Electronic Supplementary Information

Modeling the reactive properties of tandemly activated tRNAs

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Experimental

General

Reagents and solvents were purchased from Aldrich Chemical Co. or Sigma Chemical Co. and used without further purification. Anhydrous grade methylene chloride, THF, DMF and acetonitrile were purchased from VWR Scientific. All reactions involving air or moisture-sensitive reagents or intermediates were performed under an argon atmosphere. Flash column chromatography was performed using Silicycle 40-60 mesh silica gel. Analytical TLC was performed using 0.25 mm EM silica gel 60 F_{250} plates that were visualized by irradiation (254 nm) or by staining with Hanessian's stain (cerium molybdate). ^{1}H and ^{13}C NMR spectra were obtained using a 300 MHz Varian instrument. Chemical shifts are reported in parts per million (ppm, δ) referenced to the residual ^{1}H resonance of the solvent ($CDCl_3$, δ 7.26; CD_3OD, δ 3.31). ^{13}C spectra were referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, δ 77.3; DMSO-d_6, δ 39.5). Splitting patterns are designated as follows: s, singlet; br, broad; d, doublet; dd,

doublet of doublets; t, triplet; q, quartet; m, multiplet. High resolution mass spectra were obtained at the Michigan State University–NIH Mass Spectrometry Facility. Phosphoroimager analysis was performed using a Molecular Dynamics 300E Phosphorimager equipped with Image Quant software. HPLC was performed using a Varian 9012 pump coupled with a Varian 2050 UV detector and an Alltech Alltima RPC_{18} column (250 x 10 mm, 5 μm for semipreparative HPLC and 250 x 4.6 mm, 5 μm for analytical HPLC). The tetra-*n*-butylammonium (TBA) salt of pdCpA derivatives was prepared using Dowex 50Wx8, 200-400 mesh in its TBA form. The ion exchange resin (5 g) was washed with water, then stirred with a mixture of 40 mL of 20% aqueous tetra-*n*-butylammonium solution for 1 h. A column was then packed with this resin, then washed extensively with H_2O until the pH was neutral. Ten mg of pdCpA was dissolved in 500 μL of H_2O and washed through this column. The appropriate fractions were then lyophilized and the resulting salt was obtained in quantitative yield.

Mono-2'(3')-*O*-alanyl-pdCpA (4)

To a conical vial containing 1 mg (1.27 μmol) of *N*-(4-pentenoyl)-alanyl-pdCpA (**8**)[4] in 50 μL of H_2O was added 76.2 μL (7.62 μmol, 6 eq.) of 100 mM I_2 in THF. After 1 h the reaction was quenched by the addition of 50 μL of 0.1 N aq $Na_2S_2O_3$ and analyzed, then purified, by semipreparative C_{18} reversed phase column (250 x 10 mm). The column was washed with 0 → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). After lyophilization of the appropriate fractions, compound **4** was obtained as a colorless solid (retention times 13.5 and 13.8

min, for the two positional (2',3') isomers): yield 0.6 mg (66%); mass spectrum (MALDI-TOF), *m/z* 706.1370 $(M-H)^-$ ($C_{22}H_{30}N_9O_{14}P_2$ requires 706.1387).

Bis-2',3'-*O*-(alanyl)-pdCpA (1)

To a conical vial containing 1.1 mg (1.17 μmol) of bis-2',3'-*O*-[*N*-(4-pentenoyl)-*S*-alanyl]-pdCpA (**9**)[4] in 50 μL of H_2O was added 70.3 μL (7.03 μmol, 6 eq.) of 100 mM I_2 in THF. After 1 h the reaction was quenched by the addition of 50 μL of 0.1 N $Na_2S_2O_3$ and analyzed, then purified, by HPLC on a semipreparative C_{18} reversed phase column (250 x 10 mm). The column was washed with 0 → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). After lyophilization of the appropriate fractions, compound **1** was obtained as a colorless solid (retention time 17.3 min): yield 0.7 mg (77%); mass spectrum (MALDI-TOF), *m/z* 777.1784 $(M-H)^-$ ($C_{25}H_{35}N_{10}O_{15}P_2$ requires 777.1759).

***N*-Acetyl-*S*-alanine cyanomethyl ester (11)**

N-acetyl-*S*-alanine (**10**) (500 mg, 3.82 mmol) was dissolved in 5 mL of anhydrous acetonitrile and 1.20 mL (19.1 mmol, 5 eq) of chloroacetonitrile was added followed by 2.70 mL (19.1 mmol) of triethylamine. The reaction mixture was stirred at room temperature overnight, then diluted with 75 mL of ethyl acetate and washed with 30 mL of 1 N aq $NaHSO_4$, dried ($MgSO_4$), and concentrated under diminished pressure. Purification by flash chromatography on a silica gel column (15 x 2 cm), elution with 2:1 hexanes–ethyl acetate, gave **11** as a colorless solid: yield 508 mg (78%); ^1H NMR ($CDCl_3$) δ 1.40 (d, 3H, *J* = 7.5 Hz), 1.98 (s, 3H), 4.56 (q, 1H, *J* = 7.2 Hz), 4.74 (d, 2H)

and 6.56 (d, 1H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 17.7, 23.0, 48.1, 49.3, 114.4, 170.5 and 172.0; mass spectrum (FAB), *m/z* 171.0771 ($M+H^+$) ($C_7H_{11}N_2O_3$ requires 171.0770).

Propionic acid cyanomethyl ester (13)

Propionic acid (**12**) (0.500 mL, 6.73 mmol) was dissolved in 10 mL of anhydrous acetonitrile and 2.11 mL (33.6 mmol, 5 eq.) of chloroacetonitrile was added followed by 4.68 mL (33.6 mmol) of triethylamine. The reaction mixture was stirred at room temperature overnight, then diluted with 75 mL of ethyl acetate and washed with 30 mL of 1 N aq $NaHSO_4$, dried ($MgSO_4$), and concentrated under diminished pressure. Purification by flash chromatography on a silica gel column (15 x 2 cm), elution with 2:1 hexanes–ethyl acetate, gave **13** as a colorless oil: yield 760 mg (100%); ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 3H, J = 7.6 Hz), 2.38 (q, 2H, J = 7.8, 7.5 Hz) and 4.67 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 8.87, 27.0, 48.5, 114.9 and 173.1; mass spectrum (FAB), *m/z* 113.0475 (M^+) ($C_5H_7NO_2$ requires 113.0477).

2'-*O*-(*N*-Acetylalanyl)-3'-O-(N-(4-pentenoyl)alanyl)-pdCpA (14)

To a conical vial containing 5.63 mg (33.1 μmol) of *N*-acetyl-*S*-alanine cyanomethyl ester (**11**) was added a solution of 5 mg (3.31 μmol) of the tetrabutylammonium salt of *N*-(4-pentenoyl)-*S*-alanyl-pdCpA (**8**) in 100 μL of anhydrous DMF, followed by 20 μL of triethylamine. The reaction mixture was stirred at 25 °C and monitored by HPLC. After 3 days, a 5-μL aliquot of the reaction mixture was diluted with 45 μL of 1:2 CH_3CN–50 mM NH_4OAc, pH 4.5. Twenty μL of the diluted aliquot was analyzed by HPLC on a C_{18} reversed phase column (250 x 10 mm). The entire reaction mixture was then diluted with

400 μL of 1:2 CH_3CN–50 mM NH_4OAc, pH 4.5, and purified by HPLC under the same conditions described above. After lyophilization of the appropriate fractions, compound **14** was obtained as a colorless solid (retention time 21.3 min): yield 1.01 mg (34%); mass spectrum (MALDI-TOF), *m/z* 901.2330 $(M-H)^-$ ($C_{32}H_{43}N_{10}O_{17}P_2$ requires 901.2283).

2'-*O*-(*N*-Acetylalanyl)-3'-*O*-(alanyl)-pdCpA (2)

To a conical vial containing 1.01 mg (1.12 μmol) of 2'-*O*-(*N*-acetylalanyl)-3'-*O*-(*N*-(4-pentenoyl)alanyl)-pdCpA (**14**) in 50 μL of H_2O was added 67.2 μL (6.72 μmol, 6 eq.) of 100 mM I_2 in THF. After 1 h the reaction was quenched by the addition of 50 μL of 0.1 N $Na_2S_2O_3$ and analyzed, then purified, by HPLC on a semipreparative C_{18} reversed phase column (250 x 10 mm). The column was washed with 0 → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). After lyophilization of the appropriate fractions compound **2** was obtained as a colorless solid (retention time 19.6 min): yield 0.2 mg (25%); mass spectrum (MALDI-TOF), *m/z* 818.4 $(M-2H)^-$ (theoretical 818.2).

2'-*O*-Propionyl-3'-*O*-(*N*-(4-pentenoyl)alanyl)-pdCpA (15)

To a conical vial containing 3.74 mg (33.1 μmol) of propionic acid cyanomethyl ester (**13**) was added a solution of 5 mg (3.31 μmol) of the tetra-*n*-butylammonium salt of *N*-(4-pentenoyl)alanyl-pdCpA (**8**) in 100 μL of anhydrous DMF, followed by 20 μL of triethylamine. The reaction mixture was stirred at 25 °C and monitored by HPLC. After 3 days, a 5-μL aliquot of the reaction mixture was diluted with 45 μL of 1:2 CH_3CN–50 mM NH_4OAc, pH 4.5. Twenty μL of the diluted aliquot was analyzed by HPLC on a C_{18}

reversed phase column (250 x 10 mm). The entire reaction mixture was then diluted with 400 µL of 1:2 CH_3CN–50 mM NH_4OAc, pH 4.5 and purified by HPLC under the same conditions described above. After lyophilization of the appropriate fractions, compound **15** was obtained as a colorless solid (retention time 20.3 min): yield 0.6 mg (21%); mass spectrum (MALDI-TOF), *m/z* 844.2023 $(M-H)^-$ ($C_{30}H_{40}N_9O_{16}P_2$ requires 844.2068).

2'-*O*-(Propionyl)-3'-*O*-(alanyl)-pdCpA (3)

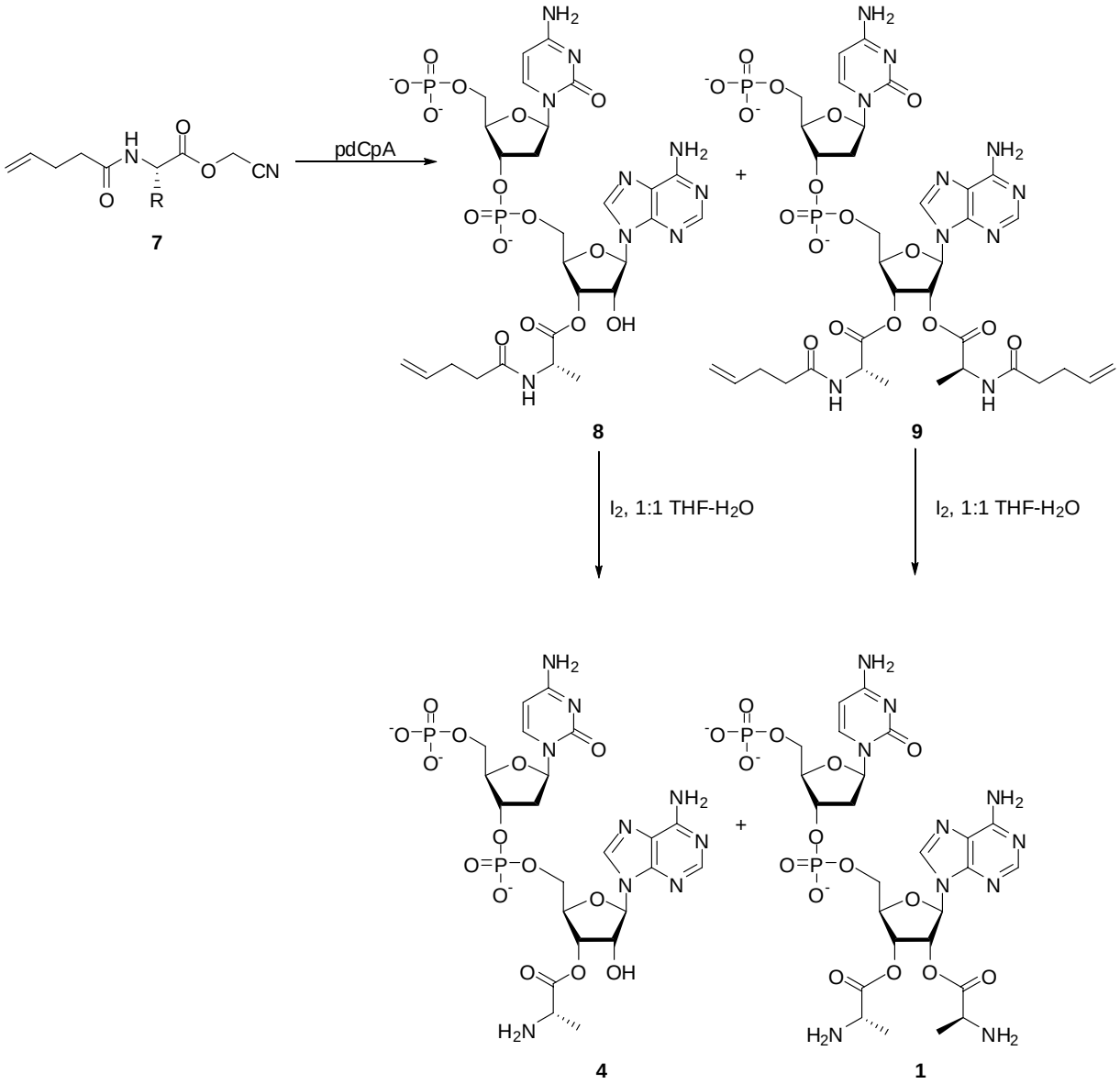
To a conical vial containing 0.6 mg (0.71 µmol) of 2'-*O*-(propionyl)-3'-*O*-(*N*-(4-pentenoyl)alanyl)-pdCpA (**15**) in 50 µL of H_2O was added 42.7 µL (4.27 µmol, 6 eq.) of 100 mM I_2 in THF. After 1 h the reaction was quenched by the addition of 50 µL of 0.1 N aq $Na_2S_2O_3$ and analyzed, then purified, by HPLC on a semipreparative C_{18} reversed phase column (250 x 10 mm). The column was washed with 0 → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). After lyophilization of the appropriate fractions, compound **3** was obtained as a colorless solid (retention time 18.7 min): yield 0.2 mg (34%); mass spectrum (MALDI-TOF), *m/z* 762.5 $(M-H)^-$ (theoretical 762.2).

2'(3')-*O*-(*N*-Acetylalanyl)-pdCpA (5) and bis-2',3'-*O*-(*N*-acetylalanyl)-pdCpA (6)

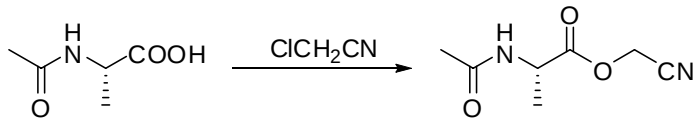
To a conical vial containing 6.24 mg (36.7 µmol) of *N*-acetyl-*S*-alanine cyanomethyl ester (**11**) was added a solution of 5 mg of the tetra-*n*-butylammonium salt of pdCpA in 100 µL of DMF. The reaction mixture was stirred at room temperature. After 24 h the reaction mixture was diluted with 1:2 CH_3CN–50 mM NH_4OAc, pH 4.5, to a total volume of 500 µL and purified by HPLC using a semi-preparative C_{18} reversed phase

column (250 x 10 mm). The column was washed with 0 → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). After lyophilization of the appropriate fractions, compound **5** was obtained as a colorless solid (retention time 16.5 min): yield 1.3 mg (47%); mass spectrum (MALDI-TOF), *m/z* 748.1496 $(M-H)^-$ ($C_{24}H_{32}N_9O_{15}P_2$ requires 748.1493), while compound **6** was obtained as a colorless solid (retention time 18.6 minutes): yield 1.2 mg (38%); mass spectrum (MALDI-TOF), *m/z* 861.1 $(M-H)^-$ (theoretical 861.2).

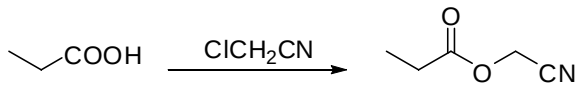
Scheme 1. Synthesis of mono-2′(3′)-*O*-alanyl-pdCpA (**4**) and bis-2′,3′-*O*-alanyl-pdCpA (**1**).



Scheme 2. Synthesis of *N*-acetyl-*S*-alanine cyanomethyl ester (**11**) and propionic acid cyanomethyl ester (**13**).

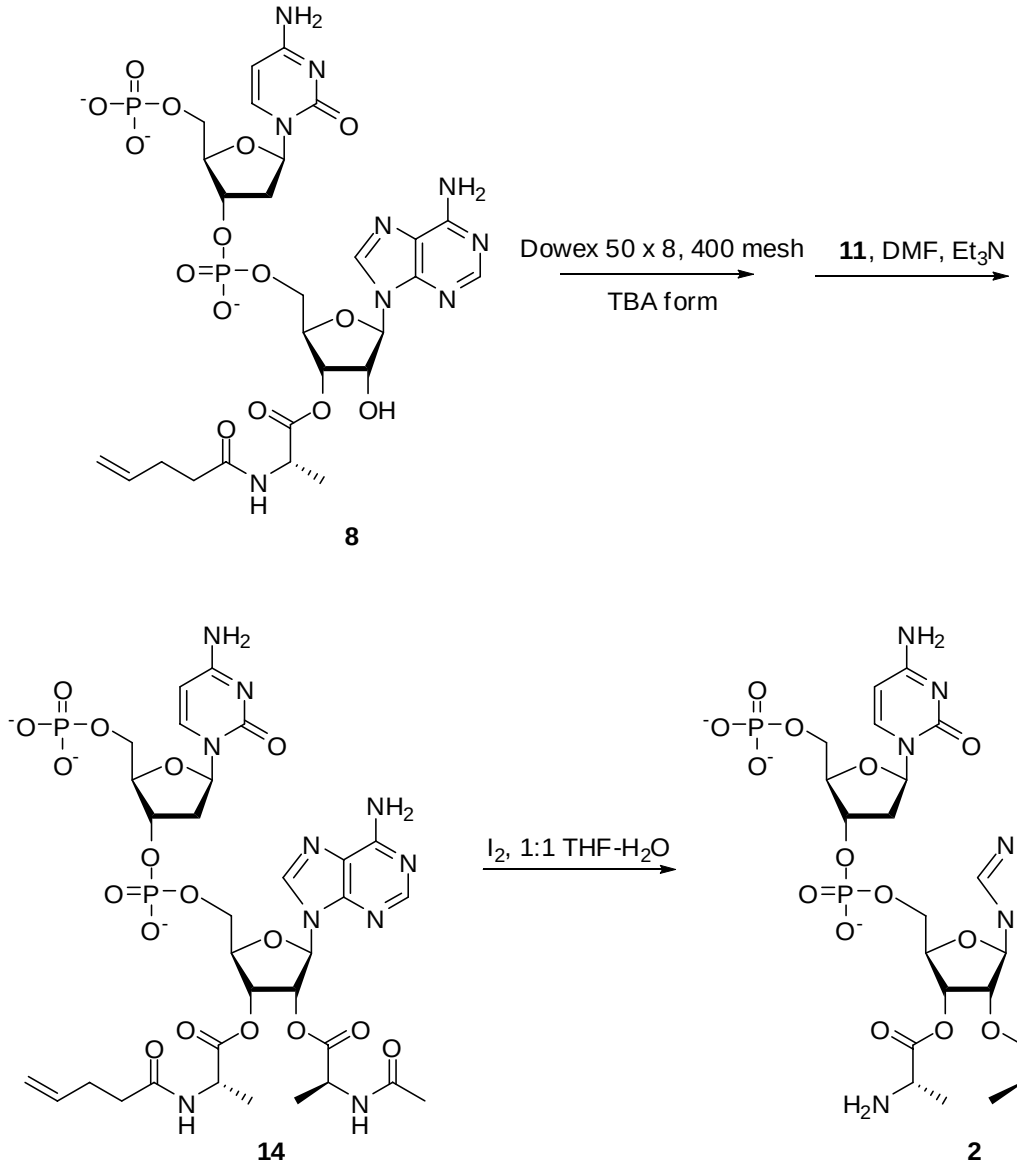


10 **11**



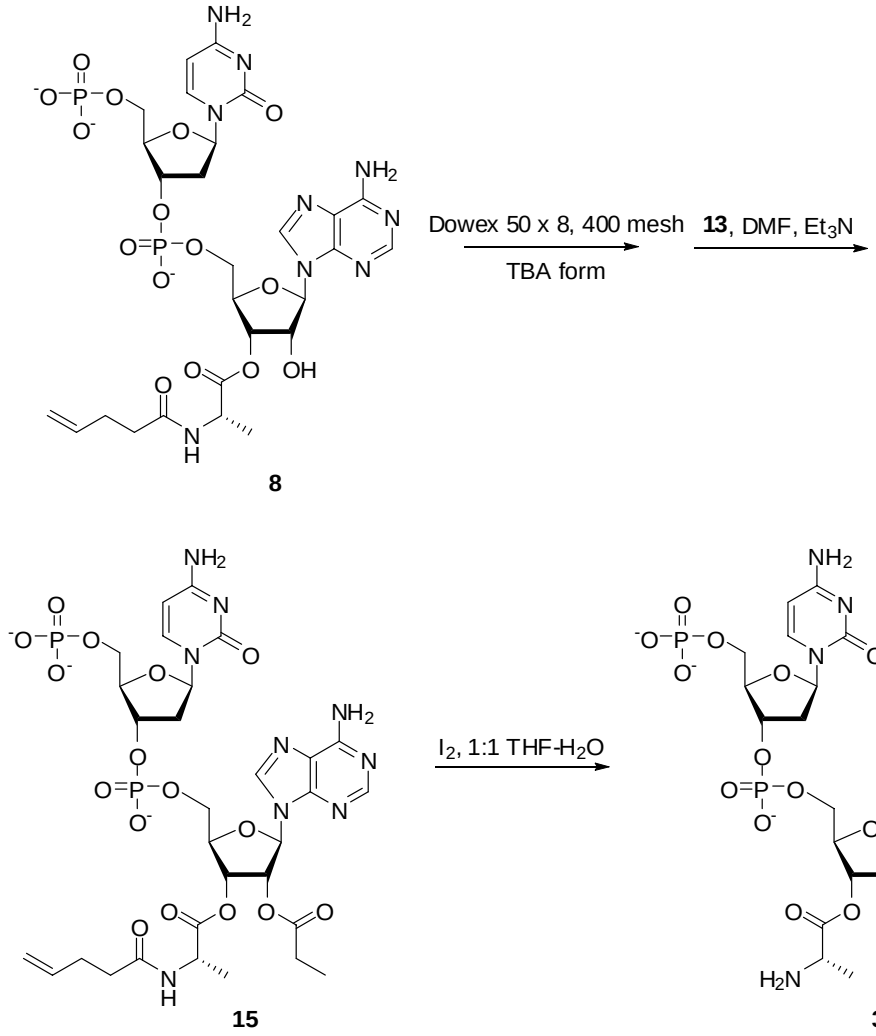
12 **13**

Scheme 3. Synthesis of 2′-*O*-(*N*-acetylalanyl)-3′-*O*-(alanyl)-pdCpA (**2**).



8 **14** **2**

Scheme 4. Synthesis of 2′-*O*-(propionyl)-3′-*O*-(alanyl)-pdCpA (**3**).



8 **15** **3**

Scheme 5. Synthesis of 2′(3′)-*O*-(*N*-acetylalanyl)-pdCpA (5) and bis-2',3'-*O*-(*N*-acetylalanyl)-pdCpA (**6**).