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SUPPLEMENTARY MATERIAL

Parameterization method

An iterative process of refinement was used to optimize parameters for the phosphoramidate backbone while keeping them compatible with regular DNA parameters using previously established protocols for the CHARMM force field (1,2). The interaction parameters optimized were the partial charges on the new modified atoms and adjacent atoms. The partial atomic charges were optimized to reproduce the *ab initio* water interaction energies and distances. *Ab initio* goal data for these interactions were obtained by HF/6-31G* calculations scaled appropriately (3) and performed with compound **A** interacting with one water molecule at a time in different orientations. Water interactions were calculated using the TIP3P water model implemented in CHARMM (4,5). A fixed HF/6-31G* optimized *gauche/trans* geometry of compound **A** and a fixed water TIP3P geometry was used for these water interaction calculations. The different interaction geometries studied are shown in Figure S1 and the water interaction energies and distances are shown in Table S1.

Bond, angle and dihedral force constants were optimized using frequency calculations to reproduce the *ab initio* vibrational spectra calculated at the MP2/6-31G* level. Analysis of the normal modes of compound **A** was done using the MOLVIB program (6) within CHARMM. Bond and angle equilibrium values were also optimized to reproduce *ab initio* optimized geometries (Table S2). The behavior of the dihedral parameters has the greatest influence on conformational properties of the molecules. The dihedral parameters for the dihedrals near the N3′-H substitution (α, ε and z) were therefore carefully optimized to reproduce both the *ab initio* vibrational frequencies and the *ab initio* dihedral surfaces. Parameters developed for the smaller model compounds were transferred to the larger model compounds and the additional new parameters specific to these compounds were optimized by similar procedures. A detailed description of our nucleic acid parameterization philosophy and its motivations are described elsewhere (1–3,7). The final parameters used in the present study are shown in Table S4.

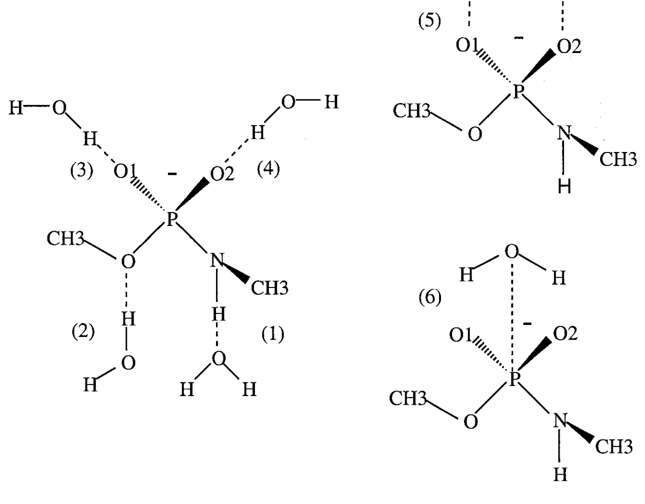

Figure S1. Water interaction geometries studied to develop partial atomic charges in model compound **A** in the *gauche/trans* orientation. Distances or hydrogen bonds along which interactions were optimized are indicated by dashed lines. In interaction 5, the plane of the water molecule is parallel to the O1–P–O2 plane. In interaction 6, the plane of the water molecule is perpendicular to the O1–P–O2 plane.

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Continued

Table S1. Empirical and *ab initio* water interaction energies and distances for compound **A** in *gauche/trans* orientation

	Ab initio		Empirical		Differences	
Interaction	E_{min}	R_{min}	E_{min}	R_{min}	ΔE	ΔR
(1) N-H...OHH	2.82	2.44	2.69	2.05	-0.13	-0.39
(2) O...HOH	-6.73	2.04	-7.35	2.05	-0.62	0.01
(3) O1...HOH	-14.01	1.84	-13.96	1.65	0.05	-0.19
(4) O2...HOH	-14.05	1.84	-13.96	1.65	0.09	-0.19
(5) P0...OHH	-17.29	3.38	-17.03	3.27	0.26	-0.11
(6) P90...OHH	-10.58	3.74	-11.07	3.59	-0.49	-0.15

Energies are in kcal/mol and distances are in Å. Interaction 5 has the O–P–O plane co-planar with the plane of the water molecule while in interaction 6 the planes are perpendicular. *Ab initio* calculations done at the HF/6-31G* level of theory.

Table S2. Comparison of the minimized *ab initio* and CHARMM geometries for model compound **A** in the *gauche/trans* conformation

	CHARMM	*Ab initio*	Difference
Bonds (in Å)			
1) N-C	1.457	1.457	0.000
2) N-P	1.715	1.717	0.002
3) N-H	1.017	1.017	0.000
Angles (in degrees)			
4) H-N-C	114.2	112.2	2.1
5) N-C-H1	110.5	113.9	-3.5
6) N-C-H2	111.7	107.2	4.5
7) N-C-H3	111.1	110.4	0.7
8) C-N-P	117.1	116.5	0.6
9) H-N-P	110.2	109.4	0.8
10)N-P-O	97.7	97.2	0.6
11)N-P-O1	109.4	109.2	0.2
12)N-P-O2	107.3	107.8	-0.5

Ab initio calculations done at the MP2/6-31+G* level.

(Opposite) Stretching modes are represented by an s followed by the atoms in the bond stretch, for example, sN-H is the stretching mode of the hydrogen attached to the N atom. Angle deformations are represented by d and a descriptor, as in dNPO, which is the N–P–O angle deformation. Wagging is represented by wag and a descriptor, as in wagNH, which is the wagging mode of the hydrogen attached to the nitrogen. Other abbreviations: tor, dihedral torsion; r, rocking; s, symmetrical at the end; as, asymmetrical at the end. *Ab initio* frequencies, at the MP2/6-31G* level, have been scaled by 0.94 as previously described (8).

Table S3. Vibrational frequency assignments and relative contributions of the assignments to the frequency for the *gauche/trans* conformation of compound **A**

	Ab initio			CHARMM		
	Frequency	Mode	Contribution	Frequency	Mode	Contribution
1	39	torNPOC	92	75	torNPOC	93
2	103	torOPNC	82	110	torOPNC	84
3	140	torPOCH	79	148	torPOCH	84
4	162	dNPO	28	168	dNPO	49
		torPNCH	25		dPOC	20
					torPNCH	17
5	205	torPNCH	61	210	torPNCH	70
		dPNC	17			
6	242	dPOC	50	295	dPOC	51
7	335	twPO2	31	369	dPNC	38
		dNPO	30		twPO2	24
					wPO2	17
8	360	twPO2	39	424	twPO2	28
		dPNC	20		rPO2	27
					sP-N	26
9	435	wPO2	48	465	wPO2	39
		dOPO	28		twPO2	33
10	479	rPO2	34	499	dOPO	58
		dOPO	24			
11	508	rPO2	23	566	wagNH	76
12	642	sP-O	38	636	dPNC	36
		wagNH	19		dOPO	19
13	693	sP-O	40	719	sP-O	23
		wagNH	19		sP-N	17
		rPO2	18		dPNC	16
		sP-N	15			
14	783	sP-N	38	810	sP-O	30
		wagNH	25		sP-N	27
					rPO2	21
15	1063	sP=O	64	1012	sN-C	36
					rch3'	15
16	1084	sO-C	26	1046	sO-C	58
		dNH	18			
17		rch3'	18			
		sN-C	17			
18	1091	sO-C	57	1053	rch3	73
		sP=O	25			
19	1131	sN-C	52	1093	rch3'	45
		rch3'	35		sN-C	19
20	1149	rch3	80	1109	sP=O	55
21	1168	rch3'	97	1141	rch3'	62
22	1188	rch3	92	1144	rch3	54
23	1275	sP=O	90	1261	sP=O	60
					dNH	28
24	1400	dNH	70	1338	dNH	52
					sP=O	28
25	1445	dch3s	110	1412	dch3as	90
26	1461	dch3s	105	1431	dch3as'	60
					dch3as	20
					rch3	19
27	1504	dch3as	65	1434	dch3as'	83
		dch3as'	33			
28	1511	dch3as'	99	1463	dch3as	58
					rch3'	25
					dch3as'	20
29	1534	dch3as	84	1561	dch3s	76
					sN-C	19
30	1535	dch3as'	65	1610	dch3s	88
		dch3as	33			
31	2958	sCH3s	87	2854	sCH3s	100
32	2967	sCH3s	98	2855	sCH3s	100
33	3017	sCH3as	72	2913	sCH3as'	52
		sCH3as'	25		sCH3as	48
34	3042	sCH3as'	60	2914	sCH3as	98
		sCH3as	38			
35	3061	sCH3as	62	2914	sCH3as	53
		sCH3as'	38		sCH3as'	46
36	3108	sCH3as'	72	2918	sCH3as'	100
		sCH3as	18			
37	3467	sN-H	100	3370	sN-H	100

Table S4. CHARMM parameters developed in this study unique to phosphoramidate

Table S4A. Bond parameters

Bond type		K_b	b_0
NN1	CN7	360.0	1.462
NN1	CN8B	360.0	1.462
NN1	P	180.0	1.792
NN1	HN2	432.5	1.025

Equilibrium bond lengths (b_0) in Å and force constants (K_b) in kcal/mol.Å

Table S4B. Angle parameters

Angle type			K_θ	θ_0	K_{UB}	S_0
HN2	NN1	CN8B	35.0	109.0		
HN2	NN1	P	30.0	123.6	40.0	2.35
HN2	NN1	CN7	35.0	109.0		
CN7	NN1	P	110.0	118.3	35.0	2.33
CN8B	NN1	P	110.0	118.3	35.0	2.33
NN1	P	ON2	60.0	103.2		
NN1	P	ON3	140.0	110.6		
NN1	P	ON4	60.0	103.2		
NN1	CN8B	CN7B	43.7	110.0		
NN1	CN7	CN7	43.7	110.0		
NN1	CN7	CN7B	43.7	110.0		
NN1	CN7	CN8	43.7	110.0		
NN1	CN7	HN7	48.0	110.0		
NN1	CN8B	CN7	43.7	110.0		
NN1	CN8B	HN8	48.0	110.0		

Equilibrium bond angles (θ_0) are given in degrees and force constants (K_θ, K_{UB}) are given in kcal/mol.Å. S_0 denotes the Urey–Bradley distance in Å.

Table S4C. Dihedral parameters

Dihedral				K_ϕ	n	ϕ
P	NN1	CN7	HN7	0.15	3	0.0
P	NN1	CN8B	HN8	0.15	3	0.0
NN1	P	ON2	CN7	0.35	1	0.0
NN1	P	ON2	CN7	0.35	2	50.0
NN1	P	ON2	CN7	0.35	3	0.0
NN1	P	ON2	CN8B	0.35	1	0.0
NN1	P	ON2	CN8B	0.35	2	50.0
NN1	P	ON2	CN8B	0.35	3	0.0
ON3	P	NN1	CN7	0.40	3	0.0
ON3	P	NN1	CN7	0.80	2	120.0
ON3	P	NN1	CN7	0.50	4	0.0
ON2	P	NN1	CN7	1.50	2	0.0
ON3	P	NN1	CN8B	0.40	3	0.0
ON3	P	NN1	CN8B	0.80	2	120.0
ON3	P	NN1	CN8B	0.50	4	0.0
ON2	P	NN1	CN8B	1.50	2	0.0
HN2	NN1	CN7	HN7	0.01	3	0.0
HN2	NN1	CN8B	HN8	0.01	3	0.0
HN2	NN1	P	ON2	0.05	3	0.0
HN2	NN1	P	ON3	0.05	3	0.0
HN2	NN1	CN7	CN7	0.30	1	0.00
HN2	NN1	CN7	CN7B	0.30	1	0.00
HN2	NN1	CN7	CN8	0.30	1	0.00
HN7	CN7	CN7	NN1	0.195	3	0.0
HN7	CN7B	CN7	NN1	0.195	3	0.0
HN8	CN8	CN7	NN1	0.195	3	0.0
ON6B	CN7	CN7	NN1	0.20	3	0.0
ON6	CN7	CN7	NN1	0.20	3	0.0
HN2	NN1	P	ON4	0.05	3	0.0
P	NN1	CN7	CN7	2.4	1	180.0
P	NN1	CN7	CN7	0.6	3	180.0
P	NN1	CN7	CN7B	2.5	1	180.0
P	NN1	CN7	CN8	2.5	1	180.0
NN1	CN7	CN7B	CN7B	1.5	1	180.0
NN1	CN7	CN7B	CN7B	1.5	2	0.0
NN1	CN7	CN7B	CN7B	1.1	3	180.0
NN1	CN7	CN8	CN7B	1.1	1	180.0
NN1	CN7	CN8	CN7B	1.1	2	0.0
NN1	CN7	CN8	CN7B	0.5	3	180.0
NN1	CN7	CN7	CN8B	0.5	2	180.0
P	NN1	CN8B	CN7	0.20	1	120.0
CN7	CN7	CN8B	NN1	0.20	4	180.0
CN7	CN7	CN8B	NN1	0.80	3	180.0
CN7	CN7	CN8B	NN1	0.40	2	0.0
CN7	CN7	CN8B	NN1	2.50	1	180.0
HN7	CN7	CN8B	NN1	0.195	3	0.0
HN2	NN1	CN8B	CN7	0.26	1	0.0
ON6B	CN7	CN8B	NN1	3.4	1	180.0
ON6	CN7	CN8B	NN1	3.4	1	180.0
CN7	CN7	ON5	HN7	0.5	3	0.0
CN7	CN7	ON5	HN7	1.0	1	0.0
CN8B	CN7	ON5	HN7	0.5	3	0.0
CN8B	CN7	ON5	HN7	1.0	1	0.0
HN7	CN7	ON5	HN7	0.1	3	0.0
ON5	CN7	CN8B	NN1	3.4	1	180.0
HN8	CN8B	CN7	ON5	0.195	1	0.0

Force constant (K_ϕ) is given in kcal/mol.radian, *n* is periodicity, ϕ is the phase angle.

Table S4D. Improper dihedral parameters

Improper dihedral				K_ω	ω_0
CN7	P	NN1	HN2	1.3	180.0
CN8B	P	NN1	HN2	1.3	180.0

Improper dihedral angle (ω_0) in degrees and force constant (K_ω) in kcal/mol.Å.