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Synthesis of 8-oxo-dGTP and its β,γ-CH_2-, β,γ-CHF-, and β,γ-CF_2-analogues

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ABSTRACT

Three novel bisphosphonate analogues of 8-oxo-dGTP **3** in which the bridging β,γ-oxygen is replaced by a methylene, fluoromethylene or difluoromethylene group (**4**–**6**, respectively) have been synthesized from 8-oxo-dGMP **2** by reaction of its morpholine 5′-phosphoramidate **14** or preferably, its *N*-methylimidazole 5′-phosphoramidate **15** with tri-*n*-butylammonium salts of the appropriate bisphosphonic acids, **11**–**13**. The latter method also provides a convenient new route to **3**. Analogues **4**–**6** may be useful as mechanistic probes for the role of **3** in abnormal DNA replication and repair.

Introduction

Oxidative DNA damage due to reactive oxygen species (ROS) has been implicated in the pathogenesis of a wide variety of diseases, including cancer [1], neurodegenerative and neurodevelopmental disorders [2], inflammatory disorders [3] and aging [4]. 8-Oxo-2′-deoxyguanosine (8-oxo-dG, **1**) is a harbinger of oxidative DNA damage [5] and has been implicated in carcinogenesis by inducing mutations [6] as well as by abnormal epigenetic modulation of gene expression [7]. The mutagenicity of **1** is attributed to a conformational shift of the N9-C1′ glycosidic bond from *anti* to *syn*, causing it to mimic a *syn* thymidine [8]. As a result of A:8-oxo-G (A:8OG) Hoogsteen base mispairing, replicative DNA polymerases (pols) often insert dATP opposite **1** instead of dCTP [9,10]. Pol β, pol η, REV1, pol ξ and pol κ have all been implicated [11] in the incorporation of 8-oxo-dGMP **2** into DNA from 8-oxo-2′-deoxyguanosine-5′-triphosphate (8-oxo-dGTP, **3**), present as an ROS in the cellular nucleotide pool [12]. Wilson and co-workers recently described the crystal structure of a pol β DNA complex in which the adenine of a DNA (*syn*)8OG:A base pair was replaced at the primer terminus by a cytosine [13]. It is apparent that complementary information about the functional mechanism and transition state (TS) is desirable.

Herein, we report the synthesis of a small toolkit of 8-oxo-dGTP bisphosphonate probes, including an alternate preparation of the reference nucleotide, **3**. The toolkit comprises three β,γ-CXY bridged 8-oxo-dGTP analogues: β,γ-methylene- **4**, β,γ-monofluoromethylene- **5**, and β,γ-difluoromethylene-8-oxo-dGTP **6** (Fig. 1).

A similar toolkit based on the natural nucleotides has been used to study leaving group effects on the nucleotidyl transfer kinetic mechanisms and fidelity of pols [14–18]and other biocatalysts [19]. As the β,γ-bridge atom X and Y substituents become more electronegative, the pK_{a4} of the corresponding bisphosphonate leaving group decreases [20,21], stabilizing the conjugate base anion. If the rate-determining step (RDS) involves P—O bond breaking, then a Brønsted plot of the log of the catalytic rate constant (k_{pol}) versus pK_{a4} is predicted to be linear (linear free energy relationship, LFER) with a negative slope reflecting the sensitivity of the TS to anion stabilization [22]. The bisphosphonates selected provide a range of pK_{a4} of 2.75 units, centered on the pK_{a4} of the leaving group in **3**, pyrophosphoric acid [20].

Results and discussion

Early oxidative methods [12,23] to prepare **3** itself directly from dGTP in low or unstated yield were not reproduced by others [24], as confirmed by own work (data not shown). Direct oxidative methods have a further limitation in that they do not give convenient access to 8-[^{17}O]- or 8-[^{18}O]-oxo-guanosine derivatives [25]. Einolf described an 8-step synthesis of **3** beginning from dG **7** involving several protection/deprotection steps, culminating in separation of the final compound from mono- and diphosphate 8-oxo-dG byproducts [26]. Nampalli and Kumar [24] subsequently outlined

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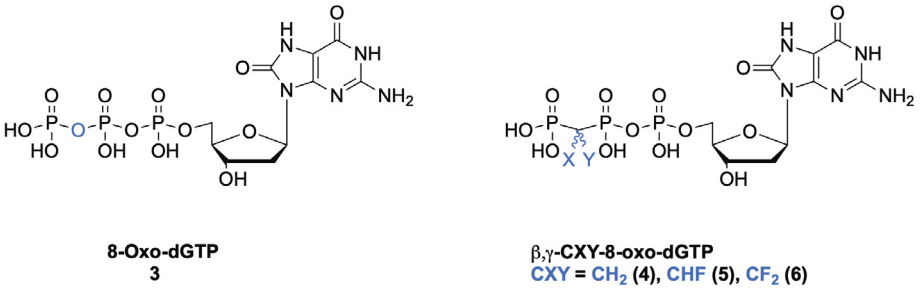
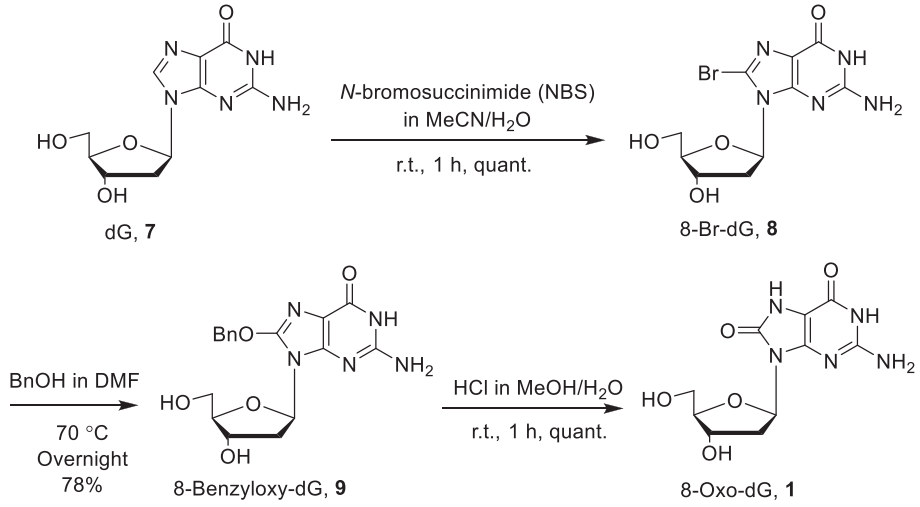


Fig. 1. Structures of 8-oxo-dGTP **3**, β,γ-CH_2- **4**, β,γ-CHF-**5**, and β,γ-CF_2-8-oxo-dGTP **6**.

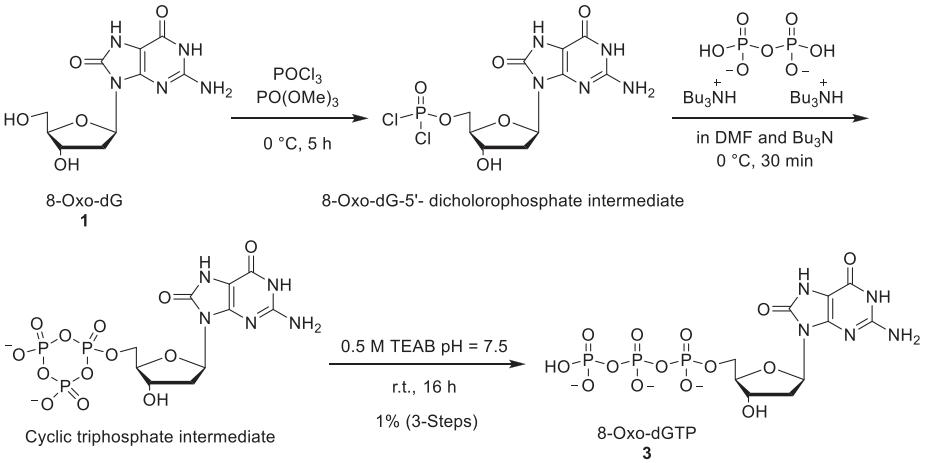
a gram-scale synthesis of **3** from 8-bromo-dG [27,28] **8** in 36% overall yield *via* conversion to the 8-benzyloxy derivative **9** using sodium/benzyl alcohol in dimethyl sulfoxide, followed by treatment with phosphorus oxychloride ($POCl_3$) [29] in trimethyl phosphate and reaction with bis(tri-*n*-butylammonium) pyrophosphate. The final product was obtained by hydrolysis of the resulting cyclic triphosphate intermediate in aqueous triethylammonium bicarbonate (TEAB) at pH 7.5 (Schemes 1 and 2) [24].

8-Benzyloxy-dG **9** can be prepared from **7** in 78% yield [27,28], and we found that formation of an 8-dimsyl-dG byproduct can be avoided by using anhydrous *N*,*N*-dimethylformamide (DMF) in place of DMSO as the solvent (Scheme 1). However, in our hands, phosphorylation of **1** or **9** on a small scale using the literature one-pot-three-step procedure [24,30,31] (Scheme 2) gave much lower yields than anticipated.

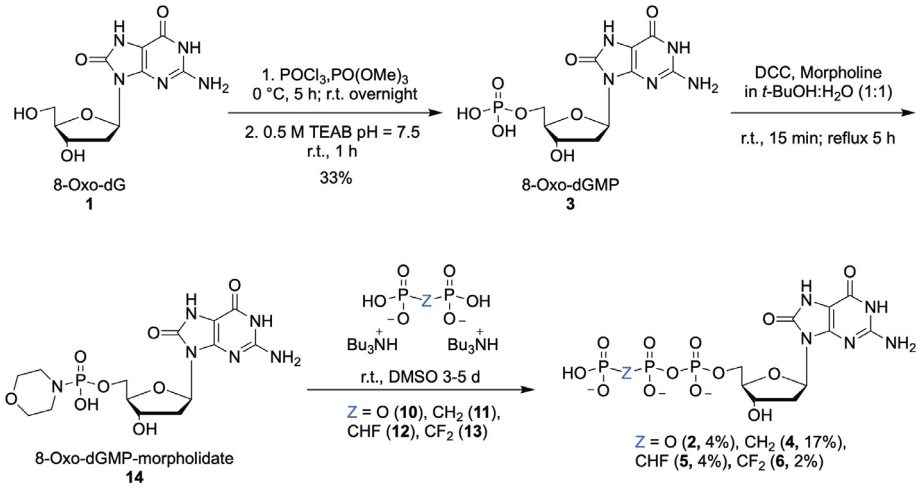
We therefore examined an alternative synthesis of **3** starting from 8-oxo-dGMP **2** (prepared in 33% yield by monophosphorylation of **1** with $POCl_3$ in $PO(OMe)_3$ [29] followed by aqueous workup with 0.5 M TEAB) after activation by morpholine [32] or *N*-methylimidazole [33,34] to facilitate coupling with pyrophosphate



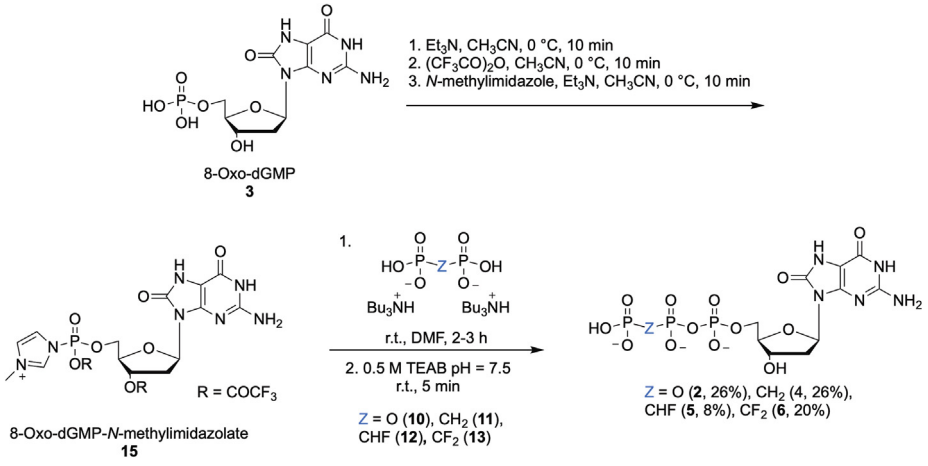
Scheme 1. Synthesis of 8-oxo-dG **1** [24,27,28].



Scheme 2. 'One-pot' phosphorylation [30,31] synthesis of 8-oxo-dGTP **3** from 8-oxo-dG **1** [24]; (TEAB: triethylammonium bicarbonate).



Scheme 3. Synthesis of **3**–**6** *via* the 5′-morpholidate **14**.



Scheme 4. Synthesis of **3**–**6** *via* the 5′-*N*-methylimidazolide **15**.

10, as a method likely to be adaptable to the synthesis of **4**–**6** from the appropriate bisphosphonate tri-*n*-butylammonium salt **11**–**13** [20], prepared by treatment of commercially available methylene-bis(phosphonic acid) or its α-fluorinated derivatives [35] with tri-*n*-butylamine in aq. ethanol. 8-Oxo-dGMP-morpholidate **14** gave **3** and the target bisphosphonate nucleotides **4**–**6**, but the reactions were sluggish, with very poor yields (Scheme 3).

Better results were obtained with *N*-methylimidazole activation [33,34]. Thus, **2** suspended in a mixture of triethylamine and excess trifluoroacetic anhydride in acetonitrile was treated with *N*-methylimidazole to give the corresponding 8-oxo-dGMP-*N*-methylimidazolide **15**, which was then added to tri-*n*-butylammonium pyrophosphate **10** or the tri-*n*-butylammonium bisphosphonate **11**, **12** or **13** in DMF (Scheme 4). Deactivated 8-oxo-dGMP **2** can be recovered during purification of the final products (characterized by ^{1}H, ^{31}P and ^{19}F NMR, LC-MS and HRMS) *via* SAX/C18 preparative HPLC.

In the coupling reactions to form **3**–**6**, the reaction time was much shorter with the *N*-methylimidazolides (2–3 h) compared to the morpholidates (3–5 d) [30–32]. It is critical to thoroughly dry the tri-*n*-butylammonium salt of the bisphosphonic acid by repeated coevaporation with anhydrous DMF before use to achieve optimal yields.

The proton-decoupled ^{31}P NMR spectra of **3**–**6** are compared in Fig. S44. As expected, a dramatic upfield shift of the P_γ and P_β resonances is seen with more electronegative substituents (CF_2 > CHF > CH_2) on the bridging β-methylene group. There is no effect on the P_α resonance which remains constant at about −10 ppm.

Consistent with published data for the β,γ-monofluoro and β,γ-difluoro analogues of dGTP [15], **5** and **6** exhibit ^{19}F NMR resonances at δ −217.32 and δ −120.95 ppm, respectively. The $^{31}P_\beta$, $^{31}P_\gamma$ and ^{19}F peaks of **5** exhibit a slight broadening consistent with a small Δδ for the *R v S* diastereomers [15] (the individual isomers should be accessible via chiral synthons derived from **12** [20]).sy).]

Conclusion

In summary, we examined several alternative approaches for the synthesis of 8-oxo-dGTP **3** and three novel bisphosphonate analogues: β,γ-methylene- **4**, (*R/S*)-β,γ-monofluoromethylene- **5**, and β,γ-difluoromethylene-8-oxo-dGTP **6**. Conjugation of the corresponding bisphosphonic acid *n*-butylammonium salts **11**–**13** with 8-oxo-dGMP-*N*-methylimidazolide **15** in anhydrous DMF is a practical route to these compounds, albeit in low (not optimized) yields. The availability of the resulting 8-oxo-dGTP analogue toolkit provides a novel means to probe leaving group effects on the binding and kinetic mechanisms of **3** interacting with nucleic acid polymerases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data (details of synthetic procedures with spectroscopic and other characterization data) to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.152890.

References

[1] T.L. Scott, S. Rangaswamy, C.A. Wicker, T. Izumi, Antioxid. Redox Signal. 20 (2014) 708.
[2] J.K. Andersen, Nat. Med. 10 (2004) 18.
[3] X. Ba, L. Aguilera-Aguirre, S. Sur, I. Boldogh, Curr. Opin. Allergy Clin. Immunol. 15 (2015) 89.
[4] M.L. Hamilton, H. Van Remmen, J.A. Drake, H. Yang, Z.M. Guo, K. Kewitt, C.A. Walter, A. Richardson, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10469.
[5] M.K. Shigenaga, E.N. Aboujaoude, Q. Chen, B.N. Ames, Meth. Enzymol. 234 (1994) 16.
[6] T. Suzuki, H. Kamiya, Genes Environ. 39 (2017) 2.
[7] P.K. Mahalingaiah, L. Ponnusamy, K.P. Singh, Oncotarget 8 (2017) 11127.
[8] M.J. Burak, K.E. Guja, E. Hambardjieva, B. Derkunt, M. Garcia-Diaz, EMBO J. 35 (2016) 2045.
[9] S. Shibutani, M. Takeshita, A.P. Grollman, Nature 349 (1991) 431.
[10] J.M. Krahn, W.A. Beard, H. Miller, A.P. Grollman, S.H. Wilson, Structure 11 (2003) 121.
[11] B. van Loon, R. Woodgate, U. Hubscher, DNA Repair 29 (2015) 1.
[12] J.Y. Mo, H. Maki, M. Sekiguchi, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 11021.
[13] V.K. Batra, S.H. Wilson, Commun. Biol. 3 (2020) 348.
[14] C.A. Sucato, T.G. Upton, B.A. Kashemirov, J. Osuna, K. Oertell, W.A. Beard, S.H. Wilson, J. Florian, A. Warshel, C.E. McKenna, M.F. Goodman, Biochemistry 47 (2008) 870.
[15] Y. Wu, V.M. Zakharova, B.A. Kashemirov, M.F. Goodman, V.K. Batra, S.H. Wilson, C.E. McKenna, J. Am. Chem. Soc. 134 (2012) 8734.
[16] K. Oertell, B.A. Kashemirov, A. Negahbani, C. Minard, P. Haratipour, K.S. Alnajjar, J.B. Sweasy, V.K. Batra, W.A. Beard, S.H. Wilson, C.E. McKenna, M.F. Goodman, Biochemistry 57 (2018) 3925.
[17] V.K. Batra, K. Oertell, W.A. Beard, B.A. Kashemirov, C.E. McKenna, M.F. Goodman, S.H. Wilson, Biochemistry 57 (2018) 3934.
[18] K.S. Alnajjar, I.S. Krylov, A. Negahbani, P. Haratipour, B.A. Kashemirov, J. Huang, M. Mahmoud, C.E. McKenna, M.F. Goodman, J.B. Sweasy, Nucleic Acids Res. 47 (2019) 11839.
[19] N.A. Setterholm, P. Haratipour, B.A. Kashemirov, C.E. McKenna, G.F. Joyce, Biochemistry 60 (2021) 1.
[20] P. Haratipour, C. Minard, M. Nakhjiri, A. Negahbani, B.T. Chamberlain, J. Osuna, T.G. Upton, M. Zhao, B.A. Kashemirov, C.E. McKenna, J. Org. Chem. 85 (2020) 14592.
[21] C.E. McKenna, P. Haratipour, M.V.V. Duro, F.H. Ebetino, Chemistry of bisphosphonatesin: Encyclopedia of Bone Biology; Saidi, M. Ed.; Elsevier, 2020. pp 551.
[22] C. Mihai, A.V. Kravchuk, M.D. Tsai, K.S. Bruzik, J. Am. Chem. Soc. 125 (2003) 3236.
[23] H. Kasai, S. Nishimura, Nucleic Acids Res. 12 (1984) 2137.
[24] S. Nampalli, S. Kumar, Bioorg. Med. Chem. Lett. 10 (2000) 1677.
[25] R.C.A. Hermanns, G. Zomer, M. Jacquemijns, J.F.C. Stavenuiter, J.G. Westra, A.J. R. Teixeira, G. Van De Werken, J. Labelled Compd. Radiopharm. 34 (1994) 191.
[26] H.J. Einolf, N. Schnetz-Boutaud, F.P. Guengerich, Biochemistry 37 (1998) 13300.
[27] T.S. Lin, J.C. Cheng, K. Ishiguro, A.C. Sartorelli, J. Med. Chem. 28 (1985) 1194.
[28] P.M. Gannett, T.P. Sura, Synth. Commun. 23 (1993) 1611.
[29] M. Yoshikawa, T. Kato, T. Takenishi, Tetrahedron Lett. (1967) 5065.
[30] J. Ludwig, Acta Biochim. Biophys. Acad. Sci. Hung. 16 (1981) 131.
[31] J.L. Ruth, Y.C. Cheng, Mol. Pharmacol. 20 (1981) 415.
[32] J.G. Moffatt, H.G. Khorana, J. Am. Chem. Soc. 83 (1961) 649.
[33] V.S. Bogachev, Bioorg. Khim. 22 (1996) 699.
[34] S. Mohamady, D.L. Jakeman, J. Org. Chem. 70 (2005) 10588.
[35] C.E. McKenna, P.D. Shen, J. Org. Chem. 46 (1981) 4573.