A Transition-State Perspective on Y-Family DNA Polymerase η Fidelity in Comparison with X-Family DNA Polymerases λ and β

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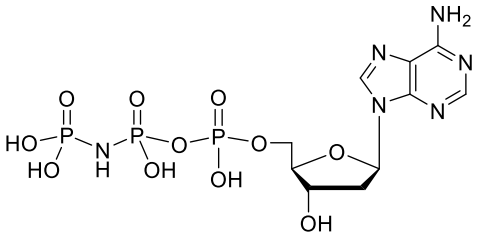
Supporting Information

Synthesis of β,γ-NH of dNTP analogues

General Method 1: The tetrasodium salt of imidodiphosphoric acid (2 equiv) was dissolved in cold water and converted to the tetraacid by passage through DOWEX H^+. An excess of TEA was added and the solution was concentrated under vacuum to obtain the tris(triethylammonium) salt. The residue was co-evaporated with anhydrous DMF (3 x 1 mL), dissolved in dry acetonitrile and cooled to 0°C in an ice-bath. In another flask, 5'-dNMP (5'-dAMP free acid, 5'-dGMP as disodium salt) (1 equiv) was dissolved in acetonitrile [0.3M] and TEA (12 equiv) and cooled to 0°C. Then, the solution of trifluoroacetic anhydride (TFAA) (15 equiv) in acetonitrile [1.2 M] cooled to 0°C in an ice-bath, then was added drop-wise using a gastight syringe to the 5'-dNMP solution under N_2. The reaction was stirred for 10 min at room temperature. Excess TFAA was removed under vacuum and the residue was cooled down to 0 °C. Then a cold solution of *N*-methylimidazole (15 equiv) in anhydrous acetonitrile [3 M] and TEA (2.5 equiv) was added dropwise under N_2 at 0 °C. The reaction monitored by ^{31}P NMR (~δ -11.5 ppm, in CD_3CN). Activated dNMP-N-methylimidazolide[1] was added dropwise to the tris(triethylammonium) salt of imidodiphosphoric acid at 0 ℃ under N_2. The solution was stirred at rt for 1 h and the reaction monitored to completion by ^{31}P NMR. The crude material was purified by dual-pass preparative HPLC: Macherey-Nagel Nucleogel SAX 1000-10 25 mm × 15 cm preparative column (8.0 mL/min, 259 nm) using a gradient (A/ H_2O and B/ 0.5 M triethylammonium bicarbonate pH 8.0 buffer: 0-7.5 min B 0-55%, 7.5-15 min B 55%, 15-20 min B 55-100%, 20-25 min B 100%) and further purified by preparative Phenomenex Luna C18 HPLC column (5 μm, 250 mm × 21 mm) (8.0 mL/min, 259 nm; isocratic mode with 6.5% acetonitrile 0.1 M triethylammonium bicarbonate pH 7.5 buffer).

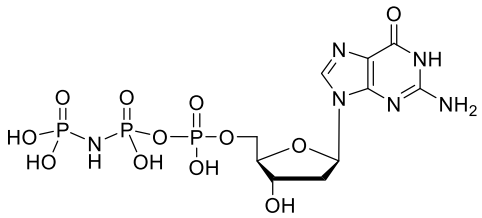


({[({[(2S,5S)-5-(6-amino-9H-purin-9-yl)-3-hydroxyoxolan-2-yl]methoxy}(hydroxy)phosphoryl)oxy](hydroxy)phosphoryl}amino)phosphonic acid – NH-dATP

According to **General Method 1**, the tris(tetraethylammonium) of imidodiphosphoric acid (2 equiv., 34 mg, 0.058 mmol) in anhydrous acetonitrile was reacted with dAMP-N-methylimidazolide (1 equiv., 0.029 mmol) for 1 h and purified by HPLC to obtain bis(triethylammonium) salt of the title compound (14.1 mg, 70% yield). ^1H NMR (500 MHz, D_2O, pH=10): δ 8.37 (s, 1H), 8.12 (d, *J* = 0.7 Hz, 1H), 6.39 (t, *J* = 6.8 Hz, 1H), 4.16 (td, *J* = 3.6, 1.3 Hz, 1H), 4.07 (m, 2H), 2.70 (dt, *J* = 13.6, 6.6 Hz, 1H), 2.47 (ddd, *J* = 13.8, 6.4, 4.1 Hz, 1H); ^{31}P NMR (202 MHz, D_2O, pH=10): δ -0.81

[1] Mohamady S, Jakeman DL. An improved method for the synthesis of nucleoside triphosphate analogues. The Journal of organic chemistry. **2005** Dec 9;70(25):10588-91.

(d, *J* = 4.5 Hz), -7.69 (dd, *J* = 21.0, 4.5 Hz), -10.65 (d, *J* = 21.3 Hz). HRMS (ESI): $[M-H]^-$, m/z calcd for $C_{10}H_{15}N_6O_{11}P_3^-$ 489.0095; found 489.0095.



({[({[(2R,5R)-5-(2-amino-6-oxo-6,9-dihydro-1H-purin-9-yl)-3-hydroxyoxolan-2-yl]methoxy}(hydroxy)phosphoryl)oxy](hydroxy)phosphoryl}amino)phosphonic acid – NH-dGTP

According to **General Method 1**, the tris(tetraethylammonium) of imidodiphosphoric acid (2 equiv., 34 mg, 0.058 mmol) in anhydrous acetonitrile was reacted with dGMP-N-methylimidazolide (1 equiv., 0.029 mmol) for 1 h and purified by HPLC to obtain bis(triethylammonium) salt of the title compound (13.5 mg, 66% yield). ^{1}H NMR (500 MHz, D_2O, pH=10): δ 7.96 (s, 1H), 6.19 (t, *J* = 6.9 Hz, 1H), 4.13 (q, *J* = 4.0 Hz, 1H), 4.06 (m, 2H), 2.70 (dt, *J* = 13.9, 6.8 Hz, 1H), 2.37 (ddd, *J* = 14.0, 6.4, 3.8 Hz, 1H). ^{31}P NMR (202 MHz, D2O, pH=10): δ -1.28 (d, *J* = 4.1 Hz), -8.23 (d, *J* = 21.7 Hz), -10.89 (d, *J* = 21.6 Hz). MS (ESI): $[M-H]^-$, m/z calcd for $C_{10}H_{16}N_6O_{12}P_3^-$ 505.00; found 505.07.

Figure S1. ^{1}H NMR (500 MHz, D_2O, pH 10.0) of β,γ-NH dATP

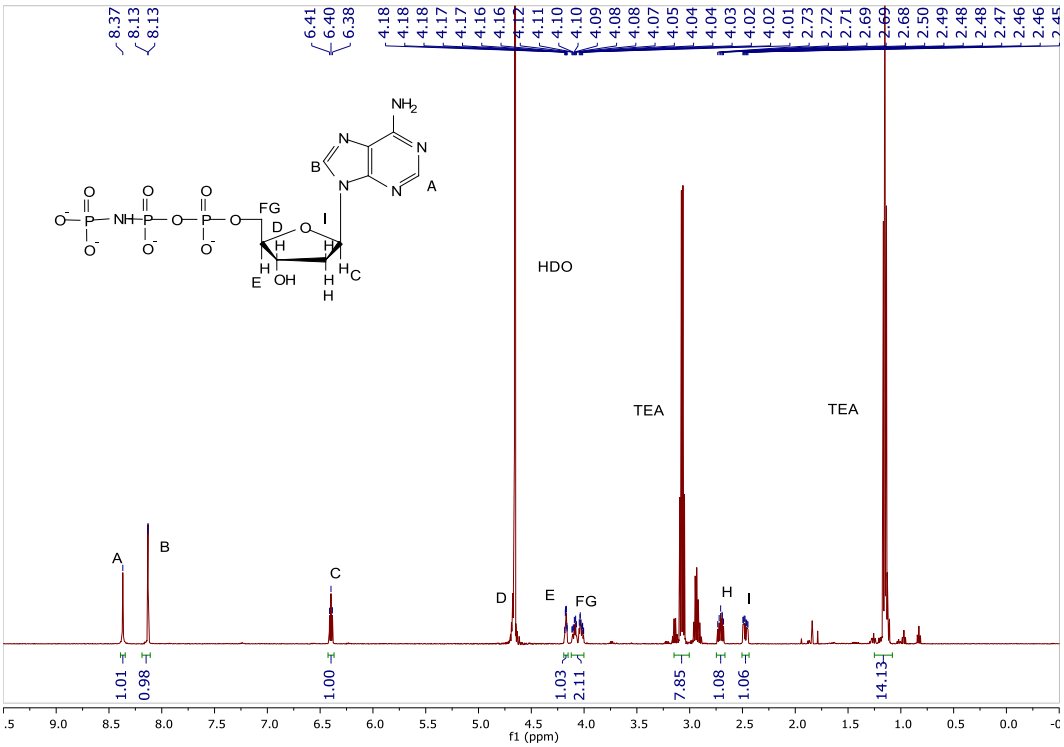


Figure S2. COSY (500 MHz, D_2O, pH=10) of β,γ-NH dATP

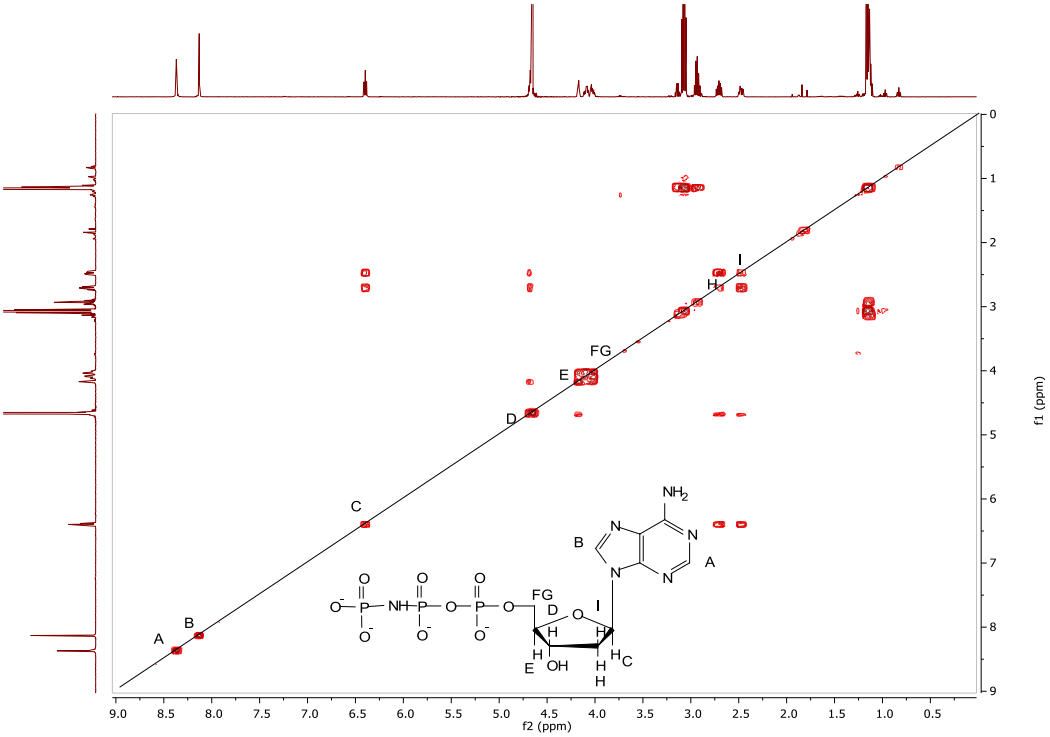


Figure S3. ^{31}P NMR (202 MHz, D_2O, pH 10.0) of β,γ-NH dATP

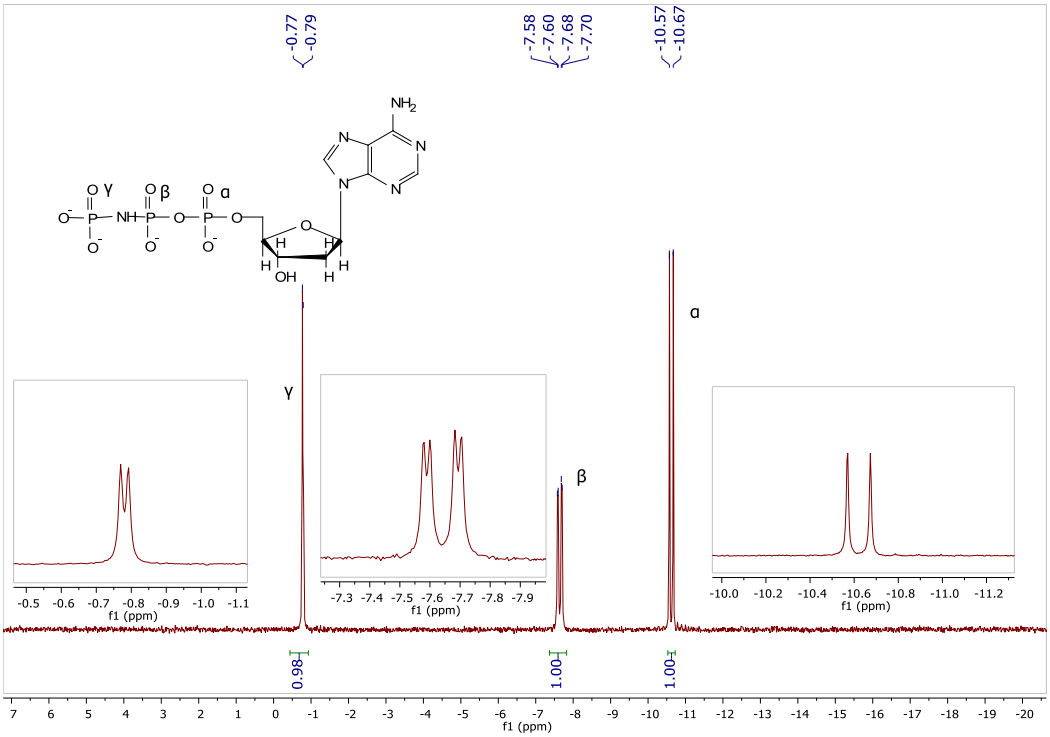


Figure S4. HRMS (ESI) $[M - H]^-$ of β,γ-NH dATP

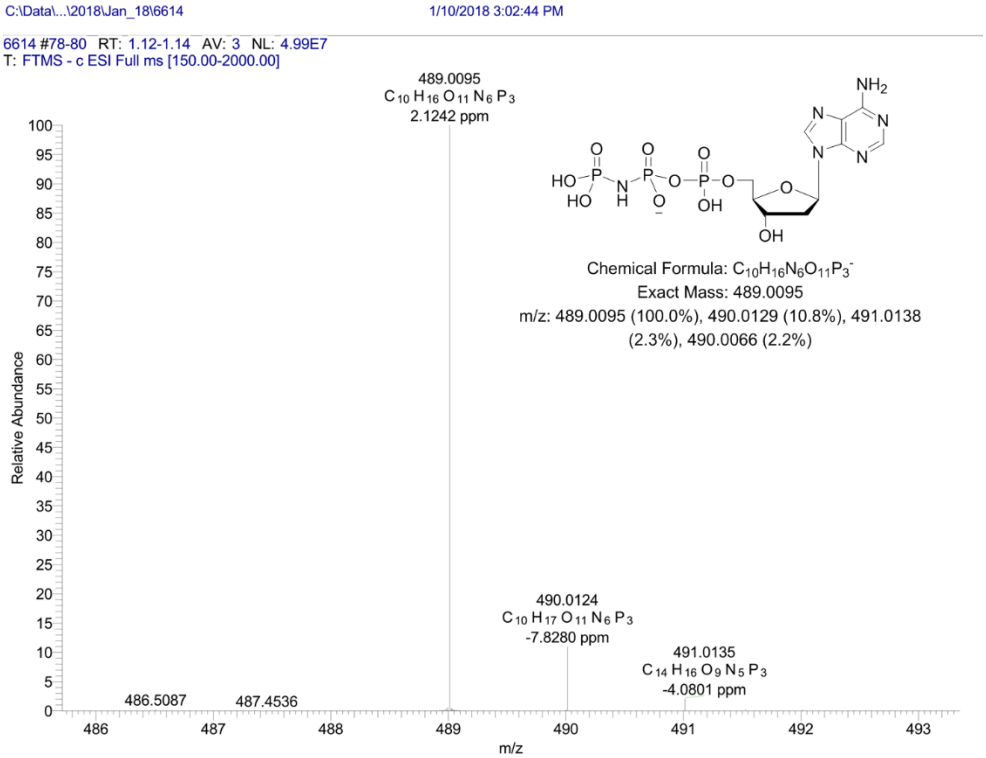


Figure S5. ^{1}H NMR (500 MHz, D_2O, pH 10.0) of β,γ-NH dGTP

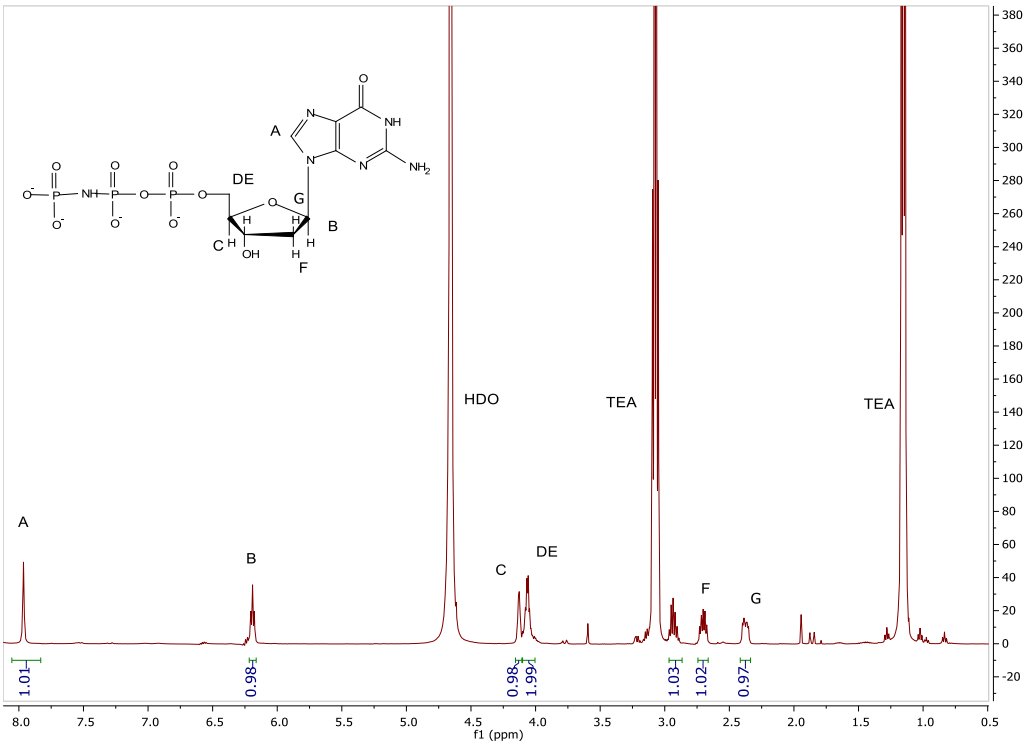


Figure S6. COSY (500 MHz, D_2O, pH=10) of β,γ-NH dGTP

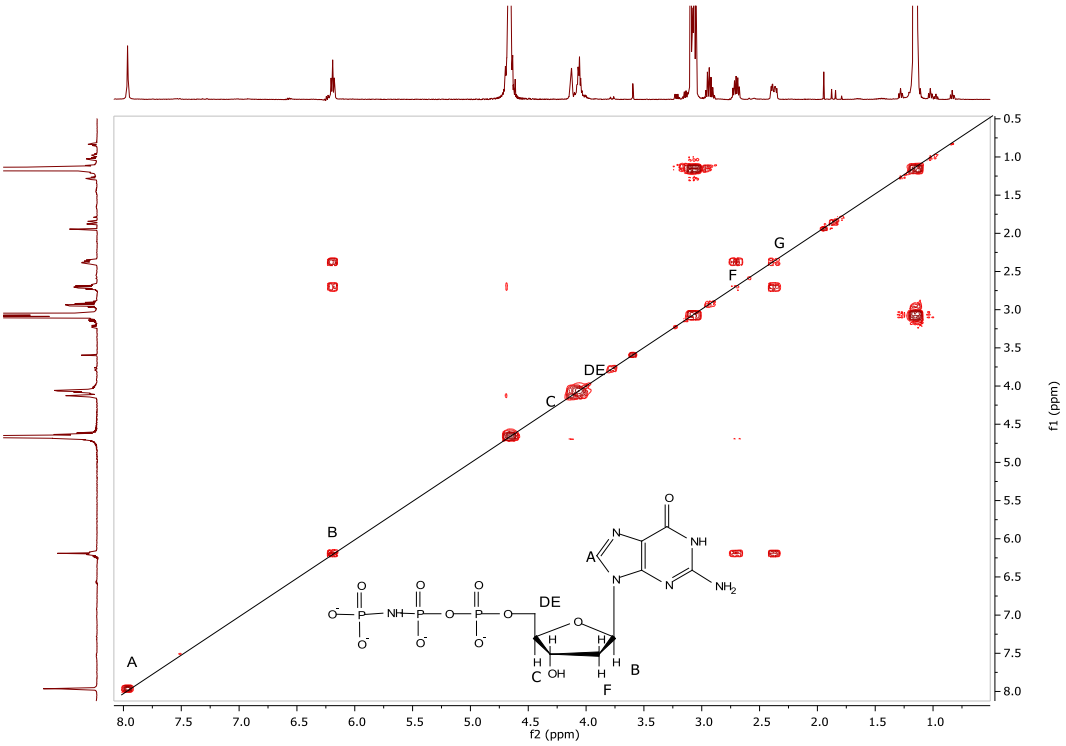


Figure S7. ^{31}P NMR (202 MHz, D_2O, pH 10.0) of β,γ-NH dGTP

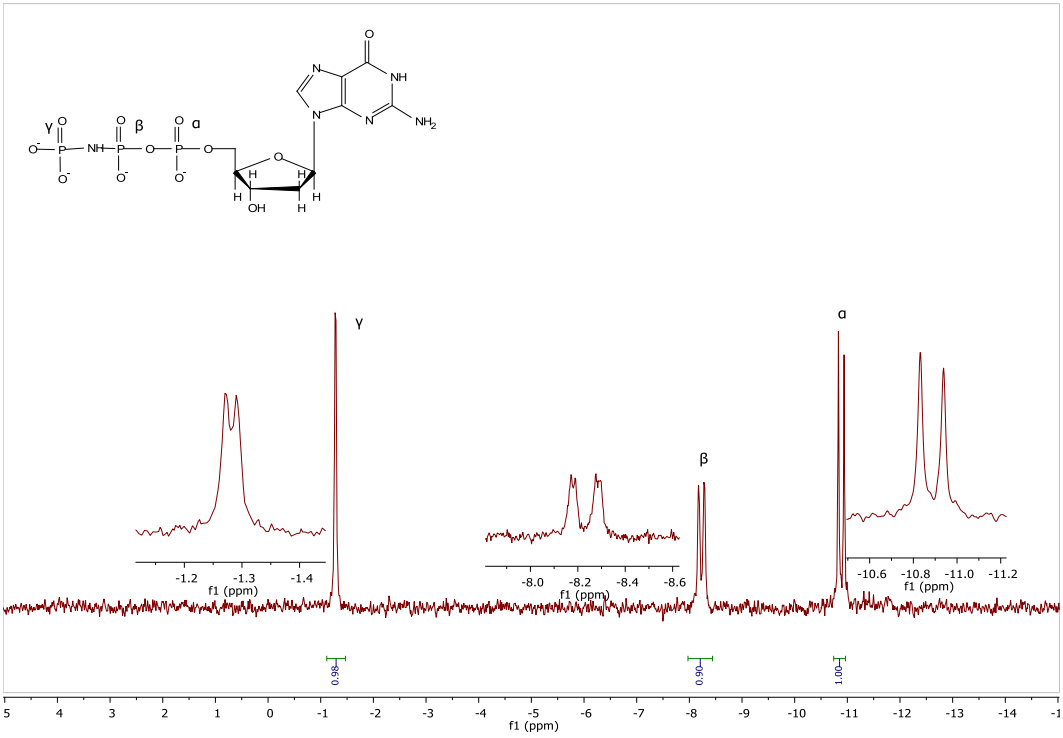


Figure S8. MS (ESI) [M – H]⁻ of β,γ-NH dGTP

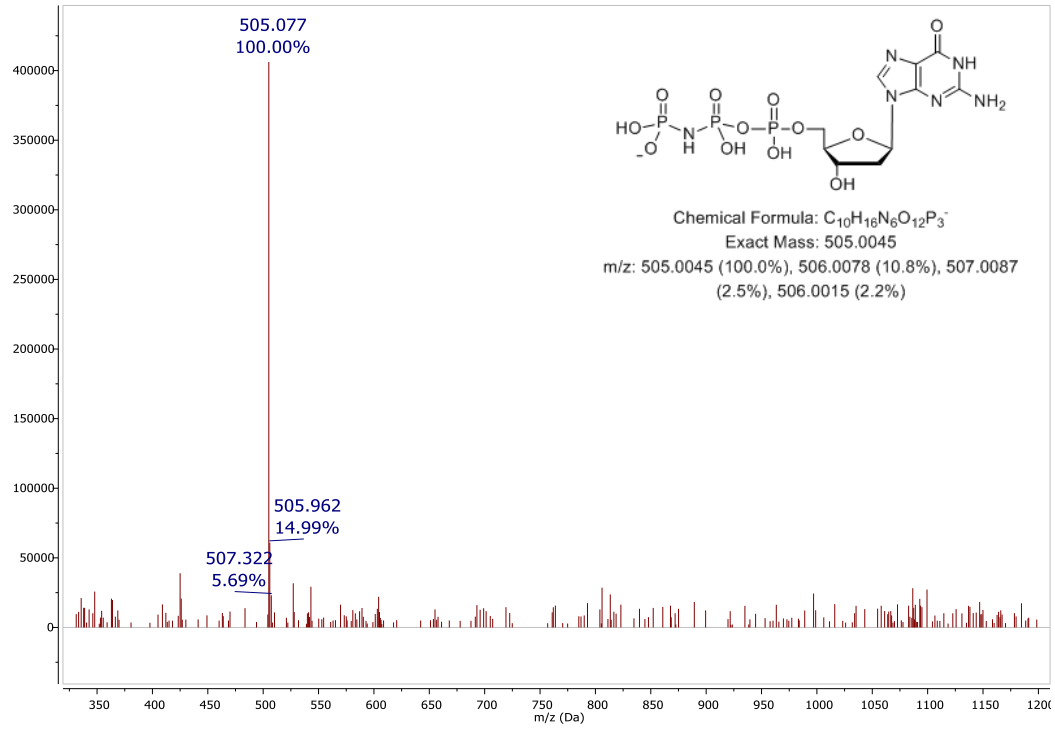
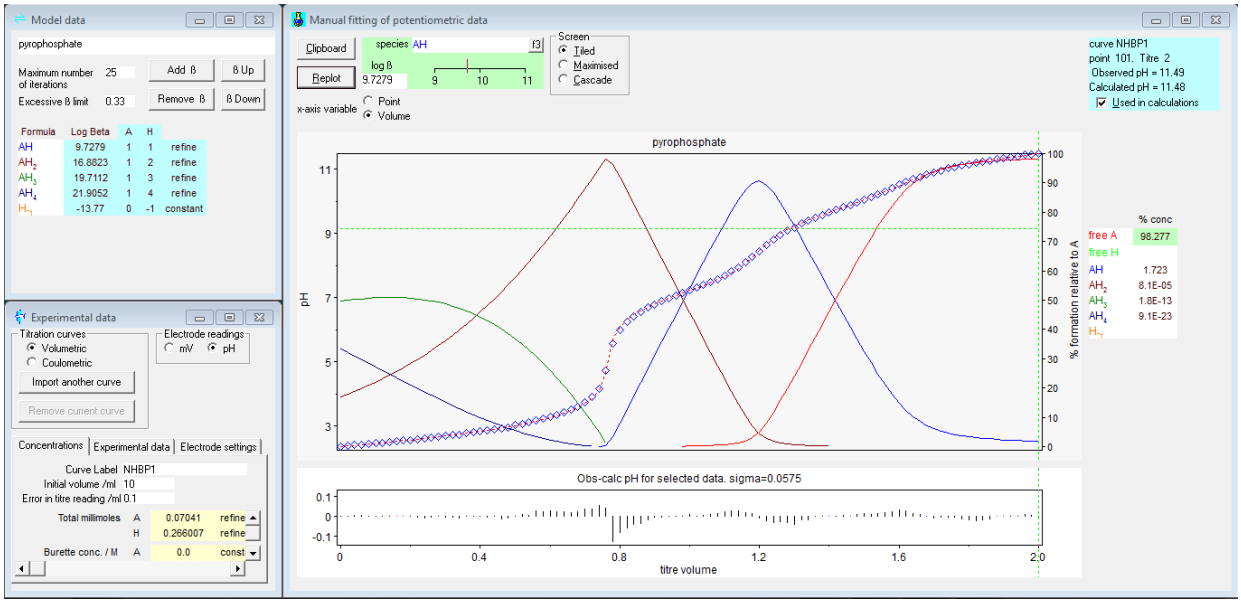


Figure S9. *Hyperquad2008* simulation for titration curve of imidodiphopsphoric acid in 0.1 M KCl solution titrated with ~0.1 M KOH in CO_2–free H_2O using a Schott Instruments Titrator Basic



Kinetic data tables

Table S1. Pol η kinetic data

		A opp. T				G opp. T			
		B Sequence		W Sequence		B Sequence		W Sequence	
-X-	pK_{a4}	k_{pol} (s^{-1})	K_d (μM)	k_{pol} (s^{-1})	K_d (μM)	k_{pol} (s^{-1})	K_d (μM)	k_{pol} (s^{-1})	K_d (μM)
CF_2	7.8	0.13 ± 0.02	150 ± 10	0.15 ± 0.02	80 ± 50			0.0053 ± 0.008	100 ± 10
CFCl	8.4	0.034 ± 0.007	560 ± 180	0.023 ± 0.001	250 ± 20				
CCl_2	8.8	0.016 ± 0.005	270 ± 10						
O	8.9	4.1 ± 1.2	9.4 ± 3.1	4.7 ± 1.0	7.1 ± 2.1	0.21 ± 0.02	30 ± 3	0.86 ± 0.02	20 ± 4
CHF (mix)	9.0	0.23 ± 0.08	190 ± 80	0.22 ± 0.03	130 ± 30	0.032 ± 0.005	370 ± 60	0.12 ± 0.00	390 ± 20
CHF (*R*)	9.0	0.24 ± 0.04	170 ± 60	0.51 ± 0.07	240 ± 30				
CHF (*S*)	9.0	0.036 ± 0.007	120 ± 30	0.076 ± 0.005	160 ± 10				
CBr_2	9.3	0.024 ± 0.003	160 ± 30	0.028 ± 0.011	370 ± 220				
CHCl (mix)	9.5	0.033 ± 0.08	610 ± 160	0.027 ± 0.003	290 ± 70				
CHCl (*R*)	9.5	0.032 ± 0.003	120 ± 70	0.025 ± 0.006	110 ± 30				
CHCl (*S*)	9.5	0.016 ± 0.004	290 ± 40	0.016 ± 0.003	310 ± 90				
NH	9.7	0.27 ± 0.06	4.8 ± 0.6	0.35 ± 0.05	3.9 ± 1.1	0.012 ± 0.001	36 ± 6	0.035 ± 0.003	44 ± 5
CH_2	10.5	0.092 ± 0.008	350 ± 22	0.11 ± 0.02	270 ± 70				
$CHCH_3$	11.59	0.0016	130	0.0024	62				

Table S2. Pol λ kinetic parameters.

		A opp. T		G opp. T	
-X-	pK_{a4}	k_{pol} (s^{-1})	K_d (μM)	k_{pol} (s^{-1})	K_d (μM)
CF_2	7.8	6.7 ± 0.9	8.1 ± 1.7	0.042 ± 0.001	68 ± 5
CFCl (mix)	8.4	2.3 ± 0.4	38 ± 7	0.017 ± 0.002	85 ± 1
CCl_2	8.8	0.81 ± 0.12	18 ± 4	0.0019 ± 0.0004	49 ± 25
O	8.9	2.6 ± 0.4	1.8 ± 0.3	0.027 ± 0.003	20 ± 5
CHF (mix)	9.0	5.4 ± 0.5	5.4 ± 2.0	0.040 ± 0.005	40 ± 9
CHF (*R*)	9.0	6.1 ± 1.0	4.3 ± 1.1	0.042 ± 0.002	17 ± 2
CHF (*S*)	9.0	2.0 ± 0.3	4.6 ± 0.4	0.012 ± 0.002	71 ± 9
CHCl (mix)	9.5	2.5 ± 0.4	4.1 ± 0.4	0.025 ± 0.001	38 ± 4
CH_2	10.5	0.38 ± 0.05	1.2 ± 0.2	0.0046 ± 0.0006	18 ± 5

Table S3. Pol β kinetic parameters.

		A opp. T		G opp. T	
-X-	pK_{a4}	k_{pol} (s^{-1})	K_d (μM)	k_{pol} (s^{-1})	K_d (μM)
CF_2	7.8	25.3 ± 1.0	5.7 ± 1.4	0.76 ± 0.01	380 ± 50
CFCl (mix)	8.4	15.7 ± 2.1	10.0 ± 2.6	0.17 ± 0.01	660 ± 40
CFCl (*R*)	8.4			0.08 ± 0.01	190 ± 40
CFCl (*S*)	8.4			0.10 ± 0.01	460 ± 60
CCl_2	8.8	6.6 ± 0.9	48.0 ± 10.6	0.05 ± 0.00	800 ± 70
O	8.9	17.5 ± 3.1	8.0 ± 1.8	1.34 ± 0.10	200 ± 7
CHF (mix)	9.0	19.3 ± 0.4	7.6 ± 2.6	1.34 ± 0.07	1300 ± 100
CHF (*R*)	9.0			2.27 ± 0.40	740 ± 140
CHF (*S*)	9.0			0.16 ± 0.01	600 ± 110
CBr_2	9.3	6.0 ± 0.7	90 ± 30	0.02 ± 0.01	500 ± 200
CHN_3	9.34			0.34 ± 0.05	540 ± 90
CHCl (mix)	9.5	9.6 ± 1.9	11.3 ± 2.4	0.22 ± 0.03	630 ± 160
CHCl (*R*)	9.5			0.26 ± 0.01	290 ± 20
CHCl (*S*)	9.5			0.051 ± 0.003	540 ± 30
CHBr	9.9			0.14 ± 0.02	470 ± 130
$CFCH_3$	10.2			0.22 ± 0.02	110 ± 10
CH_2	10.5	4.9 ± 0.7	4.7 ± 2.0	0.12 ± 0.03	470 ± 110
CCH_3N_3	10.59			0.042 ± 0.007	3300 ± 1000
$CHCH_3$	11.59	0.29 ± 0.19	2.2 ± 1.0	0.012 ± 0.001	750 ± 180
$C(CH_3)_2$	12.29			0.0042 ± 0.0002	1050 ± 70

Table S4. Effective pK_{a4} calculations.

Enzyme		η				λ				β			
Pairing		A opp. T				A opp. T		G opp. T		A opp. T		G opp. T	
Sequence		B		W		B		B		B		B	
Compound	pK_{a4} (soln)	pK_{eff}	$\Delta pK_{a4,eff}$	pK_{eff}	$\Delta pK_{a4,eff}$	pK_{eff}	$\Delta pK_{a4,eff}$	pK_{eff}	$\Delta pK_{a4,eff}$	pK_{eff}	$\Delta pK_{a4,eff}$	pK_{eff}	$\Delta pK_{a4,eff}$
CF_2	7.8	9.0	1.2	9.0	1.2	8.4	0.6	8.5	0.7	8.7	0.9	8.6	0.8
CFCl	8.4	9.6	1.2	9.8	1.4	8.9	0.5	9.0	0.6	8.9	0.5	9.3	0.9
CCl_2	8.8	9.9	1.1			9.4	0.6	10.1	1.3	9.3	0.5	9.9	1.1
O	8.9	7.3	-1.6	7.2	-1.7	8.9	0.0	8.7	-0.2	8.9	0.0	8.3	-0.6
CHF	9.0	8.7	-0.3	8.7	-0.3	8.5	-0.5	8.5	-0.5	8.8	-0.2	8.3	-0.7
R-CHF	9.0	8.6	-0.4	8.3	-0.7	8.5	-0.5	8.5	-0.5			8.0	-1.0
S-CHF	9.0	9.6	0.6	9.2	0.2	9.0	0.0	9.1	0.1			9.3	0.3
CBr_2	9.3	9.8	0.5	9.9	0.6	9.8	0.5			9.4	0.1	10.4	1.1
CHCl	9.5	9.6	0.1	9.7	0.2	8.9	-0.6	8.8	-0.7	9.2	-0.3	9.2	-0.3
R-CHCl	9.5	9.6	0.1	9.6	0.1							9.1	-0.4
S-CHCl	9.5	10.0	0.5	9.9	0.4							9.9	0.4
NH	9.7	8.6	-1.1	8.5	-1.2								
CHBr	9.9											9.4	-0.5
CH_2	10.5	9.1	-1.4	9.0	-1.5	9.8	-0.7	9.6	-0.9	9.5	-1.0	9.5	-1.0
$CHCH_3$	11.59	11.1	-0.5	10.9	-0.7					11.1	-0.5	10.6	-1.0

Potentiometric Calculations

The experimental data points were analyzed using the PSEQUAD (v.5.02) computer program (L. Zekany, I. Nagypal, in: D. Leggett (Ed.), Computational Methods for the Determination of Stability Constants, Plenum Press, New York, 1985, pp. 291-355). The protonation reactions of the ligands measured in this work are shown in eq. (1)-(2). The reaction for the calculation of the formation in this work for the MgL^{2-} complex are shown in eq 3, and the reaction for calculating the corresponding pKa value for $HMgL^{-}$ is defined in eq. 4.

$$HL^{3-} \rightleftharpoons L^{4-} + H^{+} \quad (1)$$

$$H_2L^{3-} \rightleftharpoons HL^{3-} + H^{+} \quad (2)$$

$$Mg^{2+} + L^{4-} \rightleftharpoons MgL^{2-} \quad (3)$$

$$HMgL^{-} \rightleftharpoons MgL^{2-} + H^{+} \quad (4)$$

Formation constants β_{pqr} eq. (5) in this work are reported as the reaction eq. (6) equilibrium constants

$$\beta_{pqr} = [MpLqHr^{2p+r-4q}][M^{2+}]^{-p}[L^{4-}]^{-q}[H^{+}]^{-r}. \quad (5)$$

$$pM^{2+} + qL^{4-} + rH^{+} \rightleftharpoons MpLqHr^{2p+r-4q} \quad (6)$$

Table S5. Comparison of the negative logarithms of acidity constants [Eqs. (1-2)] as determined by potentiometric titration in aqueous solution (at 25ºC, and *I*=0.4 M KCl) for bisphosphonates $(O_3P\text{-}X\text{-}PO_3)^{4-}$. Mg^{2+} complex formation constants (eq 3) with bisphosphonates, and the pK_a's values for the deprotonation reaction (eq 4).

Bridging group X	pK_{a1}	pK_{a2}	logK(MgL)	pK_a(HMgL)
CF_2	7.8	5.7	5.1	5.8
O	8.6	6.1	5.7	6.3
CH_2	9.9	6.7	5.4	7.3
NH	10.9	7.5	4.9	9.5