# Unusually Strong Binding of a Designed Transition-State Analog to a Base-Excision DNA Repair Protein

Li Deng, Orlando D. Schärer, and Gregory L. Verdine*

*Department of Chemistry and Chemical Biology
Harvard University, Cambridge, Massachusetts 02138*

DNA repair enzymes are responsible for maintaining the integrity of heritable genetic information. One class of such proteins, base-excision DNA repair (BER) enzymes, recognizes and removes from DNA aberrant bases that have arisen through the attack of exogenous or endogenous agents or through errors in replication.[1] Left unrepaired, these lesions have profound effects on cell viability and proliferation.[2] A major challenge is to identify how BER enzymes recognize their substrates, which often differ only subtly from their normal counterparts.[3] However, efforts along these lines have been hampered by the fleeting nature of the association between repair enzymes and their DNA substrates. In principle, this problem could be solved by structural alteration of either the enzyme or the substrate, so as to stall the normal reaction process and thereby generate a long-lived protein−DNA complex. Indeed, mutant versions of bacteriophage T4 endonuclease V and the human uracil DNA glycosylases have been found to bind tightly to DNA containing thymine dimer[4] and uracil, respectively.[5] X-ray crystallographic analyses of these complexes have yielded the first glimpses into the origin of substrate specificity of BER enzymes. We and others[6−8] have pursued an alternate approach based on the modification of the DNA substrate. We have focused our attention on members of a recently identified superfamily of BER enzymes, for which no cocrystal structures are yet available.[9] Our strategy for the design of altered DNA substrates that are capable of being recognized but not repaired centers on two distinct concepts, either mimicry[7] or electronic destabilization of the transition state[8,9] for the glycosyl transfer reaction leading to base excision (Figure 1).[10] Of particular relevance to the present study is the finding that pyrrolidine **1**, a transition-state mimic, binds with exceedingly high affinity and specificity to a variety of BER enzymes.[7] However, because **1** lacks a base moiety, it is unsuitable for studies aimed at elucidating the specific interactions between the substrate base and the enzyme active site. Here we report the design and synthesis of a new class of pyrrolidine-based inhibitors containing an attached base. Biochemical analysis of one such inhibitor
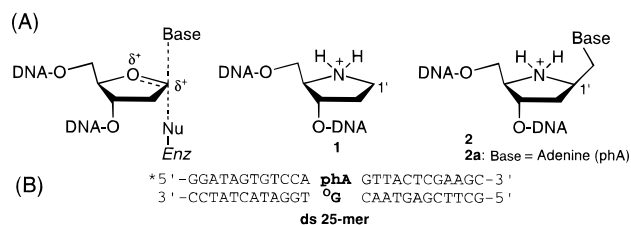
Figure 1. (A) Structural analogy between the proposed transition state for glycosidic bond cleavage catalyzed by base-excision DNA repair protein and inhibitors **1** and **2**; and (B) sequence of the double-stranded 25-mer (ds 25-mer) containing a single/centrally located phA/^{O}G pair. Asterick indicates 5′-^{32}P-label.

reveals that it binds a DNA glycosylase with a dissociation constant below 1 picomolar.

We reasoned that appropriate attachment of a DNA base to the pyrrolidine ring in **1** should lead to inhibitors possessing even stronger binding affinity and greater specificity than **1** itself. Attaching the base directly to the 1′-carbon in **1** would be expected to generate an unstable linkage,[11] thus ruling out this option. To ensure appropriate stability, we inserted a −CH$_2$− unit between the C-1′ and the base, thus generating the pyrrolidine homonucleoside containing inhibitor **2**. Although this formal insertion of a −CH$_2$− unit into the glysosidic bond lengthens the separation between the base and the pyrrolidine ring relative to that in the substrate, this bond is likewise elongated in the transition state.[10,11] Inhibitors designed along similar lines have been shown to be effective agents for glycosylases that act on monomeric nucleoside and simple carbohydrate substrates.[12] As a test system for these concepts, we decided to examine the ability of an adenine pyrrolidine homonucleoside in DNA (phA, **2a**) to inhibit the adenine DNA glycosylase MutY.[13] One component of a repair pathway specific for oxidatively damaged DNA,[14] MutY recognizes A inappropriately paired to the lesion 8-oxoguanine (^{O}G), and selectively cleaves the A residue.

The synthesis of inhibitor **2a** (Scheme 1) began with D-serine (**3**), which was converted in four steps and 60% overall yield to aldehyde **4**.[15] Allylboration of aldehyde **4** using a chiral boron reagent proceeded with excellent diastereoselectivity (94% de) to yield **5**.[16] Cleavage of the Boc-protected oxazolidine ring followed by TBS protection yielded **6**. Reaction of **6** with mCPBA in methylene chloride gave the corresponding epoxide as a mixture of diastereomers (5:1) favoring the desired stereoisomer. Acid-catalyzed intramolecular cyclization of epoxide **7** led to the formation of the key intermediate **8**. Attachment of the 6-chloropurine base to the pyrrolidine scaffold proceeded most effectively under Mitsunobu conditions[17] to afford the fully protected pyrrolidine homonucleoside **9**. Routine protecting group manipulations furnished cyclic diol **10**. Tritylation of the primary alcohol followed by phosphitylation

* To whom correspondence should be addressed.
(1) Lindahl, T. *Nature* **1993,** *362,* 709−715.
(2) Friedlberg, E. C.; Walker, G. C.; Siede, W. (1995). *DNA Repair and Mutagenesis*; ASM Press: Washington, DC, 1995.
(3) Brunner, S. D.; Verdine, G. L. *Chem. Biol.* **1997,** *4,* 329−334.
(4) Vassylyev D. G.; Kashiwagi, T.; Mikami, Y.; Ariyoshi, M.; Iwai S.; Ohtsuka, E.; Morikawa, K. *Cell* **1995,** *83,* 773−782.
(5) Slupphaug, G.; Mol, C. D.; Kavli, B.; Arvai, A. S.; Krokan, H. E.; Tainer, J. A. *Nature* **1996,** *80,* 87−92.
(6) Schärer, O. D.; Ortholand, J.-Y.; Ganesan, A.; Ezaz-Nikpay, K.; Verdine, G. L. *J. Am. Chem. Soc.* **1995,** *117,* 6623−6624. McCullough, A. K.; Schärer, O. D.; Verdine, G. L.; Lloyd, R. S. *J. Biol. Chem.* **1996,** *271,* 32147−32152.
(7) Schärer, O. D.; Verdine, G. L. *J. Am. Chem. Soc.* **1995,** *117,* 10781− 10782. Schärer, O. D.; Kawate, T.; Gallinari, P.; Jiriciny, J.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(8) Porello, S. L.; Williams, S. D.; Kuhn, H.; Michaels, M. L.; David, S. S. *J. Am. Chem. Soc.* **1996,** *118,* 10684−10692. Lu, A.-L.; Tsai-Wu, J.-J.; Cillo, J. *J. Biol. Chem.* **1995,** *270,* 23582−23588. Varaprasad, C. V.; Bulychev, N. Y.; Dorman, G.; Miller, J. H.; Eisenberg, M.; Grollman, A. P.; Johnson, F. *Biochemistry* **1996,** *35,* 13147−13156.
(9) Nash, H. M.; Bruner, S. D.; Schärer, O. D.; Kawate, T.; Addona, T.; Spooner,E.; Lane, W. S.; Verdine, G. L. *Curr. Biol.* **1996,** *6,* 1230− 1233. Labahn, J.; Schärer, O. D.; Long, A.; Ezaz-Nikpay, K.; Verdine, G. L.; Ellenberger, T. E. *Cell* **1996,** *86,* 321−329.

(10) Horenstein, B. A.; Schramm, V. L. *Biochemistry* **1993,** *32,* 7089− 7097. Horenstein, B. A.; Schramm, V. L. *Biochemistry* **1993,** *32,* 9917− 9925. Horenstein, B. A.; Zabinski, R. F.; Schramm, V. L. *Tetrahedron Lett.* **1993,** *34,* 7213−7216.
(11) Altmann, K. H. *Tetrahedron. Lett.* **1993,** *34,* 7721−7724
(12) Liu, P. S. *J. Org. Chem.* **1987,** *52,* 4717−4721. Wong, C-H.; Provencher, L.; Porco, J. A., Jr.; Jung, S.-H.; Wang, Y.-F.; Chen, L.; Wang, R.; Steensma, D. H. *J. Org. Chem.* **1995,** *60,* 1492−1501.
(13) Michaels, M. L.; Pham, L.; Nghiem, Y.; Cruz, C.; Miller, J. H. *Nucleic Acid Res.* **1990,** *18,* 3841−3845.
(14) Michaels, M. L.; Cruz, C.; Grollman, A. P.; Miller, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992,** *89,* 7022−7025. Grollman, A. P.; Moriya, M. *Trends Genet.* **1993,** *9,* 246−249. Tajiri T.; Maki, H.; Sekiguchi, M. *Mutat. Res. DNA Repair,* **1995,** *336,* 257−267. Michaels, M. L.; Tchou, J.; Grollman, A. P.; Miller, J. H. *Biochemistry* **1992,** *31,* 10964−10968.
(15) McKillop, A.; Taylor, R. J. K.; Waston, R. J.; Lewis, N. *Synthesis* **1994,** 31−33.
(16) Brown, H. C.; Bhat, K. S.; Randad, R. S. *J. Org. Chem.* **1987,** *52,* 319−320.

Scheme 1[a]
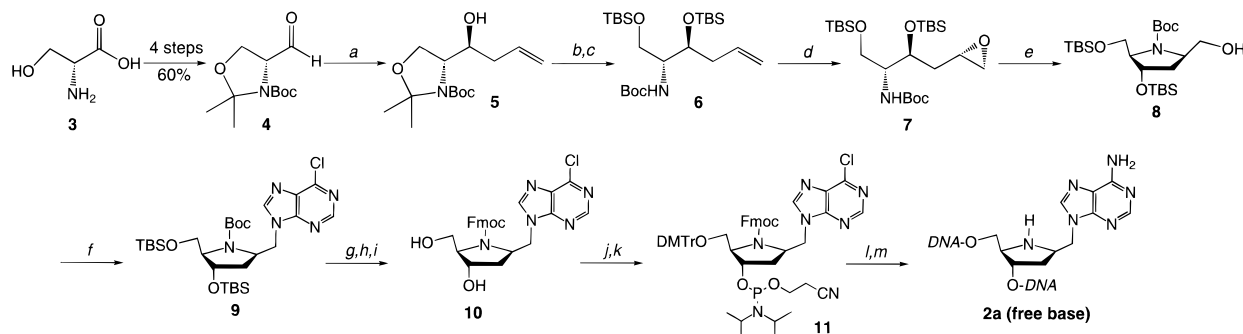
Reagents and conditions: *a*) allylB(lpc)$_2$, Et$_2$O; *b*) TsOH, MeOH, 52% 2 steps; *c*) TBSCl, DMF; *d*) mCPBA, CH$_2$Cl$_2$; *e*) AcOH; *f*) 6-chloropurine, DEAD, THF, 34% 4 steps; *g*) TBAF, THF; *h*) TFA, CH$_2$Cl$_2$; *i*) FmocCl, CH$_2$Cl$_2$/MeOH, 56% 3 steps; *j*) DMTrCl, DMAP, Et$_3$N, CH$_2$Cl$_2$; *k*) iPr$_2$NP(Cl)OC$_2$H$_4$CN, iPr$_2$NEt, CH$_2$Cl$_2$, 48% 2 steps; *l*) solid phase DNA synthesis; *m*) NH$_3$ 55 °C.
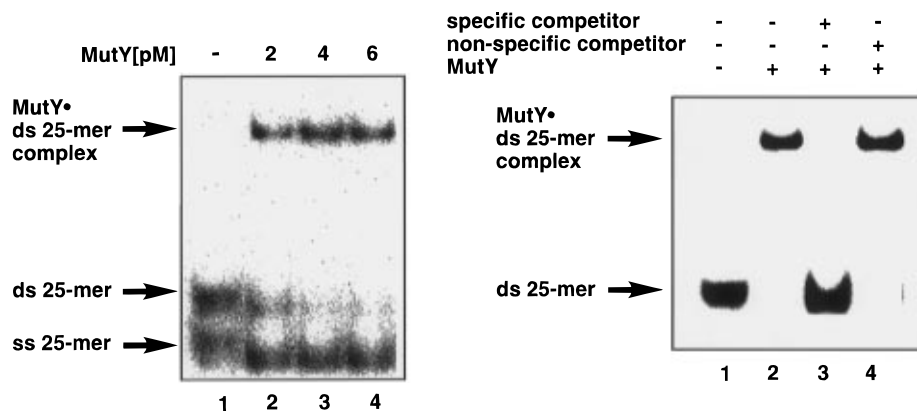
Figure 2. (A, left) EMSA assay to detect binding of **2a** to MutY. ^{32}P-labeled oligonucleotide concentration (single- plus double-stranded forms) = 3.0 pM. MutY concentrations are as indicated. (B, right) Competition EMSA assay to determine the specificity of **2a** to MutY: concentration of ^{32}P-labeled ds 25-mer, 0.2 nM; concentration of MutY, (lane 1) no protein, (lanes 2−4), 1.0 nM; concentration of unlabeled ds-25mer in lane 3, 20 nM; concentration of unlabeled non specific competitor in lane 4, 20 nM.

of the secondary alcohol provided the phosphoramidite **11**. Using phosphoramidite **11**, we synthesized a 25-mer oligonucleotide containing a centrally modified phA unit (5′-GGA TAG TGT CCA phA GTT ACT CGA AGC-3′). Deprotection of the oligonucleotide and concomitant conversion of the 6-chloropurine into adenine were accomplished by incubation of the oligonucleotide with aqueous ammonia at 55 °C for 12 h.[18] The phA-containing 25-mer was 5′-^{32}P-end labeled and annealed to a complementary 25-mer having one ^{O}G residue, thereby generating a duplex 25-mer containing a singly, centrally located phA/^{O}G pair (ds 25-mer, Figure 1B).

The binding affinity of MutY for the ds 25-mer containing phA/^{O}G was investigated with the use of the electrophoretic mobility shift assay (EMSA).[19] This assay detects the difference in electrophoretic mobility of the free ds 25-mer and the MutY·ds 25mer complex (Figure 2A). With the ds 25-mer present at a concentration of less than 3 pM, addition of 1 equiv of MutY resulted in virtually complete formation of the ds 25-mer·MutY complex (Figure 2A), thus indicating that the K_d for the protein−DNA interaction is well below 3 pM, and certainly below 1 pM.[20] By contrast, the K_d of MutY for the congener of the ds 25-mer bearing the simple pyrrolidine **1** in place of **2a** was determined to be 65 ± 11 pM. Thus, the binding of MutY is stimulated at least ~50-fold by the presence of the −CH$_2$−adenine unit in **2a**. A high degree of specificity for the inhibitor in this binding interaction was inferred on the basis of competition assays (Figure 2B), in which a 100-fold excess of nonradioactive counterpart of the ds 25-mer completely outcompeted complex formation with the radiolabeled ds 25-mer (lane 3), whereas a 100-fold excess of nonspecific DNA had

no effect (lane 4). In competition cleavage assays (data not shown), we determined that the addition of an equivalent amount of phA/^{O}G-containing DNA inhibited, by greater than 50%, MutY-catalyzed cleavage of a native A/^{O}G substrate, even when the enzyme was present in 50-fold excess over substrate.

Here we have reported a new class of mechanism-based inhibitors that bind with unprecedented strength (K_d < 1 pM) to a base-excision DNA repair protein, MutY.[21] We expect variants of **2a**, having a different base moiety, will bind specifically to other BER enzymes, especially those belonging to a recently identified superfamily of DNA glycosylases.[9] This new class of pyrrolidine homonucleosides should thus prove valuable in structural studies aimed at elucidating the molecular basis of DNA damage recognition and repair.

Supporting Information Available: General methods and analyses for the compounds discussed in this communication (11 pages). See any current masthead page for ordering and Internet access instructions.

JA970828U

(17) Mitsunobu, O. *Synthesis* **1981,** 1−54. Iwakawa, M.; Pinto, B. M.; Szarek, W. A. *Can. J. Chem.* **1978,** *56,* 326−335.
(18) Additional details are provided in the Supporting Information.
(19) Fried, M. G.; Crothers, D. M. *J. Mol. Biol.* **1984,** *172,* 263−282.

(20) Under conditions in which [DNA] ≪ K_d, the concentration of protein that affords 50% formation of a protein−DNA complex is approximately equal to K_d. As 1 equiv of MutY is sufficient to drive formation of a ds 25mer·MutY complex essentially to completion in Figure 2A, we conclude that the binding observed under these conditions is controlled by stoichiometry rather than K_d. This necessarily means that the DNA concentration in Figure 2A (3 pM) is will above K_d, hence K_d can safely be assumed to be less than 1 pM. Attempts to determine the K_d more precisely have been frustrated by the tendency of DNA to favor the single-stranded state at very low concentrations, and by the vanishingly small amounts of radioactive counts present under conditions in which binding is K_d controlled.
(21) Other known MutY inhibitors containing a base moiety bind the enzyme no more tightly than 0.2 nM.[7]