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AN EFFICIENT SYNTHESIS OF 2-[(4-AMINO-1,2-DIHYDRO-2-OXO-1-PYRIMIDINYL)METHOXY]-1,3-PROPANEDIYL-DI-L-VALINATE AN ANTI-CYTOMEGALOVIRUS AGENT

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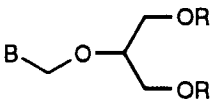
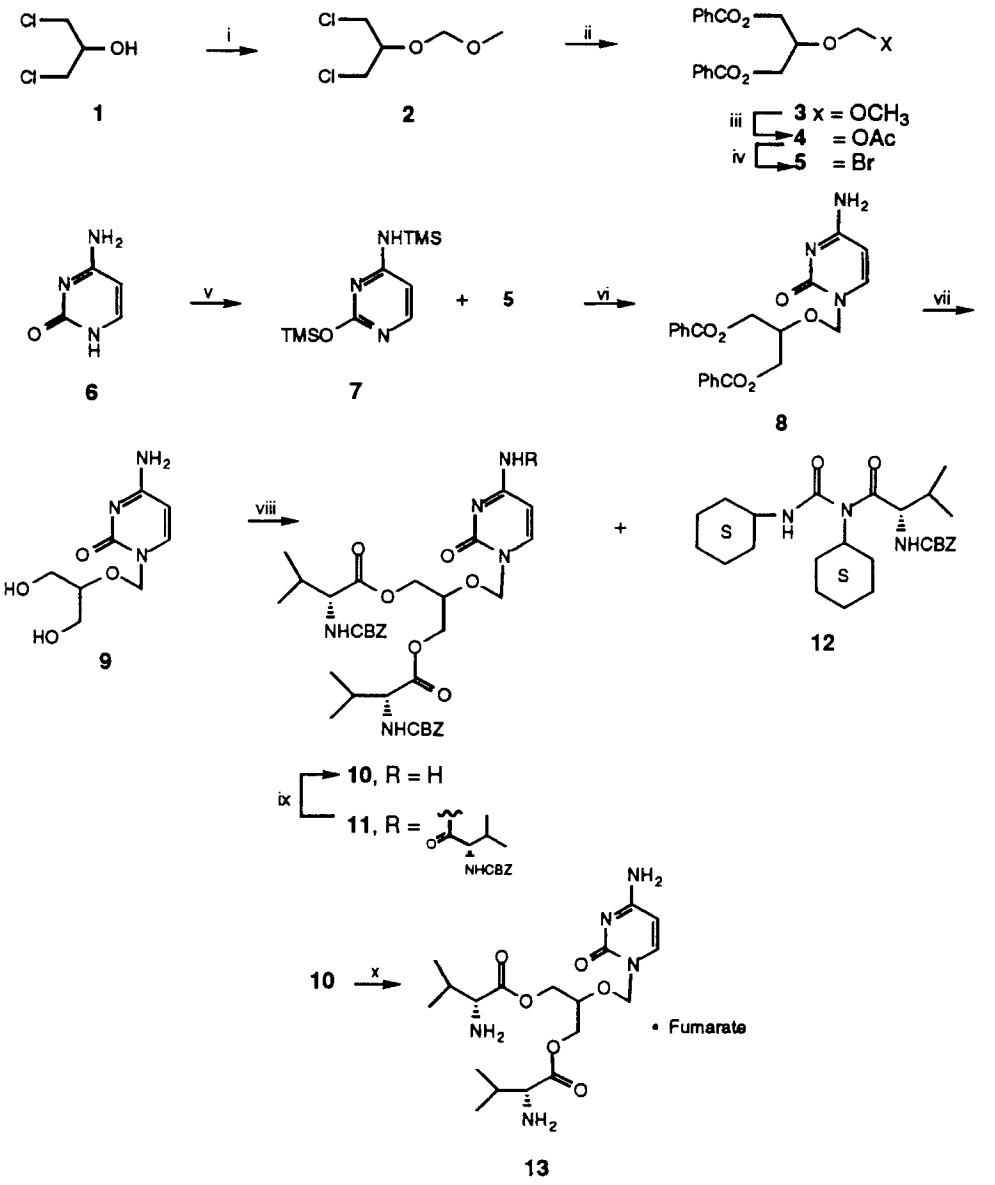
Abstract: An efficient synthesis for large scale preparation of the titled compound, a prodrug for the anti-HCMV agent 1-[2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]cytosine, **9**, has been developed. The product of each step is easily purified by either distillation or recrystallization and the final product is obtained in a high overall yield.

The cytosine analogue of ganciclovir, **9**[1] has approximately the same potency as the latter against human cytomegalovirus (HCMV) and Epstein Barr virus (EBV). To improve the oral bioavailability of **9**, several amino acid derivatives were prepared as prodrugs. The di-L-valyl ester, **13**[2], was two-fold more bioavailable compared to the parent compound. To assess the toxicological profile of **13**, multi-kilogram quantities of the compound were required.



Ganciclovir	B = guanine	R = H
9	B = cytosine	R = H
13	B = cytosine	R = L-valyl

Since the original synthesis of **13** via **9** (Scheme 1) required chromatographic purification to remove **11** and **12**, it was unsuitable for large scale preparation. Originally the dibenzoate intermediate, **8**, was prepared by heating


1
2
3 x = OCH3
4 = OAc
5 = Br
6
7
8
9
10, R = H
11, R =
12
13
i) CH2(OCH3)2, P2O5; ii) PhCO2Na; iii) Ac2O, H2SO4; iv) TMSBr; v) HMDS, (NH4)2SO4;
vi) CH2Cl2; vii) aq. CH3NH2; viii) DCC, DMAP, CBZ-L-valine; ix) ZnCl2, MeOH;
x) 10% Pd/C, Methyl-1,4-cyclohexadiene; Fumaric acid

Scheme 1

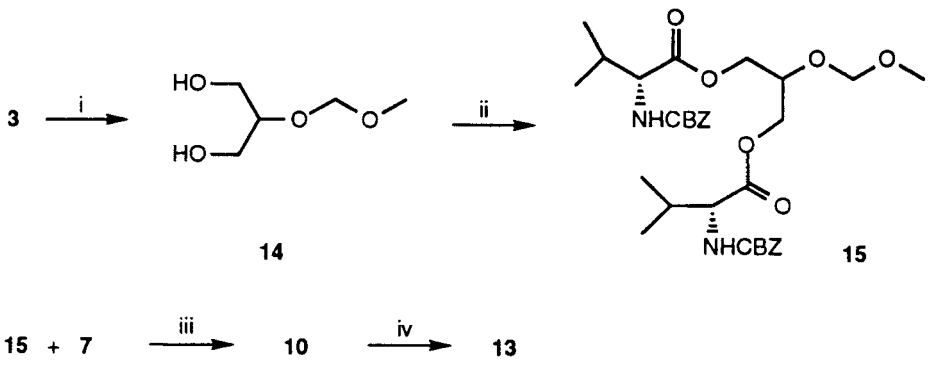
5 and **7** in CH_2Cl_2. Subsequently, **6** was silylated *in situ* with N,O-bis-(trimethylsilyl)acetamide (BSA) followed by the addition of **4** and bromotrimethylsilane (TMSBr), to give **8** in one pot in 84% yield. The procedure was further refined by using the methoxy ether **3** instead of **4** in the alkylation of silylated cytosine, in the presence of one equivalent each of TMSBr and trimethylsilyl triflate (TMS triflate) or two equivalents of TMS triflate. Hydrolysis of **8** with aqueous methylamine gave **9** as a white solid in 85% yield.

The coupling of **9** and N-CBZ-L-valine with 1,3-dicyclohexylcarbodiimide (DCC) in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) to give **10** was problematic. In addition to **10**, the impurities **11** and **12** (impurity associated with the use of DCC) were also found in the crude product. However, treatment of the crude mixture with zinc chloride[3] selectively hydrolyzed the N-4-amide group of **11** to give **10** in 79% yield after chromatographic purification to remove **12**.

The hydrogenolysis of **10** was investigated using different solvents, catalysts, and hydrogen sources. It was successful only when performed in isopropanol using Pd/C, and cyclohexene or methyl-1,4-cyclohexadiene as hydrogen sources; otherwise incomplete reaction and/or partial hydrolysis of the L-valyl ester were observed.

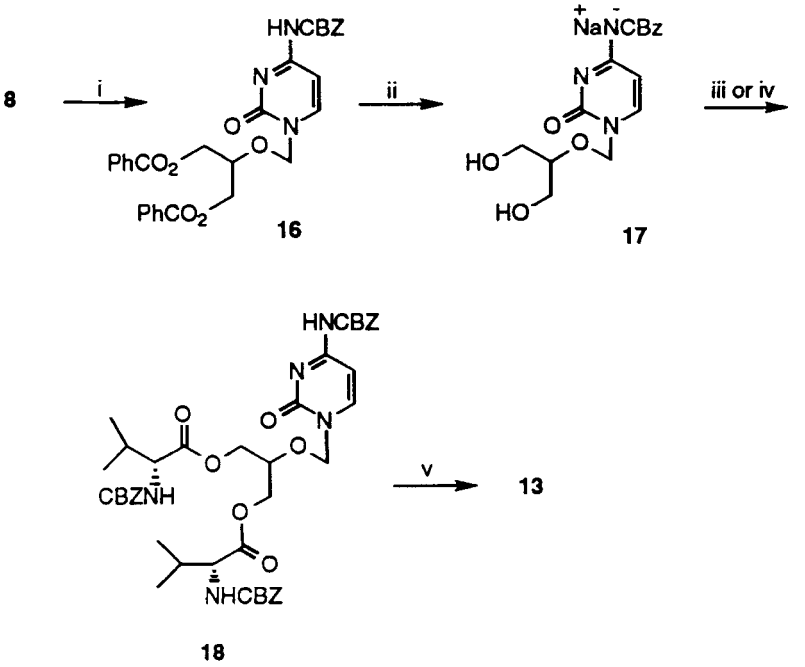
To overcome the difficulties in acylating **9**, the coupling of cytosine with the side chain **15** under Vorbruggen conditions[4] to give **10** was investigated (Scheme 2). Hydrolysis of **3** gave the diol **14**. Coupling **14** with N-CBZ-L-valine in the presence of DCC gave **15**, which was treated with silylated cytosine in the presence of t-butyldimethylsilyl triflate to give **10** in good yield. However, this method did not offer any advantage since it required chromatographic purification in each step.

Another approach to the synthesis of **13** was accomplished by protection of the 4-amino position in **8** with a CBZ group[5] to give **16** (Scheme 3). Selective hydrolysis of the benzoate esters with aqueous 1N NaOH gave the diol **17** as the sodium salt, as shown by its combustion analysis and ^1H NMR spectrum. Both **16** and **17** were solid and were isolated by filtration from the reaction mixture. Coupling **17** with N-CBZ-L-valine in the presence of DCC was sluggish and



i) aq. CH_3NH_2; ii) DCC, DMAP, CBZ-L-valine; iii) t-$BuMe_2Si$ triflate;
iv) 10% Pd/C, Methyl-1,4-cyclohexadiene; Fumaric acid

Scheme 2

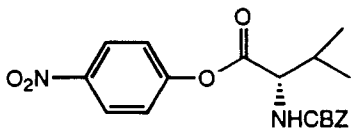


i) CBZCl; ii) 1N NaOH; iii) DCC, DMAP, CBZ-L-valine; iv) **21**;
v) 10% Pd/C, Cyclohexene; Fumaric acid

Scheme 3

required twice the amount of DMAP for completion of the reaction. However, chromatographic purification was still necessary to remove **12**.

To circumvent the chromatographic purification required to remove **12** (an impurity associated with the use of DCC), other acylating reagents (**19**[6], **20**[7], and **21**[8]) were investigated. Our best result was obtained simply by stirring N-CBZ-L-Val-N-carboxy anhydride, **21**, with **17** at ambient temprature to give **18** quantitatively in excellent purity (since CO_2 is the only byproduct of the reaction). Hydrogenolysis of **18** gave **13** as the fumarate salt in high yield.



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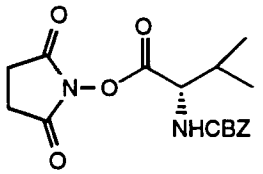

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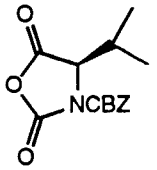

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Thus, via the acylation of **17** with **21**, a seven step synthesis of **13** from **1** with an overall 47% yield was devised. At each step the product was easily purified either by distillation or recrystallization.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover UniMelt apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs (Atlanta, Ga) or by Oneida Research Services (Whitesboro, N Y). ^1H and ^{13}C NMR spectra were recorded on Varian XL300 instrument and were relative to tetramethylsilane.

2-[(4-Amino-1,2-dihydro-2-oxo-1-pyrimidinyl)methyoxyl-1,3-propanediyl dibenzoate (8). To a mixture of cytosine (66 g, 0.60 mol) and BSA (294 mL, 1.2 mol) in 300 mL CH_3CN was added 5.5 mL of TMSBr. The reaction temperature increased from 5 to 20° C. To the resultant solution was added a solution of **3** (205 g, 0.60 mol) in 300 mL of CH_3CN, TMSBr (73 mL, 0.60 mol) and TMS triflate (130 mL, 0.67 mol). The reaction mixture was refluxed for 4.5 h, then cooled to ambient temperature. The crude product was diluted with 700 ml CH_2Cl_2, poured into 2 L of ice-water, the pH was adjusted to pH 10 with 4.5 N

aqueous NH_4OH while stirring. The product was collected by filtration to give 212 g (84%) of **8** as a white solid. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.92 (1H, d, H-6, J=7.0 Hz), 7.91-7.48 (10H, m, Ar-H), 5.63 (1H, d, H-5, J=7.0 Hz), 5.27 (2H, s, NCH_2O), and 4.53-4.31 (5H, m, CH_2CHCH_2). ^{13}C NMR (DMSO-d_6, 75.4 MHz) 166.2, 165.5, 156.1, 145.5, 133.4, 129.3, 129.2, 128.7, 94.4, 76.4, 73.8, and 63.9.

Synthesis of 8 using 4. To a mixture of cytosine (147 g, 1.32 mol), and BSA (660 mL, 2.7 mol) in 800 mL of CH_2Cl_2 was added 5 mL of TMSBr. To the resulting solution was added **4** (492 g, 1.32 mol) in 300 mL of CH_2Cl_2 followed by slow addition of TMSBr (205 mL, 1.59 mol). The reaction mixture was heated at reflux for 6 h. After work up as in the above procedure, 474 g (81%) of **8** was obtained (gave identical spectral datal as previously described).

1-[2-Hydroxy-1-(hydroxymethyl)ethoxy)methyllcytosine (9). A mixture of **8** (333 g, 0.79 mol) and 3.6 L of 20% aqueous methylamine was slurried at ambient temperature for 24 h. The resultant solution was concentrated in vacuo and triturated with 1.2 L of isopropanol to produce 144 g (85%) of **9** as a white solid. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.59 (1H, d, H-6, J=7.3 Hz), 7.21 (2H, s, NH_2), 5.67 (1H, d, H-5, J=7.3 Hz), 5.12 (2H, s, OCH_2N), 4.61 (2H, s, OH), and 3.50-3.28 (5H, m, CH_2CHCH_2). ^{13}C NMR (DMSO d-6, 75.4 MHz) 166.2, 155.9, 145.4, 94.0, 79.9, 76.8, and 60.8.

2-[(4-Amino-1,2-dihydro-2-oxo-1-pyrimidinyl)methoxyl-1,3-propanediyl bis[N-(benzyloxy)carbonyl]-L-valinate (10). To a cold (0° C) mixture of N-CBZ-L-valine (1016 g, 4.0 mol), DCC (834 g, 4.0 mol), **9** (363 g, 1.68 mol), and 4.7 L of DMF was added DMAP (51.5 g, 0.42 mol). The reaction mixture was kept at 5° C for 18 h, then filtered to remove 1,3-dicyclohexylurea. After evaporation, the filtrate was partitioned between EtOAc and water, and the organic layer was evaporated. The residual oil was dissolved in EtOAc and purified by flash chromatography eluting with EtOAc to remove both **11** and **12**. Further elution with 5% EtOH in EtOAc yielded 430 g (64%) of **10** as a cream foam. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.69-7.65 (2H, m, NHCBZ), 7.54 (1H, d, H-6, J=7.2 Hz), 7.30-7.20 (12H, m, Ar-H, and NH_2), 5.69 (1H, d, H-6, J=7.2 Hz), 5.18 (1H, d, NCHO, J=10 Hz), 5.08 (1H, d, NCHO, J=10 Hz), 5.01 (4H, s, CH_2Ph), 4.22-4.01 (5H, m, CH_2CHCH_2), 3.99-3.91 (2H, m, $HCCO_2$), 2.86-1.96 (2H, m, $CH(CH_3)_2$), 0.85 (6H, d, CH_3, J=3 Hz), and 0.84 (6H, d, CH_3, J=3 Hz). ^{13}C NMR (DMSO-d_6, 75.4 MHz) 171.6, 166.2, 156.4, 155.9, 145.3, 136.8, 128.3, 127.8, 94.3, 76.4, 73.4, 65.6, 63.4, 63.2, 59.7, 59.6, 29.6, 19.0, and 18.0.

Preparation of **10** using zinc chloride treatment: To a cold (0° C) mixture of N-CBZ-L-valine (30.4 g, 121 mmol), 130 mL of DMF, **9** (10 g, 46 mmol) and DCC (24.7 g, 121 mmol) was added DMAP (1.4 g, 11.5 mmol). The reaction mixture was stirred for 24 h at 5° C. Dicyclohexylurea was removed by filtration and the filtrate was concentrated at reduced pressure. The residual oil was partitioned between CH_2Cl_2 and water. The organic phase was concentrated to 400 mL. To this solution was added 100 mL of CH_3OH and 124 mL of 1 M solution of zinc chloride in Et_2O. The reaction mixture was stirred for 24 h at ambient temperature. After work up, the crude product was chromatographed as above to remove **12**. The later fractions gave 24.8 g (79%) of **10** (gave identical spectral data as previously described).

2-[(4-Amino-1,2-dihydro-2-oxo-1-pyrimidinyl)methoxyl-1,3-propanediyl-di-L-valinate (13). To a mixture of **10** (27 g, 0.04 mol) and 1-methyl-1,4-cyclohexadiene (31.5 mL, 0.28 mol) in 340 mL of isopropanol was added 5.4 g of 10% Pd/C. The reaction mixture was heated at reflux for 1 h. The crude product was filtered and the filtrate was treated with fumaric acid (7.0 g, 0.06 mol) in 200 mL of EtOH, diluted with EtOAc, and filtered to separate 21.6 g (92%) of **13** as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.57 (1H, d, H-6, J=7.3 Hz), 7.31-7.29 (2H, m, NH_2), 6.49 (2H, s, CH=CH), 5.69 (1H, d, H-5, J=7.3 Hz), 5.14 (2H, s, OCH_2N), 4.25-4.01 (5H, m, CH_2CHCH_2), 3.30 (1H, d, $CHNH_2$, J=5.1 Hz), 1.88-1.84 (2H, m, $CH(CH_3)_2$), 0.85 (6H, d, $C(CH_3)_2$, J=7 Hz), and 0.82 (6H, d, $C(CH_3)_2$, J=7 Hz). ^{13}C NMR (DMSO-d_6, 75.4 MHz) 172.2, 167.8, 166.2, 145.5, 135.0, 94.4, 76.5, 73.4, 63.6, 63.4, 58.4, 58.3, 30.7, 18.4, and 17.7.

2-(Methoxymethoxy)-1,3-propanediol (14). A solution of **3** (22 g, 64 mmol) in 100 mL of CH_3OH and 100 mL of 40% aqueous methylamine was stirred for 18 h at ambient temperature. The crude product was concentrated at reduced pressure, partitioned between 200 mL of Et_2O and 300 mL of water. The aqueous solution was concentrated to an oil and purified by flash chromatography to separate 7.9 g (76%) of **14** as a clear oil. ^{1}H NMR (DMSO-d_6, 300 MHZ) δ 4.61 (2H, s, OCH_2O), 4.54 (2H, s, OH), 3.45-3.26 (5H, m, CH_2CHCH_2), and 3.25 (3H, s, CH_3). ^{13}C NMR (DMSO-d_6, 75.4 MHz) 95.7, 79.3 , 61.6, and 55.1.

2-(Methoxymethoxy)-1,3-propanediyl-bis[N-[(benzyloxy)carbonyl]-L-valinate] (15). To a cold (0° C) solution of N-CBZ-L-valine (40.7 g, 162 mmol), **14**

(7.4 g, 54 mmol) and DCC (33.4 g, 162 mmol) in 500 mL of THF was added DMAP (2.0 g, 16.4 mmol). The reaction mixture was stirred for 18 h at 27° C. The solid was removed by filtration and the filtrate was diluted with 500 mL of Et_2O and washed with 100 mL of 0.1 N HCl and twice with 100 mL each of water. The organic solution was dried, concentrated to give a clear oil which was purified by flash chromatography, eluting with EtOAc: hexane (1:1) to separate 28.6 g (84%) of **15** as a wax. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.30 (10H, s, Ar-H), 5.30 (2H, d, NH, J=9 Hz), 5.10 (4H, s, $PhCH_2$), 4.67 (2H, s, OCH_2O), 4.35-4.17 (5H, m, CH_2CHCH_2), 4.00 (2H, m, $CHCO_2$), 2.28-2.08 (2H, m, $CH(CH_3)_2$), 0.97 (6H, d, $C(CH_3)_2$, J=7 Hz), and 0.88 (6H, d, $C(CH_3)_2$, J=7 Hz). ^{13}C NMR ($CDCl_3$, 75.4 MHz) 171.7, 156.1, 136.1, 128.5, 128.1, 128.0, 96.0, 72.4, 67.0, 63.9, 63.6, 58.9, 55.6, 31.1, 18.9, and 17.3.

Alternative synthesis of 10. To a cold (0° C) suspension of cytosine (4.5 g, 40 mmol), BSA (12.2 g, 60 mmol) and 100 mL of CH_3CN was added t-butyl dimethylsilyl triflate (21.2 g, 80 mmol) and **15** (25.3 g, 40 mmol) in 100 mL of CH_3CN . The reaction mixture was heated at reflux for 18 h. The crude product was partitioned between 1 L of CH_2Cl_2 and 0.5 L of water. The organic solution was dried, concentrated and chromatographed to separate 18.3 g (65%) of **10** as a foam (gave identical spectral data as previously described).

2-[(4-(((Benzyloxy)carbonyl)amino)-1,2-dihydro-2-oxo-1-pyrimidinyl) methoxyl-1,3-propanediyl dibenzoate (16). A mixture of 8 (135 g, 0.32 mol), sodium bicarbonate (148 g, 1.77 mol) and benzyl chloroformate (164 g, 0.96 mol) in 1.9 L of water were slurried at ambient temperature for 24 h. The solid was collected, slurried in toluene, and filtered to give 167 g (94%) of **16** as a white solid. ^1H NMR (DMSO-d_6, 300 MHz) δ 10.80 (1H, s, NH), 8.08 (1H, d, H-6, J=7.3 Hz), 7.90-7.32 (15H, m, Ar-H), 6.85 (1H, d, H-5, J=7.3 Hz), 5.39 (2H, s, OCH_2N), 5.17 (2H, s, OCH_2Ph), and 4.53-4.33 (5H, m, CH_2CHCH_2). ^{13}C NMR (DMSO-d_6, 75.4 MHz) 165.4, 163.4, 155.3, 152.9, 149.3, 136.0, 133.4, 129.2, 129.1, 128.7, 128.5, 128.2, 128.0, 94.6, 77.2, 74.9, 66.5, and 63.9.

2-[((4-Benzyloxy)carbonyl)amino-1,2-dihydro-2-oxo-1-pyrimidinyl)methoxyl-1,3-propanediol sodium salt (17). To a cold (0° C) solution of **16** (100 g, 0.18 mol) in 1.5 L of CH_3OH/THF (1:1) was added 390 mL of aq 1N NaOH. After 30 minutes a white precipitate was formed and collected to produce 64 g (96%) of **17** as a white solid. ^1H NMR (DMSO-d_6, 300 MHz) δ

7.42 (1H, d, H-6, J=7.5 Hz), 7.33-7.24 (5H, m, Ar-H), 6.37 (1H, d, H-5, J=7.5 Hz), 5.10(2H, s, OCH_2N), 4.94 (2H, s, OCH_2Ph), 4.66 (2H, s, OH), and 3.53-3.40 (5H, m, CH_2CHCH_2). ^{13}C NMR (DMSO-d_6, 75.4 MHz) 171.9, 162.0, 157.4, 142.9, 138.7, 128.1, 127.3, 127.1, 100.5, 79.5, 76.7, 64.7, and 60.8. Anal. Calcd for $C_{16}H_{18}N_3O_6Na$: C, 51.75; H, 4.89; N, 11.32; Na, 6.19. Found: C, 51.80; H, 4.88; N, 11.28; Na, 6.33.

[(4-(((Benzyloxy)carbonyl)amino)-1,2-dihydro-2-oxo-1-pyrimidinyl)methoxyl-1,3-propanediyl bis[N-((benzyloxy)carbonyl)-L-valinate] (18). To a mixture of N-CBZ-L-valine (14.4 g, 57 mmol), DCC (11.8 g, 57 mmol), **17** (5.0 g, 14 mmol) and 195 mL of THF was added DMAP (0.45 g, 3.6 mmol). The reaction mixture was stirred at 5° C for 1 h, filtered to remove 1,3-dicyclohexylurea, concentrated, partitioned between EtOAc and water, and evaporated. The residual oil was redissolved in EtOAc and purified by flash chromatography, eluting the target compound with 5% EtOH in EtOAc to produce 18.4 g (82%) of **18** as a yellow oil. ^1H NMR (DMSO-d_6, 300 MHz) δ 10.90 (1H, s, NH), 8.06 (1H, d, H-6, J=7.0 Hz), 7.69-7.65 (2H, m, NHCBZ), 7.38-7.26 (15H, m, Ar-H), 7.03 (1H, d, H-5, J=7.0 Hz), 5.23 (2H, m, NCH_2O), 5.16 (2H, s, OCH_2Ph), 5.01 (4H, s, OCH_2Ph) 4.24-3.88 (7H, m, CH_2CHCH_2, and $CHCO_2$) 1.98-1.93 (2H, m, $CH(CH_3)_2$), and 0.82 (12H, d, $C(CH_3)_2$) ^{13}C (DMSO-d_6, 75.4 MHz) 171.6, 163.6, 156.4, 155.2, 155.1, 153.1, 149.3, 136.8, 135.9, 128.4, 128.3, 128.1, 128.0, 127.8, 127.5, 127.4, 127.2, 94.6, 77.1, 74.3, 66.5, 65.6, 63.4, 63.2, 59.6, 29.6, 18.9, 18.6, 18.1, and 18.0.

Synthesis of 18 using N-CBZ-L-Valine-N carboxy anhydride. To a DMF (5 mL) solution of **17** (1.0 g, 2.8 mmol) was added N-CBZ-L-valine-N-carboxy anhydride (N-CBZ-L-Val NCA) **21** (1.73 g, 7.1 mmol). The reaction mixture was stirred at ambient temperature for 3.5 h. The crude product was concentrated in vacuo to give an oil which was partitioned between EtOAc and water. The organic solution was dried and evaporated to give a quantitative yield (97% AUC) of **18** (gave identical spectral data as previously described).

2-[(4-Amino-1,2-dihydro-2-oxo-1-pyrimidinyl)methoxyl-1,3-propanediyl di-L-valinate (13). A mixture of **18** (8.2 g, 10 mmol), cyclohexene (8.1 mL, 80 mmol), isopropanol (150 mL), and 1.6 g of 10% Pd/C was heated at reflux for 1 h. The palladium catalyst was removed by filtration through a celite pad and the filtrate was treated with 1.2 g of fumaric acid in 50 mL EtOH, diluted with

EtOAc, and filtered to produce 4.0 g (77%) of **13** as a white solid (gave identical spectral data as previously described).

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