Supporting Information

for

Protein Synthesis with Ribosomes Selected for the Incorporation of β-Amino Acids

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Table S1. ***In silico*** **Predicted Alterations (Partial) in the Hydrogen Bonding Interactions Network of PTC in** ***E. coli*** **Modified Ribosomes (Relax Constraints by 0.4 Å and 20.0 Degree, pdb 2WWQ)**

Interacting nucleotides	Wild type (distance Å)	040321 Mutant (distance Å)	040329 Mutant (distance Å)	0403x4 Mutant (distance Å)	040217 Mutant (distance Å)	010335 Mutant (distance Å)
2501-2450	2.75, 3.14	2.75; 3.14	2.75; 3.14	2.75; 3.14	1.92	2.75; 3.14
2501-2063	2.95	-	-	-	-	-
2504-2452	3.39; 3.58	2.03	1.57; 2.07	-	3.10; 3.31	2.03
2504-2447	-	2.55	-	-	-	2.55
2505-2506	3.35	-	-	-	-	-
2505-2610	2.75; 3.33	-	-	-	3.17	-
2506-2583	2.99; 3.16; 3.24	-	-	-	3.01; 3.28; 3.31	-
2507-2582	2.87; 2.91; 3.11	-	-	-	2.69; 2.94; 3.11	-
2058-2059	3.40	-	-	-	-	-
2061-2451	3.06; 3.10	3.45	3.45	3.45	3.45	3.45
2063-2450	3.29	2.21	2.21	2.21	2.21	-
2496-2455	2.90; 2.93; 3.07	2.90; 2.93; 3.07; 3.08	2.90; 2.93; 3.07; 3.08	2.90; 2.93; 3.07; 3.08	-	2.90; 2.93; 3.07; 3.08
2497-2498	-	3.14	3.14	3.14	-	3.14
2498-2454	2.84; 2.89; 3.09; 3.33	2.84; 2.89; 3.09; 3.33	2.84; 2.89; 3.09; 3.33	2.84; 2.89; 3.09; 3.33	-	2.84; 2.89; 3.09; 3.33
2499-2453	3.24; 3.65	3.24; 3.65	3.24; 3.65	3.24; 3.65	-	3.24; 3.65
2500-2453	2.73; 3.33	2.73; 3.33	2.73; 3.33	2.73; 3.33	1.99	2.73; 3.33

* Mutant 040321 2057GAAAGAC2063 -> 2057AGCGUGA2063, 2502GAUGUC2507 -> 2502AGAUAA2507; mutant 040329 2057GAAAGAC2063 -> 2057AGCGUGA2063, 2502GAUGUC 2507 -> 2502UGGCAG2507; mutant 0403x4 2057GAAAGAC2063 -> 2057AGCGUGA2063, 2502GAUGUC 2507 -> 2502AGCCAG2507; mutant 040217 2057GAAAGAC2063 -> 2057AGCGUGA2063, 2496CACCUC2501 -> 2496AUAGAA2501; mutant 010335 2057GAAAGAC2063 -> 2057UGCGUGG2063, 2502GAUGUC2507 -> 2502AGAUGA2507.

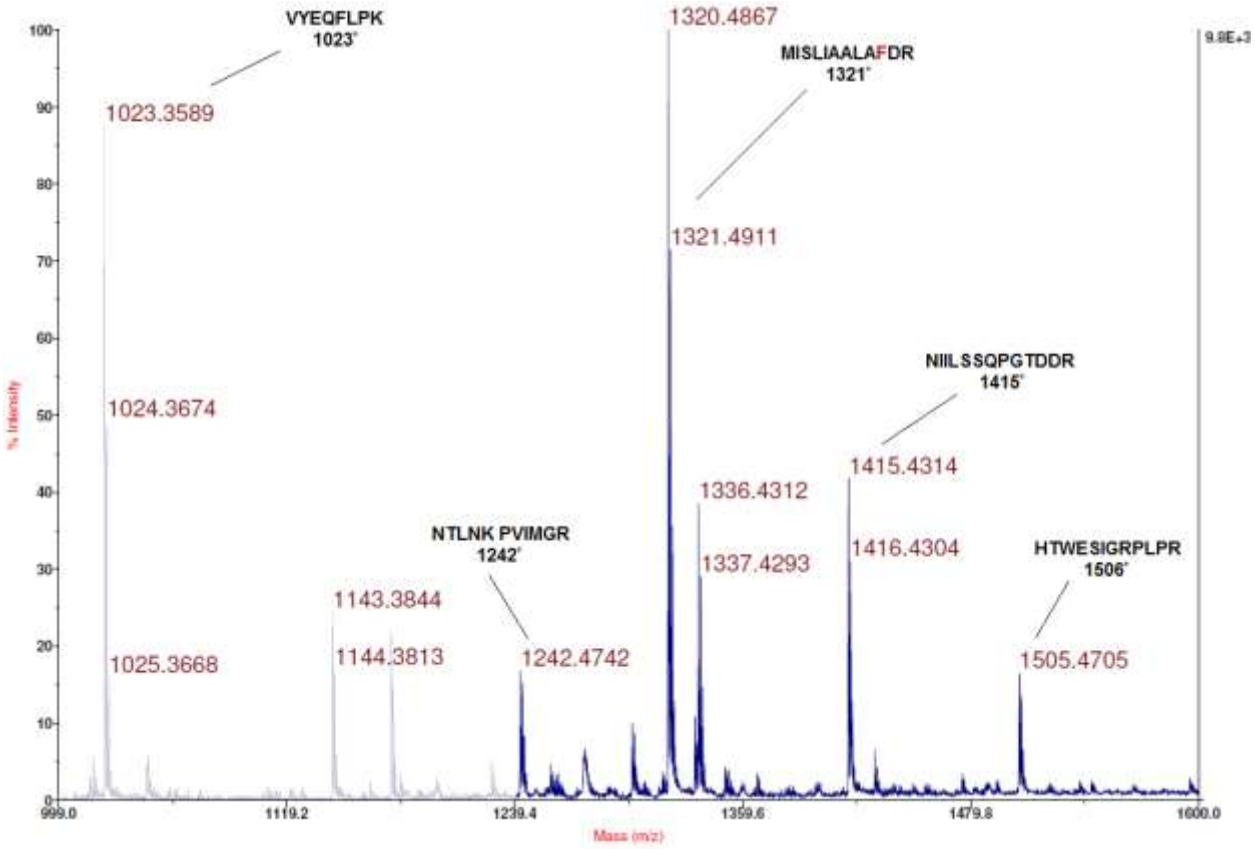


Figure S1. MALDI mass spectrum of tryptic fragments of DHFR V10F having phenylalanine at position 10 (MISLIAALA**F**DR). Mass range 1000-1600 Da (asterisk denotes an estimated value in daltons).

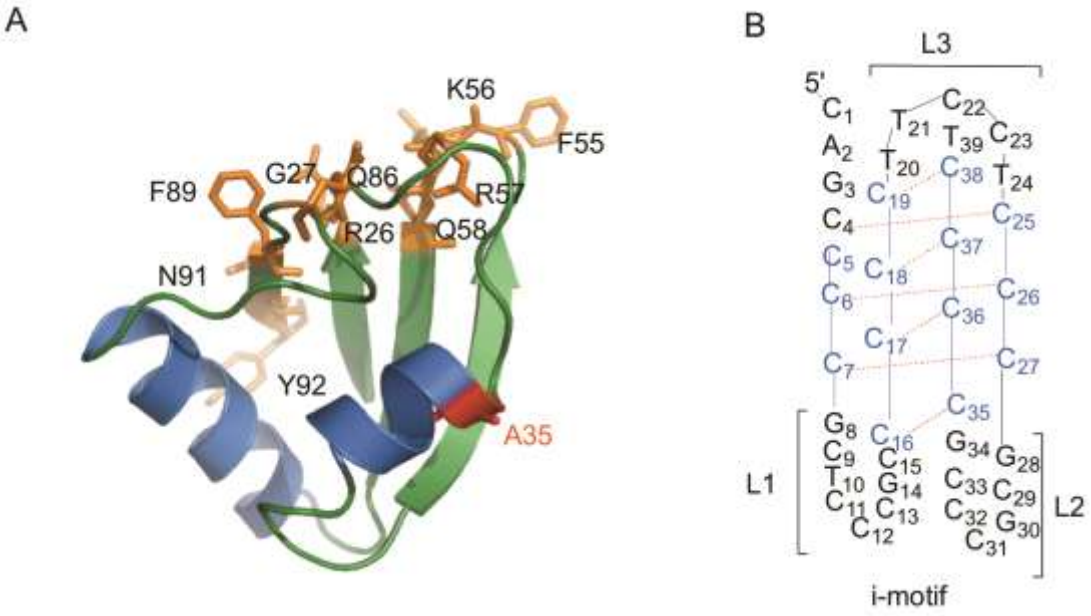
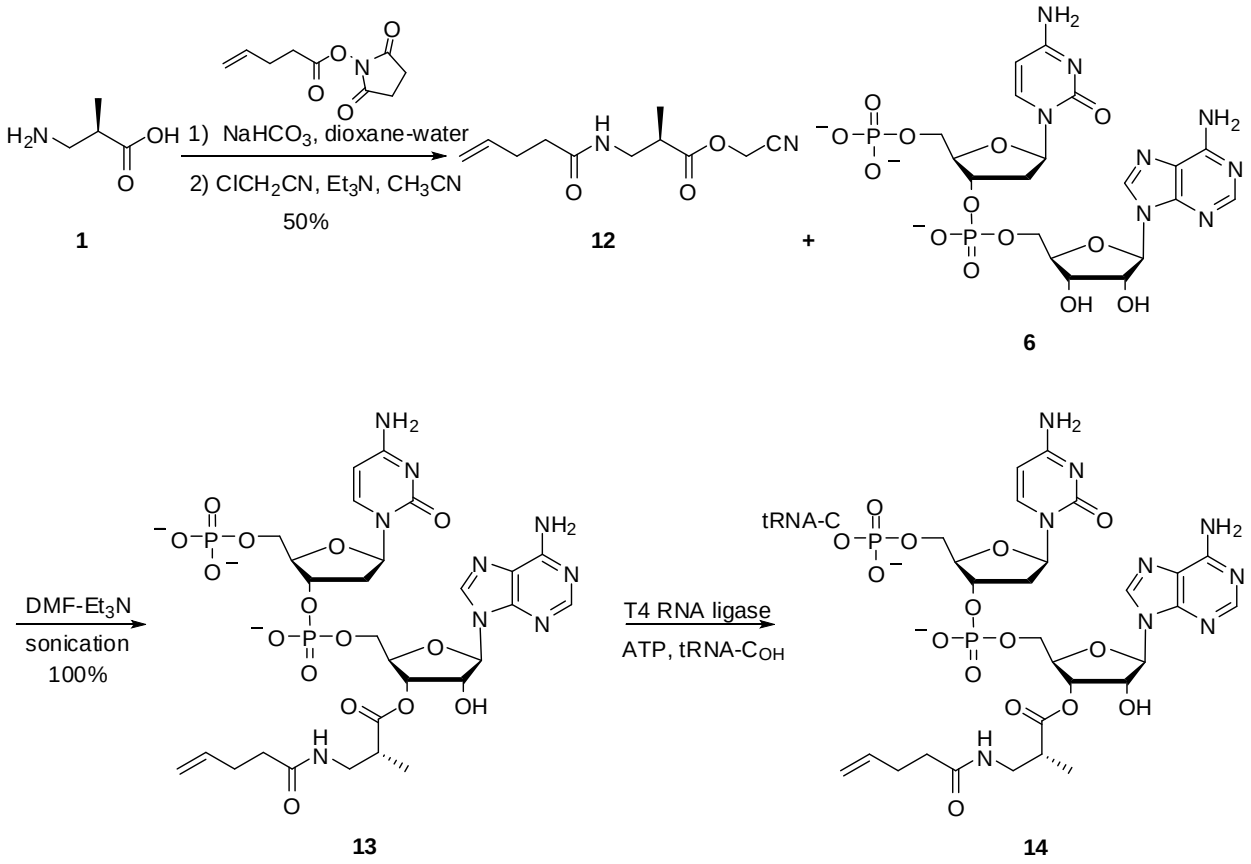
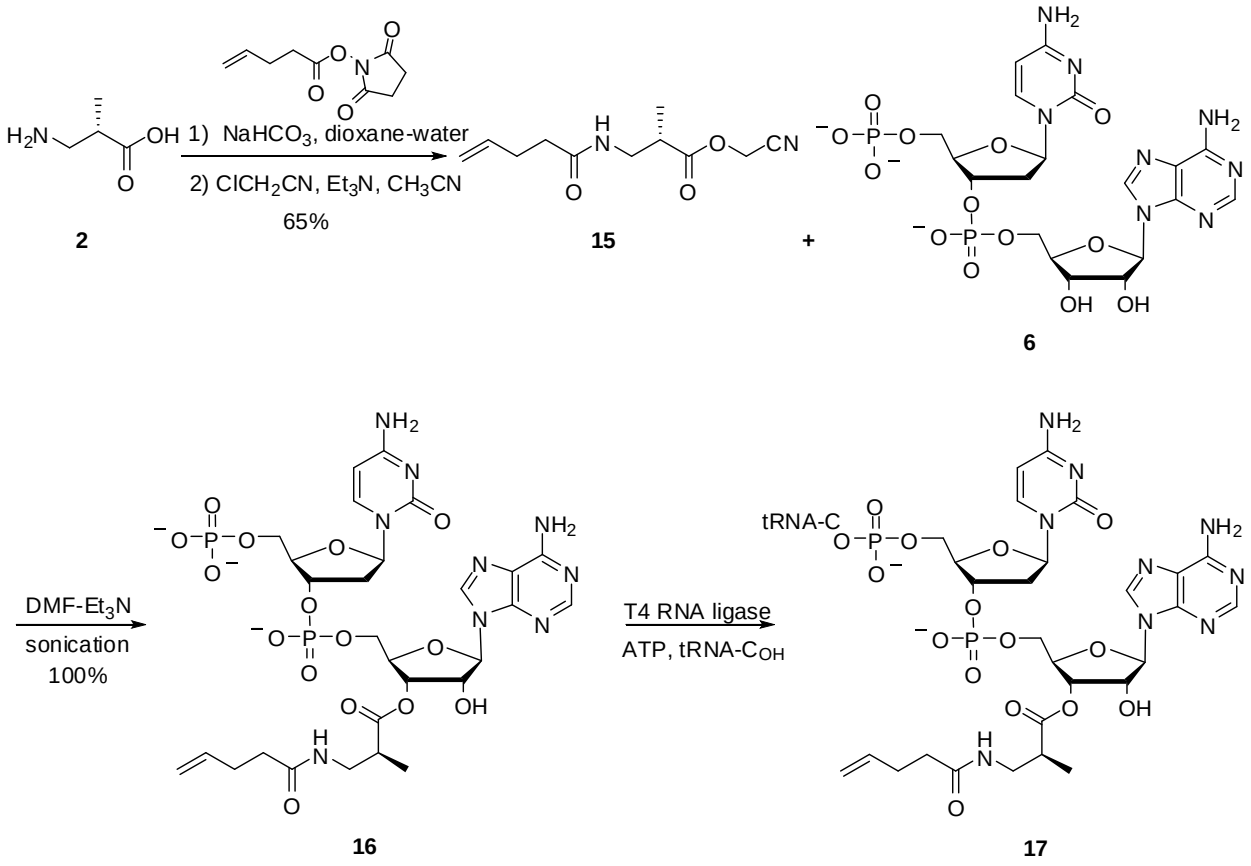


Figure S2. (A) RRM1wt displaying the α-helices (blue), β-sheets (green), position of β-amino acid incorporation (A35) and key i-motif binding residues (orange). (B) structure of the i-motif DNA with the cytidine rich stem (blue), lateral loops (L1 and L2) and the central loop (L3).

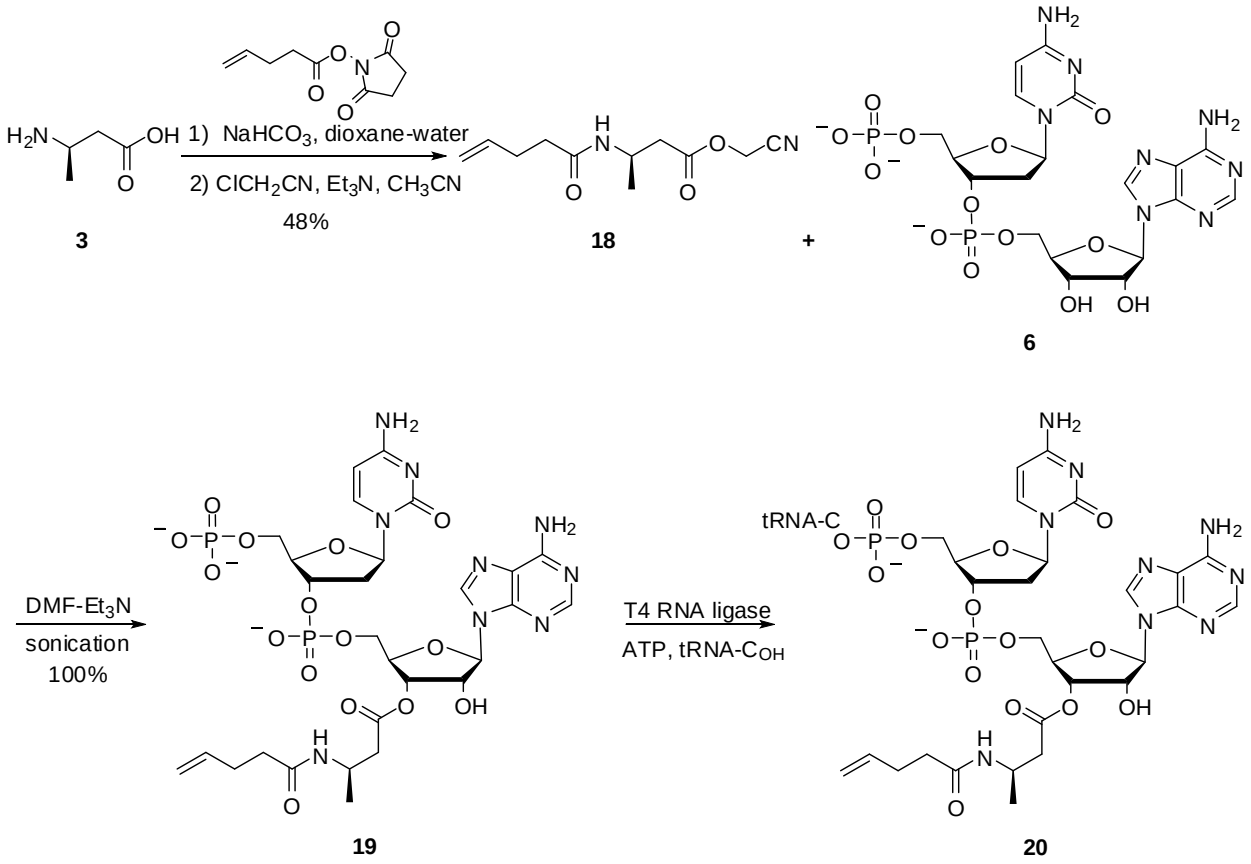
Scheme S1. Preparation of **14**, $tRNA_{CUA}$ activated with **1**.



Scheme S2. Preparation of **17**, $tRNA_{CUA}$ activated with **2**.



Scheme S3. Preparation of **20**, $tRNA_{CUA}$ activated with **3**.



MATERIALS AND METHODS

General. Reagents and solvents for chemical synthesis were purchased from Aldrich Chemical Co. or Sigma Chemical Co. and were used without further purification. Methyl-β-amino acids **1**-**4** were purchased from Peptech Corporation. All β-amino acids were used without further purification. All reactions involving air- or moisture-sensitive reagents or intermediates were performed under argon. Analytical TLC was performed using Silicycle silica gel 60 Å F254 plates (0.25 mm), and was visualized by UV irradiation (254 nm). Flash chromatography was performed using Silicycle silica gel (40–60 mesh). ^{1}H and ^{13}C NMR spectra were obtained using a Varian 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm, δ) referenced to the residual ^{1}H of the solvent ($CDCl_3$, δ 7.26). ^{13}C NMR spectra were referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, δ 77.16). Splitting patterns are designated as follows: s, singlet; br s, broad singlet, d, doublet; br d, broad doublet; dd, doublet of a doublet; t, triplet; q, quartet; m, multiplet. High resolution mass spectra were obtained at the Arizona State University CLAS High Resolution Mass Spectrometry Facility or the Michigan State University Mass Spectrometry Facility.

Synthesis and Characterization of the pdCpA Esters of β-Amino acids 1-4.

***N*-(4-Pentenoyl)-3*S*-methyl-*β*-alanine Cyanomethyl Ester (5)**. To a solution containing 100 mg (0.97 mmol) of **4** and 245 mg (2.91 mmol) of $NaHCO_3$ in 5 mL of 1:1 dioxane–H_2O was added 216 mg (1.10 mmol) of 4-pentenoyloxysuccinimide.[15,16] The reaction mixture was stirred at room temperature for 24 h under argon. The reaction was quenched by the addition of 15 mL of 1 N aq $NaHSO_4$ and the aqueous layer was extracted with two 25-mL portions of ethyl acetate. The combined organic extract was dried (anhydrous $MgSO_4$) and concentrated under diminished pressure. The crude product was then dissolved in 5 mL of acetonitrile. To this

solution were added 600 µL (435 mg, 4.30 mmol) of triethylamine and 260 µL (309 mg, 4.09 mmol) of chloroacetonitrile.[1] The reaction mixture was stirred at room temperature for 24 h at which time 20 mL of ethyl acetate was added. The organic layer was washed with 10 mL of 1 N aq $NaHSO_4$ followed by 10 mL of brine, dried (anhydrous $MgSO_4$) and concentrated to dryness under diminished pressure, affording a crude residue. The crude product was purified by flash silica gel column chromatography (15 × 2 cm) using 1:1 ethyl acetate–hexanes for elution to obtain **5** as colorless oil: yield 119 mg (55%); R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.23 (d, 3H, J = 6.8 Hz), 2.23 (t, 2H, J = 6.4 Hz), 2.35 (q, 2H, J = 6.8 Hz), 2.60 (d, 2H, J = 5.6 Hz), 4.32-4.39 (m, 1H), 4.71 (s, 2H), 4.97-5.07 (m, 2H), 5.74-5.81 (m, 1H) and 5.89 (br d, 1H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 19.9, 29.5, 35.7, 39.6, 42.0, 48.3, 114.3, 115.6, 136.9, 169.9 and 171.7; mass spectrum (APCI), *m/z* 225.1243 $(M + H)^+$ ($C_{11}H_{17}N_2O_3$ requires *m/z* 225.1239).

***N*-(4-Pentenoyl)-3*S*-methyl-*β*-alanyl-pdCpA (7)**. To a 1.5-mL Eppendorf tube containing 5.30 mg (3.70 µmol) of the tris(tetrabutylammonium) salt of pdCpA[2] (**6**) was added 10.0 mg (44.6 µmol) of **5** dissolved in 100 µL of 9:1 DMF–Et_3N. After 2 h of sonication, the reaction mixture was purified by C_{18} reversed phase HPLC (250 × 10 mm) using a gradient of 1%→65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 45 min. The fractions eluting at 13.0 and 13.6 min were collected, combined and lyophilized to afford **7** as a colorless solid: yield 3.0 mg (100%); mass spectrum (ESI), *m/z* 802.1974 $(M - H)^-$ ($C_{28}H_{38}N_9O_{15}P_2$ requires *m/z* 802.1963).

***N*-(4-Pentenoyl)-2*R*-methyl-*β*-alanine Cyanomethyl Ester (12).** To a solution containing 100 mg (0.97 mmol) of **1** and 245 mg (2.91 mmol) of $NaHCO_3$ in 5 mL of 1:1 dioxane–H_2O was added 216 mg (1.10 mmol) of 4-pentenoyloxysuccinimide.[16] The reaction mixture was stirred at

room temperature for 24 h under argon. The reaction was quenched by the addition of 15 mL of 1 N aq $NaHSO_4$ and the aqueous layer was extracted with two 25-mL portions of ethyl acetate. The combined organic extract was dried (anhydrous $MgSO_4$) and concentrated under diminished pressure. The crude product was then dissolved in 5 mL of acetonitrile. To this solution were added 600 µL (435 mg, 4.40 mmol) of triethylamine and 260 µL (309 mg, 4.09 mmol) of chloroacetonitrile. The reaction mixture was stirred at room temperature for 24 h at which time 20 mL of ethyl acetate was added. The organic layer was washed with 10 mL of 1 N aq $NaHSO_4$ followed by 10 mL of brine, dried over (anhydrous $MgSO_4$) and concentrated to dryness under diminished pressure, affording a crude residue. The crude product was purified by flash silica gel column chromatography (15 × 2 cm) using 1:1 ethyl acetate–hexanes for elution to obtain **12** as colorless oil: yield 108 mg (50%); R_f 0.5 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.16 (d, 3H, J = 7.2 Hz), 2.20-2.24 (m, 2H), 2.29-2.34 (m, 2H), 2.75-2.80 (m, 1H), 3.26-3.33 (m, 1H), 3.43-3.49 (m, 1H), 4.70 (s, 2H), 4.94-5.03 (m, 2H), 5.72-5.79 (m, 1H) and 6.16 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.5, 29.5, 35.6, 39.3, 41.6, 48.6, 114.5, 115.6, 136.9, 172.8 and 173.9; mass spectrum (APCI), *m/z* 225.1242 $(M + H)^+$ ($C_{11}H_{17}N_2O_3$ requires *m/z* 225.1239).

***N*-(4-Pentenoyl)-2*R*-methyl-*β*-alanyl-pdCpA (13).** To a 1.5-mL Eppendorf tube containing 5.30 mg (3.70 µmol) of the tris(tetrabutylammonium) salt of pdCpA (**6**) was added 10.0 mg (44.6 µmol) of **12** dissolved in 100 µL of 9:1 DMF–Et_3N. After 2 h of sonication, the reaction mixture was purified by C_{18} reversed phase HPLC (250 × 10 mm) using a gradient of 1%→65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 45 min. The fraction eluting at 13.9 min was collected, combined and lyophilized to afford **13** as a colorless solid: yield 3.0 mg (100%); mass spectrum (ESI), *m/z* 802.1937 $(M - H)^-$ ($C_{28}H_{38}N_9O_{15}P_2$ requires *m/z* 802.1963).

***N*-(4-Pentenoyl)-2*S*-methyl-*β*-alanine Cyanomethyl Ester (15).** To a solution containing 100 mg (0.97 mmol) of **2** and 245 mg (2.91 mmol) of $NaHCO_3$ in 5 mL of 1:1 dioxane–H_2O was added 216 mg (1.10 mmol) of 4-pentenoyloxysuccinimide. The reaction mixture was stirred at room temperature for 24 h under argon. The reaction was quenched by the addition of 15 mL of 1 N aq $NaHSO_4$ and the aqueous layer was extracted with two 25-mL portions of ethyl acetate. The combined organic extract was dried (anhydrous $MgSO_4$) and concentrated under diminished pressure. The crude product was then dissolved in 5 mL of acetonitrile. To this solution were added 600 µL (435 mg, 4.30 mmol) of triethylamine and 260 µL (309 mg, 4.09 mmol) of chloroacetonitrile. The reaction mixture was stirred at room temperature for 24 h at which time 20 mL of ethyl acetate was added. The organic layer was washed with 10 mL of 1 N aq $NaHSO_4$ followed by 10 mL of brine, dried (anhydrous $MgSO_4$) and concentrated to dryness under diminished pressure, affording a crude residue. The crude product was purified by flash silica gel column chromatography (15 × 2 cm) using 1:1 ethyl acetate–hexanes for elution to obtain **15** as colorless oil: yield 140 mg (65%); R_f 0.5 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.15 (d, 3H, J = 7.2 Hz), 2.19-2.31 (m, 4H), 2.74-2.77 (m, 1H), 3.29-3.32 (m, 1H), 3.41-3.44 (m, 1H), 4.69 (s, 2H), 4.93-5.02 (m, 2H), 5.71-5.78 (m, 1H) and 6.23 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.5, 29.5, 35.6, 39.3, 41.6, 48.6, 114.5, 115.6, 136.9, 172.8 and 173.9; mass spectrum (APCI), *m/z* 225.1239 $(M + H)^+$ ($C_{11}H_{17}N_2O_3$ requires *m/z* 225.1239).

***N*-(4-Pentenoyl)-2*S*-methyl-*β*-alanyl-pdCpA (16).** To a 1.5-mL Eppendorf tube containing 5.30 mg (3.70 µmol) of the tris(tetrabutylammonium) salt of pdCpA (**6**) was added 10.0 mg (44.6 µmol) of **15** dissolved in 100 µL of 9:1 DMF–Et_3N. After 2 h of sonication, the reaction mixture was purified by C_{18} reversed phase HPLC (250 × 10 mm) using a gradient of 1%→65%

acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 45 min. The fraction eluting at 13.9 min was collected, combined and lyophilized to afford **16** as a colorless solid: yield 3.0 mg (100%); mass spectrum (ESI), *m/z* 802.1945 $(M - H)^-$ ($C_{28}H_{38}N_9O_{15}P_2$ requires *m/z* 802.1963).

***N*-(4-Pentenoyl)-3*R*-methyl-*β*-alanine Cyanomethyl Ester (18).** To a solution containing 100 mg (0.97 mmol) of **3** and 245 mg (2.91 mmol) of $NaHCO_3$ in 5 mL of 1:1 dioxane–H_2O was added 216 mg (1.10 mmol) of 4-pentenoyloxysuccinimide. The reaction mixture was stirred at room temperature for 24 h under argon. The reaction was quenched by the addition of 15 mL of 1 N aq $NaHSO_4$ and the aqueous layer was extracted with two 25-mL portions of ethyl acetate. The combined organic extract was dried (anhydrous $MgSO_4$) and concentrated under diminished pressure. The crude product was then dissolved in 5 mL of acetonitrile. To this solution were added 600 µL (433 mg, 4.30 mmol) of triethylamine and 260 µL (309 mg, 4.09 mmol) of chloroacetonitrile. The reaction mixture was stirred at room temperature for 24 h at which time 20 mL of ethyl acetate was added. The organic layer was washed with 10 mL of 1 N aq $NaHSO_4$ followed by 10 mL of brine, dried (anhydrous $MgSO_4$) and concentrated to dryness under diminished pressure, affording a crude residue. The crude product was purified by flash silica gel column chromatography (15 × 2 cm) using 1:1 ethyl acetate–hexanes for elution to obtain **18** as colorless oil: yield 103 mg (48%); R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.23 (d, 3H, J = 6.8 Hz), 2.23 (t, 2H, J = 7.2 Hz), 2.35 (q, 2H, J = 7.2 Hz), 2.60 (d, 2H, J = 5.6 Hz), 4.32-4.39 (m, 1H), 4.70 (s, 2H), 4.97-5.07 (m, 2H) and 5.74-5.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 19.9, 29.5, 35.7, 39.6, 42.0, 48.3, 114.3, 115.6, 136.9, 170.0 and 171.7; mass spectrum (APCI), *m/z* 225.1239 $(M + H)^+$ ($C_{11}H_{17}N_2O_3$ requires *m/z* 225.1239).

***N*-(4-Pentenoyl)-3*R*-methyl-*β*-alanyl-pdCpA (19).** To a 1.5-mL Eppendorf tube containing 5.30 mg (3.70 μmol) of the tris(tetrabutylammonium) salt of pdCpA (**6**) was added 10.0 mg (44.6 μmol) of **18** dissolved in 100 μL of 9:1 DMF–Et_3N. After 2 h of sonication, the reaction mixture was purified by C_{18} reversed phase HPLC (250 × 10 mm) using a gradient of 1%→65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 45 min. The fractions eluting at 13.0 and 13.6 min were collected, combined and lyophilized to afford **19** as a colorless solid: yield 3.0 mg (100%); mass spectrum (ESI), *m/z* 802.1971 $(M - H)^-$ ($C_{28}H_{38}N_9O_{15}P_2$ requires *m/z* 802.1963).

Synthesis of β^2-Puromycin

2*R*-Benzyl-*β*-alanylpuromycin Aminonucleoside (11). To a solution containing 20.0 mg (0.05 mmol) of **9** in 2 mL of freshly distilled THF at 0 ^oC was added 12.4 mg (0.06 mmol) of DCC followed by 6.90 mg (0.06 mmol) of *N*-hydroxysuccinimide. The reaction mixture was stirred at room temperature for 24 h and then filtered. The filtrate was concentrated under diminished pressure and residue was dissolved in 500 μL of DMF. To this solution was added 22.3 μL (16.2 mg, 0.16 mmol) of Et_3N followed by 14.0 mg (0.05 mmol) of puromycin aminonucleoside (**10**). The reaction mixture was sonicated in a water bath for 2 h and 100 μL of piperidine was added. The resulting mixture was sonicated for 1 h, diluted with 4 mL of 2:1 acetonitrile–water and filtered. The filtrate was purified by C_{18} reversed phase HPLC (250 × 10 mm) using a gradient of 15% to 50% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 25 min. The fraction eluting at 23.5 min was collected and lyophilized to afford **11** as a white solid: yield 6.80 mg (30%); mass spectrum (ESI), *m/z* 454.2205 $(M - H)^-$ ($C_{22}H_{28}N_7O_4$ requires *m/z* 454.2203).

REFERENCES

(1) Robertson, S. A., Ellman, J. A., and Schultz, P. G. (1991) A general and efficient route for chemical aminoacylation of transfer RNAs. *J. Am. Chem. Soc. 113*, 2722–2729.

(2) Robertson, S. A., Noren, C. J., Anthony-Cahill, S. J., Griffith, M. C., and Schultz, P. G. (1989) The use of 5'-phospho-2 deoxyribocytidylylriboadenosine as a facile route to chemical aminoacylation of tRNA. *Nucleic Acids Res. 17*, 9649–9660.