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Polymerases

Selective Modulation of DNA Polymerase Activity by Fixed-Conformation Nucleoside Analogues**

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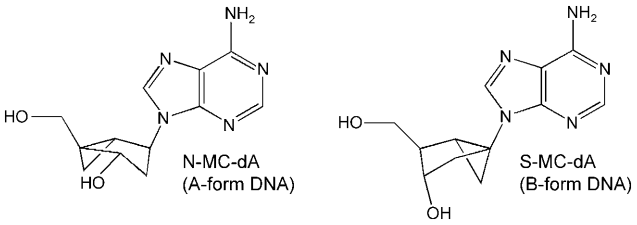
The human genome encodes multiple polymerases (pols), enzymes capable of synthesizing DNA.[1] In hindsight, it seems obvious that the complex nature of nucleic acid chemistry would necessitate a redundancy of polymerase activity that does not rely upon one single enzyme for nucleotide selectivity.[2,3] Accuracy during replication of the genetic code is vital to multicellular organisms, but the molecular constraints that facilitate high-fidelity DNA synthesis often prove inhibitory in the face of adducted (i.e., "damaged") DNA.[4,5] Evolution has resulted in many non-essential DNA polymerases (including the Y family) that are conserved as a means of bypassing damaged DNA and/or unusual secondary structures in the template DNA.[6] The deregulation of Y-family member activity has been associated with several tumor types, including breast, ovarian, colorectal, and non-small cell lung cancers.[7–11] Also, germline mutations in the human gene that encodes polymerase η result in *Xeroderma pigmentosum* variant type (XPV), which is characterized by a high susceptibility to skin cancer.[12,13]

Some members of the Y family possess distinctive mechanisms for nucleotide selection including Hoogsteen base pairing modes (e.g., pol ι during insertion opposite template purines) and protein template-directed catalysis (e.g., REV1).[6,14,15] As such, the unique properties of these enzymes represent a potential target for specific inhibition or activation by small molecules. Nucleoside analogues such as 3′-azido-2′-deoxythymidine (AZT) have been used successfully to inhibit viral genome synthesis, although the development of resistance to the drug through excision is a major obstacle to long-term efficacy.[16–18] Fixed conformation nucleoside analogues were initially created in an effort to overcome human immunodeficiency virus type-1 reverse transcriptase (HIV-1 RT) mediated excision of chain-terminating nucleoside analogues.[16,19,20] The bicyclo[3.1.0]hexane scaffold was used to generate nucleosides that are permanently locked in either the North (N) **1** or South (S) **2** envelope orientation (Scheme 1).[21] The resulting analogues



Scheme 1. Chemical structure of N-MC-dA **1** and S-MC-dA **2**, which mimic the sugar pucker observed in A- and B-form double-stranded nucleic acid helices, respectively.

have been designated as methanocarba-2′-deoxynucleoside triphosphates (MC-dNTP), with the N and S template mimicking the most common sugar pucker observed in A- (C3′-endo) and B-form (C2′-endo) double-strand DNA (dsDNA), respectively.[19] The North compound has shown antiviral activity against herpes simplex virus type 1 and orthopoxviruses; the South isomer is inactive.[22] The cytotoxic effect requires viral kinase activity to convert the nucleoside into the monophosphate form.[19,23–25] By locking the cyclopentane ring in either the N or S conformation, the 3′-OH group of the MC-dNTP is placed in either an equatorial or an axial position, respectively. The sugar pucker and the positioning of the 3′-OH group can have important consequences for both the insertion and the extension step of DNA strands by DNA polymerases but the determination of a functional preference for one conformation over the other has been difficult in the absence of the appropriate chemical probes. Previous work has shown that HIV-1 RT only utilizes the North versions of AZT and 2′,3′-dideoxynucleosides.[26,27] We decided to investigate the ability of several DNA polymerases to incorporate and extend from N- and S-oriented fixed-conformation nucleosides. Herein we report that human Y-family DNA polymerases exhibit unique properties during the insertion of fixed conformation nucleoside triphosphates and these properties were used to selectively inhibit the growth of breast

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tumor cells that overexpress one of these error-prone polymerases, namely human pol ι.

Firstly, the ability of different DNA polymerases to insert and extend from N- and S-oriented fixed-conformation nucleosides was tested. HIV-1 RT was able to utilize the N template **1** but extension of the growing DNA strand (primer) is inhibited (Figure S1 in the Supporting Information); this observation is consistent with previous results.[19] HIV-1 RT did not utilize the S template **2** under the conditions tested. Three human Y-family DNA polymerases were also tested and showed mixed results for insertion and extension of the fixed conformation nucleosides. Human DNA pol η (hpol η) appears to utilize both **1** and **2** (Figure 1b). In fact, extension appears to be slightly more favorable for S-MC-dATP, which is the triphosphate form of **2** (Figure 1b, compare 30 min time points for hpol η). In contrast to hpol η, human single dNTP into the growing DNA strand and then slowly added one nucleotide from the first insertion event. Finally, human pol κ was quite efficient at insertion and extension from N-MC-dATP **1** (Figure 1b, bottom panel). Hpol κ did not utilize S-MC-dATP.

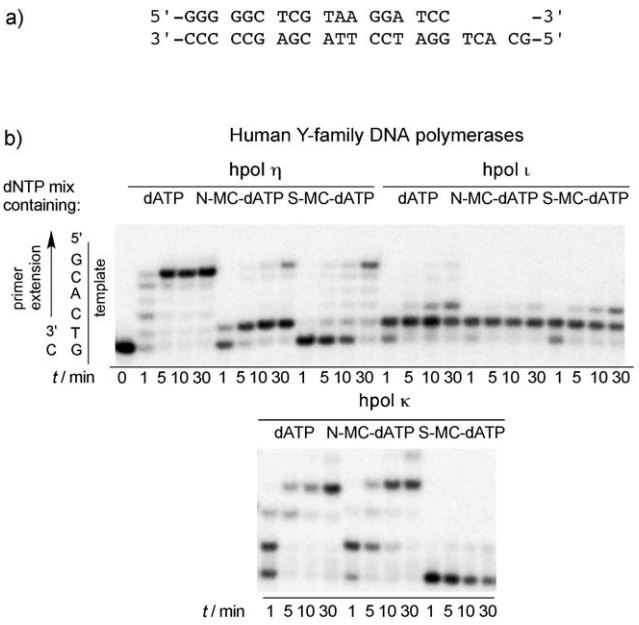

Figure 1. DNA-polymerase-catalyzed insertion and extension from fixed conformation nucleoside analogues. a) Primer-template DNA sequence. b) Full-length extension products for three human Y-family DNA polymerases. Catalysis was performed in the presence of dATP, N-MC-dATP, or S-MC-dATP and the other three dNTPs; products were analyzed by poly(acrylamide) gel electrophoresis (PAGE).

Single-nucleotide insertion experiments were then performed with N-MC-dATP **1** and S-MC-dATP **2** (Figure S2 in the Supporting Information). HIV-1 RT inserted N-MC-dATP with a velocity that was three times slower than the initial velocity relative to dATP but failed to incorporate S-MC-dATP in the time frame tested. In contrast to HIV-1 RT, hpol η inserted both the N- and S-templates with an initial velocity that was 4- and 14 times lower, respectively, relative to dATP insertion. On the other hand, hpol κ inserted only N-MC-dATP with an initial velocity that was 3.5 times slower compared to dATP. The most striking result was obtained with pol ι whose velocity of N-MC-dATP insertion was increased fivefold over that of unmodified dATP.

The concentration dependence of the dNTP insertion was determined for the fixed conformation nucleosides in order to compare the steady-state catalytic efficiencies of the human Y-family DNA polymerases to that of HIV-1 RT in a quantitative manner (Table 1). HIV-1 RT was not inhibited during insertion of N-MC-dATP opposite thymidine. Both

Table 1: Steady-state kinetic analysis of polymerase-catalyzed insertion of fixed conformation nucleoside triphosphates.

Polymerase	k_{cat} [$s^{-1}\times10^3$]	$K_{m,dNTP}$ [μM]	$k_{cat}/K_{m,dNTP}$ [$s^{-1}\times10^3$ μM^{-1}]	Δ efficiency relative to dATP
HIV-1 RT				
dATP	91 ± 15	10.2 ± 6.0	9	–
N-MC-dATP	59 ± 7	6.9 ± 2.9	8	unchanged
hpol η				
dATP	89 ± 4	0.36 ± 0.13	247	–
N-MC-dATP	39 ± 2	0.68 ± 0.21	57	4-fold decrease
S-MC-dATP	30 ± 2	10 ± 4	3	80-fold decrease
hpol ι				
dATP	121 ± 8	27 ± 9	4.5	–
N-MC-dATP	185 ± 12	8.0 ± 2.0	23	5-fold increase
dGTP	630 ± 47	17 ± 5	37	8-fold increase
hpol κ				
dATP	686 ± 14	0.76 ± 0.12	900	–
N-MC-dATP	172 ± 10	0.73 ± 0.27	240	4-fold decrease

hpol η and hpol κ showed a fourfold decrease in catalytic efficiency when utilizing the N template. The reduction in efficiency by hpol κ could be attributed to a much reduced k_{cat} value, whereas hpol η showed both a slower k_{cat} and a slightly higher $K_{m,dNTP}$ values (Table 1). Hpol η inserted S-MC-dATP 80 times less efficiently than dATP insertion opposite template thymidine, but the ability of hpol η to utilize S-MC-dATP is notable because it is different from the other enzymes tested here. The available crystal structures of both human and yeast pol η consistently show B-form dsDNA.[28–31] In these structures, the distance between the phosphate groups near the nascent base pair corresponds to the B form of DNA (6.5 to 7.5 Å). The incoming dATP remains in the C3′-endo conformation, which is compatible with the A form when paired opposite either dT or CPD.[29] The dsDNA helical structure in the pol η structures are distinct from those observed in many DNA polymerase ternary structures where the double helix near the active site is more A-form in nature with phosphate distances closer to 5.5 to 6.5 Å.[32–34] Notably, the DNA in the binding cleft of yeast pol δ is also shown by X-ray crystallography to be B form.[35]

Consistent with the single concentration time course experiments, the specificity constant of the hpol ι insertion of N-MC-dATP was five times greater than that for dATP insertion and was nearly as efficient as hpol ι catalyzed insertion of dGTP opposite thymidine. Hpol ι has the unusual tendency to insert 2′-deoxyguanosine triphosphate (dGTP)

opposite the template 2′-deoxythymidine (dT) more efficiently than any other dNTP.[36] The active site of hpol ι shortens the C1′–C1′ distance between nucleotides at the replicative site from the normal 10 or 11 Å to 8 or 9 Å. These constraints result in the preferential formation of Hoogsteen base pairs when the template base is a purine.[15,37] The exact molecular reason for the increased hpol ι activity with N-MC-dATP may be related in part to the fact that unmodified adenine adopts the *syn* orientation in the active site of hpol ι, whereas dGTP maintains the typical *anti* orientation through an interaction between the exocyclic amino group of guanine and Gln59 (Figure S3 in the Supporting Information).[34] Maintaining dGTP in the *anti* orientation causes the template dT to shear out of plane with the other template bases, while the purine ring of the incoming dGTP maintains base-stacking interactions with the nascent base pair. The purine ring system for *syn*-oriented adenine, on the other hand, is tilted out of plane with the nascent base pair. The glycosyl torsion angle χ around the C1′-N9 bond (purines) determines the orientation of the base relative to the sugar. Unrestricted furanose moieties allow the purine base to adopt both the *syn* and *anti* orientations with a small energy barrier between them (ca. 1 kcal mol^{-1}).[38,39] The bicyclo[3.1.0]hexane scaffold leads to a greater energy barrier on the interconversion between *syn* and *anti* orientations of the thymidine analogue (10–15 kcal mol^{-1}).[22] A corresponding value has not been measured for the adenosine analogue, but in the solid state N-MC-dATP is always in the *anti* orientation while S-MC-dATP adopts both *anti* and *syn* orientations.[20] It is possible that a more stable *anti*-oriented N-MC-dATP forces the template dT to adopt a conformation similar to that observed in the hpol ι/dGTP:dT ternary complex, which contributes to greater catalytic activity.

The increased catalytic activity of hpol ι with N-MC-dATP was striking. Given the fact that N-MC-dATP is a replication inhibitor we hypothesized that the growth of cells showing an overabundance of or increased reliance upon pol ι activity might be more inhibited by the presence of N-MC-dATP than the proliferation of "normal" cells. Previous reports have shown that hpol ι is overexpressed in several breast-tumor-derived cell lines, as well as in tumor biopsies.[7,11] Two syngeneic breast cell lines were treated with N-MC-dATP to test the hypothesis that N-MC-dATP might prove more effective at the inhibition of the growth of cells that overexpress hpol ι than cells with normal hpol ι levels. The malignant Hs578T cell line is derived from a triple negative metastatic invasive ductal carcinoma of the breast and has been shown to express hpol ι at levels three times as high as the nonmalignant Hs578Bst cell line.[11] We reaffirmed overexpression of hpol ι in Hs578T cells relative to Hs578Bst cells (Figure S4 in the Supporting Information). The cells were exposed to varying concentrations of N-MC-dATP by nucleofection and plated in 96-well plates. It was important to plate the cells at a density of 1000 cells per well or less in order to see effective inhibition of growth (Figure 2a). We measured cell viability after allowing the cells to grow in culture for one week. The tumor-derived Hs578T cells were found to be 16 times more sensitive to growth inhibition by N-MC-dATP than Hs578Bst cells based on IC_{50} (concentration of inhibitor at half-maximal inhibition) values of 6 ± 1 and 94 ± 10 for Hs578T and Hs578Bst, respectively (Figure 2b). Treatment with the electrophile 4-hydroxynonenal (HNE) resulted in IC_{50} values of 27 ± 3 and 38 ± 24 for Hs578T and Hs578Bst, respectively (Figure 2c). HNE is a relatively nonspecific electrophile that damages proteins, lipids, and DNA.[40] The similar IC_{50} values observed after exposure of the cells to HNE indicate that the sensitivity observed with N-MC-dATP is not due to an inherent difference in sensitivity to cytotoxic agents between the two lines.

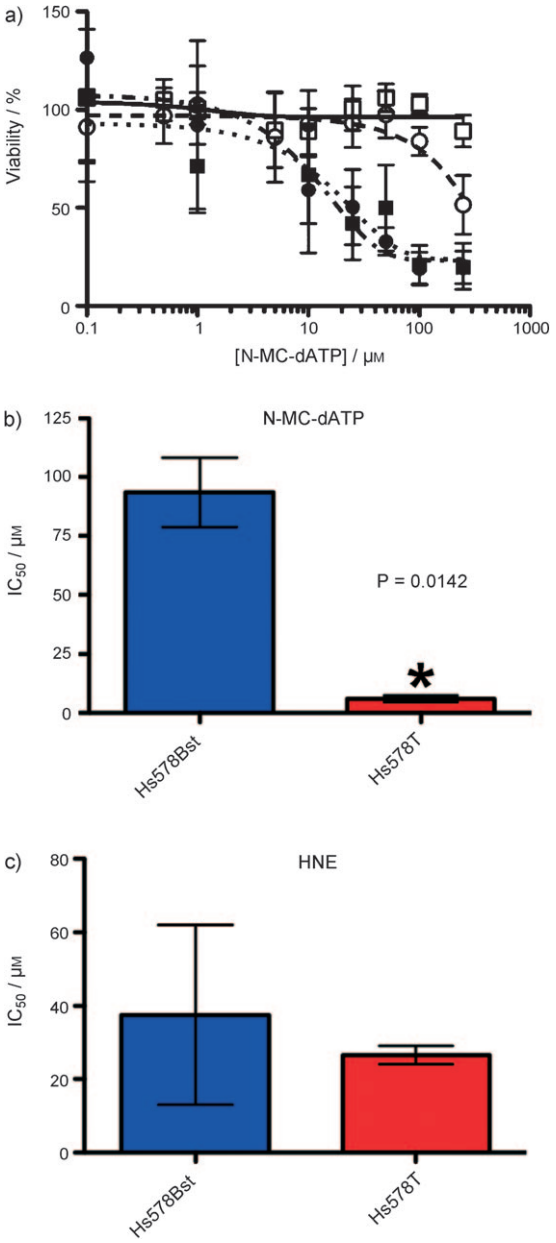

Figure 2. Selective growth inhibition of cells overexpressing human DNA polymerase ι by using fixed conformation nucleosides. a) Hs578T cells were exposed to varying concentrations of N-MC-dATP and then plated at a density of 25000 (□),10000 (○),1000 (■), and 500 (●) cells per well. Hs578Bst (blue) and Hs578T (red) were exposed to varying concentrations of b) N-MC-dATP or c) HNE in order to measure the IC_{50} value.

In conclusion, our results illustrate two important points. Firstly, different DNA polymerases (even those within a subfamily) show substantial differences in the preferences for furanose geometry of the sugar moiety during catalysis, with hpol η showing greater tolerance than the other two Y-family polymerases tested herein. The increased catalytic efficiency of hpol ι is fascinating when considering the structural determinants that influence the polymerase dNTP selectivity. Besides placing different "sugar" geometries in the polymerase active site, the N- and S-templates also influence the glycosyl torsion angle χ in different ways. The influence of the cyclopropane ring on χ most likely stabilizes the *anti* orientation of the purine in N-MC-dATP, thereby increasing the activity of hpol ι. Additionally, the results obtained with hpol ι show that C7′ does not necessarily perturb polymerase catalysis in a negative fashion. The second major conclusion derived from our work is related to targeting non-essential DNA polymerases for modulation within cells to alter biological outcomes. Other reports have illustrated that nucleoside analogues can inhibit the growth of cells overexpressing nonessential DNA polymerases with some specificity[41] and a number of inhibitors specific to certain polymerase subfamilies have been identified.[42–45] The results presented here are consistent with the idea that targeted inhibition of specialized DNA replication machinery can slow the growth of cells that have an overabundance of these enzymes. The specialized DNA polymerases β and η are known to alter the cell-killing effect of platinum-based chemotherapeutics.[46,47] Experiments are underway to investigate the potential use of S-MC-dATP to target cells that exhibit reliance upon pol η for survival. Compounds that modulate the activity of these and perhaps other nonessential polymerases may therefore represent a means of reducing the dose of toxic anti-cancer agents that are needed to achieve therapeutic benefits.

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[1] L. A. Loeb, R. J. Monnat, Jr., *Nat. Rev. Genet.* **2008**, *9*, 594.
[2] E. C. Friedberg, G. C. Walker, W. Siede, R. D. Wood, R. A. Shultz, T. Ellenberger, *DNA Repair and Mutagenesis*, 2nd ed., ASM Press, Washington, D.C., **2006**.
[3] N. Geacintov, S. Broyde, *The Chemical Biology of DNA Damage*, Wiley-VCH, Weinheim, in press.
[4] F. P. Guengerich, *Chem. Rev.* **2006**, *106*, 420.
[5] T. A. Kunkel, *J. Biol. Chem.* **2004**, *279*, 16895.
[6] W. Yang, R. Woodgate, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15591.
[7] M. R. Albertella, A. Lau, M. J. O'Connor, *DNA Repair* **2005**, *4*, 583.
[8] F. Lemée, C. Bavoux, M. J. Pillaire, A. Bieth, C. R. Machado, S. D. Pena, R. Guimbaud, J. Selves, J. S. Hoffmann, C. Cazaux, *Oncogene* **2007**, *26*, 3387.
[9] M. Wang, T. R. Devereux, H. G. Vikis, S. D. McCulloch, W. Holliday, C. Anna, Y. Wang, K. Bebenek, T. A. Kunkel, K. Guan, M. You, *Cancer Res.* **2004**, *64*, 1924.
[10] O. Wang-J, K. Kawamura, Y. Tada, H. Ohmori, H. Kimura, S. Sakiyama, M. Tagawa, *Cancer Res.* **2001**, *61*, 5366.
[11] J. Yang, Z. Chen, Y. Liu, R. J. Hickey, L. H. Malkas, *Cancer Res.* **2004**, *64*, 5597.
[12] H. Inui, K. S. Oh, C. Nadem, T. Ueda, S. G. Khan, A. Metin, E. Gozukara, S. Emmert, H. Slor, D. B. Busch, C. C. Baker, J. J. DiGiovanna, D. Tamura, C. S. Seitz, A. Gratchev, W. H. Wu, K. Y. Chung, H. J. Chung, E. Azizi, R. Woodgate, T. D. Schneider, K. H. Kraemer, *J. Invest. Dermatol.* **2008**, *128*, 2055.
[13] C. Masutani, R. Kusumoto, A. Yamada, N. Dohmae, M. Yokoi, M. Yuasa, M. Araki, S. Iwai, K. Takio, F. Hanaoka, *Nature* **1999**, *399*, 700.
[14] D. T. Nair, R. E. Johnson, L. Prakash, S. Prakash, A. K. Aggarwal, *Science* **2005**, *309*, 2219.
[15] D. T. Nair, R. E. Johnson, S. Prakash, L. Prakash, A. K. Aggarwal, *Nature* **2004**, *430*, 377.
[16] P. L. Boyer, S. G. Sarafianos, E. Arnold, S. H. Hughes, *J. Virol.* **2001**, *75*, 4832.
[17] P. L. Boyer, S. G. Sarafianos, E. Arnold, S. H. Hughes, *J. Virol.* **2002**, *76*, 9143.
[18] H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, S. Broder, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096.
[19] P. L. Boyer, J. G. Julias, V. E. Marquez, S. H. Hughes, *J. Mol. Biol.* **2005**, *345*, 441.
[20] V. E. Marquez, P. Russ, R. Alonso, M. A. Siddiqui, S. Hernandez, C. George, M. C. Nicklaus, F. Dai, H. Ford, Jr., *Helv. Chim. Acta* **1999**, *82*, 2119.
[21] V. E. Marquez, *Nucleic Acids Symp. Ser.* **2004**, *48*, 11.
[22] V. E. Marquez, T. Ben-Kasus, J. J. Barchi, Jr., K. M. Green, M. C. Nicklaus, R. Agbaria, *J. Am. Chem. Soc.* **2004**, *126*, 543.
[23] A. Ezzitouni, V. E. Marquez, *J. Chem. Soc. Perkin Trans. 1* **1997**, 1073.
[24] V. E. Marquez, M. A. Siddiqui, A. Ezzitouni, P. Russ, J. Wang, R. W. Wagner, M. D. Matteucci, *J. Med. Chem.* **1996**, *39*, 3739.
[25] W. Zhu, A. Burnette, D. Dorjsuren, P. E. Roberts, M. Huleihel, R. H. Shoemaker, V. E. Marquez, R. Agbaria, S. Sei, *Antimicrob. Agents Chemother.* **2005**, *49*, 4965.
[26] V. E. Marquez, A. Ezzitouni, P. Russ, M. A. Siddiqui, H. Ford, Jr., R. J. Feidman, H. Mitsuya, C. George, J. J. Barchi, Jr., *J. Am. Chem. Soc.* **1998**, *120*, 2780.
[27] L. Mu, S. G. Sarafianos, M. C. Nicklaus, P. Russ, M. A. Siddiqui, H. Ford, Jr., H. Mitsuya, R. Le, E. Kodama, C. Meier, T. Knispel, L. Anderson, J. J. Barchi, Jr., V. E. Marquez, *Biochemistry* **2000**, *39*, 11205.
[28] A. Alt, K. Lammens, C. Chiocchini, A. Lammens, J. C. Pieck, D. Kuch, K. P. Hopfner, T. Carell, *Science* **2007**, *318*, 967.
[29] C. Biertümpfel, Y. Zhao, Y. Kondo, S. Ramon-Maiques, M. Gregory, J. Y. Lee, C. Masutani, A. R. Lehmann, F. Hanaoka, W. Yang, *Nature* **2010**, *465*, 1044.
[30] T. Reißner, S. Schneider, S. Schorr, T. Carell, *Angew. Chem.* **2010**, *122*, 3142; *Angew. Chem. Int. Ed.* **2010**, *49*, 3077.
[31] T. D. Silverstein, R. E. Johnson, R. Jain, L. Prakash, S. Prakash, A. K. Aggarwal, *Nature* **2010**, *465*, 1039.
[32] L. S. Beese, V. Derbyshire, T. A. Steitz, *Science* **1993**, *260*, 352.
[33] L. G. Brieba, B. F. Eichman, R. J. Kokoska, S. Doublie, T. A. Kunkel, T. Ellenberger, *EMBO J.* **2004**, *23*, 3452.
[34] K. N. Kirouac, H. Ling, *EMBO J.* **2009**, *28*, 1644.
[35] M. K. Swan, R. E. Johnson, L. Prakash, S. Prakash, A. K. Aggarwal, *Nat. Struct. Mol. Biol.* **2009**, *16*, 979.
[36] A. Tissier, J. P. McDonald, E. G. Frank, R. Woodgate, *Genes Dev.* **2000**, *14*, 1642.
[37] M. G. Pence, P. Blans, C. N. Zink, T. Hollis, J. C. Fishbein, F. W. Perrino, *J. Biol. Chem.* **2009**, *284*, 1732.
[38] P. O. P. Ts'o, J. Eisinger, *Basic Principles in Nucleic Acid Chemistry*, Academic Press, New York,, **1974**.
[39] H. R. Wilson, A. Rahman, *J. Mol. Biol.* **1971**, *56*, 129.

[40] A. T. Jacobs, L. J. Marnett, *Acc. Chem. Res.* **2010**, *43*, 673.
[41] T. Louat, L. Servant, M. P. Rols, A. Bieth, J. Teissie, J. S. Hoffmann, C. Cazaux, *Mol. Pharmacol.* **2001**, *60*, 553.
[42] D. Dorjsuren, D. M. Wilson III, W. A. Beard, J. P. McDonald, C. P. Austin, R. Woodgate, S. H. Wilson, A. Simeonov, *Nucleic Acids Res.* **2009**, *37*, e128.
[43] T. Kimura, T. Takeuchi, Y. Kumamoto-Yonezawa, E. Ohashi, H. Ohmori, C. Masutani, F. Hanaoka, F. Sugawara, H. Yoshida, Y. Mizushina, *Bioorg. Med. Chem.* **2009**, *17*, 1811.
[44] Y. Mizushina, *Biosci. Biotechnol. Biochem.* **2009**, *73*, 1239.
[45] Y. Mizushina, H. Motoshima, Y. Yamaguchi, T. Takeuchi, K. Hirano, F. Sugawara, H. Yoshida, *Mar. Drugs* **2009**, *7*, 624.
[46] M. R. Albertella, C. M. Green, A. R. Lehmann, M. J. O'Connor, *Cancer Res.* **2005**, *65*, 9799.
[47] F. Boudsocq, P. Benaim, Y. Canitrot, M. Knibiehler, F. Ausseil, J. P. Capp, A. Bieth, C. Long, B. David, I. Shevelev, E. Frierich-Heinecken, U. Hubscher, F. Amalric, G. Massiot, J. S. Hoffmann, C. Cazaux, *Mol. Pharmacol.* **2005**, *67*, 1485.

