A NEW IMPROVED STRATEGY FOR THE SYNTHESIS OF THE DINUCLEOTIDE pdCpA: AN EFFICIENT METHOD FOR THE DEPROTECTION OF CYANOETHYL, TBDPS, AND BENZOYL GROUPS IN ONE-STEP AT HIGH PRESSURE

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General.
UV spectra were taken in MeOH on a Nanodrop ND-1000 spectrometer. FTIR spectra were measured in KBr on a JASCO FT/IR-6200 spectrometer. NMR spectra were obtained in CD$_3$OD or D$_2$O on a JEOL ECA-500 instrument. $^1$H NMR chemical shifts are described as δ values in ppm relative to TMS. $^{31}$P NMR chemical shifts are reported as δ values in ppm downfield from 85% H$_3$PO$_4$. High-performance liquid chromatography (HPLC) using a Waters BEH C18 column (ODS-1.7 µm, ø2.1 mm x 50 mm, 260 nm) was carried out on a Waters ACQUITY UPLC with a PDA eλ photodiode array absorption detector. Matrix-Assisted-Laser-Desorption/ionization-Time-of-flight (MALDI-TOF) mass analysis was achieved on an AB-SCIEX MALDI-TOF/TOF5800 (3-hydroxyxipicolinic acid/ammonium citrate or 2,5-dihydroxy benzoic acid were used as a matrix). Automated chromatograph system using High-Flush column (30 µm silica gel) or Prif-Pack column (30 µm ODS) carried out on Yamazen YFLC AI-580 or Shoko Scientific Purif-compact. Unless otherwise noted, all reactions were carried out at 25 °C. Powdery molecular sieves 4A (MS 4A) were used after drying the commercially supplied one (Nacalai Tesque) at 200 °C for 12 h in vacuo. Organic solvents and reagents were used as the commercially supplied ones.

Reagents.
Imidazole (Nacalai Tesque), N4-(benzoyl)-5’-O-(p,p’-dimethoxytrityl)-2’-deoxycytidine 3’-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (3) (Glen Research, 96% purity), N7-benzyoladenosine (Carbosynth, 99% purity), trichloroacetic acid (Katayama Chemical, 99% purity), 2-butanone peroxide (Sigma-Aldrich, ~31 wt% in 2,2,4-trimethyl-1,3-pentanediol diisobutyrate), bis(trimethylsilyl) peroxide (Gelest, Inc.), bis(cyanoethyl)-phosphoramidite (Toronto Research Chemicals Inc., 98.0% purity). Anhydrous DMF (Sigma-Aldrich, 99.8%), CH$_2$Cl$_2$ (Kanto Kagaku, 99.5%), AcOEt (Junsei Chemical Co., 99.0%), MeOH (Nacalai Tesque, 99.0% purity), n-hexane (Nacalai Tesque, 99.0% purity), MeCN (Nacalai Tesque, 99.0% purity), 1,4-dioxane (Nacalai Tesque, 98.0%), and n-Bu$_4$NOH (Merck, 20% solution in H$_2$O) were purchased and used as received. Analytical TLC was preformed on Silica Gel 60 F$_{254}$ and Silica Gel 60 RP-18 F$_{254}$8 (Merck).

2’,3’-O-Di(t-butyldimethylsilyl)-N6-benzyoladenosine (2),$^{[1]}$ and benzimidazolium triflate$^{[2]}$ were prepared according to the literature procedures.
Preparation of 2-cyanoethyl \([N4-(benzoyl-2'-deoxycytidylyl)(3'-5')][2',3'-O-di(t-butyl-
dimethylsilyl)-N6-benzoyladenosine] (4).

**Method A:** A mixture of 2',3'-O-di(t-butyldimethylsilyl)-N6-benzoyladenosine (2, 1.8 g, 3.0 mmol), 2'-deoxycytidine phosphoramidite (3, 3.0 g, 3.6 mmol), and MS 4A (260 mg) in dry CH\(_2\)Cl\(_2\) (9 mL) was stirred at 25 °C for 1 h. Then benzimidazolium triflate (1.2 g, 4.5 mmol) was added, and the mixture was stirred for an additional 2 h. After that, 2-butanone peroxide solution (31 wt% in 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, 1.0 mL, 1.6 mmol) was added, and stirring was continued for 30 min. An insoluble substance formed was removed by filtration through Celite, and the mother liquor was diluted with CH\(_2\)Cl\(_2\) (15 mL), which was treated with 3% CCl\(_3\)CO\(_2\)H in CH\(_2\)Cl\(_2\) (100 mL), and stirring was continued for 10 min at ambient temperature. The resulting mixture was poured into satd NaHCO\(_3\) (600 mL) with vigorous stirring, followed by separation, dried (Na\(_2\)SO\(_4\)), and concd to give the crude dinucleotide 4 as a glassy oil. The crude product was subjected to filter on 40 μm silica gel (120 g, eluted with AcOEt / MeOH = 100 : 0 → 70 : 30), then the eluent was concd and purified by reverse-phase chromatography on preparative LC system (30 μm ODS, 120 g, Shoko Scientific Co. Ltd.), eluted with distilled H\(_2\)O / MeOH = 15 : 85 → 0 : 100) to give the protected dCpA 4 (2.4 g, 75% yield) as an amorphous solid.

**Method B:** A mixture of 2',3'-O-di(t-butyldimethylsilyl)-N6-benzoyladenosine (2, 1.8 g, 3.0 mmol), 2'-deoxycytidine phosphoramidite (3, 3.0 g, 3.6 mmol), and MS 4A (260 mg) in dry CH\(_2\)Cl\(_2\) (9 mL) was stirred at 25 °C for 1 h. Then benzimidazolium triflate (1.2 g, 4.5 mmol) was added, and the mixture was stirred for an additional 2 h. After that, bis(trimethylsilyl) peroxide (2.7g, 15 mmol) was added, and stirring was continued for 1 h. An insoluble substance formed was removed by filtration through Celite and the mother liquor was concd. The residue was then treated with 3% CCl\(_3\)CO\(_2\)H in CH\(_2\)Cl\(_2\) (100 mL), and stirring was continued for 10 min at ambient temperature. The resulting mixture was poured into satd NaHCO\(_3\) (600 mL) with vigorous stirring, followed by separation, dried (Na\(_2\)SO\(_4\)), and concd to give the crude dinucleotide 4 as a glassy oil. The crude product was subjected to filter on 40 μm silica gel (120 g, eluted with AcOEt / MeOH = 100 : 0 → 70 : 30), then the eluent was concd and purified by reverse-phase chromatography on preparative LC system (30 μm ODS, 120 g, Shoko Scientific Co. Ltd.) (eluted with double-distilled H\(_2\)O / MeOH = 15 : 85 → 0 : 100) to give the protected dCpA 4 (1.4 g, 44% yield) as an amorphous solid.

**Compound 4:** FTIR (KBr) ν 3410, 1697, 1660, 1581, 1486, 1402 cm\(^{-1}\); UV (MeOH) \(\lambda_{\text{max}}\) 260 nm (ε 54,600); \(^1\)H NMR (500 MHz, CDCl\(_3\)) δ 9.23–9.12 (1H, br s), 8.79 (1H, s), 8.83–8.71 (1H, br s), 8.36, 8.29 (1H, two singlets), 8.27–8.21 (1H, two doublets, \(J = 6.3\) and 6.9 Hz), 8.06 (2H, two doublets, \(J = 6.9\) and 7.5 Hz), 7.88 (2H, d, \(J = 6.9\) Hz), 7.60 (2H, dd, \(J = 6.9\) and 8.0 Hz), 7.53–7.49 (5H, m), 6.21, 6.20 (1H, two triplets, \(J = 6.7\) and 6.7 Hz), 6.02,
6.01 (1H, two doublets, \( J = 2.3 \) and 4.0 Hz), 5.19–5.13, 5.02–4.97 (1H, two multiplets), 4.89, 4.81 (1H, two triplets, \( J = 2.3 \) and 4.0 Hz), 4.59–4.37 (2H, m), 4.36–4.18 (5H, m), 3.89–3.82 (2H, m), 3.81–3.78, 3.63–3.56 (1H, two broad singlets), 2.83–2.66 (1H, m), 2.78 (2H, t, \( J = 6.0 \) Hz), 2.49, 2.32 (1H, two ddd, \( J = 6.7, 6.7, 6.7, 6.7, 6.7, \) and 6.7 Hz), 0.94, 0.93 (9H, two singlets), 0.89, 0.84 (9H, two singlets), 0.14, 0.12 (3H, two singlets), 0.12, 0.10 (3H, two singlets), 0.08, 0.03 (3H, two singlets), and 0.02, –0.11 (3H, two singlets); \(^{31}\)P NMR (202.5 MHz, CDCl\(_3\)): \( \delta = -1.75, -1.10 \); MALDI-TOF/MS calcd for C\(_{68}H_{64}N_9O_{12}PSi_2 \) [M + H]\(^+\) m/z 1046.40, found m/z 1046.66.

Preparation of 2-cyanoethyl [5'-di(2-cyanoethyl)phosphoryl-N4-(benzoyl-2'-deoxy-cytidylyl)(3'-5')][2',3'-O-di(t-butyldimethylsilyl)-N6-benzoyladenosine] (5).

Method A: A mixture of the protected dCpA 4 (1.8g, 1.7 mmol), bis(2-cyanoethyl)-\( NN\)-diisopropylphosphoramidite 2 (680 mg, 2.5 mmol), and MS 4A (260 mg) in dry CH\(_2\)Cl\(_2\) (3.4 mL) was stirred at 25 °C for 1 h. To this mixture was added benzimidazolium triflate (900 mg, 3.4 mmol), and the mixture was stirred for an additional 1 h. Then 2-butanone peroxide solution (31 wt% in 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, 600 µL, 890 µmol) was added, and the mixture was stirred for 30 min. An insoluble substance formed was removed by filtration through Celite, and the solution was concd. The crude product was purified on silica gel (120 g), and subjected to column chromatography on 30 µm ODS (120 g, eluted with double-distilled H\(_2\)O / MeOH = 15 : 85 \(\rightarrow\) 0 : 100) to give the protected pdCpA 4 (1.9 g, 90% yield) as an amorphous solid.

Method B: A mixture of the protected dCpA 4 (1.2 g, 1.2 mmol), bis(2-cyanoethyl)-\( NN\)-diisopropylphosphoramidite 2 (500 mg, 1.8 mmol), and MS 4A (260 mg) in dry CH\(_2\)Cl\(_2\) (3.6 mL) was stirred at 25 °C for 1 h. To this mixture was added benzimidazolium triflate (640 mg, 2.4 mmol), and the mixture was stirred for an additional 1 h. Then bis(trimethylsilyl) peroxide (1.1 g, 5.9 mmol) was added, and the mixture was stirred for 1 h. An insoluble substance formed was removed by filtration through Celite, and the solution was concd. The crude product was purified on silica gel (120 g), and subjected to column chromatography on 30 µm ODS (120 g, eluted with double-distilled H\(_2\)O / MeOH = 15 : 85 \(\rightarrow\) 0 : 100) to give the protected pdCpA 4 (1.1 g, 74% yield) as an amorphous solid.

Compound 5: FTIR (KBr) v 1698, 1668, 1615, 1577, 1487, 1405 cm\(^{-1}\); UV (MeOH) \( \lambda_{\text{max}} \) 260 nm (\( \varepsilon = 40,200 \)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 9.38–9.32, 9.36–9.30 (1H, two broad singlets), 8.83–8.66 (1H, br s), 8.80, 8.79 (1H, two singlets), 8.32, 8.29 (1H, two singlets), 8.05 (2H, d, \( J = 7.5 \)), 8.06, 7.96 (1H, two doublets, \( J = 6.9 \) and 7.5 Hz), 7.88 (2H, d, \( J = 7.5 \) Hz), 7.62–7.46 (7H, m), 6.22, 6.18 (1H, two triplets, \( J = 7.0 \) and 7.3 Hz), 6.02, 6.01 (1H, two doublets, \( J = 2.9 \) and 3.4 Hz), 5.14–5.09, 5.08–5.03 (1H, two multiplets), 4.89, 4.84 (1H, two triplets, \( J = 2.9 \) and 3.4 Hz), 4.52-4.08 (13H, m), 2.88-2.68 (7H, m), 2.26, 2.20 (1H, two ddd,
$J = 7.0, 7.0, 7.0, 7.3, 7.3, 7.3$ Hz), $0.94$ (9H, s), $0.86, 0.84$ (9H, two singlets), $0.14$ (3H, s), $0.12$ (3H, s), $0.04, 0.03$ (3H, two singlets), $-0.08, -0.12$ (3H, two singlets); $^{31}$P NMR (202.5 MHz, CDCl$_3$) $\delta$ $-1.59, -1.14, -1.70, -1.75$; MALDI-TOF/MS calcd for C$_{54}$H$_{72}$N$_{11}$O$_{15}$P$_2$Si$_2$ [M + H]$^+$ m/z 1232.42, found m/z 1232.76.

**Preparation of (5'-phosphoryldeoxycytidylyl)(3’-5’)(adenosine) (1).**

**Method A:** To a solution of the protected pdCpA 5 (57 mg, 46 $\mu$mol) in MeOH (750 $\mu$L) and 1,4-dioxane (110 $\mu$L) was added 28% aq NH$_4$OH (900 $\mu$L), and the mixture was transferred to a Teflon reaction vessel and kept at 0.8 GPa and 25 °C for 14 h. After concentration, the crude product was subjected to reverse-phase short-column chromatography on 30 $\mu$m ODS (30 g) eluted with a mixture of double-distilled H$_2$O and MeCN (0 : 100 $\rightarrow$ 95 : 5) to provide the ammonium salt of pdCpA 1 as an amorphous solid (19 mg, 60% yield).

**Method B:** To a solution of the protected pdCpA 5 (370 mg, 300 $\mu$mol) in MeOH (3.7 mL), 1,4-dioxane (540 $\mu$L), and double-distilled H$_2$O (1.8 mL) was added 20% aq n-Bu$_4$NOH (2.5 mL, 1.8 mmol), and the mixture was transferred to a Teflon reaction vessel and kept at 0.8 GPa and 25 °C for 14 h. After dilution by addition of double-distilled H$_2$O, the mixture was subjected to gel filtration on Sephadex G-10 (480 mL, eluted with double-distilled H$_2$O / MeOH = 80 : 20) to give the tetra-n-butylammonium salt of pdCpA 1 (480 mg, 97% yield estimated by OD$_{260}$) as an amorphous solid.

**Compound 1** (as its tetra-n-butylammonium salt): FTIR (KBr) $\nu$ 1653, 1604, 1576, 1488, 1378 cm$^{-1}$; UV (MeOH) $\lambda_{\text{max}}$ 260 nm (e 23 800); $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.62 (1H, s, 8.17 (1H, s), 8.10 (1H, $J = 7.5$ Hz), 6.41 (1H, dd, $J = 5.4, 8.3$ Hz), 6.11 (1H, d, $J = 6.3$ Hz), 5.94 (1H, d, $J = 7.5$ Hz), 4.98–4.92 (1H, m), 4.72 (1H, dd, $J = 6.0$ and 6.9 Hz), 4.59–4.54 (1H, m), 4.32–4.28 (1H, m), 4.26–4.22 (1H, m), 4.13–3.97 (4H, m), 3.62 (1H, t, $J = 6.9$ Hz), 3.26–3.14 (24H, m), 2.65–2.56 (1H, m), 2.40 (1H, t, $J = 7.2$ Hz), 2.28–2.19 (1H, m), 1.69–1.59 (24H, m, TBA), 1.40 (24H, sexted, $J = 7.5$ Hz), 1.01 (36H, t, $J = 7.5$ Hz); $^{31}$P NMR (202.5 MHz, CD$_3$OD) $\delta$ 4.28, −0.46; MALDI-TOF/MS calcd for C$_{54}$H$_{72}$N$_{11}$O$_{15}$P$_2$ [M + H]$^+$ m/z 637.12, found m/z 637.52.
References

4700 Reflector Spec #1 MC [BP = 566.2, 3872]

[Chemical structure image]
4700 Reflector Spec #1 MC[BP = 566.2, 992]
TOF/TOF™ Reflector Spec #1 MC[BP = 563.4, 869]

DHB-Li

Mass (m/z)