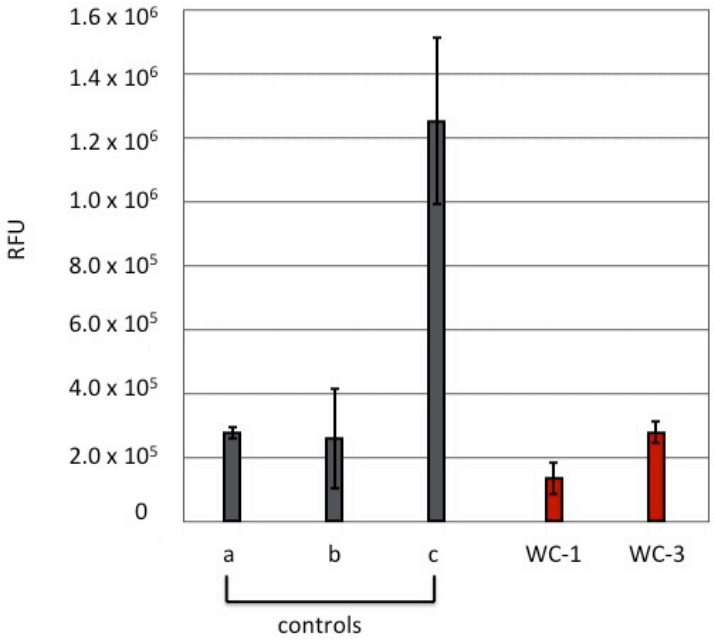
Contents of Supporting Information:

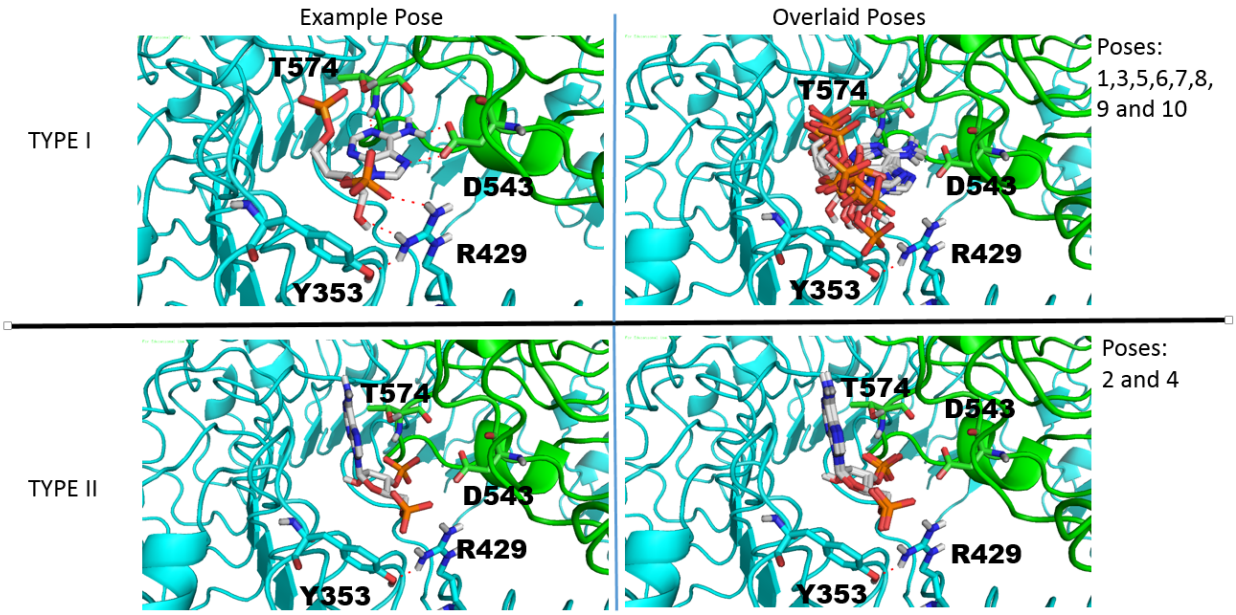
Experimental Section

Cellular Toxicity Assay

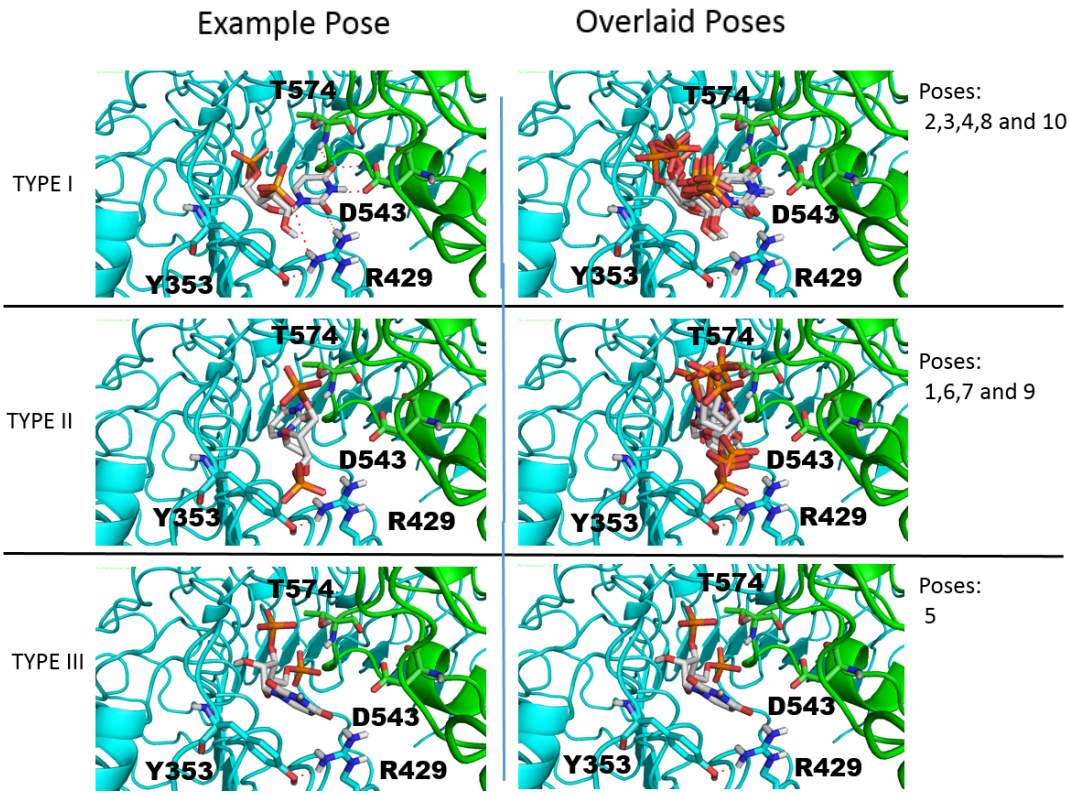
Peripheral blood mononuclear cells (PBMCs) (Sanguine Biosciences) were plated in a dark 96-well plate at 2.5-5 x 10^5 cells per well in RPMI medium (Cellgro) supplemented with 2X penicillin/streptomycin and 10% fetal bovine serum. N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP) (Roche Applied Science) was used as a liposomal transfection agent. Formulation was carried out by mixing siRNAs and DOTAP at a working concentration of 10 μg DOTAP/μg of RNA, and incubated at room temperature for 15-40 minutes. Cultured PBMCs were treated with the formulated siRNAs and incubated for 16 - 20 h at 37 °C. Cellular toxicity was quantified in triplicate by using CellTox™ Green Cytotoxicity Assay kit.



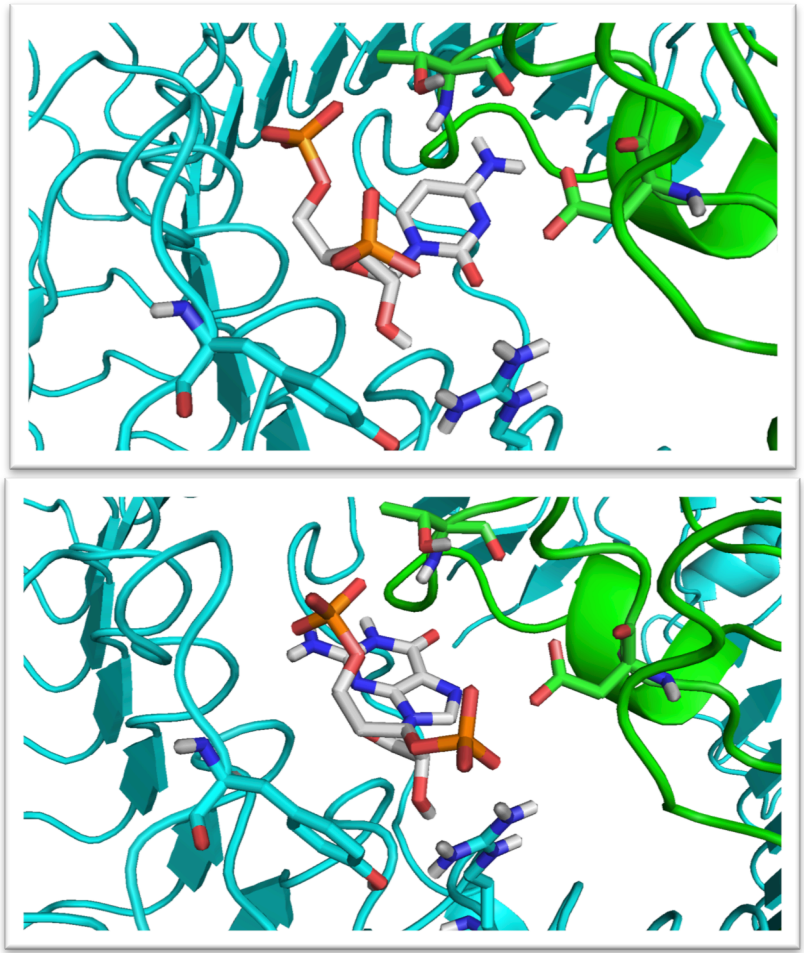
Supplementary Figure 1: Cytotoxic activity of the Watson-Crick (WC) face localized modification at guide positions 1 and 3. Modified PIK3CB siRNA (125nM) was transfected into human PBMCs. Controls: (a) cells only; (b) cells transfected with native siRNA; (c) lysed cells; Red bars: WC face localized modifications at guide positions 1 and 3. The CellTox™ Green Cytotoxicity Assay utilizes a dye that binds DNA from dead cells, resulting to an increase in fluorescence signal that is proportional to cytotoxicity. Viable cells show no substantial increases in fluorescence.



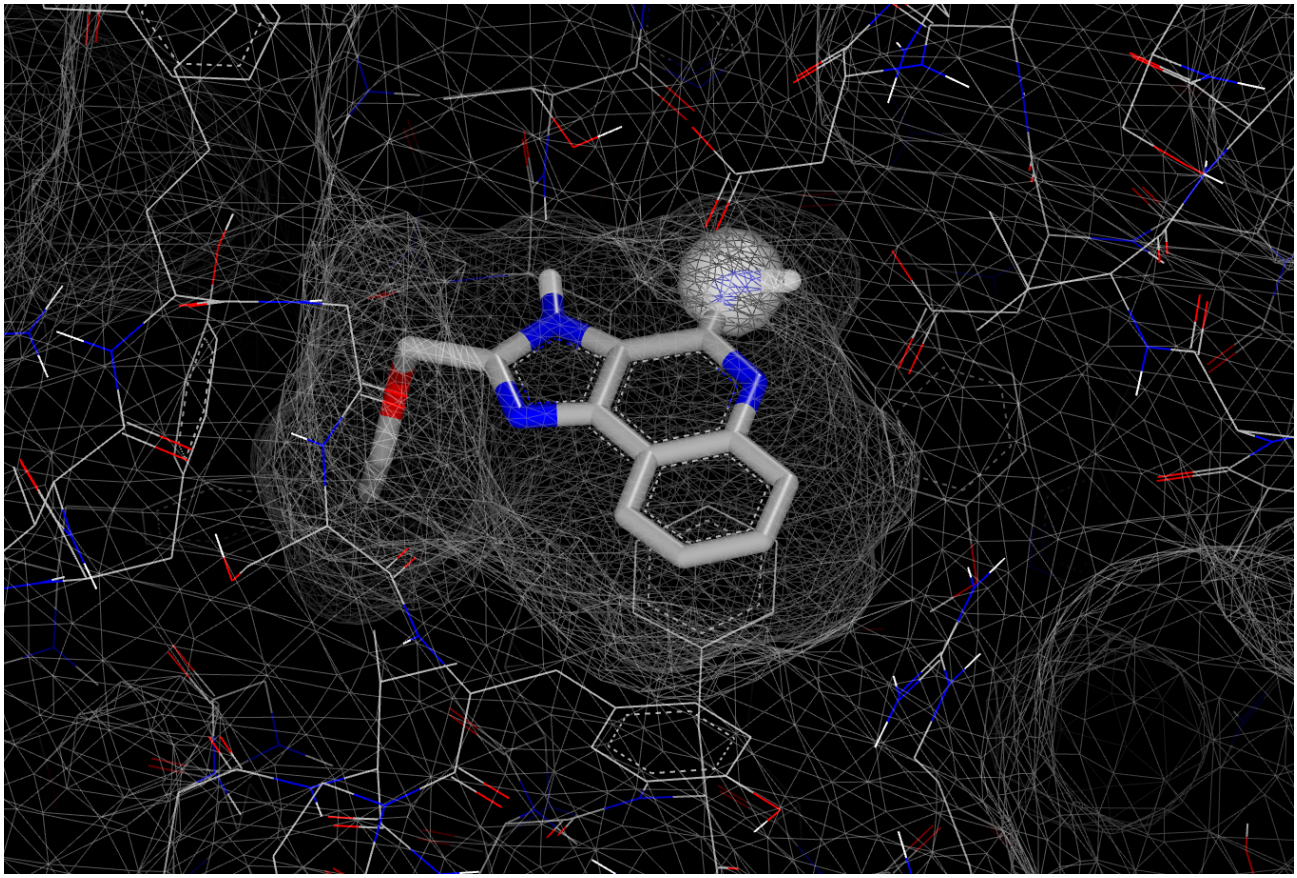
Supplementary Figure 2: The top 10 poses for adenosine-3',5'-bisphosphate bound into the CLO97 receptor of TLR8. Example poses are shown to clarify nucleobase positioning which categorizes the nucleotides in a pose type.



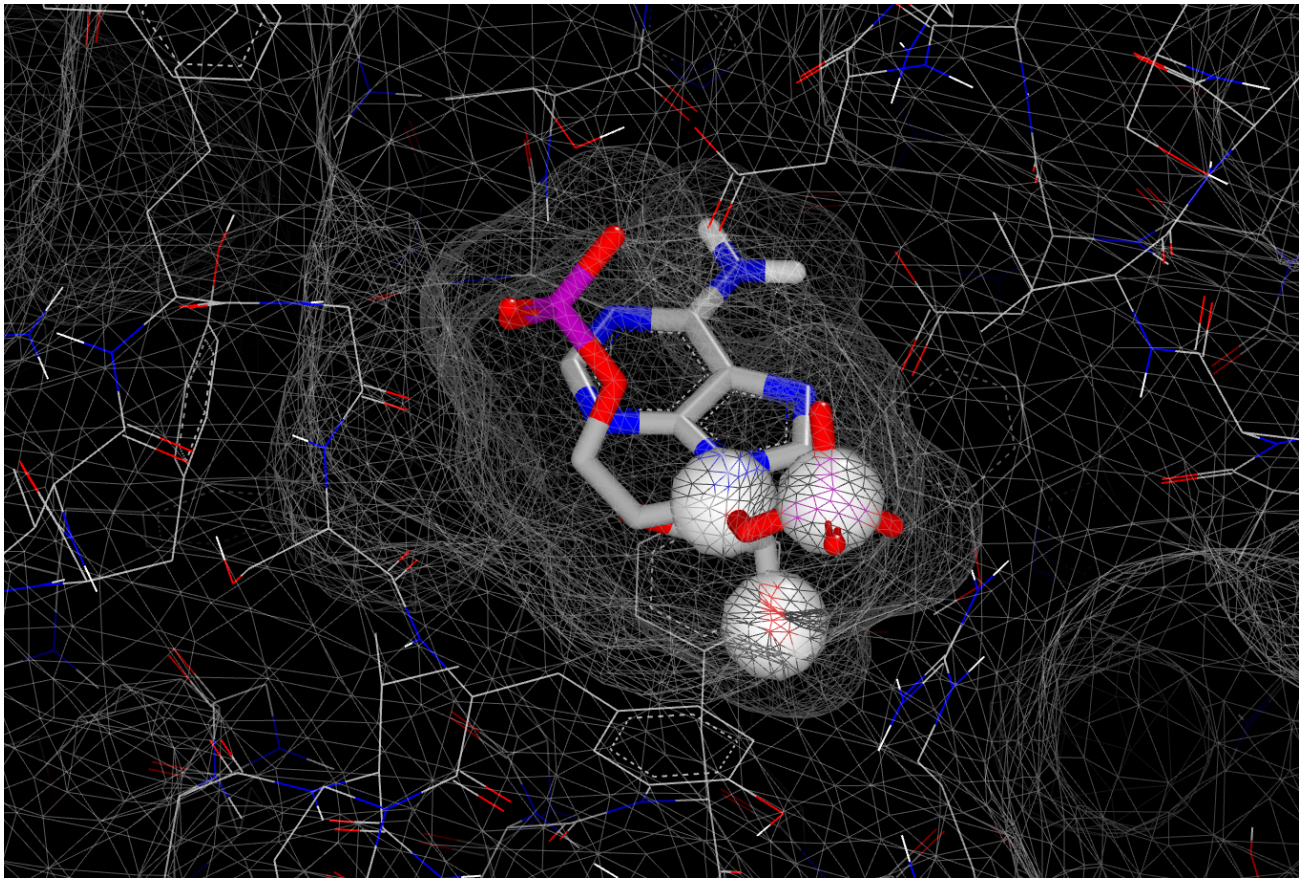
Supplementary Figure 3: The top 10 poses for uridine-3',5'-bisphosphate bound into the CLO97 receptor of TLR8. Example poses are shown to clarify nucleobase positioning which categorizes the nucleotides in a pose type.



Supplementary Figure 4: Best poses for cytidine 3', 5' bisphosphate (top) and guanosine 3', 5'-bisphosphate (bottom) which adhere to constraints placed on them during molecular docking. Neither pose was exhibited at high frequency. C pose Chemgauss4 score: -2.250368; G pose Chemgauss4 score: -1.169547.



Supplementary Figure 5: Constraint for the docking experiment with adenosine 3', 5'-bisphosphate and CLO97 binding site in TLR8. The exocyclic amine was constrained due to its important interaction with D543 in the complex with CLO97.



Supplementary Figure 6: Constraints for the docking experiments with U, C and G bisphosphates into TLR8. Constraints were placed on the best pose of adenosine 3', 5'-bisphosphate at the phosphorus atom of the 3' phosphate, the C3' carbon and C2' hydroxyl oxygen.

A : Type I		A: Type II	
Pose #	Chemgauss4 Score	Pose #	Chemgauss4 Score
1	-7.378654	2	-7.065829
3	-6.798604	4	-6.791432
5	-6.721918		
6	-6.629409		
7	-6.629411		
8	-6.607040		
9	-6.575839		
10	-6.574039		

U: Type I		U: Type II		U: Type III	
Pose #	Chemgauss4 Score	Pose #	Chemgauss4 Score	Pose #	Chemgauss4 Score
3	-5.76245	1	-6.37066	2	-5.881
4	-5.74689	6	-5.58019		
5	-5.69126	7	-5.52172		
8	-5.45346	9	-5.09956		
10	-4.96019				

CLO97 -8.827235

Supplementary Table 1: Table of pose types, poses and the Chemgauss4 scores pertaining to them. The Chemgauss4 score for the CLO97 pose from molecular docking which overlays the crystal structure is also reported.