Relative Usage Calculation. The relative usage was the ratio of the amounts of (*R*)-β,γ-CHF-dGTP vs. (*S*)-β,γ-CHF-dGTP incorporated after 5 hrs of reaction. The starting (*R*/*S*)-CHF was set to be 100 integral, 50 for each diastereomer (Fig. 1, pink). At t = 5 hr, bisphosphonate, the incorporation and hydrolysis products of (*R*/*S*)-CHF, was 31 integral; and the unreacted (*R*/*S*)-CHF was 69 (Fig. 1B, blue). ^{31}P NMR (Fig. S1.) showed 1 integral of dGMP at t = 5 hr, which was the product of (*R*/*S*)-CHF through hydrolysis assuming 0.5 from each diastereomer. Zoom-in at the unreacted (*R*/*S*)-CHF region (Fig. 1C) showing (*R*)-CHF to be 27.8 integral and (*S*)-CHF 42. The amount of (*R*)-CHF incorporated was then calculated as 50 - 0.5 – 27.8 = 21.7, and that of (*S*)-CHF was 7.5. The relative usage *R*:*S* = 21.7 / 7.5 = 2.9 ≈ 3. The relative usage for the (*R*/*S*)-CHCl analogue was calculated similarly.

Figure S1

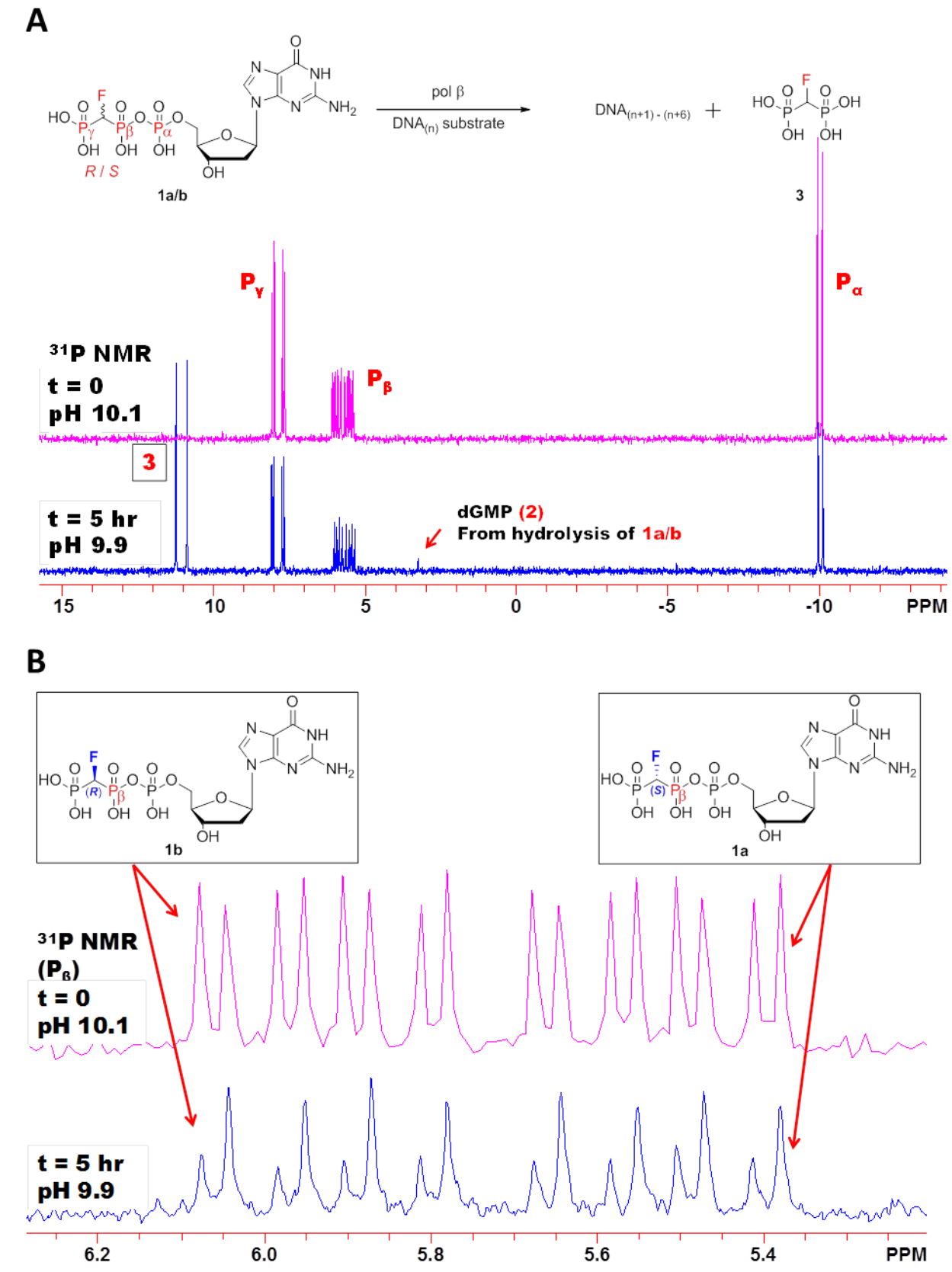
A
pol β
DNA(n) substrate
DNA(n+1) - (n+6)
1a/b
3
Pγ
Pβ
Pα
31P NMR
t = 0
pH 10.1
3
t = 5 hr
pH 9.9
dGMP (2)
From hydrolysis of 1a/b
15
10
5
0
-5
-10
PPM
B
1b
1a
31P NMR
(Pβ)
t = 0
pH 10.1
t = 5 hr
pH 9.9
6.2
6.0
5.8
5.6
5.4
PPM

Figure S1 Reaction scheme and ^{31}P NMR spectra of reaction mixtures. (**A**) β,γ-CHF-dGTP in an equal mixture of *R* and *S* is incubated with primer/template DNA with wt pol β, and ^{31}P NMR spectra are taken at time zero (lower blue trace) and after 5 h incubation (upper pink trace). A small (~1.5%) amount of dGMP is observed at t = 5 h due to hydrolysis of dGTP analogue that was not incorporated into the DNA. (**B**) Zoom in of peaks corresponding to unreacted β,γ-CHF-dGTP analogue (1a and 1b) at time zero (lower blue trace) and after five hours (upper pink trace). The outer peaks can be used to specifically identify and quantitate the individual diastereomers, with the left peak corresponding to the *R* diastereomer (1b), and the right peak corresponding to the *S* (1a).

Figure S2

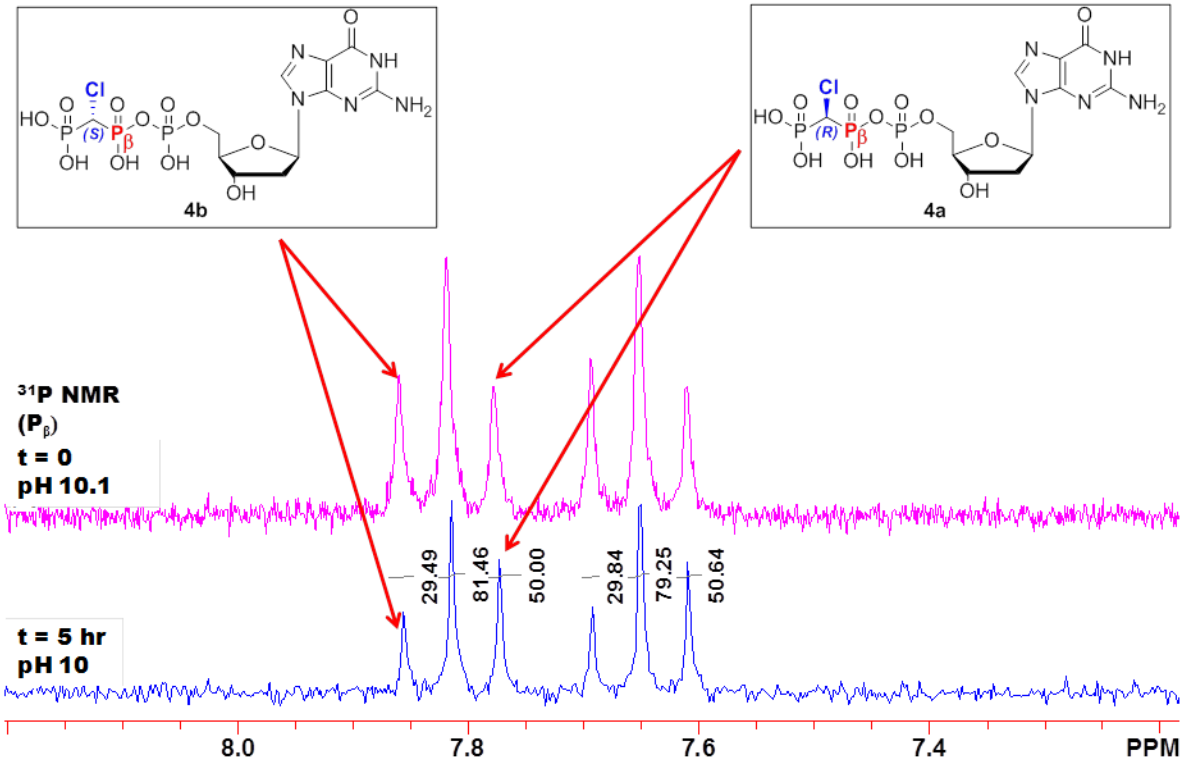

Figure S2 Zoom in of P_β from ^{31}P NMR spectrum of reaction mixtures for unreacted CHCl analogue. Lower blue trace corresponds to time zero (before reaction), and the upper blue trace corresponds to the spectrum observed after 8 h. The arrows show which peaks correspond to (*S*)-β,γ-CHCl-dGTP and (*R)*-β,γ-CHCl-dGTP (compounds 4b and 4a respectively), and can therefore be used for quantitating the reaction ratio.