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Bis(2-nitrobenzyl) Vinylphosphonate (5). Crude dichloride **4**[1] (620 mg, 2.75 mmol) was dissolved in 25 mL of pyridine at 0 °C and treated with a catalytic amount (~ 10 mg) of 4-dimethylaminopyridine and 1.40 g (9.15 mmol) of 2-nitrobenzyl alcohol. The resulting solution was stirred overnight at 25 °C and then treated with 200 mL of ethyl acetate. The solution was washed with 50 mL of saturated $NaHCO_3$ solution and then with 50 mL of saturated NaCl. The organic phase was dried ($MgSO_4$) and then concentrated under diminished pressure. The residue was purified by flash chromatography[2] on a silica gel column (40 x 3 cm); elution with 1:1 hexane-ethyl acetate afforded bis(2-nitrobenzyl) vinylphosphonate (**5**) as a colorless oil: yield 400 mg (36% for two steps); silica gel TLC R_f 0.22 (1:1 hexanes-ethyl acetate); ^1H NMR ($CDCl_3$) δ 5.52 (d, 4H, J = 7.5 Hz), 6.28 (m, 3H), 7.51 (m, 2H), 7.68 (m, 4H), and 8.07 (m, 2H); mass spectrum (FAB), *m/z* 379; mass spectrum (FAB), *m/z* 379.069 ($C_{16}H_{16}N_2O_7P$ requires 379.069).

Ethyl 2-[(Diphenylmethylene)amino]-4-bis(2-nitrobenzylphosphono)butanoate (6). Vinylphosphonate **5** (350 mg, 0.92 mmol) was dissolved in 5 mL of THF and the resulting solution was added slowly to a solution containing ethyl (N-diphenylmethylene)glycine (347 mg, 1.3 mmol) and 281 mg (1.48 mmol) of potassium bis(trimethylsilyl)amide in 15 mL of THF at -78 °C. The combined solution was stirred at -78 °C for an additional 3 h and then permitted to warm to 25 °C. Ethyl acetate (150 mL) was added and the combined solution was washed successively with 50-mL portions of saturated $NaHCO_3$ and saturated NaCl solutions. The organic phase was dried (MgSO4) and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (40 x 3 cm); elution with 2:1 ethyl acetate-hexane afforded ethyl 2-[(diphenylmethylene)amino]-4-bis-(2-nitrobenzylphosphono)butanoate (**6**) as a colorless oil: yield 240 mg (40%); silica gel TLC R_f 0.70 (ethyl acetate); ^1H NMR ($CDCl_3$) δ1.25 (t, 3H, J = 7.5 Hz), 2.06 (m, 2H), 2.27 (m, 2H), 4.12 (m, 3H), 5.47 (m, 4H), 7.16 (m, 2H), 7.50 (m, 14H) and 8.09

(m, 2H); mass spectrum (FAB), *m/z* 646; mass spectrum (FAB), *m/z* 646.194 ($C_{33}H_{33}N_3O_9P$ requires 646.195).

Ethyl 2-Amino-4-bis(2-nitrobenzylphosphono)butanoate (7). Protected amino acid derivative **6** (210 mg, 0.325 mmol) was dissolved in 10 mL of ether and the solution was treated with 2 mL of 1 N HCl. The reaction mixture was stirred at 25 °C for 30 min and the pH of the aqueous phase was then adjusted to 4 by the addition of 1N NaOH. The reaction mixture was then treated with 30 mL of 1 N $NaHSO_4$ and the aqueous phase was extracted with ethyl acetate. The combined organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. The residue was precipitated from ethyl acetate-hexane to afford **7** as a white powder: yield 72 mg (46%); silica gel TLC R_f 0.10 (1:1 hexanes-ethyl acetate); ^{1}H NMR (methanol-d_4) δ 1.26 (t, 3H, J = 7.5 Hz), 2.20 (m, 4H), 4.13 (m, 1H), 4.26 (q, 2H, J = 7.5 Hz), 5.39 (m, 4H), 7.65 (m, 6H) and 8.03 (d, 2H, J = 8.5 Hz).

Ethyl 4-Bis(2-nitrobenzylphosphono)-2-[2-(nitro-veratryloxycarbonyl)amino]butanoate (8). Amino acid derivative **7** (72 mg, 0.14 mmol) was dissolved in 5 mL of acetronitrile. This solution was treated with 98 μL (0.56 mmol) of diisopropylethylamine, and then with 51 mg (0.185 mmol) of 2-nitroveratryl chloroformate in 5 mL of acetonitrile. The reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was treated with 100 mL of ethyl acetate and washed successively with 30-mL portions of saturated $NaHCO_3$ and saturated NaCl solutions. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (25 x 3 cm); elution with 3:1 ethyl acetate-hexane afforded NVOC derivative **8** as a yellow powder: yield 80 mg (74%); silica gel TLC R_f 0.40 (1:4 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.0 Hz), 1.96 (m, 3H), 2.24 (m, 1H), 3.94 (s, 3H), 3.99 (s, 3H), 4.23 (q, 2H, J = 7.0 Hz), 4.44 (m, 1H), 5.51 (m, 6H), 5.79 (d, 1H, J = 7.0 Hz), 7.03 (s, 1H) 7.50 (m, 2H), 7.70 (m, 5H) and 8.08 (d, 2H, J = 7.5 Hz); mass

spectrum (FAB), *m/z* 721; mass spectrum (FAB), *m/z* 721.173 ($C_{30}H_{34}N_4O_{15}P$ requires 721.175).

4-Bis(2-nitrobenzylphosphono)-2-[nitroveratryloxycarbonyl)amino]butanoic acid (9). Fully protected amino acid derivative **8** (80 mg, 0.11 mmol) was dissolved in 3 mL of THF and treated with 200 μL of pyridine and 1.2 mL (0.12 mmol) of 0.1 N NaOH. The reaction mixture was stirred at 25 °C for 30 min, and the pH was then adjusted to 4 by the addition of 20 mL of 1 N $NaHSO_4$ solution. The reaction mixture was extracted twice with 50-mL portions of ethyl acetate and the combined organic extract was dried ($MgSO_4$) and concentrated. The residue was precipitated from ethyl acetate-hexane, affording 4-bis(2-nitrobenzylphosphono)-2-[2-(nitroveratryloxycarbonyl)amino]butanoic acid (**9**) as a white powder: yield 70 mg (92%); silica gel TLC R_f 0.1 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.22 (m, 4H), 3.93 (s, 3H), 3.96 (s, 3H), 4.49 (m, 1H), 5.50 (m, 6H), 5.92 (d, 1H, J = 7.5 Hz), 7.02 (s, 1H), 7.51 (m, 2H), 7.69 (m, 5H), and 8.10 (m, 2H); mass spectrum (FAB), *m/z* 693; mass spectrum (FAB), *m/z* 693.145 ($C_{28}H_{30}N_4O_{15}P$ requires 693.144).

Cyanomethyl 4-Bis(2-nitrobenzylphosphono)-2-[2-(nitroveratryloxycarbonyl)amino]butanoate (10). Amino acid derivative **9** (70 mg, 101 μmol) was dissolved in 3 mL of CH_3CN and treated with 70 μL (407 μmol) of diisopropylethylamine followed by 50 μL (115 mg, 0.69 mmol) of iodoacetonitrile. The reaction mixture was stirred at 25 °C for 12 h and then treated with 50 mL of ethyl acetate. The organic phase was washed successively with 15-mL portions of saturated $NaHCO_3$ and saturated NaCl solutions, then dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (15 x 2 cm). Elution with ethyl acetate afforded cyanomethyl ester **10** as a white powder: yield 52 mg (70%); silica gel TLC R_f 0.66 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 3H), 2.32 (m, 1H), 3.95 (s, 3H), 4.00 (s, 3H), 4.40 (m, 1H), 4.76 (d, 1H, J = 16 Hz), 4.85 (d, 1H, J = 16 Hz), 5.50 (m, 6H), 5.90 (d, 1H,

J = 8.5 Hz), 7.02 (s, 1H), 7.51 (m, 2H), 7.70 (m, 5H) and 8.09 (m, 2H); mass spectrum (FAB), *m/z* 732, mass spectrum (FAB), *m/z* 732.153 ($C_{30}H_{31}N_5O_{15}P$ requires 732.155).

4-Bis(2-nitrobenzylphosphono)-2-[2-nitroveratryloxycarbomyl)amino]butanoic Acid pdCpA Ester (11). A solution of the tris(tetrabutylammonium) salt of pdCpA[3] (4.1 mg, 3 μmol) dissolved in 50 μL of freshly distilled DMF was added to a conical, flame-dried vial containing activated ester **10** (20 mg, 27 μmol). The reaction mixture was stirred at μm 25 °C and monitored by hplc. Four-μL aliquots were removed periodically and diluted with 40 μL of 50 mM NH_4OAc, pH 4.5; 10 μL of each aliquot was analyzed on a 3 μm, C_{18} reverse phase column (100 x 4.6 mm). The column was washed with 1 → 65% CH_3 CN in 50 mM NH_4OAc, pH 4.5, over 45 min at a flow rate of 1 mL/min (monitoring at 260 nm); the desired product had a retention time of 28.7 min. After 1 h, the reaction mixture was diluted with 600 μL of 1:1 CH_3CN-50 mM NH_4OAc, pH 4.5, and then separated by C18 reverse phase hplc on a semi-preparative column (250 x 10 mm) using the same gradient described above at a flow rate of 4 mL/min. Fully protected dinucleotide derivative **11** was recovered from the relevant fractions by lyophilization as a white solid: yield 3.2 mg (80%); mass spectrum (ESI, negative mode), *m/z* 1330.

2-Amino-4-phosphonobutanoic Acid pdCpA Ester (12). The photochemical deprotection of aminoacyl-pdCpA derivative **11** was carried out following literature precedent[4] by the use of a 500 W mercury-xenon lamp using Pyrex and water filters. The deblocking of **11** was monitored by C_{18} reverse phase hplc as described above for the formation of **11**. Dinucleotide **2** had a retention time of 6.9 min in this system; the deblocking was complete within 7.5 min.

1,2,3,4,6-Penta-*O*-allyloxycarbonyl-D-glucopyranose (13). To a solution of 1.0 g (5.6 mmol) of D-glucopyranose in 15 mL of THF was added 2.63 mL (2.7 mg, 33 mmol) of pyridine and 4.3 g (33 mmol) of allyl chloroformate at 0 °C with stirring. The reaction mixture was stirred at 0 °C for 1 h, then at reflux for an additional 5 h. The

cooled reaction mixture was filtered and the filtrate was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (25 x 2 cm); elution was effected with 1:3 ethyl acetate-hexanes. 1,2,3,4,6-Penta-*O*-allyloxycarbonyl-D-glucopyranose (**13**) was isolated as a colorless oil from the appropriate fractions: yield 2.0 g (60%); silica gel TLC R_f 0.45 (2:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 4.28 (m, 3H), 4.63 (d, 10H, J = 5.5 Hz), 4.94 (dd, 1H, J = 10.5, 3.5 Hz), 5.03 (t, 1H, J = 10.5 Hz), 5.35 (m, 11H), 5.90 (m, 5H) and 6.30 (d, 1H, J = 3.5 Hz); mass spectrum (chemical ionization), *m/z* 559 $(M\text{-}C_3H_5)^+$ and 515 $(M\text{-}CO_2C_3H_5)^+$; mass spectrum (FAB), *m/z* 623.158 ($C_{26}H_{32}O_{16}Na$ requires 623.159).

2,3,4,6-Tetra-*O*-allyloxycarbonyl-α-D-glucopyranosyl Bromide (14). A solution containing 0.3 g (0.5 mmol) of 1,2,3,4,6-penta-*O*-allyloxycarbonyl-D-glucopyranose (**13**) in 5 mL of 33% HBr in glacial acetic acid was stirred at 0 °C for 4 h. The reaction mixture was then diluted with 100 mL of cold CH_2Cl_2 and partitioned against 50 mL of ice water. The organic layer was washed successively with 25-mL portions of cold $NaHCO_3$ solution, brine and water, and then dried (Na_2SO_4). Following concentration of the organic phase under diminished pressure, the residue was purified by flash chromatography on a silica gel column (25 x 2 cm); elution with 1:3 ethyl acetate-hexanes provided glucopyranosyl bromide **14** as a viscous, colorless oil: yield 0.22 g (72%); silica gel TLC R_f 0.48 (2:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 4.12 (dd, 1H, J = 14, 7 Hz), 4.35 (m, 2H), 4.64 (d, 8H, J = 5 Hz), 4.74 (dd, 1H, J = 10, 4 Hz), 5.05 (t, 1H, J = 9.5 Hz), 5.33 (m, 9H), 5.90 (m, 4H) and 6.57 (d, 1H, J = 4 Hz); mass spectrum (chemical ionization), *m/z* 581 and 579.

[*N*-(2-Nitroveratryloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-allyloxycarbonyl-α-D-glucopyranosyl)]-L-serine Cyanomethyl Ester (15). A stirred solution containing 0.05 g (0.13 mmol) of *N*-(2-nitroveratryloxycarbonyl)-L-serine cyanomethyl ester[5] in 2 mL of CH_2Cl_2 at -20 °C containing 4 Å molecular sieves was treated with 110 mg (0.4 mmol) of silver trifluoromethanesulfonate. After 15 min at -20 °C, the reaction mixture was cooled to -78 °C and treated with a cold solution containing 70

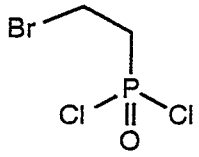
mg (0.13 mmol) of glucopyranosyl bromide **14** in 3 mL of CH_2Cl_2 with stirring. After 4 h, the stirred reaction mixture was diluted with 15 mL of CH_2Cl_2 and filtered through a layer of silica gel, which was washed with ethyl acetate. The filtrate was concentrated under diminished pressure and the residue was applied to a silica gel flash chromatography column (15 x 1 cm). Elution with 1:2 ethyl acetate-hexanes provided glucosylated serine derivative **15** as a viscous, yellow oil: yield 58 mg (68%); silica gel TLC R_f 0.39 (1:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 3.80 (m, 2H), 3.95 (s, 3H), 4.01 (s, 3H), 4.30 (m, 2H), 4.62 (m, 10H), 4.76 (m, 2H), 4.86 (d, 1H, J = 10 Hz) 4.92 (d, 1H, J = 10 Hz), 5.10 (t, 1H, J = 10 Hz), 5.30 (m, 8H), 5.47 (d, 1H, J = 15 Hz), 5.58 (d, 1H, J = 15 Hz), 5.63 (d, 1H, J = 15 Hz), 5.77 (d, 1H, J = 8.5 Hz), 5.85 (m, 4H), 7.05 (s, 1H) and 7.72 (s, 1H); mass spectrum (FAB), *m/z* 882.242 ($C_{37}H_{44}N_3O_{22}$ requires 882.242).

[*N*-(2-Nitroveratryloxycarbonyl)-*O*-(α-D-glucopyranosyl)]-L-serine pdCpA Ester (17). The reaction mixture contained 4 mg (2.75 μmol) of the tris(tetrabutylammonium) salt of pdCpA[3] and 11.6 mg (13.7 μmol) of glucosylated serine derivative **15** in 50 μL of dry DMF under argon at 25 °C. The course of the reaction was monitored by hplc. Ten-μl aliquots were removed periodically and diluted with 90 μL of 1:1 CH_3CN-50 mM NH_4OAc, pH 4.5; 10 μL of each diluted aliquot was assayed on a 3 μm, C_{18} reverse phase column (Alltech). The column was washed with 1 → 65% CH_3CN in 50 mM NH_4OAc, pH 4.5, over 45 min at a flow rate of 1 mL/min (monitoring at 260 nm); the desired product had a retention time of 28 min. After 4 h, the reaction mixture was purified on a semi-preparative C_{18} reverse phase column (10 μm) using the same gradient. The fully blocked *O*-glucosylseryl pdCpA derivative **16** was obtained as a pale yellow foam: yield 0.6 mg (14 A_{260} units); λ_{max} (H_2O) 345, 260 (sh) and 249 nm.

A suspension containing 0.6 mg of *O*-glucosylseryl pdCpA derivative **16** and 0.6 mg of palladium tetrakis(triphenylphosphine) in 50 μL of THF was treated with 2 μL (2.3 mg, 0.05 mmol) of formic acid and 5 μL (3.7 mg, 0.05 mmol) of n-butylamine. The

reaction mixture was stirred at room temperature; aliquots were removed periodically, diluted 10-fold with 1:1 CH_3 CN-50 mM NH_4OAc, pH 4.5, and assayed for removal of the allyloxycarbonyl protecting groups by C_{18} reverse phase hplc as described above. The fully *O*-deblocked product had a retention time of 20 min in this system and its formation was judged to be optimal after 12 h. The reaction mixture was purified by hplc on a semi-preparative C_{18} reverse phase column using the same conditions described above. Deblocked *O*-glucosylseryl pdCpA derivative **17** was isolated as a pale yellow foam, yield 3 A_{260} units; λ_{max} (H_2O) 345, 260 (sh) and 249 nm; ^1H NMR (D_2O) δ 1.8 (m, 2H), 2.17 (m, 2H), 3.55 (m, 2H), 3.72 (s, 3H), 3.8 (m, 3H), 3.90 (s, 3H), 3.95-4.4 (m, 11H), 5.47 (m, 2H), 6.0 (m, 4H), 7.06 (s, 0.5H), 7.60 (s, 0.5H), 7.62 (s, 1H), 7.81 (m, 1H), 7.96 (s, 1H), 8.14 (s, 0.5H) and 8.47 (s, 0.5H); mass spectrum (ESI, negative mode), *m/z* 1123.

[*O*-(α-D-Glucopyranosyl)]-L-serine pdCpA Ester. *N*-Deblocking of aminoacylated dinucleotide **16** was carried out immediately prior to ligation of the deprotected product to an abbreviated tRNA transcript. Dinucleotide **17** (0.5 A_{260} unit) was dissolved in 50 µL of 1 mM KOAc, pH 5.0, and irradiated with a 500 W mercury-xenon lamp using Pyrex and water filters. The deblocking of **17** was carried out for 2 min; formation of the desired product was verified by hplc analysis.



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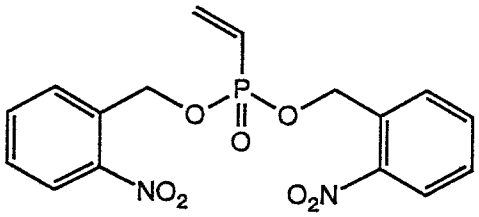

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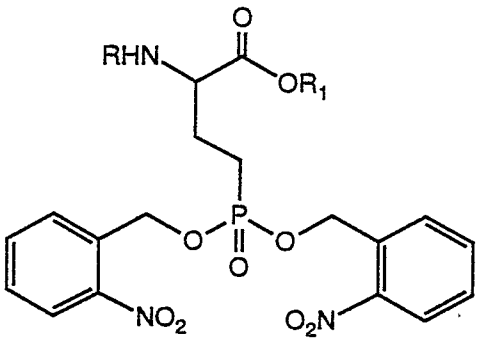
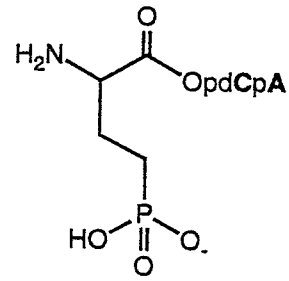




12

6 R = $(C_6H_5)_2CH=$, R_1 = Et

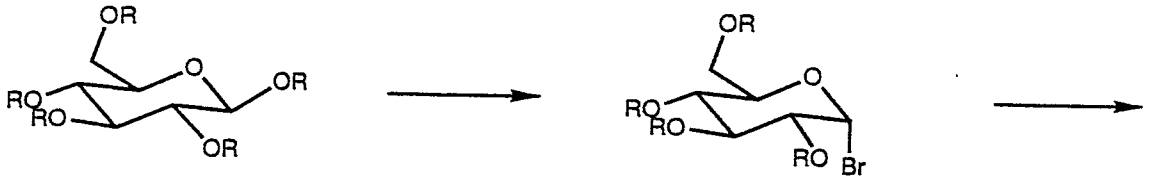
7 R = H , R_1 = Et

8 R = NVOC , R_1 = Et

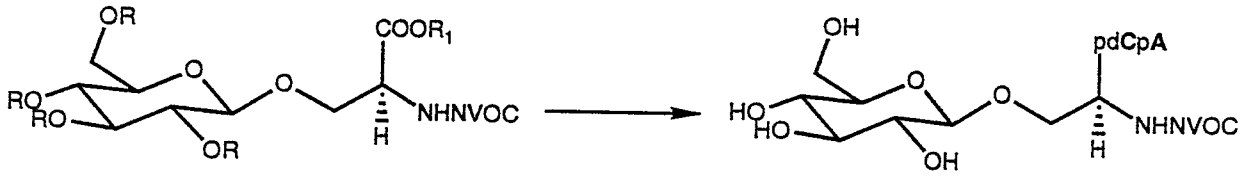
9 R = NVOC , R_1 = H

10 R = NVOC , R_1 = CH_2CN

11 R = NVOC , R_1 = pd**C**p**A**



13 R = CH_2=$CHCH_2OCO$ **14** R = CH_2=$CHCH_2OCO$



15 R = CH_2=$CHCH_2OCO$, R = CH_2CN

16 R = CH_2=$CHCH_2OCO$, R = pd**CpA**

17

References

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