Supporting information

Phosphine containing oligonucleotides for the development of metallodeoxyribozymes

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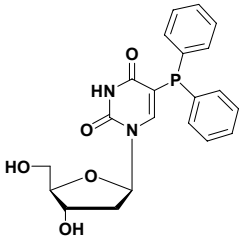
General Procedures

Chemicals were purchased from Aldrich Chemical Co., Link Technologies Ltd. and Fluka and were used as received. *N,N*-dimethylformamide was degassed and stored under nitrogen (Aldrich Chemical Co). THF was distilled from sodium/benzophenone, petroleum ether was distilled from sodium/ benzophenone/triglyme, and acetonitrile, triethylamine, dichloromethane, pyridine and diisopropylethylamine were distilled from CaH_2. $CDCl_3$ was distilled from CaH_2 and stored under argon over K_2CO_3. Other deuteriated solvents were used as received and kept under argon. Silica gel 60 purchased from Fluka was used for column chromatography.

All air- and water-sensitive reactions were carried out under dry, air free conditions using dry degassed solvents and standard Schlenk techniques under an atmosphere of purified argon. NMR spectra were recorded at room temperature on a Bruker Avance 300 spectrometer. Positive chemical shifts (δ) are given (in ppm) for high-frequency shifts relative to a TMS reference (^1H and ^{13}C) or an 85% H_3PO_4 reference (^{31}P). ^{13}C and ^{31}P spectra were measured with ^1H decoupling. FAB mass spectra was recorded on a JOEL JMS SX/SX102A four sector mass spectrometer coupled to a JOEL MS-MP7000 data system. MALDI-TOF mass spectra were collected on a Voyager-DE Biospectrometry Workstation (PerSeptive Biosystems Inc. Framingham, MA, USA) mass spectrometer using HPA and ammonium citrate dibasic as matrix (8/1). Oligonucleotides were synthesised by an Applied Biosystems 392 DNA/RNA synthesiser using protocols obtained from Applied Biosystems.

5-diphenylphosphino-2'-deoxyuridine

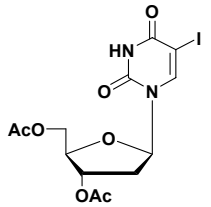


5-iodo -2'-deoxyuridine (0.5 g, 1.41 mmol), DMF (10 mL), Et_3N (0.22 mL, 1.55 mmol) and diphenylphosphine (0.26 mL, 1.55 mmol), were charged into a Schlenk tube followed by addition of $Pd(OAc)_2$ (9.5 mg, 42 μmol). The deep purple solution was heated to 60°C until completion (monitored by TLC, *c.a.*30 minutes). The solvent was removed under vacuum and the residue loaded to a silica gel column chromatography (eluant hexane/EtOAc/methanol 50/48/2 to 40/50/10) to obtain a white crystalline compound (0.553 g, 95 %).

^{1}H NMR (CD_3OD) δ (ppm): 7.32-7.20 (m, 10 H, C_6H_5) ; 6.88 (d, J = 2.7 Hz, 1 H, H 6); 6.10 (t, J = 6.6 Hz, 1 H, H 1'); 4.79 (br s, 3 H); 3.88 (dt, ^{3}J = 6.0 Hz, J = 3.6 Hz , 1 H, H4'); 3.78 (dt, ^{3}J = 5.0 Hz, 3.6 Hz, 1 H, H3'); 3.23-3.19 and 3.10-2.99 both (m, 1 H, 5 H'); 2.20- 2.11 and 1.85-1.74 both (m, 1 H, H2')
^{13} C NMR (CD_3OD) δ (ppm): 166.05 (d, J_{C-P} = 19.3 Hz); 150.93; 144.18; 135.16; 135.03; 134.98; 134.85; 133.70; 133.43; 129.22; 128.79; 128.69; 110.80 (d, J_{C-P} = 12.2 Hz); 87.49; 85.55; 71.25; 62.04; 39.80
^{31}P NMR (CD_3OD) δ (ppm): -20.88
MS (FAB+) 413.13 $[M+H]^+$, 429.12 $[M+O]^+$; HMRS calculated for $C_{21}H_{22}O_5N_2P$: 413.1261, found 413.1260

Crystallography: A full hemisphere of data was collected at 93 K using a Rigaku MM007 high brilliance RA (Mo- Kα radiation λ = 0.71073 Å) and a Mercury CCD detector. Colourless platelet 0.2 x 0.1 x 0.01 mm, $C_{21}H_{21}N_2O_5P.(H20)_{0.25}$, M = 416.87, monoclinic, space group *C*2, a = 18.165(4), b = 5.1771(9), c = 22.063(5) Å β = 108.12(3) °, U = 1972.0(7) Å^3, Z = 4, D_c = 1.404 Mgm^{-3}, μ = 0.177 mm^{-1}, F(000) = 874. Of 6287 measured data, 3282 were unique (R_{int} = 0.0256) and 2868 observed ($I > 2\sigma(I)$]) to give R_1= 0.0527 and wR_2 = 0.1244. Flack parameter 0.02(14). Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 626863. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

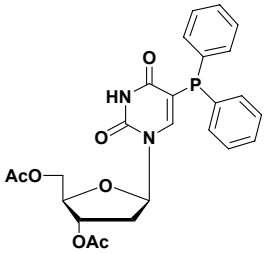
5-iodo -5',3'-di-*O*-acetyl -2'-deoxyuridine



To a solution of 5-iodo-2'-deoxyuridine (0.5 g, 1.41 mmol) in pyridine was added acetic anhydride (3 mmol) in pyridine/THF (10 mL). After 2 hours, the solvent was removed under vacuum and the residue loaded to a silica gel column chromatography (eluant petroleum ether/ethyl acetate 1/1 to 3/7) to obtain a white crystalline compound (0.568 g, 92 %).

^{1}H NMR ($CDCl_3$) δ (ppm): 9.01 (br s, 1 H, N-H), 7.90 (s, 1 H, H 6); 6.22 (dd, ^{3}J = 5.7 Hz, ^{3}J = 8.2 Hz, 1 H, H1'); 5.16 (dt, ^{3}J = 6.5 Hz, J = 2.0 Hz, 1 H, H3'); 4.38-4.22 (m, 3 H); 2.47 (ddd, ^{2}J = 14.2 Hz, ^{3}J = 5.7 Hz, ^{3}J = 2.0 Hz, 1 H); 2.14 (s, 3 H, CH_3); 2.05 (s, 3 H, CH_3); 2.15- 2.05 (m, 1 H).
^{13} C NMR ($CDCl_3$) δ (ppm): 170.73; 170.50; 160.28; 150.37; 144.09; 134.64; 134.51; 133.85; 85.69; 82.86; 74.39; 64.13; 38.46; 21.41;21.18
MS (FAB+) 437.99 $[M+H]^+$

5-diphenylphosphine -3',5'-di-*O*-acetyl-2'-deoxyuridine

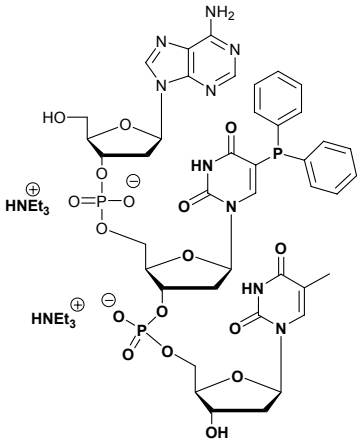


5-iodo-3', 5'-di-*O*-acetyl-2'-deoxyuridine (0.616 g, 1.41 mmol), DMF (10 mL), Et_3N (0.22 mL, 1.55 mmol) and diphenylphosphine (0.26 mL, 1.55 mmol), were charged into a Schlenk tube followed by addition of $Pd(OAc)_2$ (9.5 mg, 42 μmol). The deep purple solution was heated to 60°C until completion (monitored by TLC, *c.a.*30 minutes). The solvent was removed under vacuum and the residue loaded to a silica gel column chromatography under argon (eluant 1/4 petroleum ether (40/60) / EtOAc) to obtain an air sensitive white crystalline compound (0.630 g, 90 %).

^{1}H NMR ($CDCl_3$) δ (ppm): 9.16 (br s, 1 H, N-H), 7.31-7.20 (m, 10 H, C_6H_5) ; 6.94 (d, J_{P-H} = 3.8 Hz, 1 H, H 6); 6.11 (dd, ^{3}J = 5.7 Hz, ^{3}J= 8.2 Hz, 1 H, H1'); 4.93 (dt, ^{3}J = 2.1 Hz, ^{3}J = 6.4 Hz, 1 H, H3'); 4.06 (m, 1 H, H4'); 3.86 (dd, ^{3}J = 4.7 Hz, ^{2}J = 12.0 Hz, 1 H, H5'); 3.71 (dd, ^{3}J = 4.7 Hz, ^{2}J = 12.0 Hz, 1 H, H5'); 2.40 (ddd, ^{2}J = 14.2 Hz, ^{3}J = 5.7 Hz, ^{3}J = 2.1 Hz, 1 H, H2'); 2.00 (s, 3 H, CH_3); 1.97 (s, 3 H, CH_3); 1.95-1.94 (m, 1 H, H2')
^{13} C NMR ($CDCl_3$) δ (ppm): 170.73 (CO_2Me); 170.50 (CO_2Me); 162.40 (d, J_{C-P} = 17.9 Hz, C=O); 150.20 (C=O); 143.26 (d, J_{C-P} = 17.9 Hz, CH=); 134.64; 134.51; 133.85; 13375; 133.57; 133.48; 129.69; 129.60; 129.09; 129.5; 129.00; 128.95; 112.07 (d, J_{C-P} = 15.9 Hz); 85.99; 82.44; 76.83; 74.40; 63.90 (CH_2); 37.97 (CH_2); 21.12 (2 CH_3)
^{31}P NMR ($CDCl_3$) δ (ppm): -18.77
MS (FAB+) 497.15 $[M+H]^+$, 513.14$[M+O]^+$; HMRS calculated for $C_{25}H_{26}O_7N_2P$: 497.1472; found 497.1463

5-iodo-5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite- 2'-deoxyuridine
This compound was prepared following literature procedure.[1]

Trimer dAdppdUdT

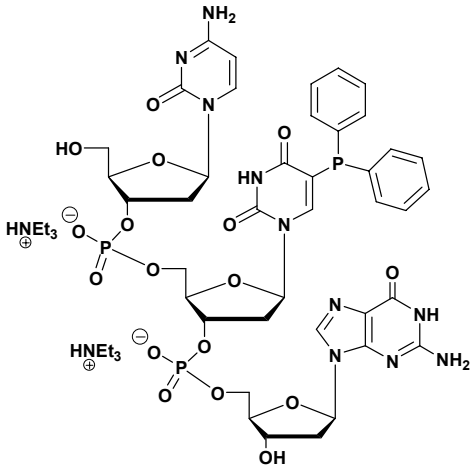


To the supported trinucleotide on CPG (10 μmol obtained by standard automated DNA synthesis) was added acetonitrile (2 mL), Et_3N (0.1 mL) and diphenylphosphine (12 μL, 130 μmol) followed by addition of $Pd(OAc)_2$ (2.25 mg, 10 μmol). The deep purple solution was heated to 60°C for 72 hours. The organic phase was removed by filtration and the silica supported oligonucleotide was carefully washed by acetonitrile and a mixture of dppe in dichloromethane (0.25 M). The oligonucleotide was then isolated after cleavage and deprotection in a methyl amine/NH_4OH solution at 55°C (3 hours). Purification was obtained through FPLC (Amersham Biosciences equipped a HiTrap Q Sepharose column) using a gradient of triethylammonium acetate as mobile phase (0.2 M and 2 M TEAAc in water). The overall yield was 40% based on original nucleotide loading of 10 μmol on cpg.

^{31}P NMR (D_2O) δ (ppm): -0.91, -22.15

MALDI-TOF: $[M+H]^+$ calculated 1030.23; found 1030.20

Trimer dCdppdUdG



^{31}P NMR (D_2O) δ (ppm): -0.81, -22.97

MALDI-TOF: $[M+H]^+$ calculated 1032.22; found 1033.55

Asymmetric Allylic Alkylation Reaction (Typical Procedure).

In a Schlenk tube $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (0. 5 μmol) and the respective ligand (1 μmol) were dissolved in 1 ml of THF. (*E*)-1,3-diphenylprop-2-ene-1-yl acetate (1 mmol) was added as the substrate and after 20 min of stirring at room temperature, dimethyl malonate (3mmol), BSA (N,O-bis(trimethylsilyl) acetamide, 3 mmol) and a catalytic amount of KOAc (0.024 mmol) were added consecutively. The reaction mixture was stirred at the given temperature. If TLC indicated no further conversion, the reaction was quenched by dilution with Et_2O (2 ml) and washed with saturated NH_4Cl solution (1 ml) and dried over Na_2SO_4. Filtration and removal of solvent left a red oil, which was chromatographed (SiO_2; petroleum ether/CH_2Cl_2 = 1:1) to give analytically pure products. Determination of e.e. values was performed by chiral HPLC (Chiralcel OD-H, n-hexane/2-propanol = 98:2, 0.5 ml·min^{-1}, t_R (*R*) = 15.4 min, t_R (*S*) = 16.8 min).

Asymmetric Allylic Amination Reaction (Typical Procedure). In a Schlenk tube $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (7.5 μmol) and the respective ligand (15 μmol) were dissolved in 2 ml of the relevant solvent and stirred for desired time. (E)-1,3-diphenylprop-2-ene-1-yl acetate (0.3 mmol) was added and the solution was stirred for 20 min at ambient temperature. Subsequently, benzylamine or N-methylbenzylamine (0.9 mmol) was added, and the reaction was kept at the given temperature until TLC indicated no further progress. The solvent was removed *in vacuo* and the residue was purified by column chromatography (SiO_2; petroleum ether/ethyl acetate = 95:5 to give pure products. Determination of e.e. values was performed by chiral HPLC (Chiralcel-OD-H; n-hexane/2-propanol = 99.5:0.5 at 0.5 ml·min^{-1}, t_R (*R*) = 15.6 min, t_R (*S*) = 17.2 min).

[1] S. I. Khan, M. W. Grinstaff, *J. Am. Chem.*, 1999, 121, 4704.