Supplementary Data

Accommodation of an N-(deoxyguanosin-8-yl)-2-acetylaminofluorene adduct in the active site of human DNA polymerase ι: Hoogsteen or Watson-Crick base pairing?†

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Running Title: Bypassing a bulky major groove dG-AAF adduct in polι

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Table S1: Torsion angles for initial dG-AAF structures.

Structure	χ(°)	α′(°)	β′(°)	γ′(°)
HG Unmodified	64.9	N/A	N/A	N/A
HG-AAF1	61.2	138.3	357.5	195.9
HG-AAF2	61.2	138.3	214.8	195.9
HG-AAF3	61.2	183.9	6.8	204.3
HG-AAF4	61.2	183.9	186.9	204.3
WC Unmodified 1	254.1	N/A	N/A	N/A
WC Unmodified 2	169.8	N/A	N/A	N/A
WC-AAF1	192.7	260.7	203.9	160.7
WC-AAF2	192.7	82.1	163.1	160.7
WC-AAF3	192.7	260.7	344.4	160.7
WC-AAF4	192.7	82.1	339.1	160.7

Table S2: Atom types, topologies, and partial charges for dG-AAF.

Atom Name	Atom Type	Topology Type	Partial Charge
P	P	M	1.135035
O1P	O2	E	-0.802764
O2P	O2	E	-0.802764
O5′	OS	M	-0.494340
C5′	CT	M	0.045138
H5′1	H1	E	0.063861
H5′2	H1	E	0.063861
C4′	CT	M	0.180138
H4′	H1	E	0.108174

O4′	OS	S	-0.450678
C1′	CT	3	0.041166
H1′	H2	E	0.138883
C2′	CT	B	-0.164433
H2′1	HC	E	0.105277
H2′2	HC	E	0.105277
N9	N*	B	0.075941
C4	CB	S	-0.028326
N3	NC	S	-0.182524
C2	CA	B	0.431628
N2	N2	B	-0.919351
H21	H	E	0.440359
H22	H	E	0.440359
N1	NA	B	-0.332623
H1	H	E	0.321305
C6	C	B	0.402236
O6	O	E	-0.556027
C5	CB	S	0.195340
N7	NB	E	-0.537343
C8	CK	S	0.237276
N14	N*	B	0.048353
C18	C	B	0.504024
OAce	O	E	-0.549076
C19	CT	3	-0.262061
H191	HC	E	0.081507
H192	HC	E	0.081507
H193	HC	E	0.081507
C15	CA	S	0.017160
C14	CA	B	-0.309590

H14	HA	E	0.232032
C13	CB	S	0.026232
C12	CT	3	-0.001508
H121	HC	E	0.043781
H122	HC	E	0.043781
C11	CB	S	0.042214
C10	CA	B	-0.200667
H10	HA	E	0.140661
C9	CA	B	-0.140804
H9	HA	E	0.139289
C7	CA	B	-0.156272
H7	HA	E	0.142396
C3	CA	B	-0.179500
H3	HA	E	0.152272
C	CB	S	0.020935
C1	CB	S	0.032255
C17	CA	B	-0.192244
H17	HA	E	0.163321
C16	CA	S	-0.155998
H16	HA	E	0.201481
C3′	CT	M	0.118551
H3′	H1	E	0.074903
O3′	OS	M	-0.500524

Table S3: Atom types, topologies, and partial charges for dCTP$^+$.

Atom Name	Atom Type	Topology Type	Partial Charge
O1G	O2	M	-0.93283
PG	P	M	1.35374
O2G	O2	E	-1.01588
O3G	O2	E	-0.98337
O3B	OS	M	-0.53323
PB	P	M	1.28778
O1B	O2	E	-0.92812
O2B	O2	E	-0.78988
O3A	OS	M	-0.56804
PA	P	M	1.11343
O1A	O2	E	-0.70413
O2A	O2	E	-0.85764
O5′	OS	M	-0.52751
C5′	CT	M	0.05539
H5′1	H1	E	0.07799
H5′2	H1	E	0.06574
C4′	CT	M	0.42413
H4′	H1	E	-0.00175
C3′	CT	3	0.54226
H3′	H1	E	-0.07755
C2′	CT	B	-0.03056
H2′1	HC	E	0.00359
H2′2	HC	E	0.04303
O3′	OH	S	-0.78480

HO3′	HO	E	0.45144
O4′	OS	M	-0.57857
C1′	CT	M	0.11238
H1′	H2	E	0.17464
N1	N*	M	0.11879
C6	CM	M	0.06361
H6	H4	E	0.23845
C5	CM	M	-0.37365
H5	HA	E	0.20924
C4	CA	M	0.46441
N4	N2	B	-0.93663
H41	H	E	0.42684
H42	H	E	0.48657
N3	N3	M	-0.14236
H3	H	E	0.31250
C2	C	S	0.30474
O2	O	E	-0.56418

Table S4: AMBER parameters for dG-AAF and dCTP$^+$.

Bond	K_r kcal/(mol Å^2)	r_{eq} Å
C-N3	255.5	1.41
CA-N*	449.0	1.42
CA-N3	325.6	1.36
CT-CB	331.3	1.51

Angle	K_θ kcal/(mol radian^2)	θ degrees
CT-C-N*	68.2	115.38
N*-C-N3	68.3	113.84
N3-C-O	74.1	122.00
CM-CA-N3	67.4	117.17
N2-CA-N3	72.6	119.52
CA-CA-N*	69.3	119.50
CA-CB-CT	63.2	129.10
CB-CB-CB	64.5	108.05
CB-CB-CT	63.3	110.80
N*-CK-N*	72.9	119.87
HC-CT-CB	47.0	128.96
CB-CT-CB	65.5	102.11
C-N*-CA	65.6	125.26
CK-N*-C	65.6	117.13
CK-N*-CA	64.3	117.46
C-N3-H	46.4	112.50
CA-N3-C	65.4	126.11
CA-N3-H	49.1	121.39

Torsion	**# of paths**	$V_n/2$ kcal/mol	γ degrees	**n**
X-CA-N*-X	4	4.2	180.0	2
X-CT-CB-X	6	0.0	0.0	2

Table S5: Hydrogen bond occupancies between the templating dG/dG-AAF and the incoming dCTP/dCTP$^+$ for the stable region (last 4 ns) of each trajectory. All bonds are represented as donor residue(donor heavy atom)…acceptor residue(acceptor heavy atom), followed by percent occupancy.

HG Unmodified	dCTP$^+$(N3)…dG(N7)	99.32
	dCTP$^+$(N4)…dG(N7)	4.72
	dCTP$^+$(N4)…dG(O6)	99.72
HG-AAF1	dCTP$^+$(N3)…dG-AAF(N7)	1.00
HG-AAF2	None	
HG-AAF3	dCTP$^+$(N4)…dG-AAF(N7)	42.73
	dCTP$^+$(N4)…dG-AAF(O6)	4.28
	dCTP$^+$(N4)…dG-AAF(O6)	73.03
HG-AAF4	dCTP$^+$(N3)…dG-AAF(N7)	33.40
	dCTP$^+$(N4)…dG-AAF(N7)	16.93
	dCTP$^+$(N4)…dG-AAF(O6)	86.59
WC Unmodified 1	dCTP(N4)…dG(O6)	92.95
	dG(N1)…dCTP(N3)	99.93
	dG(N2)…dCTP(N3)	63.11
	dG(N2)…dCTP(O2)	99.93
WC Unmodified 2	dCTP(N4)…dG(O6)	98.35
	dG(N1)…dCTP(N3)	100.0
	dG(N2)…dCTP(N3)	34.29
	dG(N2)…dCTP(O2)	99.76
WC-AAF1	dCTP(N4)…dG-AAF(O6)	58.78
	dG-AAF(N1)…dCTP(N3)	81.70
	dG-AAF(N1)…dCTP(O2)	68.80
	dG-AAF(N2)…dCTP(N3)	8.03
	dG-AAF(N2)…dCTP(O2)	99.98

WC-AAF2	dCTP(N4)…dG-AAF(O6)	99.08
	dG-AAF(N1)…dCTP(N3)	100.00
	dG-AAF(N1)…dCTP(O2)	4.95
	dG-AAF(N2)…dCTP(N3)	19.55
	dG-AAF(N2)…dCTP(O2)	99.80
WC-AAF3	dCTP(N4)…dG-AAF(O6)	93.75
	dG-AAF(N1)…dCTP(N3)	100.00
	dG-AAF(N1)…dCTP(O2)	18.05
	dG-AAF(N2)…dCTP(N3)	11.75
	dG-AAF(N2)…dCTP(O2)	100.00
WC-AAF4	dCTP(N4)…dG-AAF(O6)	89.05
	dG-AAF(N1)…dCTP(N3)	99.78
	dG-AAF(N1)…dCTP(O2)	1.45
	dG-AAF(N2)…dCTP(N3)	39.83
	dG-AAF(N2)…dCTP(O2)	99.95

Table S6: Hydrogen bond occupancies between the incoming dCTP/dCTP$^+$ and polι for the stable region (last 4 ns) of each trajectory. Amino acid numbering scheme taken from the ternary crystal structure(Nair et al., 2005), PDB ID: 2ALZ. All bonds are represented as donor residue(donor heavy atom)…acceptor residue(acceptor heavy atom), followed by percent occupancy.

HG Unmodified	ARG61(N1)…dCTP$^+$(O2G)	76.61
	ARG61(N1)…dCTP$^+$(O3G)	70.03
	ARG61(N2)…dCTP$^+$(O2G)	7.93
	ARG61(N2)…dCTP$^+$(O3B)	97.77
	ARG61(N2)…dCTP$^+$(O3G)	83.28
	dCTP$^+$(O3′)…dCTP$^+$(O1B)	100.00
	CYS37(N)…dCTP$^+$(O1G)	6.20
	CYS37(N)…dCTP$^+$(O3B)	39.30
	CYS37(N)…dCTP$^+$(O3G)	99.00
	LYS214(NZ)…dCTP$^+$(O1G)	3.50
	LYS214(NZ)…dCTP$^+$(O2G)	16.03
	LYS214(NZ)…dCTP$^+$(O3G)	11.57
	LYS214(NZ)…dCTP$^+$(O1G)	4.63
	LYS214(NZ)…dCTP$^+$(O2G)	17.18
	LYS214(NZ)…dCTP$^+$(O3G)	18.67
	LYS214(NZ)…dCTP$^+$(O1G)	2.28
	LYS214(NZ)…dCTP$^+$(O2G)	3.73
	LYS214(NZ)…dCTP$^+$(O3G)	4.75
	LYS77(NZ)…dCTP$^+$(O2A)	27.27
	LYS77(NZ)…dCTP$^+$(O2G)	26.05
	LYS77(NZ)…dCTP$^+$(O3A)	12.28
	LYS77(NZ)…dCTP$^+$(O2A)	45.25

HG Unmodified	LYS77(NZ)…dCTP^{+}(O2G)	27.92
	LYS77(NZ)…dCTP^{+}(O3A)	19.88
	LYS77(NZ)…dCTP^{+}(O2A)	25.83
	LYS77(NZ)…dCTP^{+}(O2G)	45.83
	LYS77(NZ)…dCTP^{+}(O3A)	11.78
	PHE38(N)…dCTP^{+}(O1B)	2.97
	PHE38(N)…dCTP^{+}(O2B)	60.37
	THR65(OG1)…dCTP^{+}(O1B)	99.97
	THR65(OG1)…dCTP^{+}(O3′)	2.61
	TYR39(N)…dCTP^{+}(O3′)	16.30
HG-AAF1	ARG61(N1)…dCTP^{+}(O2G)	79.30
	ARG61(N1)…dCTP^{+}(O3G)	80.60
	ARG61(N2)…dCTP^{+}(O2G)	3.08
	ARG61(N2)…dCTP^{+}(O3B)	39.08
	ARG61(N2)…dCTP^{+}(O3G)	96.23
	CYS37(N)…dCTP^{+}(O3B)	7.70
	CYS37(N)…dCTP^{+}(O3G)	99.90
	LYS77(NZ)…dCTP^{+}(O2A)	32.39
	LYS77(NZ)…dCTP^{+}(O2G)	29.20
	LYS77(NZ)…dCTP^{+}(O3A)	1.50
	LYS77(NZ)…dCTP^{+}(O3B)	11.85
	LYS77(NZ)…dCTP^{+}(O2A)	60.85
	LYS77(NZ)…dCTP^{+}(O2G)	32.55
	LYS77(NZ)…dCTP^{+}(O3A)	2.63

HG-AAF1	LYS77(NZ)…dCTP^{+}(O3B)	37.45
	LYS77(NZ)…dCTP^{+}(O2A)	28.88
	LYS77(NZ)…dCTP^{+}(O2G)	62.03
	LYS77(NZ)…dCTP^{+}(O3A)	1.73
	LYS77(NZ)…dCTP^{+}(O3B)	37.98
	PHE38(N)…dCTP^{+}(O1B)	3.78
	PHE38(N)…dCTP^{+}(O2B)	30.78
	THR65(OG1)…dCTP^{+}(O1B)	100.00
HG-AAF2	ARG61(N1)…dCTP^{+}(O2G)	12.83
	ARG61(N1)…dCTP^{+}(O3G)	97.62
	ARG61(N2)…dCTP^{+}(O1B)	21.48
	ARG61(N2)…dCTP^{+}(O3B)	39.60
	ARG61(N2)…dCTP^{+}(O3G)	87.98
	dCTP^{+}(O3′)…TYR39(OH)	2.80
	CYS37(N)…dCTP^{+}(O3B)	37.03
	CYS37(N)…dCTP^{+}(O3G)	99.03
	LYS77(NZ)…dCTP^{+}(O2A)	16.95
	LYS77(NZ)…dCTP^{+}(O2G)	59.23
	LYS77(NZ)…dCTP^{+}(O3A)	1.62
	LYS77(NZ)…dCTP^{+}(O3B)	38.93
	LYS77(NZ)…dCTP^{+}(O2A)	43.23
	LYS77(NZ)…dCTP^{+}(O2G)	17.45
	LYS77(NZ)…dCTP^{+}(O3A)	2.78
	LYS77(NZ)…dCTP^{+}(O3B)	9.70

HG-AAF2	LYS77(NZ)…dCTP^{+}(O2A)	33.55
	LYS77(NZ)…dCTP^{+}(O2G)	68.37
	LYS77(NZ)…dCTP^{+}(O3A)	5.08
	LYS77(NZ)…dCTP^{+}(O3B)	22.63
	PHE38(N)…dCTP^{+}(O1B)	5.43
	PHE38(N)…dCTP^{+}(O2B)	81.00
	THR65(OG1)…dCTP^{+}(O1B)	99.43
	TYR39(OH)…dCTP^{+}(O3′)	60.18
	TYR68(OH)…dCTP^{+}(O3G)	22.95
	ARG61(N1)…dCTP^{+}(O2G)	55.43
HG-AAF3	ARG61(N1)…dCTP^{+}(O3B)	2.98
	ARG61(N1)…dCTP^{+}(O3G)	78.90
	ARG61(N2)…dCTP^{+}(O2G)	5.05
	ARG61(N2)…dCTP^{+}(O3B)	99.77
	ARG61(N2)…dCTP^{+}(O3G)	45.30
	dCTP^{+}(O3′)…dCTP^{+}(O1B)	100.00
	CYS37(N)…dCTP^{+}(O3B)	23.55
	CYS37(N)…dCTP^{+}(O3G)	99.70
	LYS214(NZ)…dCTP^{+}(O1G)	34.30
	LYS214(NZ)…dCTP^{+}(O2G)	18.75
	LYS214(NZ)…dCTP^{+}(O1G)	30.70
	LYS214(NZ)…dCTP^{+}(O2G)	37.59

HG-AAF3	LYS214(NZ)…dCTP^{+}(O1G)	23.68
	LYS214(NZ)…dCTP^{+}(O2G)	18.60
	LYS77(NZ)…dCTP^{+}(O2A)	19.05
	LYS77(NZ)…dCTP^{+}(O2G)	28.43
	LYS77(NZ)…dCTP^{+}(O3A)	8.05
	LYS77(NZ)…dCTP^{+}(O2A)	51.58
	LYS77(NZ)…dCTP^{+}(O2G)	19.30
	LYS77(NZ)…dCTP^{+}(O3A)	17.85
	LYS77(NZ)…dCTP^{+}(O2A)	27.62
	LYS77(NZ)…dCTP^{+}(O2G)	52.20
	LYS77(NZ)…dCTP^{+}(O3A)	37.23
	PHE38(N)…dCTP^{+}(O1B)	7.95
	PHE38(N)…dCTP^{+}(O2B)	70.83
	THR65(OG1)…dCTP^{+}(O1B)	100.00
	THR65(OG1)…dCTP^{+}(O3′)	2.62
	TYR39(N)…dCTP^{+}(O3′)	28.78
	TYR39(OH)…dCTP^{+}(O2)	74.48
HG-AAF4	ARG61(N1)…dCTP^{+}(O2G)	9.30
	ARG61(N1)…dCTP^{+}(O3G)	97.80
	ARG61(N2)…dCTP^{+}(O1B)	68.28
	ARG61(N2)…dCTP^{+}(O3B)	3.78
	ARG61(N2)…dCTP^{+}(O3G)	97.39
	CYS37(N)…dCTP^{+}(O1G)	38.65
	CYS37(N)…dCTP^{+}(O3G)	99.83

HG-AAF4	LYS214(NZ)…dCTP^{+}(O1G)	3.58
	LYS214(NZ)…dCTP^{+}(O2G)	25.68
	LYS214(NZ)…dCTP^{+}(O1G)	3.48
	LYS214(NZ)…dCTP^{+}(O2G)	11.37
	LYS214(NZ)…dCTP^{+}(O1G)	3.88
	LYS214(NZ)…dCTP^{+}(O2G)	38.43
	LYS77(NZ)…dCTP^{+}(O2A)	27.55
	LYS77(NZ)…dCTP^{+}(O2G)	16.95
	LYS77(NZ)…dCTP^{+}(O3A)	1.70
	LYS77(NZ)…dCTP^{+}(O3B)	38.25
	LYS77(NZ)…dCTP^{+}(O2A)	34.88
	LYS77(NZ)…dCTP^{+}(O2G)	12.39
	LYS77(NZ)…dCTP^{+}(O3A)	1.59
	LYS77(NZ)…dCTP^{+}(O3B)	12.10
	LYS77(NZ)…dCTP^{+}(O2A)	34.45
	LYS77(NZ)…dCTP^{+}(O2G)	17.23
	LYS77(NZ)…dCTP^{+}(O3A)	2.33
	LYS77(NZ)…dCTP^{+}(O3B)	38.98
	PHE38(N)…dCTP^{+}(O2B)	4.23
	THR65(OG1)…dCTP^{+}(O1B)	99.98
WC Unmodified 1	ARG61(N1)…dCTP(O2G)	26.39
	ARG61(N1)…dCTP(O3B)	5.48
	ARG61(N1)…dCTP(O3G)	88.05
	ARG61(N2)…dCTP(O1B)	7.30

WC Unmodified 1	ARG61(N2)…dCTP(O2G)	2.98
	ARG61(N2)…dCTP(O3B)	99.55
	ARG61(N2)…dCTP(O3G)	49.30
	dCTP(O3′)…dCTP(O1B)	2.77
	CYS37(N)…dCTP(O1G)	1.32
	CYS37(N)…dCTP(O3B)	46.50
	CYS37(N)…dCTP(O3G)	67.65
	LYS214(NZ)…dCTP(O1G)	10.73
	LYS214(NZ)…dCTP(O2G)	5.39
	LYS214(NZ)…dCTP(O3G)	8.55
	LYS214(NZ)…dCTP(O1G)	5.07
	LYS214(NZ)…dCTP(O2G)	7.80
	LYS214(NZ)…dCTP(O3G)	10.64
	LYS214(NZ)…dCTP(O1G)	9.43
	LYS214(NZ)…dCTP(O2G)	8.61
	LYS214(NZ)…dCTP(O3G)	6.73
	LYS77(NZ)…dCTP(O2A)	28.39
	LYS77(NZ)…dCTP(O2G)	23.16
	LYS77(NZ)…dCTP(O3A)	8.25
	LYS77(NZ)…dCTP(O2A)	45.23
	LYS77(NZ)…dCTP(O2G)	25.43
	LYS77(NZ)…dCTP(O3A)	11.65
	LYS77(NZ)…dCTP(O2A)	23.11
	LYS77(NZ)…dCTP(O2G)	65.30

WC Unmodified 1	LYS77(NZ)…dCTP(O3A)	10.32
	PHE38(N)…dCTP(O1B)	1.77
	PHE38(N)…dCTP(O2B)	77.18
	THR65(OG1)…dCTP(O1B)	100.00
	TYR39(N)…dCTP(O3′)	88.16
	TYR39(OH)…dCTP(O2)	4.02
WC Unmodified 2	ARG61(N1)…dCTP(O2G)	46.57
	ARG61(N1)…dCTP(O3G)	78.35
	ARG61(N2)…dCTP(O3B)	99.37
	ARG61(N2)…dCTP(O3G)	49.90
	CYS37(N)…dCTP(O3B)	45.10
	CYS37(N)…dCTP(O3G)	86.89
	LYS214(NZ)…dCTP(O1G)	19.21
	LYS214(NZ)…dCTP(O2G)	18.62
	LYS214(NZ)…dCTP(O3G)	11.10
	LYS214(NZ)…dCTP(O1G)	19.17
	LYS214(NZ)…dCTP(O2G)	16.34
	LYS214(NZ)…dCTP(O1G)	12.00
	LYS214(NZ)…dCTP(O2G)	15.10
	LYS77(NZ)…dCTP(O2A)	18.00
	LYS77(NZ)…dCTP(O2G)	53.79
	LYS77(NZ)…dCTP(O2A)	22.13
	LYS77(NZ)…dCTP(O2G)	19.33

WC unmodified 2	LYS77(NZ)…dCTP(O2A)	52.75
	LYS77(NZ)…dCTP(O2G)	23.54
	LYS77(NZ)…dCTP(O3A)	13.08
	PHE38(N)…dCTP(O2B)	63.41
	THR65(OG1)…dCTP(O1B)	100.00
	TYR39(N)…dCTP(O3′)	82.90
WC-AAF1	ARG61(N1)…dCTP(O2G)	1.73
	ARG61(N1)…dCTP(O3B)	7.60
	ARG61(N1)…dCTP(O3G)	99.10
	ARG61(N2)…dCTP(O1B)	37.65
	ARG61(N2)…dCTP(O2G)	2.58
	ARG61(N2)…dCTP(O3B)	99.70
	ARG61(N2)…dCTP(O3G)	60.58
	dCTP(O3′)…dCTP(O1B)	8.43
	CYS37(N)…dCTP(O3B)	62.70
	CYS37(N)…dCTP(O3G)	50.10
	LYS214(NZ)…dCTP(O1G)	26.59
	LYS214(NZ)…dCTP(O2G)	5.55
	LYS214(NZ)…dCTP(O3G)	17.77
	LYS214(NZ)…dCTP(O1G)	23.62
	LYS214(NZ)…dCTP(O2G)	3.68
	LYS214(NZ)…dCTP(O3G)	39.55
	LYS214(NZ)…dCTP(O1G)	23.45
	LYS214(NZ)…dCTP(O2G)	5.73

WC-AAF1	LYS214(NZ)…dCTP(O3G)	39.18
	LYS77(NZ)…dCTP(O2A)	60.90
	LYS77(NZ)…dCTP(O2G)	26.59
	LYS77(NZ)…dCTP(O3A)	9.68
	LYS77(NZ)…dCTP(O2A)	26.58
	LYS77(NZ)…dCTP(O2G)	39.59
	LYS77(NZ)…dCTP(O3A)	10.10
	LYS77(NZ)…dCTP(O2A)	24.18
	LYS77(NZ)…dCTP(O2G)	29.68
	LYS77(NZ)…dCTP(O3A)	8.95
	PHE38(N)…dCTP(O1B)	1.65
	PHE38(N)…dCTP(O2B)	55.18
	THR65(OG1)…dCTP(O1B)	100.00
	TYR39(N)…dCTP(O3′)	98.55
WC-AAF2	ARG61(N1)…dCTP(O2G)	62.18
	ARG61(N1)…dCTP(O3B)	5.80
	ARG61(N1)…dCTP(O3G)	84.48
	ARG61(N2)…dCTP(O1B)	7.88
	ARG61(N2)…dCTP(O2G)	3.65
	ARG61(N2)…dCTP(O3B)	98.78
	ARG61(N2)…dCTP(O3G)	49.80
	dCTP(O3′)…dCTP(O1B)	1.55
	CYS37(N)…dCTP(O1G)	2.70
	CYS37(N)…dCTP(O3B)	61.05

WC-AAF2	CYS37(N)…dCTP(O3G)	83.10
	LYS214(NZ)…dCTP(O1G)	9.59
	LYS214(NZ)…dCTP(O2G)	16.80
	LYS214(NZ)…dCTP(O3G)	37.59
	LYS214(NZ)…dCTP(O1G)	12.68
	LYS214(NZ)…dCTP(O2G)	23.77
	LYS214(NZ)…dCTP(O3G)	37.30
	LYS214(NZ)…dCTP(O1G)	7.73
	LYS214(NZ)…dCTP(O2G)	38.55
	LYS214(NZ)…dCTP(O3G)	10.45
	LYS77(NZ)…dCTP(O2A)	27.60
	LYS77(NZ)…dCTP(O2G)	19.58
	LYS77(NZ)…dCTP(O3A)	9.18
	LYS77(NZ)…dCTP(O2A)	60.50
	LYS77(NZ)…dCTP(O2G)	26.83
	LYS77(NZ)…dCTP(O3A)	11.93
	LYS77(NZ)…dCTP(O2A)	25.39
	LYS77(NZ)…dCTP(O2G)	60.00
	LYS77(NZ)…dCTP(O3A)	10.30
	PHE38(N)…dCTP(O1B)	2.23
	PHE38(N)…dCTP(O2B)	64.88
	THR65(OG1)…dCTP(O1B)	100.00
	TYR39(N)…dCTP(O3′)	87.68

WC-AAF3	ARG61(N1)…dCTP(O2G)	7.08
	ARG61(N1)…dCTP(O3B)	38.75
	ARG61(N1)…dCTP(O3G)	92.75
	ARG61(N2)…dCTP(O1B)	27.39
	ARG61(N2)…dCTP(O2G)	1.30
	ARG61(N2)…dCTP(O3B)	99.33
	ARG61(N2)…dCTP(O3G)	18.83
	CYS37(N)…dCTP(O3B)	65.78
	CYS37(N)…dCTP(O3G)	46.68
	LYS214(NZ)…dCTP(O1G)	19.78
	LYS214(NZ)…dCTP(O2G)	17.18
	LYS214(NZ)…dCTP(O3G)	4.85
	LYS214(NZ)…dCTP(O1G)	19.58
	LYS214(NZ)…dCTP(O2G)	38.00
	LYS214(NZ)…dCTP(O3G)	8.65
	LYS214(NZ)…dCTP(O1G)	16.18
	LYS214(NZ)…dCTP(O2G)	39.70
	LYS214(NZ)…dCTP(O3G)	5.73
	LYS77(NZ)…dCTP(O2A)	48.18
	LYS77(NZ)…dCTP(O2G)	27.78
	LYS77(NZ)…dCTP(O3A)	10.08
	LYS77(NZ)…dCTP(O2A)	22.55
	LYS77(NZ)…dCTP(O2G)	48.60
	LYS77(NZ)…dCTP(O3A)	7.45

WC-AAF3	LYS77(NZ)…dCTP(O2A)	27.80
	LYS77(NZ)…dCTP(O2G)	22.95
	LYS77(NZ)…dCTP(O3A)	7.40
	PHE38(N)…dCTP(O1B)	2.18
	PHE38(N)…dCTP(O2B)	93.58
	THR65(OG1)…dCTP(O1B)	100.00
	TYR39(N)…dCTP(O3′)	91.45
	TYR39(OH)…dCTP(O2)	65.98
WC-AAF4	ARG61(N1)…dCTP(O2G)	64.28
	ARG61(N1)…dCTP(O3B)	3.25
	ARG61(N1)…dCTP(O3G)	87.62
	ARG61(N2)…dCTP(O1B)	1.43
	ARG61(N2)…dCTP(O2G)	1.80
	ARG61(N2)…dCTP(O3B)	99.62
	ARG61(N2)…dCTP(O3G)	79.70
	dCTP(O3′)…dCTP(O1B)	2.73
	CYS37(N)…dCTP(O1G)	4.05
	CYS37(N)…dCTP(O3B)	59.03
	CYS37(N)…dCTP(O3G)	98.23
	LYS77(NZ)…dCTP(O2A)	38.60
	LYS77(NZ)…dCTP(O2G)	59.65
	LYS77(NZ)…dCTP(O3A)	7.70
	LYS77(NZ)…dCTP(O2A)	40.00
	LYS77(NZ)…dCTP(O2G)	18.68

WC-AAF4	LYS77(NZ)…dCTP(O3A)	37.39
	LYS77(NZ)…dCTP(O2A)	28.80
	LYS77(NZ)…dCTP(O2G)	45.30
	LYS77(NZ)…dCTP(O3A)	11.65
	PHE38(N)…dCTP(O1B)	1.62
	PHE38(N)…dCTP(O2B)	57.43
	THR65(OG1)…dCTP(O1B)	100.00
	TYR39(N)…dCTP(O3′)	62.60

Table S7: Hydrogen bond occupancies between the templating dG/dG-AAF and polι for the stable region (last 4 ns) of each trajectory. Amino acid numbering scheme taken from the ternary crystal structure(Nair et al., 2005), PDB ID: 2ALZ. All bonds are represented as donor residue(donor heavy atom)…acceptor residue(acceptor heavy atom), followed by percent occupancy.

HG Unmodified	dG(N2)…dG(O2P)	3.22
	LYS60(NZ)…dG(O1P)	3.77
	LYS60(NZ)…dG(O1P)	9.18
	LYS60(NZ)…dG(O1P)	5.90
	SER307(OG)…dG(O1P)	32.52
	SER307(OG)…dG(O2P)	64.88
HG-AAF1	dG-AAF(N2)…dG-AAF(O2P)	59.73
	dG-AAF(N2)…dG-AAF(O5′)	1.33
	GLN59(NE2)…dG-AAF(O3′)	37.18
	TYR61(OH)…dG-AAF(O1P)	77.88
HG-AAF2	dG-AAF(N2)…dG-AAF(O2P)	61.60
	LYS309(NZ)…dG-AAF(O1P)	39.03
	LYS309(NZ)…dG-AAF(O2P)	16.23
	LYS309(NZ)…dG-AAF(O1P)	11.40
	LYS309(NZ)…dG-AAF(O2P)	10.98
	LYS309(NZ)…dG-AAF(O1P)	11.48
	LYS309(NZ)…dG-AAF(O2P)	38.40
	SER307(OG)…dG-AAF(O1P)	97.10
HG-AAF3	dG-AAF(N2)…dG-AAF(O2P)	3.08
	HIS354(NE2)…dG-AAF(O1P)	7.10
	HIS354(NE2)…dG-AAF(O2P)	17.20
	TYR355(OH)…dG-AAF(O2P)	2.43

HG-AAF4	LYS309(NZ)…dG-AAF(O1P)	5.93
	LYS309(NZ)…dG-AAF(O1P)	8.43
	LYS309(NZ)…dG-AAF(O1P)	6.78
WC Unmodified 1	ARG347(N1)…dG(O2P)	52.73
	ARG347(N2)…dG(O2P)	88.50
	dG(N2)…GLN59(OE1)	97.84
	SER307(OG)…dG(O3′)	1.32
WC Unmodified 2	dG(N2)…GLN59(OE1)	76.76
	dT5(O5′)…dG(N7)	13.90
	TYR61(OH)…dG(O1P)	13.76
WC-AAF1	ARG347(N2)…dG-AAF(O2P)	1.39
	dG-AAF(N2)…GLN59(OE1)	97.78
	GLN59(NE2)…dG-AAF(O3′)	65.80
	HIS354(NE2)…dG-AAF(O2P)	3.65
	LYS60(NZ)…dG-AAF(O1P)	39.50
	LYS60(NZ)…dG-AAF(O5′)	3.18
	LYS60(NZ)…dG-AAF(O1P)	11.95
	LYS60(NZ)…dG-AAF(O5′)	3.28
	LYS60(NZ)…dG-AAF(O1P)	11.90
	LYS60(NZ)…dG-AAF(O5′)	2.00
	SER307(OG)…dG-AAF(O1P)	20.60
	SER307(OG)…dG-AAF(O2P)	88.03

WC-AAF2	ARG347(N1)…dG-AAF(O1P)	1.05
	ARG347(N1)…dG-AAF(O2P)	59.75
	ARG347(N1)…dG-AAF(O5′)	6.45
	ARG347(N2)…dG-AAF(O1P)	30.59
	ARG347(N2)…dG-AAF(O2P)	51.28
	ARG347(N2)…dG-AAF(O5′)	1.08
	dG-AAF(N2)…GLN59(OE1)	97.10
	SER307(OG)…dG-AAF(O1P)	92.05
	SER307(OG)…dG-AAF(O2P)	3.25
	SER307(OG)…dG-AAF(O5′)	4.59
WC-AAF3	dG-AAF(N2)…GLN59(OE1)	92.73
	GLN59(NE2)…dG-AAF(O3′)	19.78
	LYS309(NZ)…dG-AAF(O1P)	3.59
	LYS309(NZ)…dG-AAF(O2P)	7.88
	LYS309(NZ)…dG-AAF(O1P)	3.25
	LYS309(NZ)…dG-AAF(O2P)	3.60
	LYS309(NZ)…dG-AAF(O1P)	4.40
	LYS309(NZ)…dG-AAF(O2P)	3.62
	LYS60(NZ)…dG-AAF(O1P)	12.03
	LYS60(NZ)…dG-AAF(O3′)	2.08
	LYS60(NZ)…dG-AAF(O5′)	6.55
	LYS60(NZ)…dG-AAF(O1P)	10.70
	LYS60(NZ)…dG-AAF(O3′)	2.80

WC-AAF3	LYS60(NZ)…dG-AAF(O5′)	4.80
	LYS60(NZ)…dG-AAF(O1P)	10.28
	LYS60(NZ)…dG-AAF(O3′)	2.55
	LYS60(NZ)…dG-AAF(O5′)	3.80
	SER307(OG)…dG-AAF(O2P)	1.93
	SER307(OG)…dG-AAF(O3′)	1.20
WC-AAF4	ARG347(N2)…dG-AAF(O2P)	4.37
	dG-AAF(N2)…GLN59(OE1)	99.80
	LEU62(N)…dG-AAF(OAce)	1.63
	LYS60(NZ)…dG-AAF(O3′)	1.77
	LYS60(NZ)…dG-AAF(O3′)	1.93
	LYS60(NZ)…dG-AAF(O3′)	2.63
	SER307(OG)…dG-AAF(O1P)	43.37
	SER307(OG)…dG-AAF(O2P)	21.62
	TYR61(N)…dG-AAF(OAce)	5.60

Table S8: Mg^{2+} coordination distances for the stable region (last 4 ns) of each trajectory, presented as mean values ± standard deviation.

	Mg_A-Asp126 Oδ1 (Å)	Mg_A–Gln127 Oε2 (Å)	Mg_A–Asp34 Oδ2 (Å)	Mg_A –O3′ (Å)	Mg_A–dCTP O1α (Å)	Mg_A – H_2O (Å)
Initial Model[a]	3.14	2.32	2.85	2.84	2.37	2.14
HG Unmodified	1.9±0.05	1.9±0.04	1.9±0.05	2.1±1.04	2.0±0.23	2.0±0.06
HG-AAF1	1.9±0.05	1.9±0.04	1.9±0.05	2.1±0.09	3.8±0.16	2.0±0.05
HG-AAF2	1.9±0.05	1.9±0.06	1.9±0.05	8.4±1.42	3.1±0.33	2.0±0.06
HG-AAF3	1.9±0.05	1.9±0.04	1.9±0.05	5.3±0.93	2.2±0.25	2.0±0.07
HG-AAF4	1.9±0.05	1.9±0.04	1.9±0.05	2.1±0.09	2.0±0.09	2.0±0.07
WC Unmodified 1	1.9±0.05	1.9±0.04	1.9±0.05	2.1±0.09	2.0±0.26	2.0±0.06
WC Unmodified 2	1.9±0.05	1.9±0.04	1.9±0.05	2.1±0.08	2.0±0.13	2.0±0.07
WC-AAF1	1.9±0.05	1.9±0.04	1.9±0.05	2.1±0.09	2.2±0.38	2.0±0.06
WC-AAF2	1.9±0.05	1.9±0.04	1.9±0.05	2.1±0.09	2.0±0.12	2.0±0.06
WC-AAF3	1.9±0.04	2.0±0.30	1.9±0.05	2.1±0.09	3.3±0.34	2.0±0.06
WC-AAF4	1.9±0.05	1.9±0.04	1.9±0.05	2.1±0.08	1.9±0.17	2.0±0.07

[a] Coordination distances in the initial model are as in crystal structure PDB ID: 2ALZ, except for the primer terminal O3′ , which has been modeled due to its absence in the crystal.

Table S8 continued: Mg^{2+} coordination distances for the stable region (last 4 ns) of each trajectory, presented as mean values ± standard deviation.

	Mg_B-Asp126 Oδ2 (Å)	Mg_B–Asp34 Oδ1 (Å)	Mg_B–Leu35 O (Å)	Mg_B–dCTP O1α (Å)	Mg_B–dCTP O1γ (Å)	Mg_B–dCTP O2β (Å)
Initial Model[a]	2.08	2.15	2.12	2.36	2.37	2.12
HG Unmodified	1.9±0.05	1.9±0.04	1.9±0.05	3.0±0.43	1.8±0.04	1.9±0.05
HG-AAF1	1.9±0.52	1.9±0.05	2.0±0.08	2.0±0.13	1.8±0.04	1.9±0.05
HG-AAF2	1.9±0.53	1.9±0.05	2.0±0.07	2.0±0.17	1.8±0.04	1.9±0.06
HG-AAF3	1.9±0.05	1.9±0.05	1.9±0.06	2.6±0.32	1.8±0.04	1.8±0.05
HG-AAF4	1.9±0.05	1.9±0.04	1.9±0.05	3.2±0.24	1.8±0.04	1.8±0.04
WC Unmodified 1	1.9±0.05	1.9±0.04	1.9±0.05	2.8±0.35	1.8±0.04	1.9±0.05
WC Unmodified 2	1.9±0.05	1.9±0.04	1.9±0.05	2.8±0.26	1.8±0.04	1.9±0.05
WC-AAF1	1.9±0.05	1.9±0.04	1.9±0.70	2.5±0.44	1.8±0.04	1.9±0.06
WC-AAF2	1.9±0.05	1.9±0.04	1.9±0.05	2.9±0.39	1.8±0.04	1.9±0.05
WC-AAF3	1.9±0.05	1.9±0.05	2.0±0.07	1.9±0.12	1.8±0.04	2.0±0.08
WC-AAF4	1.9±0.05	1.9±0.05	1.9±0.05	3.0±0.25	1.8±0.03	1.9±0.05

[a] Coordination distances in the initial model are as in crystal structure PDB ID: 2ALZ, except for the primer terminal O3′ , which has been modeled due to its absence in the crystal.

Table S9: Torsion angles χ, α′, β′, γ′, the pseudorotation phase angle *P* of the sugar pucker of dG/dG-AAF, and the in-line attack angle O3′-Pα-O3α for the stable region (last 4 ns) of each trajectory. Data presented as mean ± standard deviation.

Structure	**χ(°)**	***P*(°)**	**α′(°)**	**β′(°)**	**γ′(°)**	**O3′-Pα-O3α(°)**
HG Unmodified	30.8±15.3	102.7±15.9	N/A	N/A	N/A	161.8±5.2
HG-AAF1	52.2±9.2	50.4±16.3	127.3±13.2	171.8±13.3	316.0±10.4	70.22±7.5
HG-AAF2	2.6±21.4	175.2±23.7	83.7±16.7	243.9±9.6	192.7±13.4	87.7±26.62
HG-AAF3	52.2±11.7	159.0±13.4	215.0±9.7	63.7±10.7	203.0±10.1	119.8±18.0
HG-AAF4	56.9±8.2	64.5±18.1	235.1±9.9	191.6±12.3	318.5±17.8	124.4±10.3
WC Unmodified 1	199.1±15	156.2±31.4	N/A	N/A	N/A	167.2±5.6
WC Unmodified 2	247.2±25	164.4±32.0	N/A	N/A	N/A	167.7±5.4
WC-AAF1	195.0±8.7	137.7±13.5	239.8±11.2	258.9±17.5	186.0±10.9	170.2±5.2
WC-AAF2	175.4±15.6	120.3±25.8	130.3±11.6	126.7±12.0	159.2±9.5	165.2±5.8
WC-AAF3	200.8±9.8	110.5±23.6	241.0±93	72.0±17.7	185.7±11.9	172.2±4.3
WC-AAF4	171.0±18.7	82.11±31.9	134.9±14.9	306.0±18.5	157.2±10.6	163.4±5.4