Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1677–1679

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Efficient Synthesis of 8-Oxo-dGTP: A Mutagenic Nucleotide

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Received 24 March 2000; accepted 23 May 2000

Abstract—An efficient synthesis of mutagenic and oxidative DNA damage product, 8-oxo-dGTP (**4**) has been achieved in high yield, along with a serendipitous generation of 8-dimsyl-dG (**2**). In combination with dPTP (**5**), 8-oxo-dGTP (**4**) can be formulated into a kit for investigating DNA random mutagenesis.

8-Oxoguanosine, an oxidative DNA damage product, is believed to play a significant role in aging and cancer.[1] In the cellular nucleotide pool, 8-oxo-2′-deoxyguanosine-5′-triphosphate is generated by the oxidation of 2′-deoxyguanosine during normal cellular metabolism.[2] When added to PCR reactions, 8-oxo-dGTP **4** (Fig. 1) is incorporated opposite adenine and cytosine bases with almost equal efficiency (Fig. 2), which results in two transversion mutations, A→C and T→G. Another nucleotide analogue, dPTP (**5**), can be incorporated in place of TTP and dCTP causing any of the four possible transition mutations. In PCR based random mutagenesis[3] of DNA, utilization of these two nucleotide analogues—8-oxo-dGTP (**4**) and dPTP (**5**)—not only helps understand protein structure/function, but also sets the requirement for some knowledge of the most important regions of the gene to study. While there was easy access to the synthesis of dPTP[4] (**5**), for it to be part of the mutagenesis kit there existed no reliable/reproducible literature method for the synthesis of 8-oxo-dGTP (**4**). A direct oxidative method[5] on dGTP using hydrogen peroxide/ascorbic acid in phosphate buffer produces poor yields, and in our hands it could not be reproduced. Recently, Guengerich et al.[6] reported a cumbersome 7-step synthesis of 8-oxo-dGTP starting from 2′-deoxy-dG. This method involved unnecessary protection/de-protection strategy, in addition to multistage purification of the final compound with no mention of yields. Herein, we report on a short, efficient, and high-yield synthesis of 8-oxo-dGTP (**4**), mechanism of formation along with the unexpected generation of 8-dimsyl-2′-deoxyguanosine (**5**).

Results and Discussion

In an attempt to achieve the synthesis of 8-oxo-dGTP (**4**) in commercial quantities for the formulation of mutagenesis kit, 8-benzyloxy-2′-deoxyguanosine[7] **3** (Scheme 1) was envisaged to be the suitably protected starting material derivable from 8-bromo-2′-deoxyguanosine[7] (**1**). Initial experiments to convert 8-bromo-2′-deoxyguanosine (**1**) to 8-benzyloxy-2′-deoxyguanosine (**3**) under the Na/BnOH/DMSO (1/3), 65 °C, 16 h heating conditions resulted in a serendipitous formation of 8-dimsyl-dG **2**,[8] (major) and the desired 8-bezyloxy-dG **3** (minor). It was expected that changing the ratio of the solvents BnOH/DMSO from 1/3 to 3/2 would result in a product outcome in favor of the desired 8-benzyloxy-dG (**3**) exclusively or as the major compound. True to the expectation, exclusive formation of 8-bezyloxy-dG (**3**) occurred in an isolated yield of 80% by addition of 8-bromo-dG (**1**) to the clearly dissolved solution of sodium metal in BnOH/DMSO (3/2) mixture, at room temperature and heating at 65 °C for 16 h. Note that dissolution of sodium metal in BnOH takes about 3 h at rt. DMSO was used not only to help dissolve the solid 8-bromo-dG, but also for its powerful solvating effect of cations (Na^+) with consequently enhanced reactivity[9] of the counter ions (BnO^-), for nucleophilic displacement. It is extremely

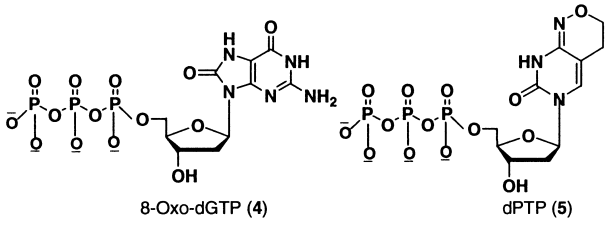

Figure 1.

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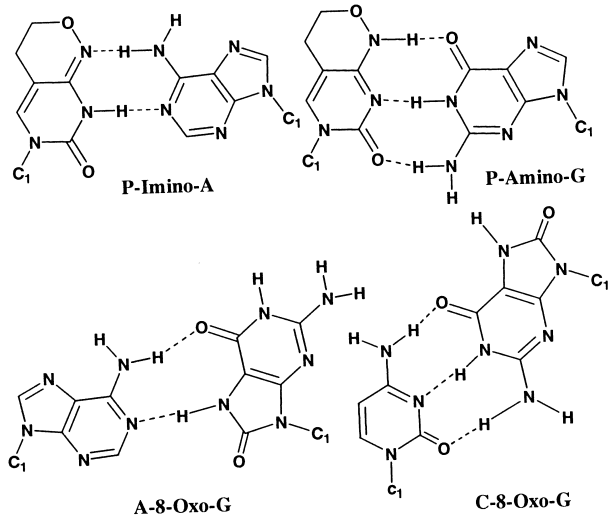
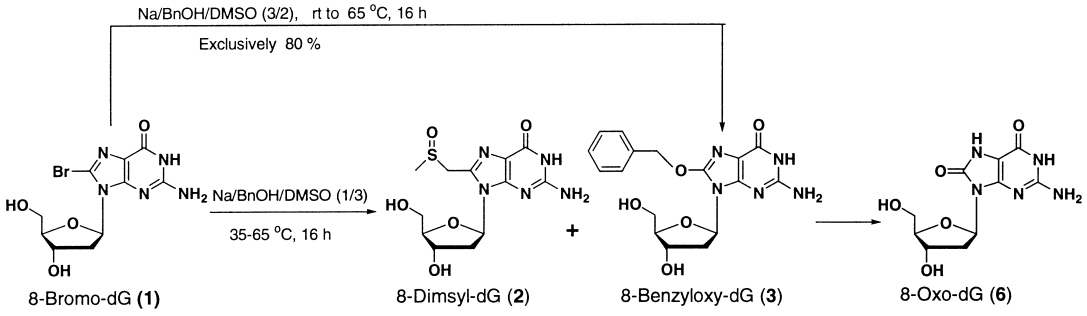


Figure 2. The base pairing properties of dPTP and dGTP: dPTP can base pair with dA or dGTP and 8-Oxo-dGTP can base pair with dA or dC.

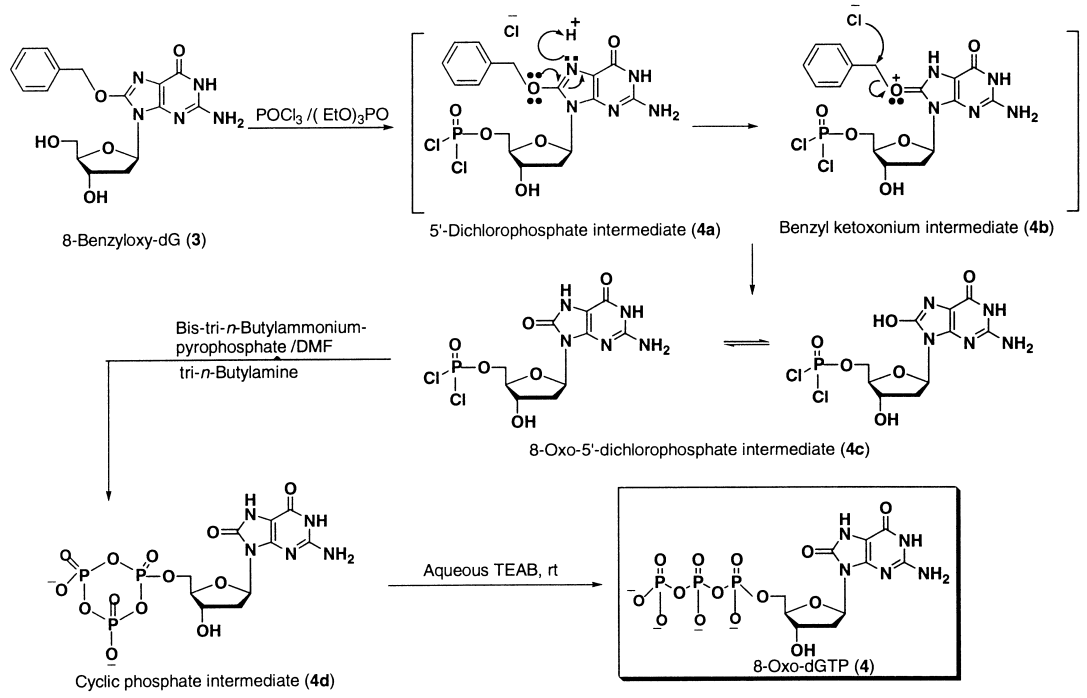
difficult to get the 8-bromo-dG (**1**) dissolved in BnOH without using DMSO.

Having obtained the desired 8-benzyloxy-dG **3** (Scheme 2) in an excellent yield,[10] it was phosphorylated[11] at 0–5 °C with $POCl_3$ in $(EtO)_3PO$, followed by simultaneous addition of tri-*n*-butylamine and bis-tri-*n*-butylammonium-pyrophosphate. This treatment directly produced 8-oxo-dGTP (**4**) in 45% isolated yield[12] after being stirred with 1.0 M TEAB (triethylammoniumbicarbonate) and RP-HPLC purification, without requiring removal of benzyl group under catalytic hydrogenolysis conditions.

Mechanistically, in situ generated HCl from the reaction of **3** with $POCl_3$ is believed to have catalyzed the cleavage of benzyl iminol ether in 5′-phosphorodichloridate intermediate **4a**, giving rise to a benzylketoxonium intermediate **4b**. Finally, cleavage of benzyl moiety from **4b** occurs as soon as chloride anion attacks the benzylic position to give intermediate **4c**, which then reacts with bis-tri-*n*-



Scheme 1.



Scheme 2.

butylammoniumpyrophosphate in the presence of tri-*n*-butylamine affording 8-oxo-dGTP(**4**). The formation of **4** occurs via the usual cyclic intermediate **4d**, after being hydrolyzed by aqueous TEAB at pH 8. As enol ethers are readily hydrolyzed by acids,[13] we have experimented and demonstrated that the iminol benzyl ether (**3**) collapses to 8-oxo-dG (**6**)[14] upon treatment with 1.0 N HCl/MeOH.

In summary, the synthesis of 8-benzyloxy-dG (**3**) could be achieved in an improved yield (80%), and converted directly to 8-oxo-dGTP (**4**) in high yield (45%), which helps in studying the random DNA mutagenesis in formulation with dPTP (**5**) analogue. In order to help explain the direct generation of 8-oxo-dGTP (**4**) from **3** in the phosphorylation reaction, an HCl catalyzed mechanistic pathway for the cleavage of benzyl group in **3** has been invoked.

References and Notes

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10. 8-Benzyloxy-dG (**3**). Freshly cut Na metal (0.96 g, 41.74 mmol, 5 equiv) was dissolved in BnOH (30 mL) at rt by magnetic stirring under Argon atmosphere, solid 8-bromo-dG **1** (2.9 g, 8.3 mmol) was added followed by anhyd DMSO (20 mL) to ensure complete dissolution of the solid 8-bromo-dG. The reaction mixture was heated at 65 °C for 16 h, after cooling, the reaction was poured into magnetically stirred ether (800 mL) solvent for precipitation of the desired compound. The precipitated solid was filtered and adsorbed onto silica gel by rotary evaporation from methanol under reduced pressure. Silica gel column chromatography of the adsorbed solid eluting with 5–10% MeOH in $CHCl_3$ plus a few drops of NH_4OH eliminated the impurities. 8-Benzyloxy-dG **3** (2.5 g, 80%) was isolated as a white solid eluting with 15–20% MeOH in $CHCl_3$ plus a few drops of NH_4OH. Compound **3** showed similar spectral properties as reported in the literature.[7]
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12. 8-Oxo-dGTP (**4**). To a magnetically stirred and cooled (0–5 °C) solution of 8-benzyloxy-dG **3** (2.1 g, 5.62 mmol) in triethylphosphate (25 mL) under Argon atmosphere was added $POCl_3$ (1.29 g, 8.41 mmol, 1.5 equiv) drop-wise. After 2 h, simultaneously were added 5 equiv each of 0.5 M DMF solution of bis-tri-*n*-butylammonium pyrophosphate (15.42 g, 28.10 mmol) and tri-*n*-butylamine (5.21 g, 28.10 mmol). The cyclic phosphate formed was stirred at rt for 30 min, then hydrolyzed with 1.0 M TEAB (~500 mL, pH 7.2) overnight. TEAB and DMF were evaporated from the reaction mixture under reduced pressure, the residue obtained was dissolved in S.Q. (super quality) water, and filtered through 0.4 μ filterware. The filtrate containing the desired 8-oxo-dGTP (**4**) was subjected to Sephadex column purification using 0.05 to 1.0 M TEAB. The fractions containing the desired compound were pooled, evaporated, and the residue obtained was further subjected to RP-HPLC, on a Delta Pak C18 preparative column (5×30 cm), using gradient 0.1 M TEAB (buffer A, pH 7.0) and 25% CH_3CN in 0.1 M TEAB (buffer B, pH 7.0) at 130 mL/min. Fractions containing the desired compound were pooled, evaporated and lyophilized to obtain 8-oxo-dGTP **4** (2.24 g, 45.5%) as an amorphous powder. ^{31}P NMR (D_2O): −8.1 (d, γ-P), −10.1 (d, α-P), −22.22 (t, β-P) ppm. ^1H NMR (D_2O) δ 6.13 (1H, dd, J=6.0 Hz, 9.0 Hz, 1′-H), 4.22 (1H, m, 3′-H), 4.05 (1H, m, 4′-H), 3.18 (2H, m, 5′-H2), 2.16 (1H, m, 2′-H_a), 2.85 (1H, m, 2′-H_b), NH_2, NH, 3′-OH, and 5′-OH, protons have been exchanged with D_2O. UV (10 mM Tris, pH 6.9) λ_{max} 247, 296 nm.
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14. 8-Oxo-dG (**6**). To a stirred solution of 8-benzyloxy-dG **3** (50 mg) in MeOH (5 mL) at rt was added 1.0 M HCl (0.5 mL). The reaction was stirred for 1 h. Removal of water and MeOH under reduced pressure followed by trituration of the residue from MeOH/ether afforded quantitative yield of the known[7] 8-oxo-dG (**6**). ^1H NMR (DMSO-d_6) δ 6.55 (2H, bs, D_2O exch. NH_2), 6.07 (1H, dd, J=6.0 Hz, 9.0Hz, 1′-H), 5.18 (1H, bs, D_2O exch. 3′-OH), 4.86 (1H, bs, D_2O exch. 5′-OH), 4.60 (1H, bs, 8-OH), 3.79 (1H, m, 3′-H), 3.56 (1H, m, 4′-H), 3.56 (2H, m, 5′-H2), 2.97 (1H, m, 2′-H_a 2.08 (1H, m, 2′-H_b).