Supplementary Material for:

Fluorescent Properties of DNA Base Analogue tC upon Incorporation into DNA - Negligible Influence of Neighboring Bases on Fluorescence Quantum Yield

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Synthesis of 3-[2-deoxy-3-O-[2-cyanoethoxy-(*N,N*-diisopropylamino)-phosphino]-5-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-1,3-diaza-2-oxophenothiazine

Experimentals:

^{1}H and ^{31}P NMR spectra were recorded on a Varian 400 MHz UNITY-VXR 5000 and a Bruker AC300 spectrometer, respectively.

Low-resolution mass spectra were recorded using electrospray technique on a Fisons VG platform instrument in acetonitrile. High-resolution mass spectra were recorded in acetonitrile using electrospray technique on a Bruker APEX III FT-ICR mass spectrometer.

3-(2-deoxy-β-D-ribofuranosyl)-1,3-diaza-2-oxophenothiazine (3)

1,3-diaza-2-oxophenothiazine(1,2) (**1**) (1.0 g, 4.6 mmol) was suspended in DMF (60 ml) and NaH (60% in mineral oil, 203 mg, 5.1 mmol) was added in one portion. The suspension was stirred at room temperature under a nitrogen atmosphere until a clear yellow solution was obtained. Solid 2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride(3) (2.06 g, 5.3 mmol) was added in small portions to the solution over a period of 15 min. The reaction was stirred for 2 h, after which traces of undissolved **1** were filtered off and the filtrate was evaporated. The residue was dissolved in ethyl acetate and washed twice with saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product was dissolved in toluene, applied to a silica gel column, washed with a solution of 20% ethylacetate in toluene and then eluted with a solution of 60% ethylacetate in toluene to give the toluoyl-protected nucleoside (**2**) as a mixture of the α- and β-anomers.

Compound **2** was dissolved in a solution of sodium (17 mg) in anhydrous methanol (17 ml) and stirred at room temperature over night. The reaction was neutralized with acetic acid and evaporated. The crude product was dissolved in ethanol and evaporated with silica gel which was then applied to a silica gel column [9-12% ethanol in chloroform]. The anomerically pure title compound **3** (β-anomer) was isolated as a yellow powder (218 mg, 14% from **1**).

^{1}H NMR spectrum was identical to what has been earlier reported in the literature.(4)

3-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-1,3-diaza-2-oxophenothiazine (4)

Compound **3** (389 mg, 1.17 mmol) was coevaporated twice with freshly distilled pyridine and then dissolved in the same solvent (10 ml). 4,4'-dimethoxytrityl chloride (474 mg, 1.40 mmol) was dissolved in freshly distilled pyridine (2.5 ml) and added dropwise to the solution which was stirred at room temperature for 2 h. Methanol (~1 ml) was added and the solution was evaporated and coevaporated twice with toluene. The residue was added to a silica gel column and eluted with 5% methanol in dichloromethane containing 1% pyridine to give the title product **4** as a yellow powder (524 mg, 70%).

^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H), 7.59 (s, 1H), 7.2-7.4 (m, 9H), 7.07 (m, 1H), 6.91 (m, 7H), 6.09 (t, 1 H, *J* = 6.4 Hz), 5.32 (d, 1H, *J* = 4,8 Hz), 4.26 (m, 1H), 3.91 (m, 1H), 3.72 (2s, 6H), 3.23 (m, 1H), 3.14 (m, 1H), 2.18 (m, 2H).

3-[2-deoxy-3-O-[2-cyanoethoxy-(*N,N*-diisopropylamino)-phosphino]-5-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-1,3-diaza-2-oxophenothiazine (5)

Under argon atmosphere, 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.14 ml, 628 μmol) was added dropwise to a stirred solution of **4** (398 mg, 626 μmol) and diisopropylethylamine (0.27 ml, 1.57 mmol) in distilled tetrahydrofuran (5 ml). The reaction mixture was stirred at room temperature for 30 min. and then partitioned between ethyl acetate and saturated KCl. The organic layer was separated, dried over Na_2SO_4 and evaporated. Purification by column chromatography (ethyl acetate plus 1% pyridine) under argon afforded the diasteroisomeric mixture (1:1) of compound **5** as bright yellow foam (472 mg, 92%).

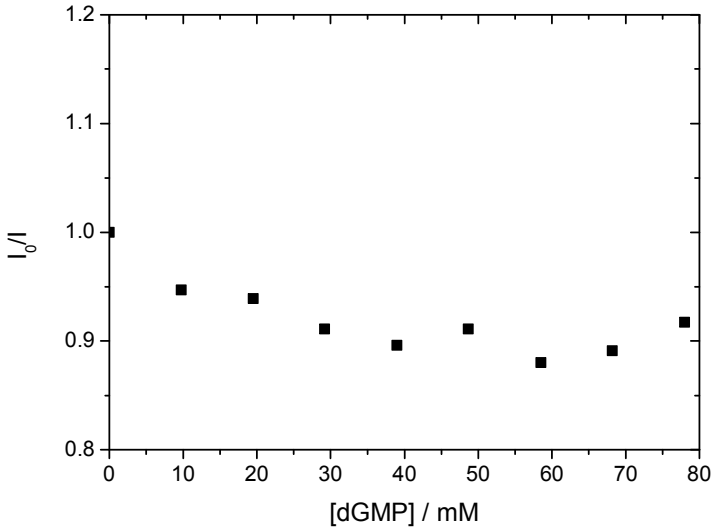
^{31}P NMR spectrum: δ_P(162 MHz, $CDCl_3$) 149.7, 149.2.

LRMS (ES+, CH_3CN) $C_{45}H_{50}N_5O_7PSNa$ requires: 858. Found: 858 ($M+Na^+$, 100%).

HRMS (ES+, CH_3CN) $C_{45}H_{51}N_5O_7PS$ requires: 858.3061, found: 858.3073 ($M+H^+$).

Quenching titration of tC with dGMP

Figure S-1. Stern-Volmer plot for fluorescence quenching titration of tC with dGMP. The initial intensity (I_0) of a tC solution (20 μM) compared to intensity for increasing concentration of dGMP (I) was monitored by steady-state spectroscopy at 25°C in a 50 mM sodium phosphate buffer (pH 7.5). Excitation wavelength was 375 nm, emission spectra were corrected for concentration and integrated from 380 to 750 nm.



References

1. Roth, B. and Schloemer, L.A. (1663) 5-Arylthiopyrimidines. III. Cyclization of 4-Hydroxy Derivatives to 10H-Pyrimido[5,4-b][1,4]benzothiazines (1,3-diazaphenothiazines). *J. Org. Chem*, **28**, 2659-2672.

2. Roth, B. and Hitchings, G.H. (1961) 5-Arylthiopyrimidines. II. 2- and 4-Alkylamino and 4-Amino Derivatives. *J. Org. Chem.*, **26**, 2770-2778.

3. Rolland, V., Kotera, M. and Lhomme, J. (1997) Convenient preparation of 2-deoxy-3,5-di-O-p-toluoyl-alpha-D-erythro-pentofuranosyl chloride. *Synth. Com.*, **27**, 3505-3511.

4. Lin, K.Y., Jones, R.J. and Matteucci, M. (1995) Tricyclic 2'-Deoxycytidine Analogs - Syntheses and Incorporation into Oligodeoxynucleotides Which Have Enhanced Binding to Complementary Rna. *J. Am. Chem. Soc.*, **117**, 3873-3874.