Synthesis and Evaluation of the First *cis*-Cyclobutane Containing Receptor for Lipid A

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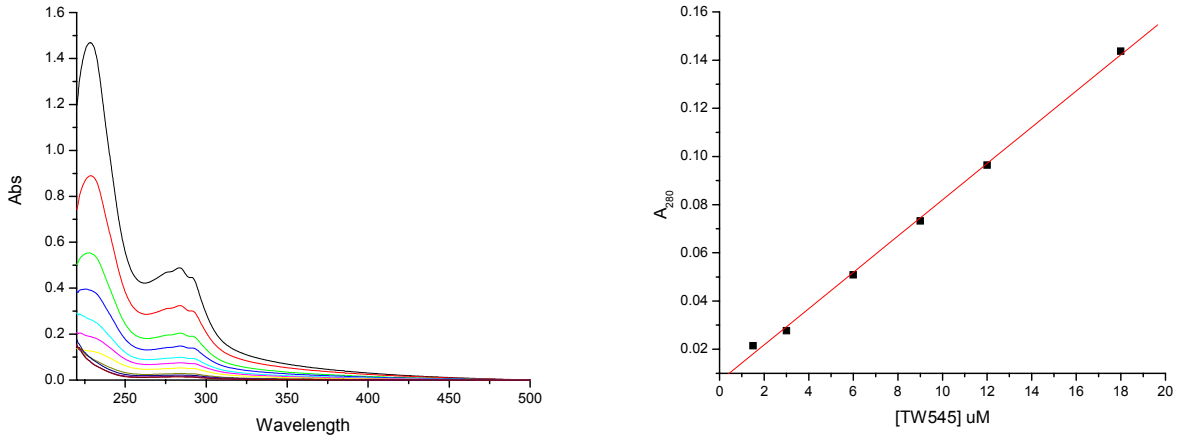
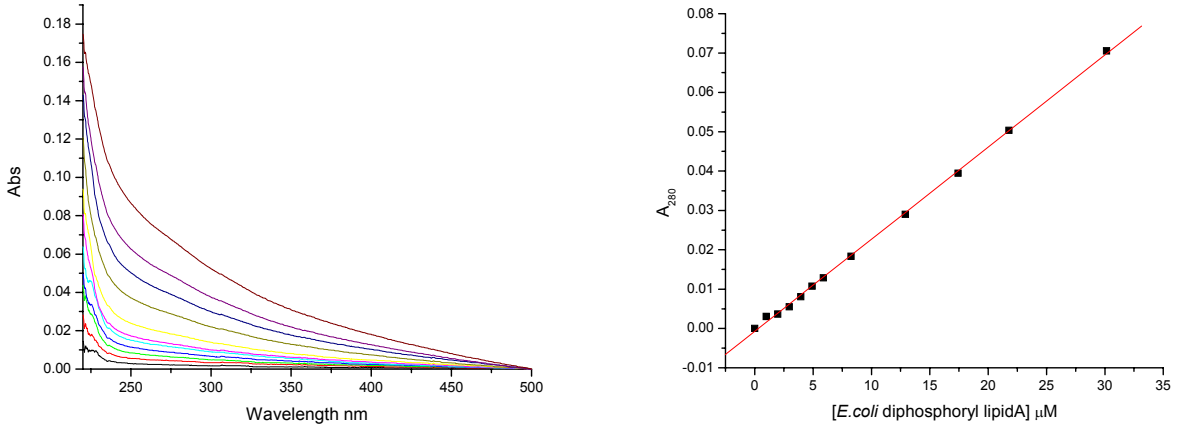
Supplementary Material - I

Test of Receptor A_{280} Linearity Throughout Titration Concentration Range



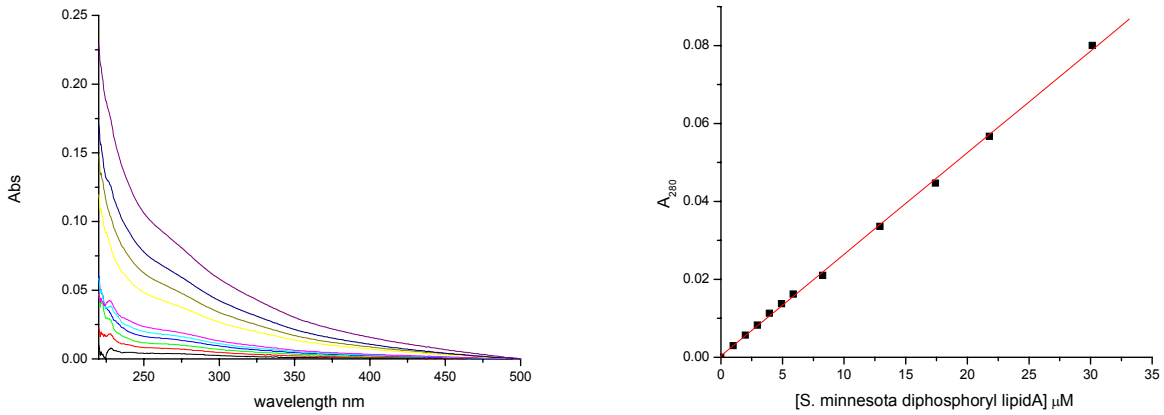
Left: UV-Vis titration of TW545 (**2**) into buffer (20 mM HEPES, pH 7.0); Right: Linear fit of the UV-Vis titration of TW545 (**2**) into buffer (20 mM HEPES, pH 7.0) throughout titration concentration range.

E. coli Diphosphoryl Lipid A into Buffer Control Titration



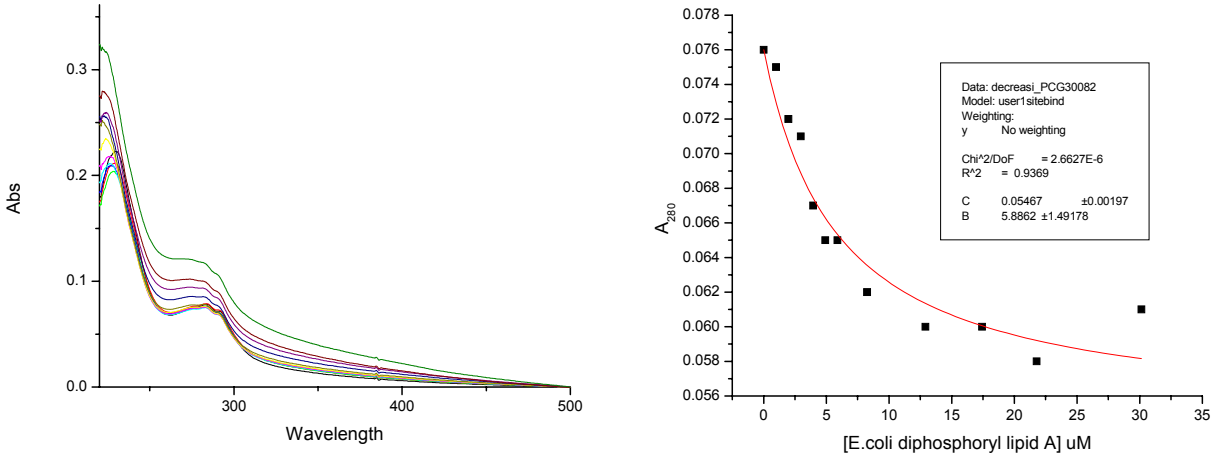
Left: UV-Vis control titration of 300 μM *E. coli* diphosphoryl lipid A into buffer (20 mM HEPES, pH 7.0); Right: Linear fit of the average of three control titrations of 300 μM *E. coli* diphosphoryl lipid A into buffer (20 mM HEPES, pH 7.0). Data fit the linear equation (y = .00234x – 0.00075) where y = A_{280} and x = [lipid A] μM.

S. minnesota Diphosphoryl Lipid A into Buffer Control Titration



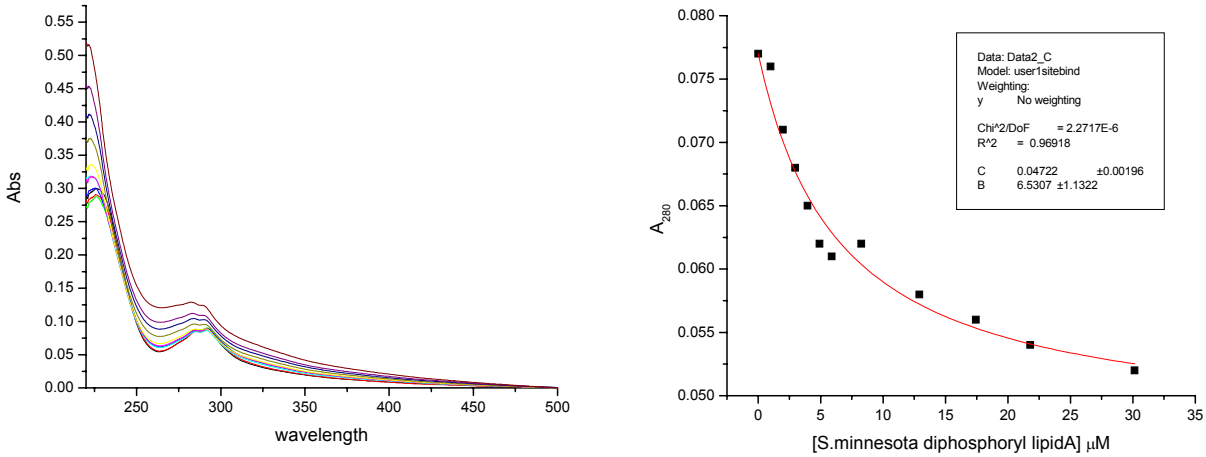
Left: UV-Vis control titration of 300 μM *S. minnesota* diphosphoryl lipid A into buffer (20 mM HEPES, pH 7.0); Right: Linear fit of the average of three control titrations of 300 μM *S. minnesota* diphosphoryl lipid A into buffer (20 mM HEPES, pH 7.0). Data fit the linear equation ($y = 0.00261x + 0.00034$) where $y = A_{280}$ and x = [lipid A] μM.

E. coli Diphosphoryl Lipid A into TW545 (2) UV-Vis vTitration



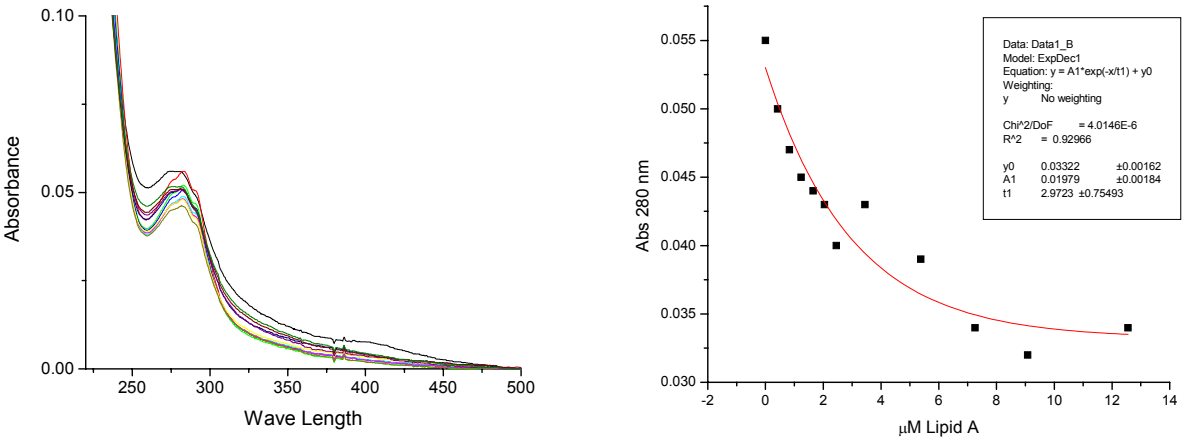
Left: UV-Vis titration of 6.0 μM TW545 (**2**) with 300 μM *E. coli* diphosphoryl lipid A in 20 mM HEPES at pH 7.0; Right: Extracted A_{280} values, corrected for lipid A absorbance and dilution of **2**. A K_d of 5.9 μM is derived from nonlinear least-squares fit (red line) to a one-site binding model.

S. minnesota Diphosphoryl Lipid A into TW545 (2) UV-Vis Titration



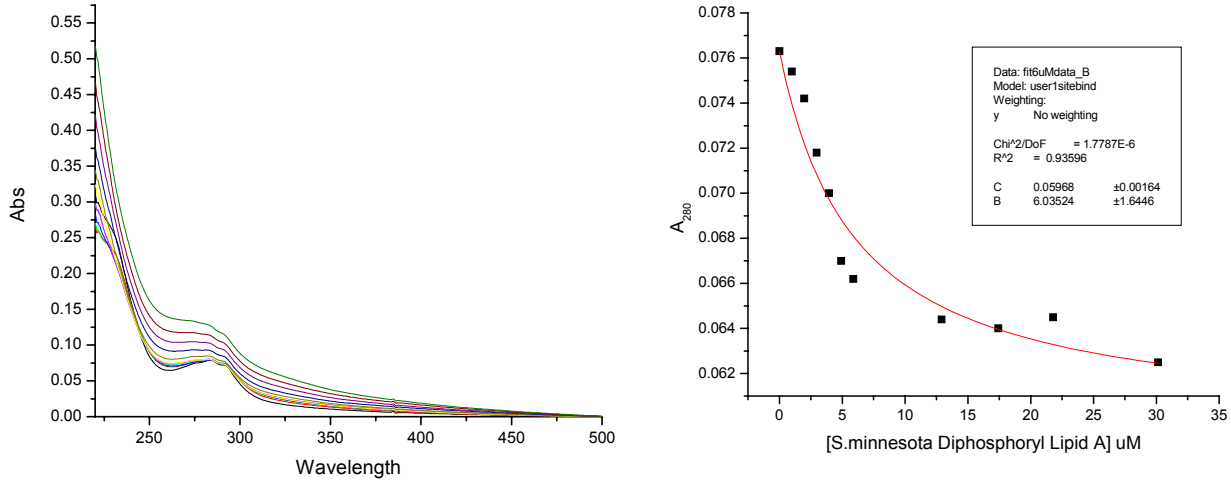
Left: UV-Vis titration of 6.0 μM TW545 (**2**) with 300 uM *S. minnesota* diphosphoryl lipid A in 20 mM HEPES at pH 7.0; Right: extracted A_{280} values, corrected for lipid A absorbance and dilution of **2**. A K_d of 6.5 μM is derived from nonlinear least-squares fit (red line) to a one-site binding model.

E. coli Diphosphoryl Lipid A into TWTCP (1) UV-Vis Titration



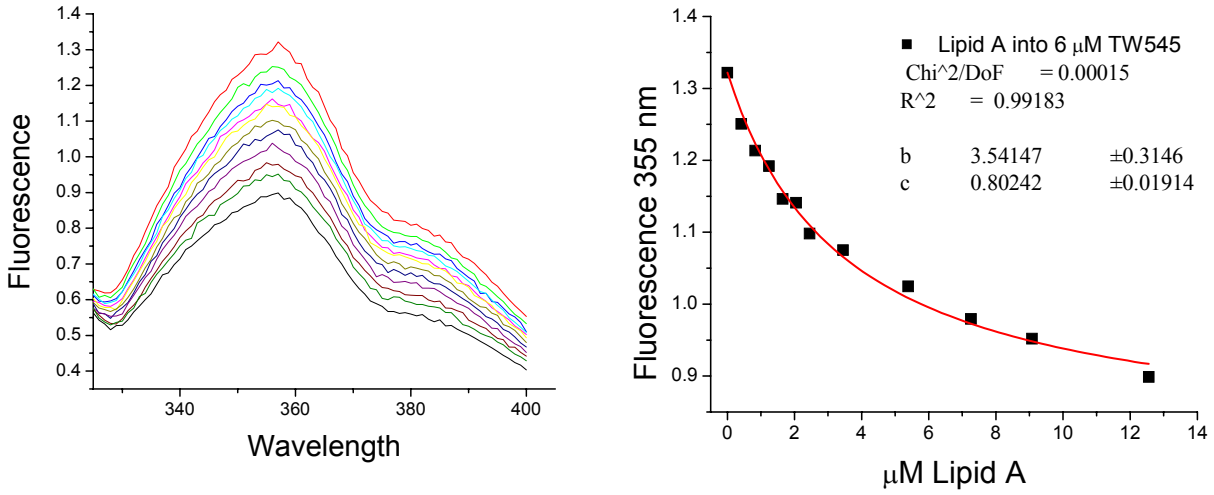
Left: UV-Vis titration of 6.0 μM TWTCP (**1**) with 300 μM *E. coli* diphosphoryl lipid A in 20 mM HEPES at pH 7.0; Right: extracted A_{280} values, corrected for lipid A absorbance and dilution of **1**. A K_d of 3.0 μM is derived from nonlinear least-squares fit (red line) to a one-site binding model.

S. minnesota Diphosphoryl Lipid A into TWTCP (1) UV-Vis Titration



Left: UV-Vis titration of 6.0 uM TWTCP (**1**) with 300 µM *S. minnesota* diphosphoryl lipid A in 20 mM HEPES at pH 7.0; Right: extracted A_{280} values, corrected for lipid A absorbance and dilution of **1**. A K_d of 6.0 µM is derived from nonlinear least-squares fit (red line) to a one-site binding model.

E. coli Diphosphoryl Lipid A into TW545 (2) Fluorescence Titration



Left: Fluorescence titration of 6.0 µM TWTCP (**1**) with 300 µM *E. coli* diphosphoryl lipid A in 20 mM HEPES at pH 7.0.; Right: Extracted E_{355} values reveal a K_d of 3.5 µM derived from nonlinear least-squares fit (red line) to a one-site binding model.

Crystallographic Data for 7.

Crystals were grown from the reaction mixture as described in the main text. A colorless fragment of approximate dimensions 0.10 x 0.14 x 0.19 mm^3 was cut from a cluster of plate-like block under Paratone-8277, mounted under the oil on a glass fiber, and immediately placed in a cold nitrogen stream at -80 °C on the X-ray diffractometer. The X-ray intensity data were collected on a standard Siemens SMART CCD Area Detector System equipped with a normal focus molybdenum-target X-ray tube operated at 2.0 kW (50 kV, 40 mA). A total of 1321 frames of data (1.3 hemispheres) were collected employing a narrow frame method with scan widths of 0.3° in ω and exposure times of 60 sec/frame using a detector-to-crystal distance of 5.09 cm (maximum 2θ angle of 46.6°). Frames were integrated to a maximum 2θ angle of 46.6° with the Siemens SAINT program to yield a total of 9334 reflections, of which 5919 were independent (R_{int} = 2.91 %, R_{sig} = 3.26 %)[1] and 2157 were above 2σ(I). Laue symmetry revealed a orthorhombic crystal system, and the final unit cell parameters (at -80 °C) were determined from the least-squares refinement of three dimensional centroids of 4858 reflections.[2] Data were corrected for absorption using the program SADABS.[3]

The space group was assigned as *Pca2₁* and the structure was solved by using direct methods and refined employing full-matrix least-squares on F^2 (Siemens, SHELXTL[4], version 5.04). For a Z value of 8, there are two independent molecules in the asymmetric unit. All atoms were refined anisotropically, with the hydrogens included in idealized positions giving a data : parameter ratio of approximately 9 : 1. The structure refined to a goodness of fit (GOF)[5] of .981 and final residuals[6] of R_1 = 5.39 % (I > 2σ(I)), wR_2 = 14.74 % (I > 2σ(I)).

Note: C92 & C102 are disordered with SOFs of disordered atom pairs @ 50% & 60% respectively

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[1] $R_{int} = \sum\left|F_o^2 - F_o^2(\text{mean})\right| / \sum\left[F_o^2\right]$; $R_{sigma} = \sum\left[\sigma\left(F_o^2\right)\right] / \sum\left[F_o^2\right]$

[2] It has been noted that the integration program SAINT produces cell constant errors that are unreasonably small, since systematic error is not included. More reasonable errors might be estimated at 10x the listed value.

[3] The SADABS program is based on the method of Blessing; see Blessing, R.H. "An Empirical Correction for Absorption Anistropy." *Acta Crystallogr., Sect A* , **1995**, *51*, 33-38.

[4] *SHELXTL: Structure Analysis Program, version 5.04*; Siemens Industrial Automation Inc.: Madison, WI, 1995.

[5] $GOF = \left[\sum\left[w\left(F_o^2 - F_c^2\right)^2\right] / \left(n - p\right)\right]^{1/2}$, where n and p denote the number of data and parameters.

[6] $R_1 = \left(\sum\left\|F_o\right| - \left|F_c\right\|\right) / \sum\left|F_o\right|$; $wR_2 = \left[\sum\left[w\left(F_o^2 - F_c^2\right)^2\right] / \sum\left[w\left(F_o^2\right)^2\right]\right]^{1/2}$

where $w = 1 / \left[\sigma^2\left(F_o^2\right) + \left(a \cdot P\right)^2 + b \cdot P\right]$ and $P = \left[\left(\text{Max};0, F_o^2\right) + 2 \cdot F_c^2\right] / 3$.

Table 1. Crystal data and structure refinement for **7**.
Note: 120s/Frame data; 2 molecules in asymmetric unit

Identification code	sad1/milkb01
Empirical formula	C14 H17 N O3
Formula weight	247.29
Temperature	293(2) K
Wavelength	0.71073 A
Crystal system	Orthorhombic
Space group	Pca2(1)
Unit cell dimensions	a = 11.7699(8) A alpha = 90 deg. b = 9.9771(7) A beta = 90 deg. c = 21.9102(15) A gamma = 90 deg.
Volume, Z	2572.9(3) A^3, 8
Density (calculated)	1.277 Mg/m^3
Absorption coefficient	0.090 mm^-1
F(000)	1056
Crystal size	0.10 x 0.14 x 0.19 mm
Theta range for data collection	1.86 to 23.30 deg.
Limiting indices	-12<=h<=13, -11<=k<=11, -24<=l<=13
Reflections collected	10446
Independent reflections	2830 [R(int) = 0.0291]
Reflections > 2Sig(I)	2157
Absorption correction	SADABS
Max. and min. transmission	0.928 and 0.847
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2830 / 1 / 326
Goodness-of-fit on F^2	0.981
Final R indices [I>2sigma(I)]	R1 = 0.0539, wR2 = 0.1474
R indices (all data)	R1 = 0.0712, wR2 = 0.1582
Absolute structure parameter	0(3)
Largest diff. peak and hole	0.394 and -0.233 e.A^-3