH_2Cl_2 and purified by flash chromatography (SiO_2, 50% EtOAc–hexanes) to provide **12** (0.8 g, 94%) as an amorphous white solid: $[\alpha]^{23}_D$ +3.0 (*c* 1.0, $CHCl_3$); ^1H NMR (CD_3CN, 500 MHz, 60 °C) δ 7.70 (s, 1H), 7.28 (m, 15H), 3.97 (dd, *J* = 5.7, 7.6 Hz, 1H), 3.88 (m, 2H), 3.77 (d, *J* = 7.9 Hz, 1H), 3.02 (m, 1H), 2.70 (m, 3H), 1.53 (s, 3H), 1.47 (s, 9H), 1.46 (s, 3H); IR (film) ν_{max} 3305, 3056, 2978, 2928, 2873, 1690, 1660, 1597, 1527, 1492, 1448, 1389, 1367, 1259, 1171, 1087, 847, 700 cm^{-1}; HRESI-TOF *m/z* 569.3121 ($C_{34}H_{40}N_4O_4$ + H^+ requires 569.3122).

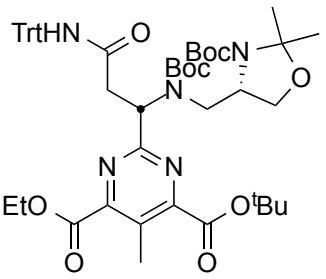


(*S*)-*tert*-Butyl-4-((((*S*)-1-Amino-1-(hydroxyimino)-4-oxo-4-(tritylamino)butan-2-yl)amino)methyl)-2,2-dimethyloxazolidine-3-carboxylate (13). A solution of **12** (0.03 mmol, 17 mg) dissolved in absolute EtOH (0.5 mL) at 25 °C was treated with a 50% aqueous solution of NH_2OH (0.12 mmol, 8 μL). The resulting mixture was stirred at 25 °C until complete conversion of starting material was observed by TLC (~12 h). The reaction mixture was concentrated in vacuo and the residue dissolved in CH_2Cl_2 and purified by PTLC (SiO_2, 5% MeOH–CH_2Cl_2). The product **13** was isolated as a white amorphous solid (16 mg, 90%): $[\alpha]^{23}_D$ +24 (*c* 1.0, $CHCl_3$); ^1H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.15 (s, 1H), 7.28 (m, 15H), 4.84 (s, 2H), 3.79 (m, 2H), 3.70 (m, 1H), 3.41 (dd, *J* = 5.8, 7.3 Hz, 1H), 2.78 (m, 1H), 2.51 (m, 3H), 1.50 (s, 3H), 1.45 (s, 9H), 1.43 (s, 3H); IR (film) ν_{max} 3320, 3057, 2980, 2930, 1663, 1597, 1492, 1448, 1392, 1366, 1257, 1173, 1103, 851, 753, 701 cm^{-1}; HRESI-TOF *m/z* 602.3337 ($C_{34}H_{43}N_5O_5$ + H^+ requires 602.3337).



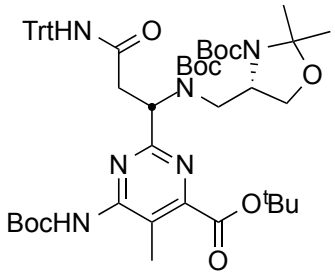
4-*tert*-Butyl-6-Ethyl-2-((*S*)-1-((((*S*)-3-(*tert*-Butyloxycarbonyl)-2,2-dimethyloxazolidin-4-yl)methyl)amino)-3-oxo-3-(tritylamino)propyl)-5-methylpyrimidine-4,6-dicarboxylate (14). ***Amidine preparation:*** A solution of **13** (0.083 mmol, 50 mg) in MeOH (3 mL) was treated with glacial AcOH (0.170 mmol, 10 μL) followed by a spatula tip of Raney® 2800 Nickel as an aqueous cake. This suspension was stirred at 25 ºC until complete consumption of the amidoxime was evident by TLC. The reaction mixture was filtered through a Celite plug and concentrated in vacuo without allowing the temperature of the sample to increase above 30 °C. The residue was redissolved in CH_2Cl_2 and concentrated two additional times to remove residual MeOH. The resulting residue was treated with CH_2Cl_2 (2 mL) followed by 1 N aqueous NaOH (2 mL). This mixture was shaken briefly before the organic layer was extracted, dried over Na_2SO_4, and concentrated in vacuo, without allowing the temperature of the sample to increase above 30 °C, to yield the free-based amidine **4** (41 mg, 84%). The resulting residue was then briefly dried under high vaccuum and used within 1 h.

Inverse electron demand Diels-Alder reaction: A solution of powdered 4 Å molecular sieves and 1,2,3-triazine **5a** (0.034 mmol, 9.0 mg) in anhydrous CH_3CN (35 μL) cooled to 5 °C was treated with a solution of crude amidine **4** (0.017 mmol, 10 mg) in anhydrous CH_3CN (50 μL) dropwise. This mixture was stirred at 5 °C for 14 h at which time the reaction mixture was allowed to warm to ambient temperature and stir for 6 h. The reaction mixture was filtered through Celite and concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2 and purified by flash chromatography (SiO_2, 30% EtOAc–hexanes) to yield **14** as a tan film (7.4 mg, 54%): $[\alpha]^{23}_D$ +0.32 (*c* 0.48, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.80 (s, 1H), 7.20 (m, 15H), 4.43 (q, *J* = 7.1 Hz, 2H), 4.20 (dd, *J* = 4.9, 7.5 Hz, 1H), 3.73 (m, 2H), 3.67 (d, *J* = 3.8 Hz, 2H), 2.70 (m, 5H), 2.43 (s, 3H), 1.60 (s, 9H), 1.45 (s, 3H), 1.40 (bs, 12H), 1.36 (t, *J* = 7.1 Hz, 3H); IR (film) ν_{max} 2975, 2928, 1735, 1690, 1550, 1491, 1447, 1367, 1250, 1155, 1082, 1034, 844, 734, 699 cm^{-1}; HRESI-TOF *m/z* 808.4278 ($C_{46}H_{57}N_5O_8 + H^+$ requires 808.4280).

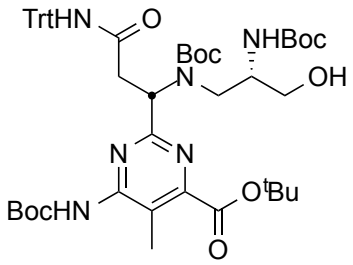


4-*tert*-Butyl-6-Ethyl-2-((*S*)-1-((*tert*-Butyloxycarbonyl)(((*S*)-3-(*tert*-butyloxycarbonyl)-2,2-dimethyloxazolidin-4-yl)methyl)amino)-3-oxo-3-(tritylamino)propyl)-5-methylpyrimidine-4,6-dicarboxylate (15). A solution of **14** (0.021 mmol, 17 mg) in anhydrous THF was treated with Et_3N (0.084 mmol, 16 mg) followed by Boc_2O (0.074 mmol, 16 mg) at 25 °C. The reaction

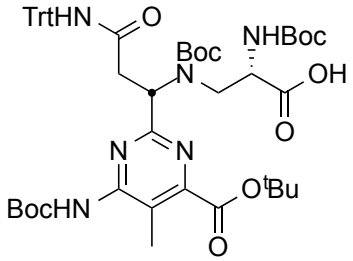
mixture was warmed at 50 °C and stirred for 12 h, upon which complete conversion was noted by TLC. The reaction mixture was concentrated, redissolved in $CHCl_3$ and purified by PTLC (SiO_2, 30% EtOAc–hexanes). Product **15** was isolated as a white film (17 mg, 90%): $[\alpha]^{23}_D$ –22 (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 7.33–7.17 (m, 16H), 4.94 (bs, 1H), 4.40 (qd, *J* = 2.7, 7.1 Hz, 2H), 4.15 (m, 2H), 3.88 (ddd, *J* = 3.0, 5.7, 8.1 Hz, 1H), 3.77 (bs, 1H), 3.48 (m, 1H), 3.07 (bs, 2H), 2.43 (s, 3H), 1.59 (s, 9H), 1.55 (s, 3H), 1.44 (s, 3H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.35 (bs, 9H), 1.20 (bs, 9H); IR (film) ν_{max} 2975, 2931, 1737, 1694, 1559, 1488, 1424, 1390, 1368, 1254, 1162, 1074, 851, 701 cm^{-1}; HRESI-TOF *m/z* 908.4824 ($C_{51}H_{65}N_5O_{10}$ + H^+ requires 908.4804).



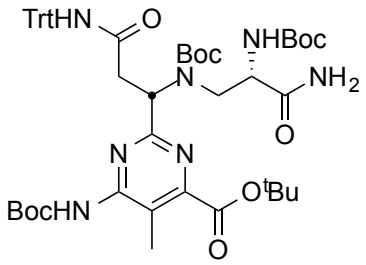
(*S*)-*tert*-Butyl-4-(((*tert*-Butyloxycarbonyl)((*S*)-1-(4-(*tert*-butyloxycarbonyl)-6-((*tert*-butyloxycarbonyl)amino)-5-methylpyrimidin-2-yl)-3-oxo-3-(tritylamino)propyl)amino)methyl)-2,2-dimethyloxazolidine-3-carboxylate (16). A solution of **15** (0.018 mmol, 16 mg) in THF:MeOH (800 μL, 3:1) was treated with 1 N aqueous NaOH (135 μL) dropwise at 0 °C. The reaction mixture was stirred at 0 °C. After 10 min, complete conversion was noted by TLC. The reaction mixture was quenched with addition of 1 N aqueous HCl to a pH < 4 and the product was extracted with CH_2Cl_2. The organic layer was concentrated and lyophilized. The residue was redissolved in anhydrous ^{t}BuOH (200 μL), to which Et_3N (0.040 mmol, 6 μL) and DPPA (0.036 mmol, 8 μL) were added at 25 °C. The reaction mixture was warmed at reflux and monitored by TLC. After 4 h, complete conversion was noted and the reaction mixture was concentrated. The residue was redissolved in CH_2Cl_2 and purified by flash chromatography (SiO_2, 30% EtOAc–hexanes). Product **16** was isolated as a white amorphous solid (13 mg, 78% for 2-steps): $[\alpha]^{23}_D$ –24 (*c* 0.52, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 7.51 (s, 1H), 7.32–7.19 (m, 15H), 5.00–4.73 (m, 1H), 4.26–4.15 (m, 1H), 4.15–4.06 (m, 1H), 3.93–3.83 (m, 1H), 3.83–3.67 (m, 1H), 3.56–3.43 (m, 1H), 3.13–2.87 (m, 2H), 2.21 (s, 3H), 1.58 (s, 9H), 1.54 (s, 3H), 1.47 (s, 9H), 1.43 (s, 3H), 1.42–1.10 (m, 18H); IR (film) ν_{max} 2978, 2960, 2928, 1688, 1578, 1563, 1490, 1392, 1325, 1285, 1251, 1161, 1090, 1021, 847, 768, 700 cm^{-1}; HRESI-TOF *m/z* 951.5219 ($C_{53}H_{70}N_6O_{10}$ + H^+ requires 951.5226).



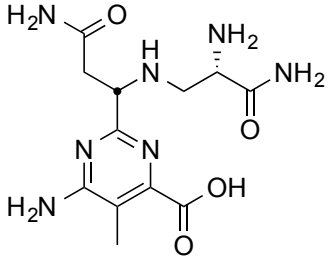
***tert*-Butyl-2-((5*S*,8*S*)-6-(*tert*-Butyloxycarbonyl)-8-(hydroxymethyl)-12,12-dimethyl-3,10-dioxo-1,1,1-triphenyl-11-oxa-2,6,9-triazatridecan-5-yl)-6-((*tert*-butyloxycarbonyl)amino)-5-methylpyrimidine-4-carboxylate (17)**. Compound **16** (0.013 mmol, 12 mg) was dissolved in MeOH **(**200 μL) and *p*-TsOH monohydrate (0.006 mmol, 1.2 mg) was added in one portion at 25 °C. The reaction mixture was stirred at 25 °C for 3 h, upon which time complete conversion was noted by TLC. The reaction was quenched with the addition of saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic phases were combined, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by PTLC (SiO_2, 40% EtOAc–hexanes) to yield **17** as a film (8.3 mg, 72%): $[\alpha]^{23}_D$ –27 (*c* 0.42, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 7.47 (s, 1H), 7.32–7.18 (m, 15H), 5.69–5.53 (m, 1H), 4.94–4.85 (m, 1H), 3.82–3.73 (m, 1H), 3.73–3.61 (m, 1H), 3.61–3.47 (m, 3H), 3.15 (dd, *J* = 6.1, 14.5 Hz, 1H), 2.95–2.81 (m, 1H), 2.22 (s, 3H), 1.58 (s, 9H), 1.47 (s, 9H), 1.38 (s, 9H), 1.23 (bs, 9H); IR (film) ν_{max} 3315 (broad), 2979, 2931, 1679, 1559, 1493, 1394, 1248, 1157, 1096, 1053, 846, 752, 702, 631, 584 cm^{-1}; HRESI-TOF *m/z* 911.4909 ($C_{50}H_{66}N_6O_{10}$ + H^+ requires 911.4913).



(*S*)-3-((*tert*-Butyloxycarbonyl)((*S*)-1-(4-(*tert*-Butyloxycarbonyl)-6-((*tert*-butyloxycarbonyl)amino)-5-methylpyrimidin-2-yl)-3-oxo-3-(tritylamino)propyl)amino)-2-((*tert*-butyloxycarbonyl)amino)propanoic Acid (18). A solution of **17** (0.011 mmol, 10 mg) in acetone (200 μL) cooled to 0 °C was treated with a solution of 2.5 M Jones' reagent (12 μL). The reaction mixture was stirred at 0 °C for 1 h, upon which time complete conversion was noted by TLC. Isopropanol (100 μL) was added dropwise to quench the reaction. Concentration under a stream of nitrogen provided a residue that was redissolved in a mixture of H_2O and EtOAc. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting residue was purified by PTLC (SiO_2, 50% EtOAc–hexanes containing 1–2% AcOH). The carboxylic acid **18** was isolated as a white amorphous solid (9 mg, 88%): $[\alpha]^{23}_D$ –7.5 (*c* 0.53, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 7.49 (s, 1H), 7.28–7.19 (m, 15H), 4.99–4.88 (m, 1H), 4.32–4.21 (m, 1H), 3.88–3.79 (m, 1H), 3.61–3.54 (m, 1H), 3.37–3.25 (m, 3H), 3.06–2.87 (m, 2H), 2.23 (s, 3H), 1.58 (s, 9H), 1.47 (s, 9H), 1.38 (s, 9H), 1.26 (bs, 9H); IR (film) ν_{max} 2962, 2925, 2852, 1692, 1561, 1492, 1452, 1393, 1367, 1317, 1258, 1156, 1096, 1021, 844, 799, 700 cm^{-1}; HRESI-TOF *m/z* 925.4687 ($C_{50}H_{64}N_6O_{11}$ + H^+ requires 925.4706).



***tert*-Butyl-2-((5*S*,8*S*)-6-(*tert*-Butyloxycarbonyl)-8-carbamoyl-12,12-dimethyl-3,10-dioxo-1,1,1-triphenyl-11-oxa-2,6,9-triazatridecan-5-yl)-6-((*tert*-butyloxycarbonyl)amino)-5-methylpyrimidine-4-carboxylate (19)**. A solution of **18** (0.005 mmol, 4.6 mg) and HOBt (0.006 mmol, 0.8 mg) in anhydrous CH_2Cl_2 (125 μL) at 0 °C was treated with EDCI (0.006 mmol, 1.2 mg). Upon complete addition of EDCI, the reaction mixture was warmed to 25 °C and stirred for 30 min, before being re-cooled to 0 °C and the addition of 0.5 M NH_3 in THF (40 μL). Precipitation formed as a consequence of the addition. The reaction mixture was warmed to 25 °C and stirred for 30 min, upon which time complete conversion was noted by TLC. The reaction mixture was loaded directly onto a PTLC plate (SiO_2) and purified using 3% MeOH–CH_2Cl_2 as the eluent. Compound **19** was isolated as a white film (4.4 mg, 95%): $[\alpha]^{23}_D$ –8.9 (*c* 0.35, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 7.45 (s, 1H), 7.31–7.19 (m, 15H), 4.96–4.86 (m, 1H), 4.24 (q, *J* = 6.8 Hz, 1H), 3.88–3.80 (m, 1H), 3.59 (dd, *J* = 7.2, 15.3 Hz, 1H), 3.38–3.26 (m, 1H), 3.00–2.77 (m, 1H), 2.24 (s, 3H), 1.58 (s, 9H), 1.47 (s, 9H), 1.39 (s, 9H), 1.36–1.16 (m, 9H); IR (film) ν_{max} 2976, 2925, 2854, 1728, 1689, 1558, 1492, 1452, 1412, 1393, 1368, 1247, 1158, 700 cm^{-1}; HRESI-TOF *m/z* 924.4862 ($C_{50}H_{65}N_7O_{10}$ + H^+ requires 924.4865).



6-Amino-2-((*S*)-3-amino-1-(((*S*)-2,3-diamino-3-oxopropyl)amino)-3-oxopropyl)-5-methylpyrimidine-4-carboxylic Acid ((–)-Pyrimidoblamic Acid, 2). Compound **19** (0.006 mmol, 6 mg) was dissolved in TFA:CH_2Cl_2 (500 μL, 3:2). This reaction mixture was stirred at room temperature and monitored by LC/MS. After 16 h, complete conversion to the desired product was observed. The reaction was quenched by dropwise addition of MeOH and concentrated under a nitrogen stream. The resulting residue was purified by reverse-phase HPLC (C18, 25 x 100 mm, 8 mL/min, R_T = 9.6 min) utilizing a H_2O:CH_3CN gradient to yield the TFA salt of **2**. The isolated residue was treated with 1 N aqueous HCl to provide the desired HCl salt of (–)-pyrimidoblamic acid (**2**) as an off white amorphous solid (1.9 mg, quant.) identical in all respects with authentic material: $[\alpha]^{23}_D$ –30 (*c* 0.07, H_2O); lit[1] $[\alpha]^{25}_D$ –27 (*c* 0.12, H_2O); ^{1}H NMR (D_2O, 600 MHz) δ 4.22 (dd, *J* = 5.6, 8.0 Hz, 1H), 4.13 (dd, *J* = 4.5, 6.8 Hz, 1H), 3.19 (dd, *J* = 4.6, 13.7 Hz, 1H), 3.07 (dd, *J* = 6.8, 13.6 Hz, 1H), 2.89 (dd, *J* = 5.4, 15.8 Hz, 1H), 2.82 (dd, *J* = 7.7, 15.8 Hz, 1H), 2.23 (s, 3H); ^{13}C NMR (D_2O, 150 MHz) δ 175.6, 174.4, 172.4, 171.1, 167.0, 162.3, 111.6, 57.8, 53.2, 47.7, 39.0, 11.9; IR (neat) ν_{max} 3456, 3247, 1695, 1681, 1557, 1161, 1078, 820 cm^{-1}; HRESI-TOF *m/z* 326.1563 ($C_{12}H_{19}N_7O_4$ + H^+ requires 326.1571).

[1]Boger, D.L.; Honda, T.; Dang, Q. *J. Am. Chem. Soc.* **1994**, *116*, 5619.

^{1}H NMR Comparison

Synthetic Pyrimidoblamic Acid (600 MHz, D_2O)	***Authentic Pyrimidoblamic Acid*** [2] (400 MHz, D_2O)
4.22 (dd, *J* = 5.6, 8.0 Hz, 1H)	4.24 (dd, *J* = 7.9, 7.9 Hz, 1H)
4.13 (dd, *J* = 4.5, 6.8 Hz, 1H)	4.12 (dd, *J* = 4.5, 6.6 Hz, 1H)
3.19 (dd, *J* = 4.6, 13.7 Hz, 1H)	3.21 (dd, *J* = 4.5, 13.7 Hz, 1H)
3.07 (dd, *J* = 6.8, 13.6 Hz, 1H)	3.08 (dd, *J* = 6.6, 13.7 Hz, 1H)
2.89 (dd, *J* = 5.4, 15.8 Hz, 1H)	2.89 (dd, *J* = 5.4, 15.8 Hz, 1H)
2.82 (dd, *J* = 7.7, 15.8 Hz, 1H)	2.82 (dd, *J* = 7.4, 15.8 Hz, 1H)
2.23 (s, 3H)	2.22 (s, 3H)

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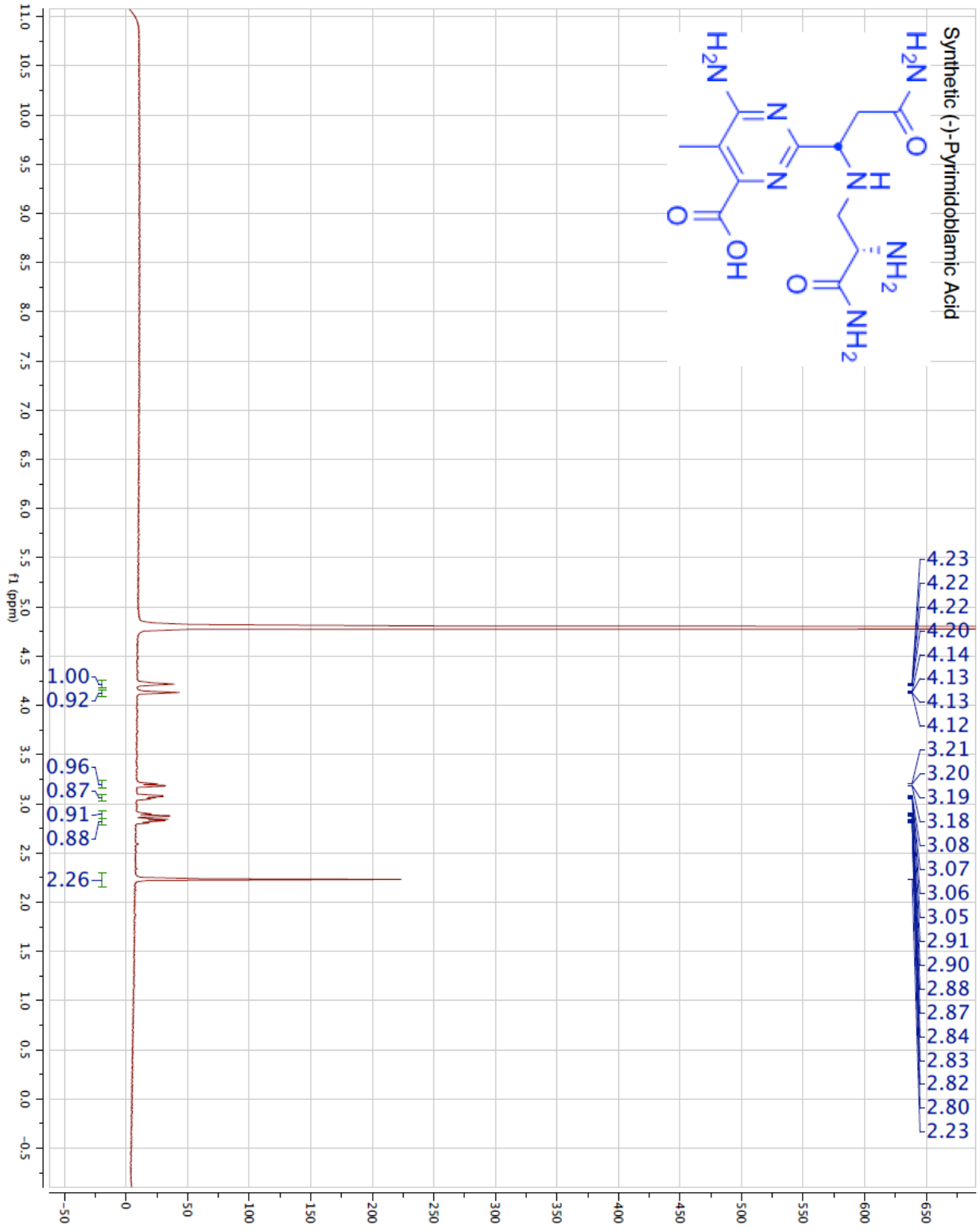
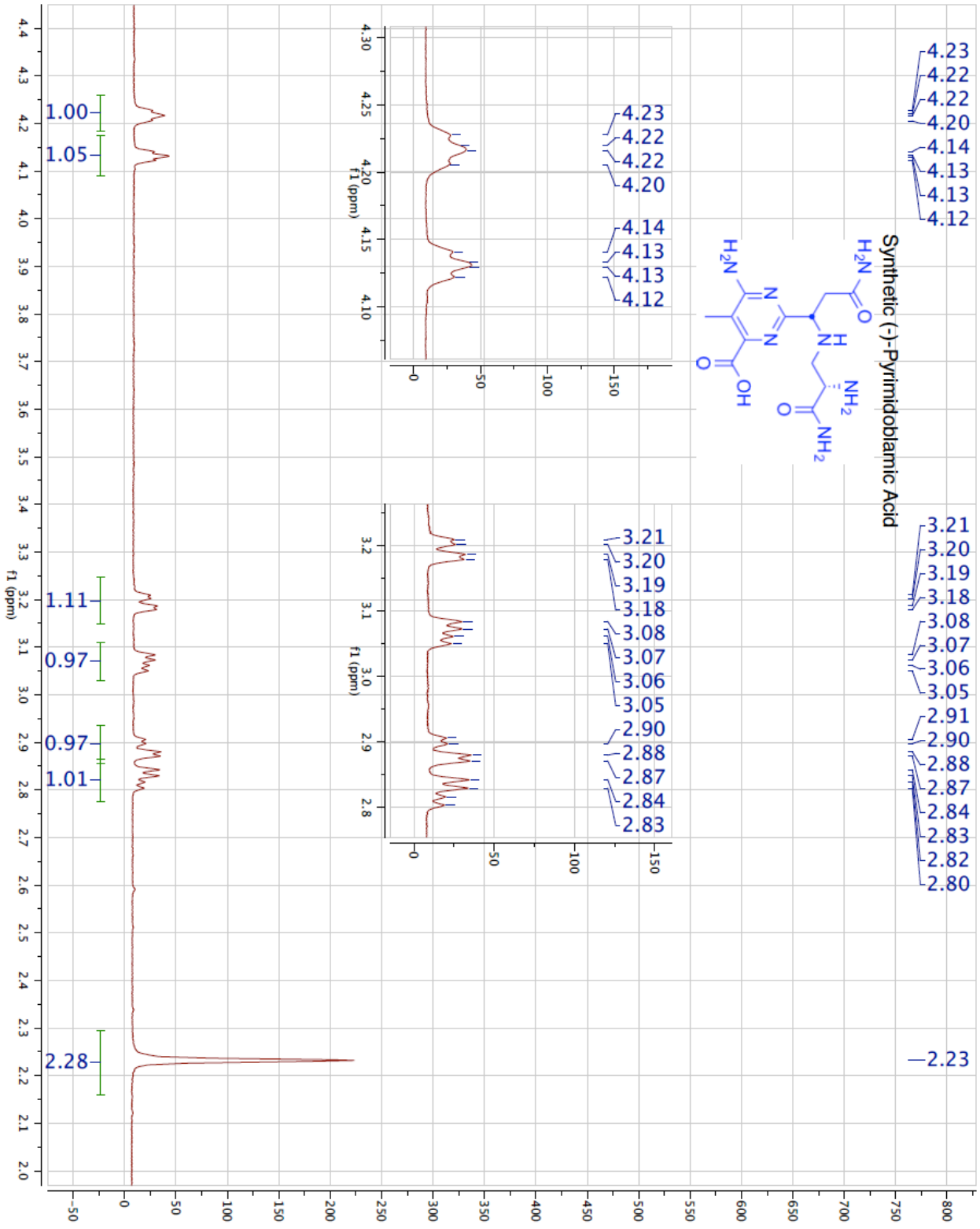
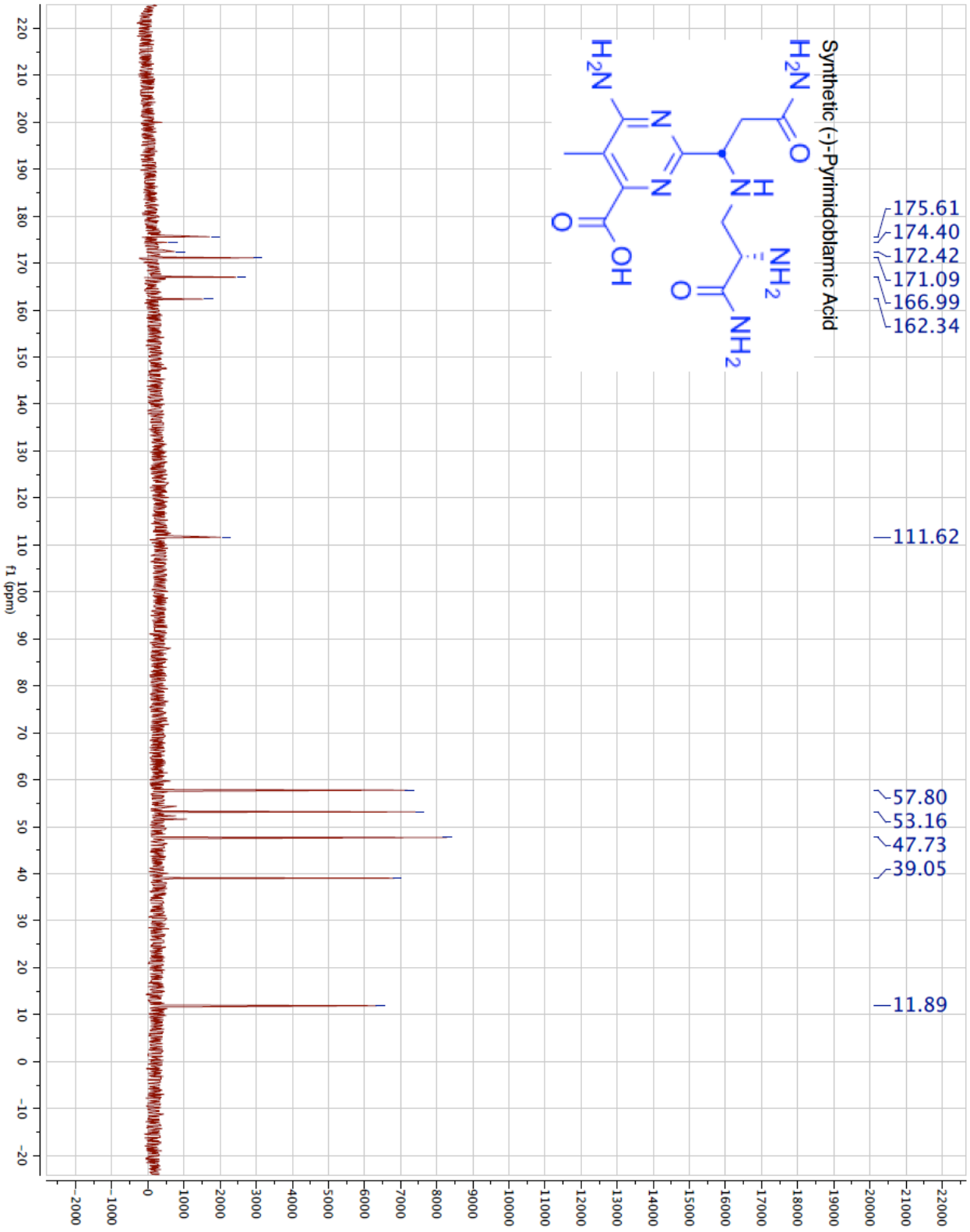
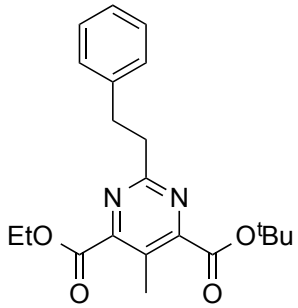
^{13}C NMR Comparison

Synthetic Pyrimidoblamic Acid (150 MHz, D_2O)	***Authentic Pyrimidoblamic Acid*** [2] (100 MHz, D_2O)
175.6	
174.4	
172.4	
171.1	
167.0	Not Reported
162.3	
111.6	
57.8	
53.2	
47.7	
39.0	
11.9	

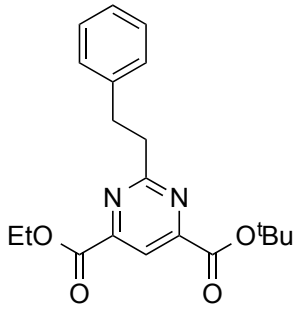

Synthetic (-)-Pyrimidoblamic Acid
f1 (ppm)
1.00
0.92
0.96
0.87
0.91
0.88
2.26
4.23
4.22
4.22
4.20
4.14
4.13
4.13
4.12
3.21
3.20
3.19
3.18
3.08
3.07
3.06
3.05
2.91
2.90
2.88
2.87
2.84
2.83
2.82
2.80
2.23


Synthetic (-)-Pyrimidoblamic Acid
4.23
4.22
4.22
4.20
4.14
4.13
4.13
4.12
3.21
3.20
3.19
3.18
3.08
3.07
3.06
3.05
2.91
2.90
2.88
2.87
2.84
2.83
2.82
2.80
2.23
1.00
1.05
1.11
0.97
0.97
1.01
2.28
f1 (ppm)

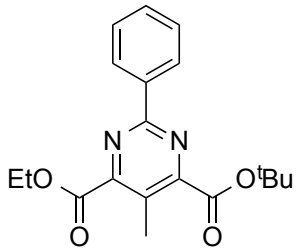

Synthetic (-)-Pyrimidoblamic Acid
175.61
174.40
172.42
171.09
166.99
162.34
111.62
57.80
53.16
47.73
39.05
11.89
f1 (ppm)



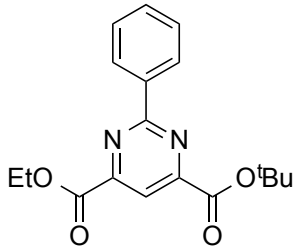
4-(*tert*-Butyl) 6-Ethyl 5-Methyl-2-phenethylpyrimidine-4,6-dicarboxylate (20). A solution of **5a** (0.075 mmol, 20 mg) in anhydrous CH_3CN (160 μL) at 25 ºC was treated with a solution of free-based 3-phenylpropanimidamide (0.037 mmol, 5.5 mg) in anhydrous CH_3CN (125 μL). The reaction mixture was stirred at 25 ºC for 16 h, upon which time the mixture was filtered through Celite and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 10% EtOAc–hexanes) to yield **20** as a clear oil (9.0 mg, 66%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.31–7.23 (m, 4H), 7.20–7.17 (m, 1H), 4.48 (q, J = 7.1 Hz, 2H), 3.36–3.29 (m, 2H), 3.19–3.12 (m, 2H), 2.44 (s, 3H), 1.64 (s, 9H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 168.1, 165.4, 164.7, 160.3, 158.1, 141.4, 128.7 (2C), 128.5 (2C), 126.1, 123.0, 84.4, 62.6, 40.4, 34.4, 28.3 (3C), 14.3, 14.2; IR (neat) ν_{max} 2977, 2927, 1736, 1556, 1453, 1406, 1370, 1253, 1224, 1163, 1135, 1045, 845, 751, 700 cm^{-1}; HRESI-TOF *m/z* 371.1963 ($C_{21}H_{26}N_2O_4$ + H^+ requires 371.1965).



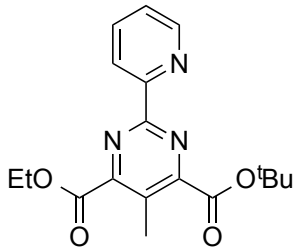
4-(*tert*-Butyl) 6-Ethyl 2-Phenethylpyrimidine-4,6-dicarboxylate (21). A solution of **5b** (0.079 mmol, 20 mg) in anhydrous CH_3CN (170 μL) at 25 ºC was treated with a solution of free-based 3-phenylpropanimidamide (0.04 mmol, 6.0 mg) in anhydrous CH_3CN (135 μL). The reaction mixture was stirred at 25 ºC for 4 h, upon which time the mixture was filtered through Celite and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 20% EtOAc–hexanes) to yield **21** as a clear oil (12.7 mg, 89%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.30 (s, 1H), 7.32–7.23 (m, 4H), 7.21–7.16 (m, 1H), 4.52 (q, J = 7.1 Hz, 2H), 3.54–3.45 (m, 2H), 3.27–3.17 (m, 2H), 1.65 (s, 9H), 1.46 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.3, 164.1, 162.6, 158.8, 157.5, 141.2, 128.7 (2C), 128.5 (2C), 126.2, 117.5, 84.1, 63.0, 41.0, 34.4, 28.1 (3C), 14.4; IR (neat) ν_{max} 2980, 2931, 1746, 1727, 1555, 1386, 1454, 1386, 1370, 1276, 1256, 1202, 1157, 1103, 1025, 844, 756, 701 cm^{-1}; HRESI-TOF *m/z* 357.1816 ($C_{20}H_{24}N_2O_4$ + H^+ requires 357.1809).



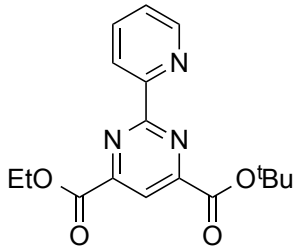
4-(*tert*-Butyl) 6-Ethyl 5-Methyl-2-phenylpyrimidine-4,6-dicarboxylate (22). A solution of **5a** (0.094 mmol, 25 mg) in anhydrous CH_3CN (200 µL) at 25 ºC was treated with a solution of free-based benzamidine (0.062 mmol, 7.5 mg) in anhydrous CH_3CN (200 µL). The reaction mixture was stirred at 60 ºC for 16 h, upon which time the mixture was cooled to 25 ºC, filtered through Celite, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 10% EtOAc–hexanes) to yield **22** as a clear oil (13.2 mg, 62%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.54–8.39 (m, 2H), 7.54–7.39 (m, 3H), 4.50 (q, J = 7.1 Hz, 2H), 2.49 (s, 3H), 1.66 (s, 9H), 1.46 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.5, 164.8, 162.4, 160.5, 158.4, 136.4, 131.2, 128.64 (2C), 128.59 (2C), 123.5, 84.3, 62.5, 28.3 (3C), 14.4, 14.3; IR (neat) ν_{max} 2981, 2930, 1736, 1555, 1454, 1397, 1373, 1292, 1254, 1161, 1134, 1095, 1044, 846, 748, 721, 695 cm^{-1}; HRESI-TOF *m/z* 343.1658 ($C_{19}H_{22}N_2O_4 + H^+$ requires 343.1652).



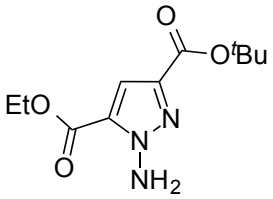
4-(*tert*-Butyl) 6-Ethyl 2-Phenylpyrimidine-4,6-dicarboxylate (23). A solution of **5b** (0.090 mmol, 22.7 mg) in anhydrous CH_3CN (200 µL) at 25 ºC was treated with a solution of free-based benzamidine (0.066 mmol, 8.0 mg) in anhydrous CH_3CN (200 µL). The reaction mixture was stirred at 60 ºC for 16 h, upon which time the mixture was cooled to 25 ºC, filtered through Celite, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 15% EtOAc–hexanes) to yield **23** as a clear oil (12.7 mg, 49%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.61–8.59 (m, 2H), 8.35 (s, 1H), 7.55–7.50 (m, 3H), 4.54 (q, J = 7.1 Hz, 2H), 1.68 (s, 9H), 1.49 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.0, 164.3, 162.8, 159.0, 157.8, 136.4, 131.8, 129.0 (2C), 128.8 (2C), 117.6, 84.0, 62.9, 28.2 (3C), 14.4; IR (neat) ν_{max} 2981, 2930, 1746, 1727, 1555, 1458, 1377, 1279, 1256, 1216, 1187, 159, 1104, 1020, 845, 737, 694 cm^{-1}; HRESI-TOF *m/z* 329.1497 ($C_{18}H_{20}N_2O_4 + H^+$ requires 329.1496).



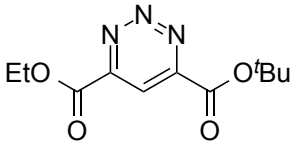
4-(*tert*-Butyl)-6-Ethyl-5-Methyl-2-(pyridin-2-yl)pyrimidine-4,6-dicarboxylate (24). A solution of **5a** (0.094 mmol, 25 mg) in anhydrous CH_3CN (200 μL) at 25 ºC was treated with a solution of free-based picolinimidamide (0.062 mmol, 7.5 mg) in anhydrous CH_3CN (200 μL). The reaction mixture was stirred at 60 ºC for 16 h, upon which time the mixture was cooled to 25 ºC, filtered through Celite, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 50% EtOAc–hexanes) to yield **24** as a clear oil (16.3 mg, 77%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.84 (ddd, *J* = 0.9, 1.7, 5.0 Hz, 1H), 8.53 (dt, *J* = 1.0, 8.0 Hz, 1H), 7.85 (td, *J* = 1.8, 7.8 Hz, 1H), 7.40 (ddd, *J* = 1.2, 4.8, 7.6 Hz, 1H), 4.50 (q, *J* = 7.2 Hz, 2H), 2.53 (s, 3H), 1.66 (s, 9H), 1.44 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.1, 164.3, 161.1, 160.8, 159.1, 153.6, 150.2, 137.3, 125.3, 124.3, 84.6, 62.7, 53.6, 28.3 (3C), 14.4, 14.3; IR (neat) ν_{max} 2980, 2930, 1732, 1553, 1443, 1395, 1373, 1289, 1257, 1161, 1138, 1091, 1045, 844, 751 cm^{-1}; HRESI-TOF *m/z* 344.1616 ($C_{18}H_{21}N_3O_4$ + H^+ requires 344.1605).



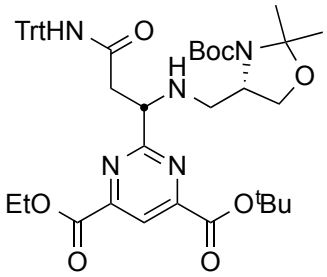
4-(*tert*-Butyl) 6-Ethyl 2-(Pyridin-2-yl)pyrimidine-4,6-dicarboxylate (25). A solution of **5b** (0.099 mmol, 25 mg) in anhydrous CH_3CN (200 μL) at 25 ºC was treated with a solution of free-based picolinimidamide (0.066 mmol, 8.0 mg) in anhydrous CH_3CN (200 μL). The reaction mixture was stirred at 60 ºC for 16 h, upon which time the mixture was cooled to 25 ºC, filtered through Celite, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 45% EtOAc–hexanes) to yield **25** as a clear oil (13.6 mg, 63%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.90–8.84 (m, 1H), 8.63 (d, *J* = 7.9 Hz, 1H), 8.48 (s, 1H), 7.88 (tt, *J* = 5.2, 10.7 Hz, 1H), 7.47–7.41 (m, 1H), 4.55 (q, *J* = 7.2 Hz, 2H), 1.68 (s, 9H), 1.48 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.0, 164.1, 162.4, 159.4, 158.5, 153.6, 150.5, 137.2, 125.7, 124.7, 119.1, 84.3, 63.2, 28.1 (3C), 14.3; IR (neat) ν_{max} 2981, 2932, 1729, 1555, 1446, 1377, 1258, 1224, 1191, 1159, 1108, 1018, 842, 753, 676 cm^{-1}; HRESI-TOF *m/z* 330.1454 ($C_{17}H_{19}N_3O_4$ + H^+ requires 330.1448).



3-(*tert*-Butyl) 5-Ethyl 1-Amino-1*H*-pyrazole-3,5-dicarboxylate (9b). Following the N-amination procedure described in the formation of **9a**, compound **9b** was generated from **8b** (4.6 mmol, 1.1 g). The residue was purified by flash chromatography (SiO_2, 20% EtOAc–hexanes) to yield **9b** as a mixture of regioisomers as a white amorphous solid (0.84 g, 72%). Isomer A: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.16 (s, 1H), 6.08 (bs, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.56 (s, 9H), 1.37 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 161.6, 159.3, 137.3, 128.6, 111.9, 83.9, 61.3, 28.3 (3C), 14.5. Isomer B: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.25 (s, 1H), 6.08 (bs, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.57 (s, 9H), 1.37 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 160.6, 160.1, 139.0, 127.6, 111.7, 82.1, 61.9, 28.3 (3C), 14.3; IR for mixture (film) ν_{max} 3334, 3243, 2979, 2935, 1708, 1588, 1528, 1448, 1392, 1369, 1283, 1221, 1158, 1104, 1026, 845, 764, 721 cm^{-1}; HRESI-TOF *m/z* 256.1285 ($C_{11}H_{17}N_3O_4$ + H^+ requires 256.1292).



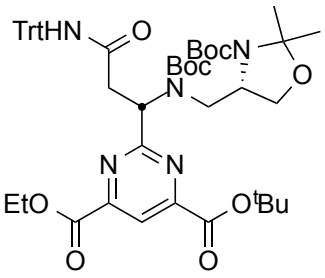
4-(*tert*-Butyl) 6-Ethyl 1,2,3-Triazine-4,6-dicarboxylate (5b). Compound **5b** was prepared from **9b** (0.78 mmol, 0.20 g) following the procedure used to prepare **5a**. The resulting residue was purified by flash chromatography (SiO_2, 15% EtOAc–hexanes) to yield **5b** as a yellow oil (135 mg, 68%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, 1H), 4.60 (q, *J* = 7.1 Hz, 2H), 1.69 (s, 9H), 1.50 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5, 161.1, 152.1, 151.0, 117.2, 85.8, 63.9, 28.1 (3C), 14.3; IR (film) ν_{max} 2981, 2937, 1729, 1573, 1459, 1370, 1333, 1258, 1160, 1096, 1018, 955, 840, 799, 764, 721 cm^{-1}; HRESI-TOF *m/z* 254.1135 ($C_{11}H_{15}N_3O_4$ + H^+ requires 254.1135).



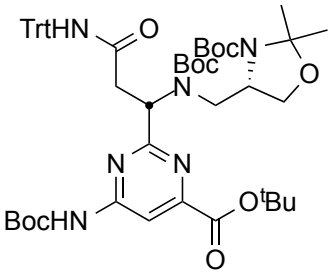
4-(*tert*-Butyl) 6-Ethyl 2-((*S*)-1-((((*S*)-3-(*tert*-Butyloxycarbonyl)-2,2-dimethyloxazolidin-4-yl)methyl)amino)-3-oxo-3-(tritylamino)propyl)pyrimidine-4,6-dicarboxylate (26). A solution of **13** (0.166 mmol, 100 mg) in MeOH (6 mL) was treated with glacial AcOH (0.34 mmol, 20 μL) followed by a spatula tip of Raney® 2800 Nickel as an aqueous cake. This suspension was stirred at 25 ºC until complete consumption of the amidoxime was evident by TLC. The reaction was filtered through a Celite plug and concentrated in vacuo without

allowing the temperature of the sample to increase above 30 ºC. The residue was redissolved in CH_2Cl_2 and concentrated two additional times to remove residual MeOH. The resulting residue was treated with CH_2Cl_2 (4 mL) followed by 1 N aqueous NaOH (4 mL). This mixture was shaken briefly before the organic layer was extracted, dried over Na_2SO_4, and concentrated in vacuo, without allowing the temperature of the sample to increase above 30 ºC, to yield the free-based amidine **4** (82 mg, 84 %). The resulting residue was then briefly dried under high vacuum and used within 1 h.

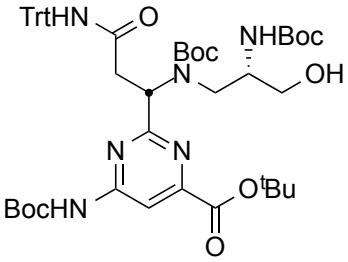
Inverse electron demand Diels–Alder reaction: A solution of powdered 4 Å molecular sieves and 1,2,3-triazine **5b** (0.136 mmol, 35 mg) in anhydrous CH_3CN (280 µL) at 25 °C was treated with a solution of crude amidine **4** (0.068 mmol, 40 mg) in anhydrous CH_3CN (175 µL) dropwise. This mixture was stirred at 25 °C for 12 h at which time the reaction mixture was filtered through Celite and concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2 and purified by flash chromatography (SiO_2) using a gradient elution of 30% EtOAc–hexanes to elute unreacted 1,2,3-triazine **5b** and then 60% EtOAc–hexanes to yield **26** (41 mg, 76%): $[\alpha]^{23}_D$ +0.40 (*c* 1.00, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.71 (s, 1H), 8.23 (s, 1H), 7.35–7.07 (m, 15H), 4.45 (q, *J* = 7.1 Hz, 2H), 4.32 (dd, *J* = 4.9, 7.6 Hz, 1H), 3.80–3.65 (m, 3H), 2.81 (dd, *J* = 7.7, 15.4 Hz, 2H), 2.74 (dd, *J* = 5.0, 15.4 Hz, 1H), 2.65 (dd, *J* = 8.6, 11.6 Hz, 1H), 1.62 (s, 9H), 1.44 (s, 3H), 1.39 (m, 15H); IR (film) ν_{max} 2979, 2930, 1689, 1551, 1492, 1391, 1257, 1155, 1086, 1021, 843, 803, 738, 700 cm^{-1}; HRESI-TOF *m/z* 794.4115 ($C_{45}H_{55}N_5O_8$ + H^+, requires 794.4123).



4-(*tert*-Butyl) 6-Ethyl 2-((*S*)-1-((*tert*-Butyloxycarbonyl)(((*S*)-3-(*tert*-butyloxycarbonyl)-2,2-dimethyloxazolidin-4-yl)methyl)amino)-3-oxo-3-(tritylamino)propyl)pyrimidine-4,6-dicarboxylate (27). A solution of **26** (0.026 mmol, 21 mg) in anhydrous THF was treated with Et_3N (0.104 mmol, 10.5 mg) followed by Boc_2O (0.091 mmol, 20 mg) at 25 °C. The reaction mixture was warmed at 50 °C and stirred for 12 h, upon which complete conversion was noted by TLC. The reaction mixture was concentrated, redissolved in $CHCl_3$ and purified by PTLC (SiO_2, 30% EtOAc–hexanes). Product **27** was isolated as a white film (20 mg, 87%): $[\alpha]^{23}_D$ –25 (*c* 1.00, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.23 (s, 1H), 7.33–7.18 (m, 15H), 5.25–4.96 (m, 1H), 4.51–4.34 (m, 2H), 4.23 (bs, 1H), 4.18 (d, *J* = 9.1 Hz, 1H), 4.00 (bs, 1H), 3.79 (bs, 1H), 3.60 (bs, 1H), 3.11 (m, 2H), 1.60 (s, 9H), 1.55 (s, 3H), 1.45 (s, 3H), 1.45–1.15 (m, 18H), 1.38 (t, *J* = 7.1 Hz, 3H); IR (film) ν_{max} 2979, 1687, 1556, 1490, 1423, 1392, 1257, 1159, 1081, 1021, 847, 753, 700 cm^{-1}; HRESI-TOF *m/z* 894.4644 ($C_{50}H_{63}N_5O_{10}$ + H^+ requires 894.4647).

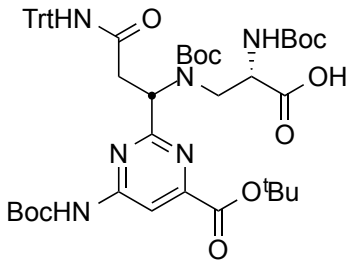


***tert*-Butyl-(*S*)-4-(((*tert*-Butyloxycarbonyl)((*S*)-1-(4-(*tert*-butyloxycarbonyl)-6-((*tert*-butyloxycarbonyl)amino)pyrimidin-2-yl)-3-oxo-3-(tritylamino)propyl)amino)methyl)-2,2-dimethyloxazolidine-3-carboxylate (28)**. A solution of **27** (0.017 mmol, 15 mg) in THF:^{t}BuOH (400 μL, 3:1) was treated with 1 N aqueous NaOH (17 μL) dropwise at 25 °C. After 12 h, complete conversion was observed by TLC. The reaction was quenched with the addition of 1 N aqueous HCl to a pH < 4 and the product was extracted with CH_2Cl_2. The organic layer was concentrated and lyophilized. The residue was redissolved in anhydrous ^{t}BuOH (500 μL), to which Et_3N (0.025 mmol, 3.6 μL) and DPPA (0.023 mmol, 5.0 μL) were added at 25 °C. The reaction mixture was warmed at reflux and monitored by TLC. After 12 h, complete conversion was noted and the reaction mixture was concentrated. The residue was redissolved in CH_2Cl_2 and purified by flash chromatography (SiO_2, 30% EtOAc–hexanes) to provide **28** as a white amorphous solid (9.8 mg, 82% for 2-steps): $[\alpha]^{23}_D$ –19 (*c* 0.83, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.18 (s, 1H), 7.99 (s, 1H), 7.38–7.13 (m, 16H), 5.16–4.68 (m, 1H), 4.30–4.06 (m, 2H), 3.98–3.85 (m, 1H), 3.80–3.66 (m, 1H), 3.55–3.44 (m, 1H), 3.11–2.97 (m, 2H), 1.57 (s, 9H), 1.55 (s, 9H), 1.48–1.09 (m, 24H); IR (film) ν_{max} 2978, 2932, 1740, 1693, 1566, 1493, 1391, 1368, 1326, 1244, 1159, 1102, 1078, 848, 768, 701 cm^{-1}; HRESI-TOF *m/z* 937.5061 ($C_{52}H_{68}N_6O_{10}$ + H^+ requires 937.5069).

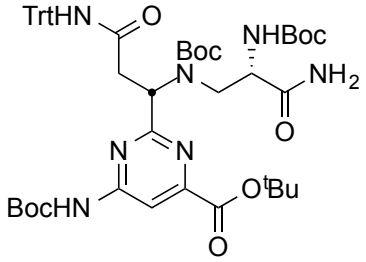


***tert*-Butyl 2-((5*S*,8*S*)-6-(*tert*-Butoxycarbonyl)-8-(hydroxymethyl)-12,12-dimethyl-3,10-dioxo-1,1,1-triphenyl-11-oxa-2,6,9-triazatridecan-5-yl)-6-((*tert*-butoxycarbonyl)amino)pyrimidine-4-carboxylate (29)**. Compound **28** (0.0096 mmol, 9.0 mg) was dissolved in MeOH (200 μL) and *p*-TsOH monohydrate (0.0048 mmol, 1.0 mg) was added in one portion at 25 °C. The reaction mixture was stirred at 25 °C for 3 h, upon which time complete conversion was noted by TLC. The reaction was quenched with the addition saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic phases were combined, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by PTLC (SiO_2, 40% EtOAc–hexanes) to yield **29** as a film (7.3 mg, 85%): $[\alpha]^{23}_D$ –36 (*c* 0.64, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.22 (s, 1H), 7.42 (s, 1H), 7.29–7.21 (m, 15H), 5.64 (bs, 1H), 4.95–4.86 (m, 1H), 3.93–3.82 (m, 1H), 3.68–3.49 (m, 4H), 3.21–3.10 (m, 1H), 2.99–2.83 (m, 1H), 1.56 (s, 9H), 1.55 (s, 9H), 1.40 (s, 9H), 1.35–1.08 (m, 9H); IR (film)

ν_{max} 2978, 2930, 1739, 1691, 1569, 1496, 1456, 1394, 1368, 1327, 1248, 1159, 774, 700 cm^{-1}; HRESI-TOF *m/z* 897.4751 ($C_{49}H_{64}N_6O_{10}$ + H^+ requires 897.4756).

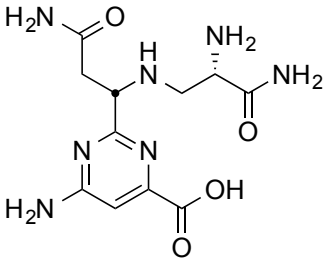


(*S*)-3-((*tert*-Butyloxycarbonyl)((*S*)-1-(4-(*tert*-butyloxycarbonyl)-6-((*tert*-butyloxycarbonyl)-amino)pyrimidin-2-yl)-3-oxo-3-(tritylamino)propyl)amino)-2-((*tert*-butyloxycarbonyl)-amino)propanoic Acid (30). A solution of **29** (0.0055 mmol, 5.0 mg) in acetone (200 μL) cooled to 0 °C was treated with a solution of 2.5 M Jones' reagent (6.5 μL). The reaction mixture was stirred at 0 °C for 1 h, upon which time complete conversion was noted by TLC. Isopropanol (100 μL) was added dropwise to quench the reaction. Concentration under a stream of nitrogen provided a residue that was redissolved in a mixture of H_2O and EtOAc. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting residue was purified by PTLC (SiO_2, 50% EtOAc–hexanes containing 1–2% AcOH). The carboxylic acid **30** was isolated as a white amorphous solid (4.9 mg, 98%): $[\alpha]^{23}_D$ –15 (*c* 0.49, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.24 (s, 1H), 7.49 (s, 1H), 7.30–7.20 (m, 15H), 5.02–4.84 (m, 1H), 4.44–4.34 (m, 1H), 3.90–3.79 (m, 1H), 3.60–3.47 (m, 1H), 3.47–3.28 (m, 1H), 3.06–2.89 (m, 1H), 1.57 (s, 9H), 1.55 (s, 9H), 1.39 (s, 9H), 1.35–1.13 (m, 9H); IR (film) ν_{max} 2974, 2928, 2855, 1737, 1692, 1568, 1511, 1493, 1451, 1393, 1367, 1245, 1153, 1097, 1024, 994, 841, 799, 767, 735, 699 cm^{-1}; HRESI-TOF *m/z* 911.4549 ($C_{49}H_{62}N_6O_{11}$ + H^+ requires 911.4549).

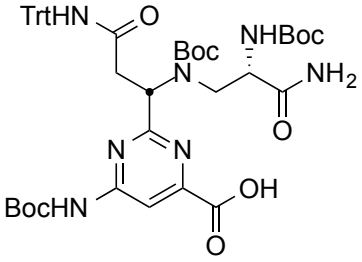


***tert*-Butyl-2-((5*S*,8*S*)-6-(*tert*-Butyloxycarbonyl)-8-carbamoyl-12,12-dimethyl-3,10-dioxo-1,1,1-triphenyl-11-oxa-2,6,9-triazatridecan-5-yl)-6-((*tert*-butyloxycarbonyl)amino)-pyrimidine-4-carboxylate (31)**. A solution of **30** (0.0065 mmol, 6.0 mg) and HOBt (0.0078 mmol, 1.1 mg) in anhydrous CH_2Cl_2 (175 μL) at 0 °C was treated with EDCI (0.0078 mmol, 1.5 mg). Upon complete addition of EDCI, the reaction mixture was warmed to 25 °C and stirred for 30 min, before being re-cooled to 0 °C and addition of 0.5 M NH_3 in THF (52 μL). Precipitation formed as a consequence of the addition. The reaction mixture was warmed to 25 °C and stirred for 30 min, upon which time complete conversion was noted by TLC. The reaction mixture was loaded directly onto a PTLC plate (SiO_2) and purified using 5% MeOH–CH_2Cl_2 as the eluent. Compound **31** was isolated as a white film (5.5 mg, 93%): $[\alpha]^{23}_D$ –20 (*c* 0.37, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.25 (s, 1H), 7.41 (s, 1H), 7.32–7.20 (m,

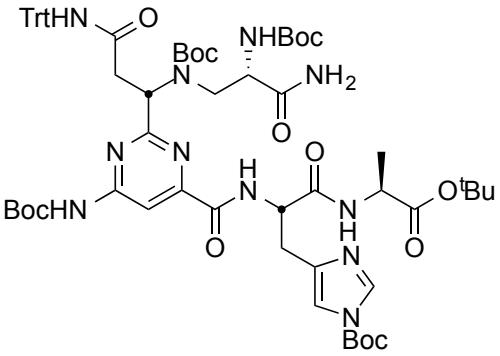
15H), 5.00–4.80 (m, 1H), 4.38–4.29 (m, 1H), 3.90–3.79 (m, 1H), 3.62–3.49 (m, 1H), 3.44–3.25 (m, 1H), 3.04–2.82 (m, 1H), 1.58 (s, 9H), 1.55 (s, 9H), 1.41 (s, 9H), 1.37–1.09 (m, 9H); IR (film) ν_{max} 2963, 2927, 2855, 1738, 1663, 1567, 1493, 1455, 1393, 1366, 1258, 1151, 1090, 1019, 868, 798, 769, 736, 699 cm^{-1}; HRESI-TOF *m/z* 910.4704 ($C_{49}H_{63}N_7O_{10}$ + H^+ requires 910.4709).



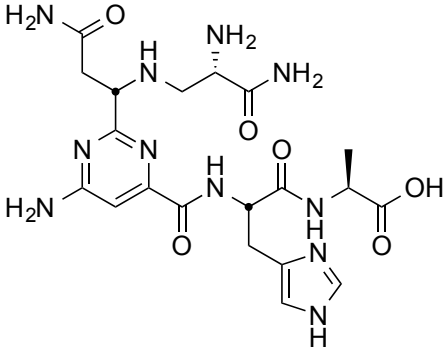
6-Amino-2-((*S*)-3-Amino-1-(((*S*)-2,3-diamino-3-oxopropyl)amino)-3-oxopropyl)pyrimidine-4-carboxylic Acid (32). Compound **31** (0.005 mmol, 4.5 mg) was dissolved in TFA:CH_2Cl_2 (400 µL, 3:1). This reaction mixture was stirred at room temperature and monitored by LC/MS. After 16 h, complete conversion to the desired product was observed. The reaction was quenched by dropwise addition of MeOH and concentrated under a nitrogen stream. The resulting residue was redissolved in H_2O and flushed through a pipette column (C18, 100% H_2O) to yield the TFA salt of **32**. The isolated residue was treated with 1 N aqueous HCl to provide the desired HCl salt of **32** as a white amorphous solid (1.3 mg, 87%): $[\alpha]^{23}_D$ –28 (*c* 0.16, H_2O); lit[2] $[\alpha]^{25}_D$ –23 (*c* 0.065, H_2O); ^{1}H NMR (CD_3CN, 600 MHz) δ 7.09 (s, 1H), 4.35 (dd, *J* = 5.3, 7.7 Hz, 1H), 4.19 (dd, *J* = 4.9, 6.6 Hz, 1H), 3.27 (dd, *J* = 4.8, 13.7 Hz, 1H), 3.19–3.15 (m, 1H), 2.94 (dd, *J* = 5.4, 16.0 Hz, 1H), 2.86 (dd, *J* = 7.7, 16.0 Hz, 1H); IR (neat) ν_{max} 3442, 3236, 1700, 1678, 1498, 1152, 1062, 819 cm^{-1}; HRESI-TOF *m/z* 312.1415 ($C_{11}H_{17}N_7O_4$ + H^+ requires 312.1415).



2-((5*S*,8*S*)-6-(*tert*-Butyloxycarbonyl)-8-carbamoyl-12,12-dimethyl-3,10-dioxo-1,1,1-triphenyl-11-oxa-2,6,9-triazatridecan-5-yl)-6-((*tert*-butyloxycarbonyl)amino)pyrimidine-4-carboxylic Acid (33). A solution of **31** (0.0087 mmol, 8.0 mg) in THF:MeOH (220 µL, 3:1) was treated with 1 N aqueous NaOH (17.4 µL) at 25 ºC. After 4 h, complete conversion was noted by TLC. The reaction was quenched with addition of 1 N aqueous HCl and the product was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to provide pure **33** as a white amorphous solid (7.2 mg, 97%): $[\alpha]^{23}_D$ –31 (*c* 0.26, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 8.36 (bs, 1H), 7.46 (bs, 1H), 7.30–7.22 (m, 15H), 5.66 (bs, 1H), 4.99 (bs, 1H), 4.33 (bs, 1H), 3.84–3.80 (m, 1H), 3.52 (bs, 1H), 2.98 (bs, 1H), 1.55 (s, 9H), 1.42 (s, 9H), 1.30 (bs, 9H); IR (film) ν_{max} 2971, 2927, 1740, 1686, 1570, 1507, 1494, 1448, 1420, 1367, 1329, 1232, 1156, 701 cm^{-1}; HRESI-TOF *m/z* 854.4082 ($C_{45}H_{55}N_7O_{10}$ + H^+ requires 854.4083).



***tert*-Butyl-4-((*S*)-3-(((*S*)-1-(*tert*-Butyloxy)-1-oxopropan-2-yl)amino)-2-(2-((5*S*,8*S*)-6-(*tert*-butoxycarbonyl)-8-carbamoyl-12,12-dimethyl-3,10-dioxo-1,1,1-triphenyl-11-oxa-2,6,9-triazatridecan-5-yl)-6-((*tert*-butyloxycarbonyl)amino)pyrimidine-4-carboxamido)-3-oxopropyl)-1*H*-imidazole-1-carboxylate (34)**. A solution of **33** (0.004 mmol, 3.5 mg) in DMF (75 μL) was treated with a solution of $N^{(Im)}$-Boc-L-His-L-Ala-O^{t}Bu (0.008 mmol, 3.0 mg) in DMF (25 μL), HOBt monohydrate (0.0044 mmol, 0.6 mg), and EDCI (0.0044 mmol, 0.8 mg) at room temperature. The mixture was stirred for 20 h and then concentrated under a nitrogen stream. The resulting residue was redissolved in CH_2Cl_2 and rinsed with 1 N aqueous HCl. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by PTLC (SiO_2, 3% MeOH–CH_2Cl_2). Compound **34** was isolated as a white amorphous solid (4.0 mg, 89%): $[\alpha]^{23}_D$ –11 (*c* 0.27, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, 60 °C) δ 9.65 (s, 1H), 8.57 (s, 1H), 8.41 (s, 1H), 7.47–7.36 (m, 15H), 5.81 (bs, 1H), 5.56 (t, *J* = 4.6 Hz, 1H), 4.84–4.80 (m, 1H), 4.48–4.42 (m, 1H), 4.25–4.15 (m, 1H), 4.06–3.93 (m, 1H), 3.83–3.63 (m, 1H), 3.17–3.05 (m, 2H), 2.53–2.40 (m, 1H), 1.79 (s, 9H), 1.74 (s, 9H), 1.59 (s, 9H), 1.49 (bs, 9H), 1.34 (d, *J* = 7.1 Hz, 3H); IR (film) ν_{max} 2927, 1691, 1508, 1394, 1251, 1154, 717, 686, 672 cm^{-1}; HRESI-TOF *m/z* 1218.6191 ($C_{63}H_{83}N_{11}O_{14}$ + H^+ requires 1218.6193).



(6-Amino-2-((*S*)-3-amino-1-(((*S*)-2,3-diamino-3-oxopropyl)amino)-3-oxopropyl)pyrimidine-4-carbonyl)-L-histidyl-L-alanine (P-3A, 3). Compound **34** (0.003 mmol, 3.5 mg) was treated with 1.2 mL TFA:CH_2Cl_2 (3:1) and stirred at room temperature. After 5 h, the reaction mixture was concentrated under a nitrogen stream. The residue was redissolved in H_2O and passed through a C18 pipette column. The fractions containing product were lyophilized to provide the TFA salt of **3**. The residue was redissolved in 1 N aqueous HCl and lyophilized to provide **3** as the HCl salt (2.0 mg, quant.): ^{1}H NMR (D_2O, 600 MHz) δ 8.61 (s, 1H), 7.33 (s, 1H), 7.08 (s, 1H), 4.90 (t, *J* = 7.3 Hz, 1H), 4.67 (t, *J* = 6.1 Hz, 1H), 4.49 (t, *J* = 5.4 Hz, 1H), 4.39 (q, *J* = 7.3

[2]Boger, D.L.; Honda, T.; Menezes, R.F.; Colletti, S.L.; Dang, Q.; Yang, W. *J. Am. Chem. Soc.* **1994**, *116*, 82.

Hz, 1H), 3.71 (dd, *J* = 5.2, 14.0 Hz, 1H), 3.61 (dd, *J* = 5.7, 14.0 Hz, 1H), 3.41 (dd, *J* = 7.1, 15.4 Hz, 1H), 3.32 (dd *J* = 7.6, 15.3 Hz, 1H), 3.14 (dd, *J* = 5.3, 16.7 Hz, 1H), 3.08 (dd, *J* = 7.0, 16.6 Hz, 1H), 1.40 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (D_2O, 125 MHz) δ 178.6, 176.2, 173.4, 170.8, 167.5, 166.8, 164.5, 155.6, 136.0, 130.4, 120.0, 106.3, 62.1, 55.0, 52.6, 51.3, 48.8, 38.2, 29.3, 18.5; IR (neat) ν_{max} 3307, 3188, 1684, 1654, 1634, 1559, 1538, 1517, 1457, 1414, 1359, 1265, 1162, 1098 cm^{-1}; HRESI-TOF *m/z* 520.2371 ($C_{20}H_{29}N_{11}O_6 + H^+$ requires 520.2375).

^{1}H NMR Comparison

Synthetic P-3A (600 MHz, D_2O)	***Authentic P-3A***[2] (400 MHz, D_2O)
8.61 (s, 1H)	8.60 (s, 1H)
7.33 (s, 1H)	7.32 (s, 1H)
7.08 (s, 1H)	7.07 (s, 1H)
4.90 (t, *J* = 7.3 Hz, 1H)	4.90 (t, *J* = 7.3 Hz, 1H)
4.67 (t, *J* = 6.1 Hz, 1H)	4.64 (t, *J* = 6.7 Hz, 1H)
4.49 (t, *J* = 5.4 Hz, 1H)	4.49 (t, *J* = 5.5 Hz, 1H)
4.39 (q, *J* = 7.3 Hz, 1H)	4.37 (q, *J* = 7.4 Hz, 1H)
3.71 (dd, *J* = 5.2, 14.0 Hz, 1H)	3.71 (dd, *J* = 5.2, 14.0 Hz, 1H)
3.61 (dd, *J* = 5.7, 14.0 Hz, 1H)	3.58 (dd, *J* = 5.8, 14.0 Hz, 1H)
3.41 (dd, *J* = 7.1, 15.4 Hz, 1H)	3.41 (dd, *J* = 6.8, 14.0 Hz, 1H)
3.32 (dd, *J* = 7.6, 15.3 Hz, 1H)	3.32 (dd, *J* = 7.6, 14.0 Hz, 1H)
3.14 (dd, *J* = 5.3, 16.7 Hz, 1H)	3.10 (dd, *J* = 7.0, 14.0 Hz, 1H)
3.08 (dd, *J* = 7.0, 16.6 Hz, 1H)	3.06 (dd, *J* = 5.5, 14.0 Hz, 1H)
1.40 (d, *J* = 7.4 Hz, 3H)	1.41 (d, *J* = 7.2 Hz, 3H)

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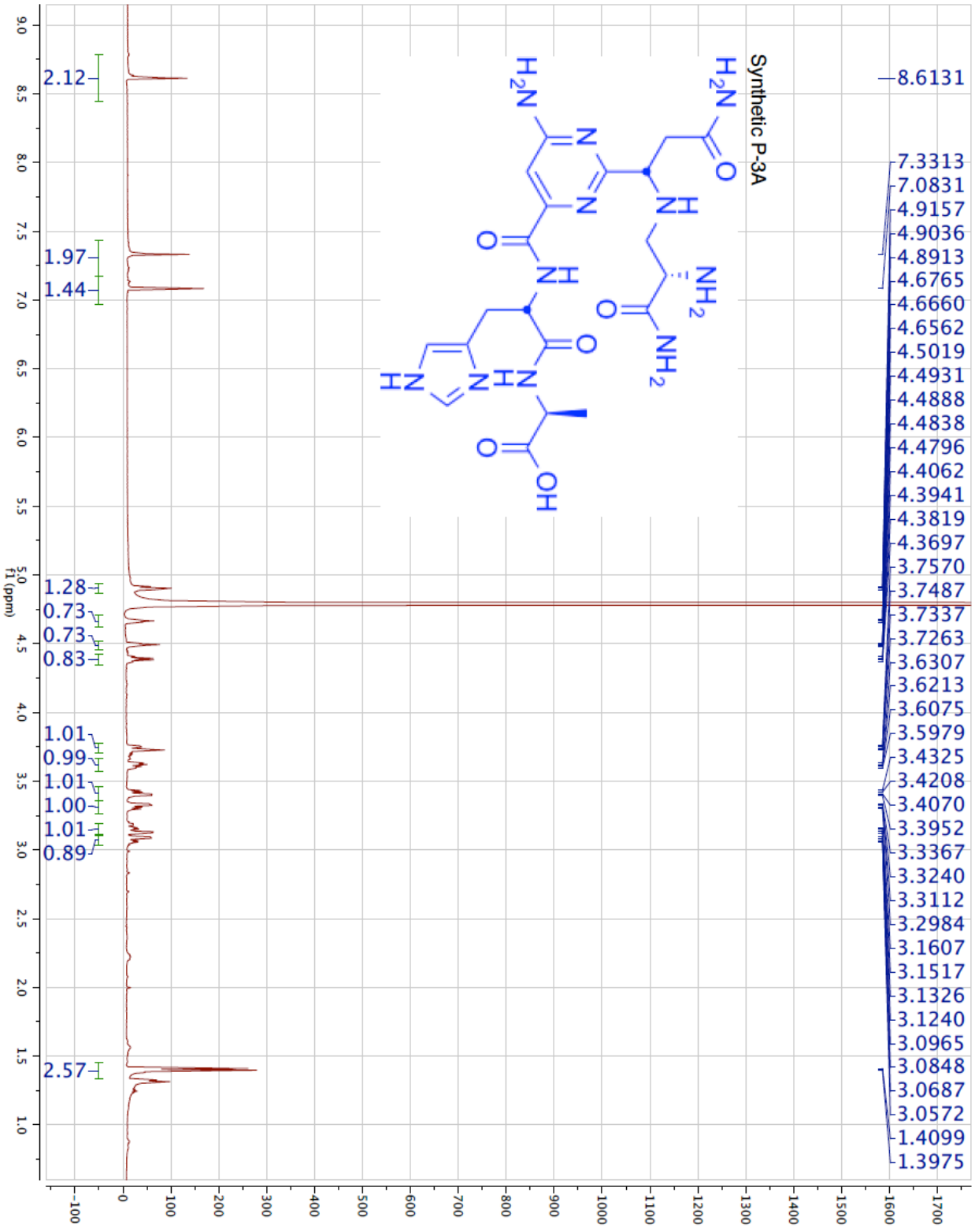
^{13}C NMR Comparison

Synthetic P-3A (150 MHz, D_2O)	***Authentic P-3A***[2] (100 MHz, D_2O)
178.6	178.8
176.2	176.3
173.4	173.4
170.8	170.9
167.5	167.6
166.8	166.8
164.5	164.8
155.6	155.6
136.0	136.0
130.4	130.5
120.0	120.0
106.3	106.2
62.1	62.1
55.0	55.1
52.6	52.7
51.3	51.6
48.8	48.8
38.2	38.4
29.3	29.3
18.5	18.5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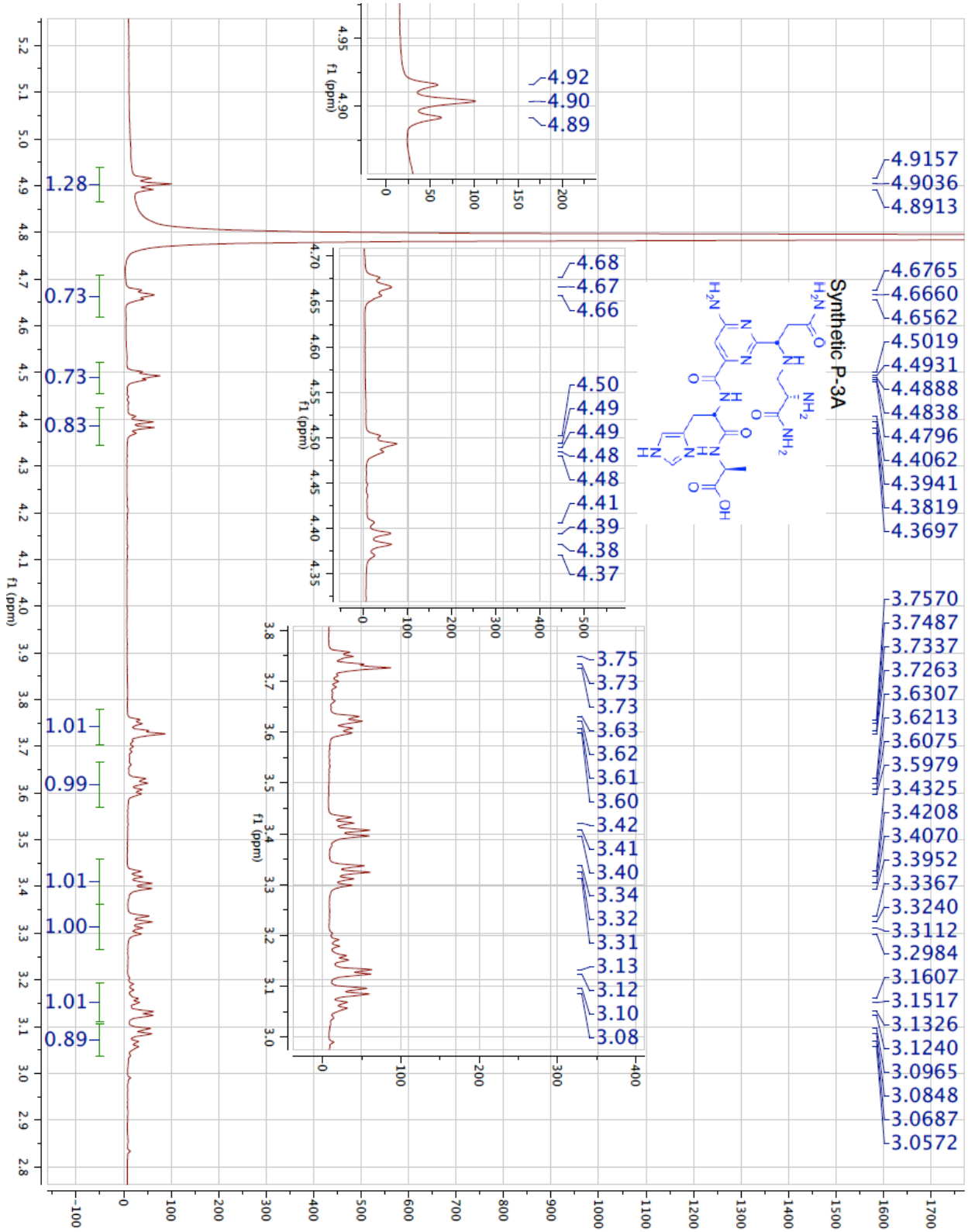
7.3313
7.0831
4.9157
4.9036
4.8913
4.6765
4.6660
4.6562
4.5019
4.4931
4.4888
4.4838
4.4796
4.4062
4.3941
4.3819
4.3697
3.7570
3.7487
3.7337
3.7263
3.6307
3.6213
3.6075
3.5979
3.4325
3.4208
3.4070
3.3952
3.3367
3.3240
3.3112
3.2984
3.1607
3.1517
3.1326
3.1240
3.0965
3.0848
3.0687
3.0572
1.4099
1.3975
2.12
1.97
1.44
1.28
0.73
0.73
0.83
1.01
0.99
1.01
1.00
1.01
0.89
2.57
f1 (ppm)


Synthetic P-3A
4.9157
4.9036
4.8913
4.6765
4.6660
4.6562
4.5019
4.4931
4.4888
4.4838
4.4796
4.4062
4.3941
4.3819
4.3697
3.7570
3.7487
3.7337
3.7263
3.6307
3.6213
3.6075
3.5979
3.4325
3.4208
3.4070
3.3952
3.3367
3.3240
3.3112
3.2984
3.1607
3.1517
3.1326
3.1240
3.0965
3.0848
3.0687
3.0572
1.28
0.73
0.73
0.83
1.01
0.99
1.01
1.00
1.01
0.89
4.92
4.90
4.89
4.68
4.67
4.66
4.50
4.49
4.49
4.48
4.48
4.41
4.39
4.38
4.37
3.75
3.73
3.73
3.63
3.62
3.61
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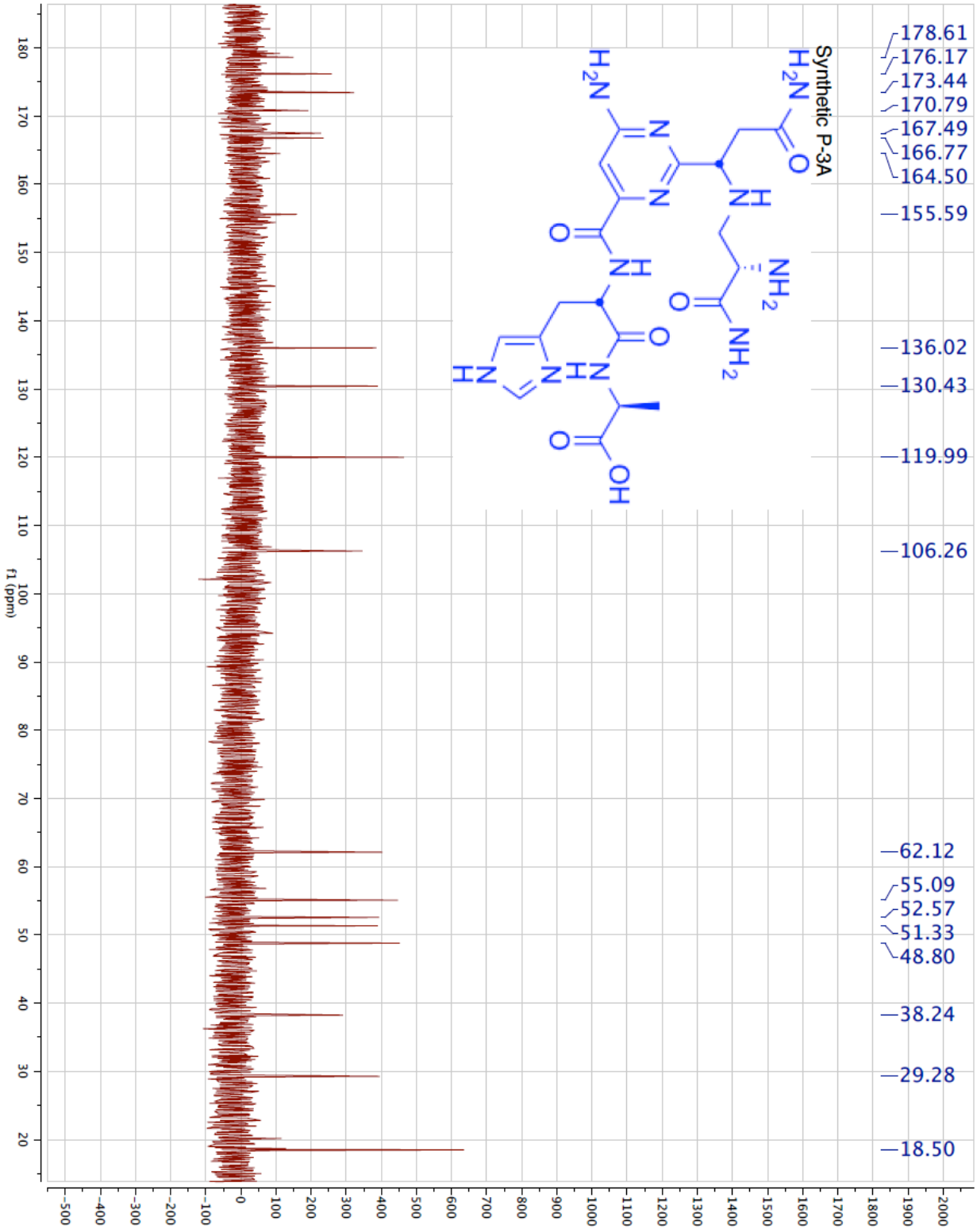
3.13
3.12
3.10
3.08
f1 (ppm)


Synthetic P-3A
178.61
176.17
173.44
170.79
167.49
166.77
164.50
155.59
136.02
130.43
119.99
106.26
62.12
55.09
52.57
51.33
48.80
38.24
29.28
18.50
f1 (ppm)