Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Reaction mechanism of nucleoside 2'-deoxyribosyltransferases: the free-energy landscape supports an oxocarbenium ion as the reaction intermediate

SUPPORTING INFORMATION

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Table S1. Available crystallographic structures in the Protein Data Bank			
PDB id.	**Resolution (Å)**	**Organism**	**Small molecules**
1F8X	2.5	*L. leichmannii*	-
1F8Y	2.4		5MD
1S3F	2.2	*L. helveticus*	SNI
1S2L	2.1		-
1S2I	2.24		BP1
1S2G	2.1		3D1
1S2D	2.1		ADE, AR4
4MEJ	2.1		28Y, SO4
4MCJ	2.4	*P. distasonic*	CL, MSE
2F67	1.6	*T. brucei*	12B
2F64	1.6		12Q
2F62	1.5		12M
2F2T	1.7		5IQ
2AOK	1.8		GOL
5NBR	1.66	*L. mexicana*	GOL

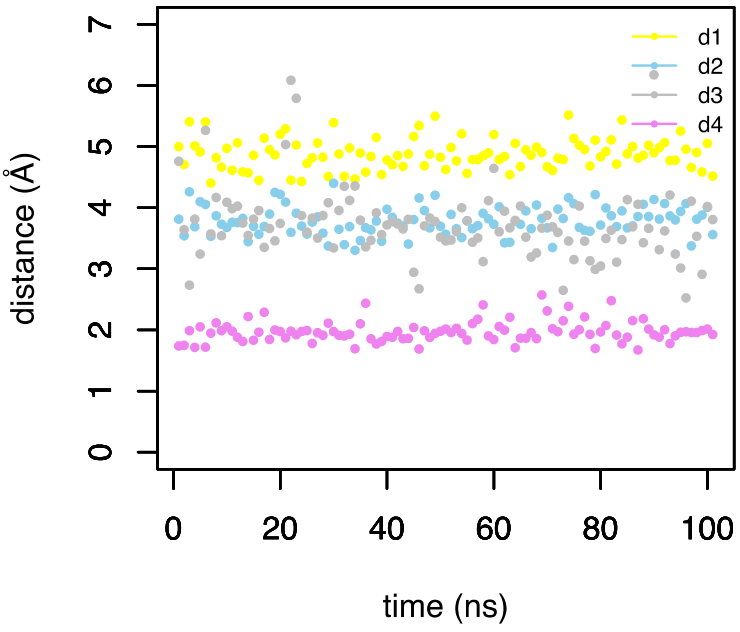


Figure S1. Evolution of d1-d4 (Å) distances along 100 ns of MD simulation of the E98D variant of LINDT in complex with dIno. The sampling of the conformational space of the variants is one of the main challenges

in the MD-based methods. In our case, we stablished a time of 10 ns in order to get equilibrated structures and remove possible steric crashes. As example, we show the simulation of Glu98Asp variant until 100 ns. We measured the key d1-d4 distances as shown in Figure 1 of the manuscript. No significant changes were found.

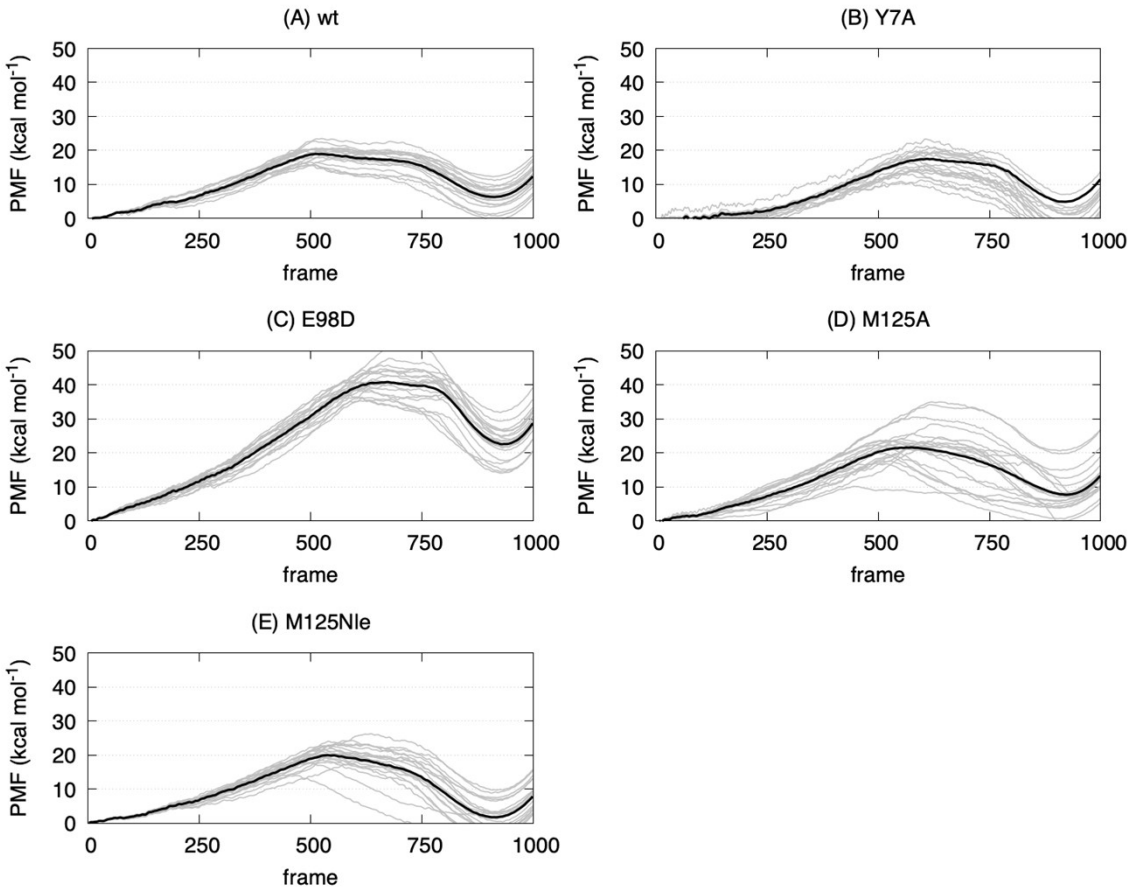


Figure S2. PMF (kcal mol^{-1}) of the 20 steered QM/MM MD simulations of the first half reaction catalyzed by the wild type, Y7A, E98D and M125Nle variants of *Ll*NDT. RC was defined using a linear combination of two variables: the shortening of the distance between C1' and OE1 of Glu98 and the shortening of the distance between the proton of the carboxylic acid of Tyr157# and the N7 of dIno. The mean value of the PMF profiles is shown with a bold black line.

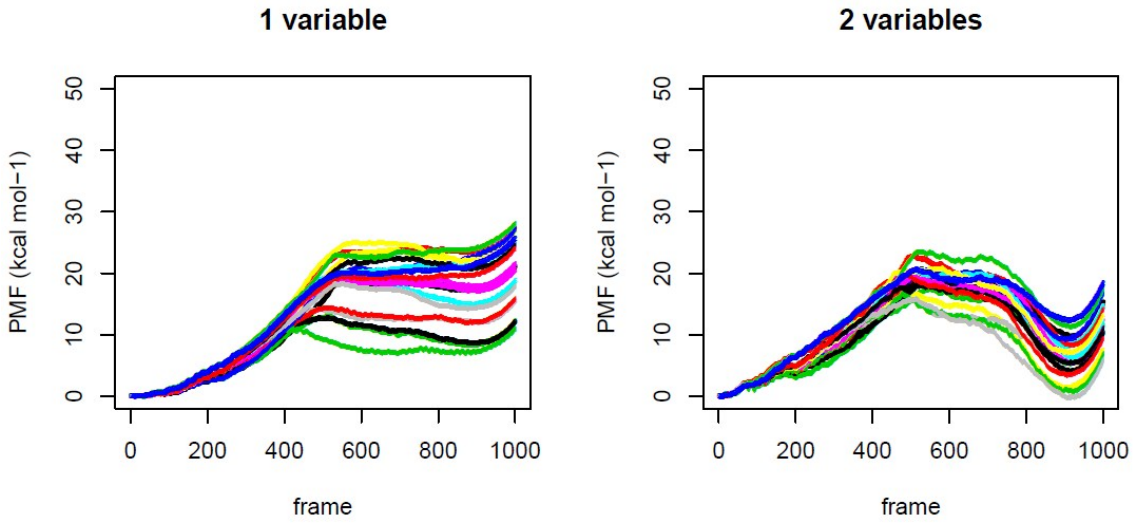


Figure S3. PMF (kcal mol^{-1}) of the 20 steered QM/MM MD simulations of the first half reaction with no explicitly defined proton transfer (RC_1, 1 variable) and including the proton transfer (RC_1 + RC_2, 2 variables) for the reaction catalyzed by the wild type enzyme. The x-axis stands for the snapshot along the steered QM/MM MD simulation.

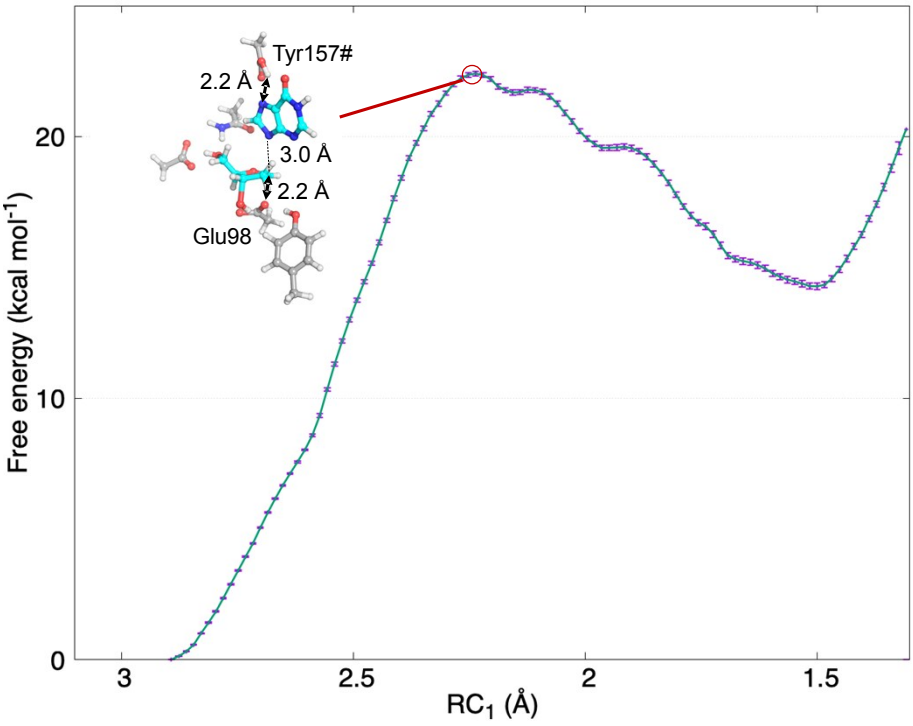


Figure S4. Umbrella-sampling free energy profile (kcal mol^{-1}) obtained for the first half reaction catalyzed by the wild-type *Ll*NDT using dIno as the donor nucleoside. The energy profile has been computed using WHAM and the error bars were computed by applying a bootstrap error analysis. The structure for the free energy maximum is highlighted.

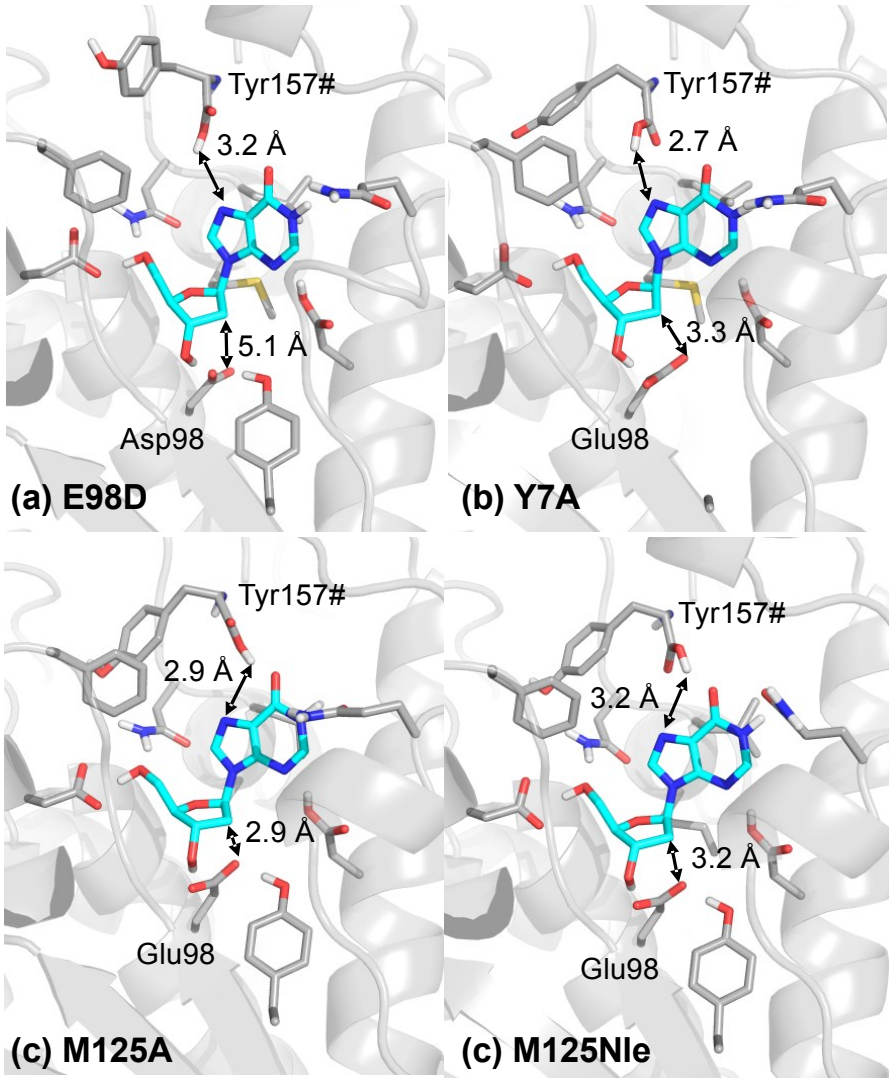


Figure S5. Near-attack conformations of the (a) E98D, (b) Y7A, (c) M125A and (d) M125Nle variants with dIno as the substrate. The two distances included in the collective variable (d_1 and d_2) are highlighted.

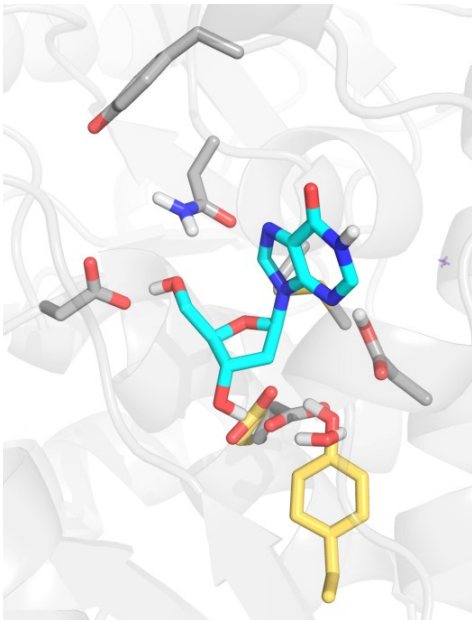


Figure S6. Detail of the active site of the Tyr7Ala *Ll*NDT variant where some of the main residues are shown as sticks (C-atoms colored in grey). Residues Tyr7 and Glu98 of the wild-type counterpart (C-atoms colored in yellow) are also shown after 3D structural superimposition. A water molecule spatially overlaps with the OH of Tyr7.

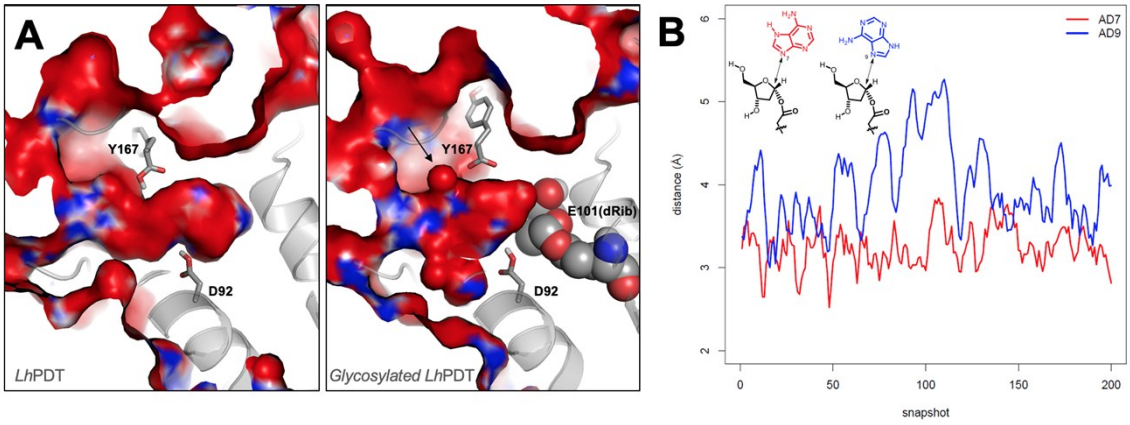
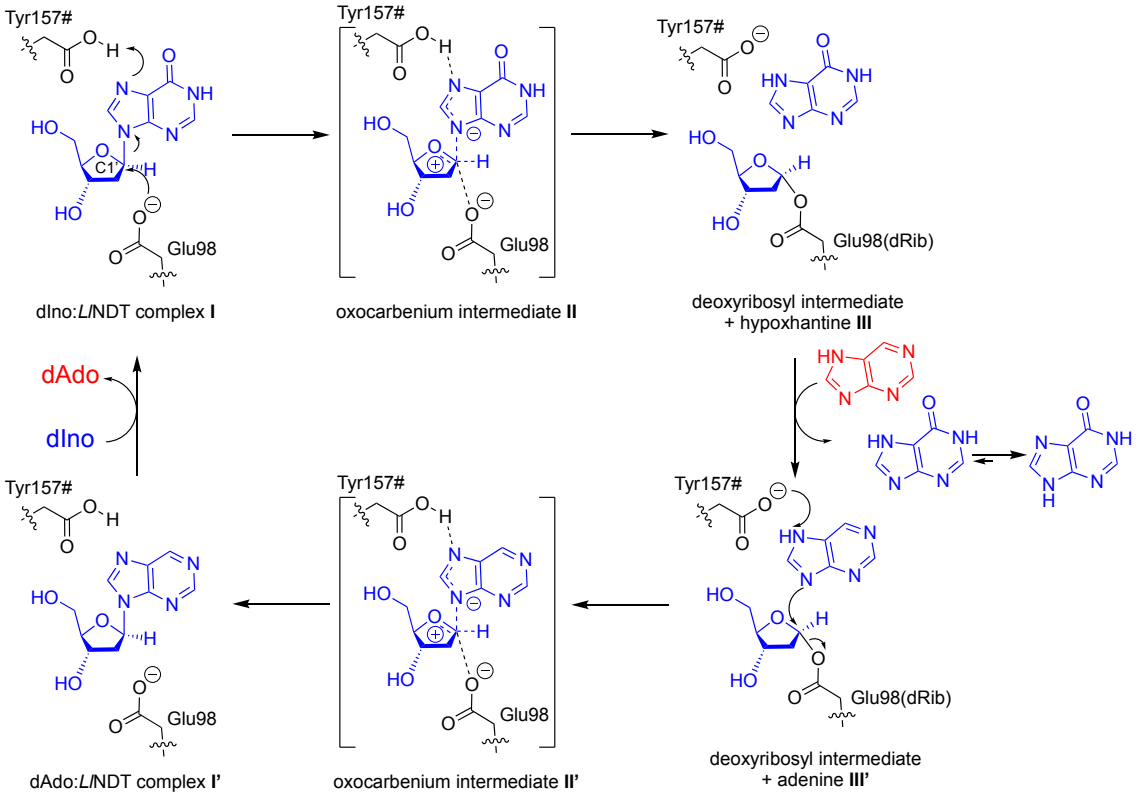


Figure S7. Binding of an incoming adenine protonated either on N7 (AD7) or N9 (AD9) into the active site of *Ll*NDT with Glu98 2'-deoxyribosylated. (A) Electrostatic potential map on the active site of *Ll*NDT with the catalytic Glu98 as carboxylate (left) and deoxyribosylated (right). (B) Evolution of the distance (Å) between the anomeric carbon C1' of the 2'-deoxyribosyl moiety bound to Glu98 and the N7 (tautomer AD7, red line) and N9 (tautomer AD9, blue line) nitrogen atoms.



Scheme S1. Full reaction mechanism of the transglycosylation between dIno and adenine catalyzed by *L*NDT.