Supporting Information for

# Exploring the Strength of a Hydrogen Bond as a Function of Steric Environment using 1,2-Azaborine Ligands and Engineered T4 Lysozyme Receptors

Julia Liu and Shih-Yuan Liu

*Department of Chemistry, Boston College, Chestnut Hill, Massachusetts, 02467, USA*

## Index

**Protein Expression and Purification**

*Escherichia coli strain* RR1 (ATCC® 31343™) was transformed with the sub-cloned plasmids (Addgene plasmids # 18476 and 18477 from Brian Matthews lab, for T4 lysozyme WT* (**L99A**) and T4L mutant (S38D **L99A M102Q** N144D), respectively. Ampicillin-resistant transformants were isolated and stored in 20% glycerol stock solution in (lysogeny broth) LB media. The E.coli RR1 were grown in 4.0 liters of LB media with 100mg/liter ampicillin at 37 °C within an agitating fermentor at 250 rpm. When the optical density of broth reached 0.7 at 600 nm, isopropyl-β-D-1-thiogalatopyranoside (IPTG) was added to a final concentration of 0.7mM to induce protein expression. Cell culture was incubated at 25 °C at 110 rpm shaking speed for 21hrs. Then the resulting pellet was suspended in 300 mL of a buffer solution containing 0.1 M sodium phosphate (pH= 6.6) and 0.2 M NaCl. 30 mL of 0.5 M EDTA (pH 8.0) solution was added, and the suspension was stirred for 17 h at 4 °C. To the suspension was then added 30 mL of 0.5 M EDTA (pH 8.0), and the mixture was stirred at room temperature for 8 h at 4 °C. The supernatant was collected and dialyzed into 20 mM phosphate buffer (pH 7.3) and then loaded onto a CM Sepharose Fast Flow (GE healthcare) column that was pre-equilibrated with equilibration buffer of 50 mM Tris, 1.0 mM EDTA, pH 7.3 and eluted with a linear gradient of 0 mM to 300 mM NaCl within equilibration buffer. The fractions containing pure protein were pooled and dialyzed against the degassed ITC buffer (5% to 10% PEG400, 0.5M NaCl, 0.1M sodium phosphate (pH 6.8). Specifically, 5% PEG (for NHBMe / toluene pair) and 10% PEG (for NHBnPr / n-Propylbenzene pair, and NHBiPr / i-Propylbenzene pair) was added into the degassed ITC buffer to make the ligand more soluble.

**ITC Experiments**

Isothermal titration calorimetry experiments (in a Nano ITC calorimeter from TA instruments) were carried out at 10 °C with 310 rpm stirring rate with a data collection interval of 4 min/injection. A total of volume of 100 µl (2.5 µl x 40 injections) ligand solution (2.66 - 3.74 mM) were injected into 320 µl of 0.3 mM protein solution. The ligand was directly dissolved in the degassed ITC buffer from protein dialysis and ligand concentration was determined by UV-Vis against a standard calibration curve. The heat of ligand dilution was measured by titrating the ligand into the ITC buffer for each titration pairs, and the heat of dilution has been subtracted from each titration.[1] The resulting reaction heat profiles were fit to the one binding site independent binding model with the stoichiometry n fixed to 1.0. All data were analyzed using NanoAnalyze software. Experiments (except i-Propylbenzene and NHBiPr pair only repeated for 2 times) were repeated five times and averaged with standard deviation error of the mean. Representative ITC traces are shown below.

**Notes for displacement titration of *i*-Propylbenzene and NHBiPr.**

The competition-based method[2,3] was utilized here for determining $K_a$ of the low-affinity ligand (*i*-Propylbenzene and NHBiPr) binding interactions with L99A and L99A/M102Q. The high-affinity ligand in syringe was titrated into the protein in the cell in the presence of a large excess amount of the low-affinity ligand. The heat of dilution was accounted for by background subtraction of the heat generated by injecting the high affinity ligand into ITC buffer in the presence of the low-affinity ligand in the cell in the absence of the protein.

Phenol was used for the displacement of NHBiPr and *i*-Propylbenzene from the L99A/M102Q cavity. The binding affinity constant for phenol binding to L99A/M102Q was determined to be $5.03 \times 10^4$ $M^{-1}$ and the concentration of the ligands (*i*-Propylbenzene and NHBiPr) was 9.4 mM.

Toluene was used for displacement of *n*-Propylbenzne and *i*-Propylbenzne from L99A cavity, and the binding affinity constant for toluene binding to L99A was determined to be $3.57 \times 10^4$ $M^{-1}$. The ligand concentration of the low affinity ligand (*i*-Propylbenzene and NHBiPr) in the cell was 10 mM.

The data was analyzed using the standard one binding site model supplied by Sigurkjold's displacement model.[3,4] Binding constant Kb for our low affinity ligand was determined based on the apparent binding constant Kapp (i.e., measured binding constant of the higher-affinity ligand to the protein in the presence of the lower-affinity ligand) and Ka (i.e., inherent binding constant of the higher-affinity ligand to the protein that was determined from independent titration experiments). [B] is the concentration of the lower-affinity ligand in the titration cell.

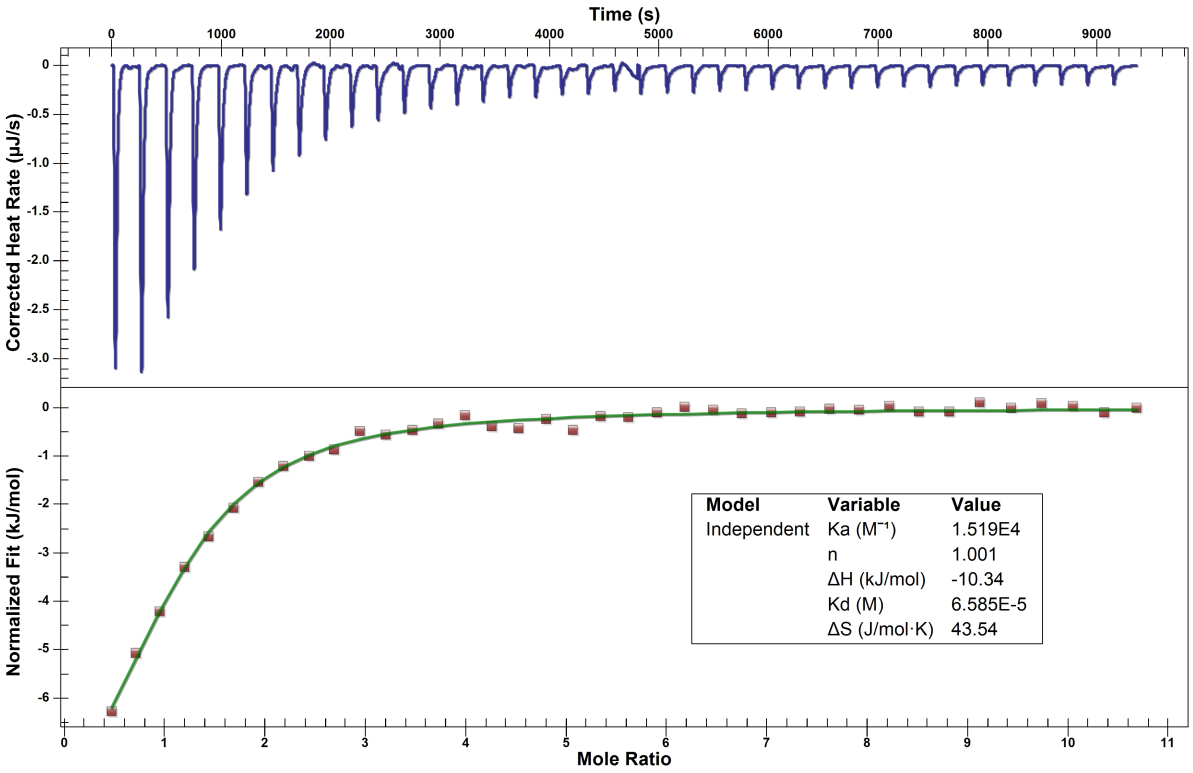
$$\mathrm{Kapp} = \frac{\mathrm{Ka}}{(1 + \mathrm{Kb} * [\mathrm{B}])}$$

Table S1. Affinity Constant ($K_a$) and Binding Free Energy ($\Delta G$) Determined by ITC.

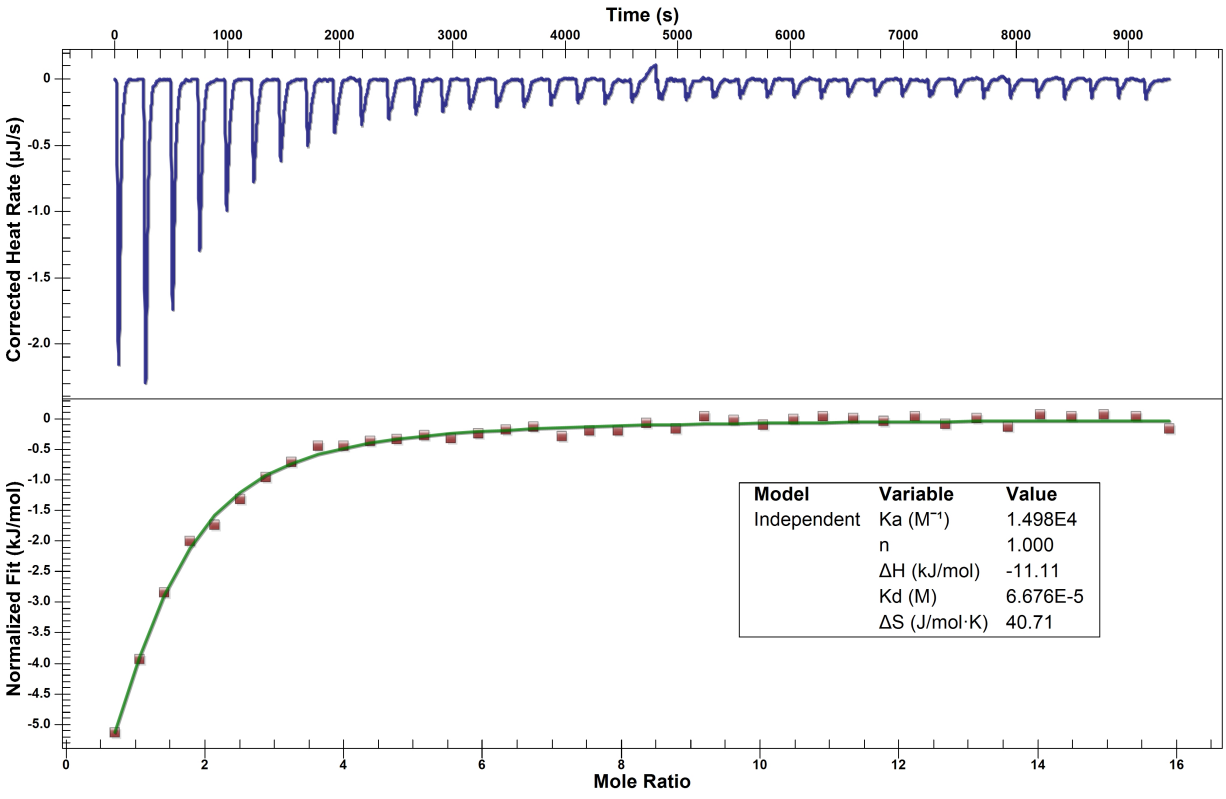
| | L99A | | L99A/M102Q | |
|---|---|---|---|---|
| | $K_a$ (x $10^4$ $M^{-1}$) | $\Delta G$(kcal/mol) | $K_a$ (x $10^4$ $M^{-1}$) | $\Delta G$(kcal/mol) |
| Toluene | 1.59±0.15 | -5.44±0.05 | 1.25±0.05 | -5.30±0.02 |
| NHBMe | 0.93±0.09 | -5.13±0.02 | 4.3±0.5 | -5.98±0.07 |
| n-Propylbenzene | 2.4±0.2 | -5.66±0.06 | 1.07±0.09 | -5.21±0.02 |
| NHBnPr | 3.3±0.2 | -5.85±0.05 | 4.3±0.1 | -6.00±0.02 |
| i-Propylbenzene | 57 $M^{-1}$ | -2.27 | 87$M^{-1}$ | -2.51 |
| NHBiPr | 34 $M^{-1}$ | -1.96 | 69$M^{-1}$ | -2.38 |

## Representative ITC Traces

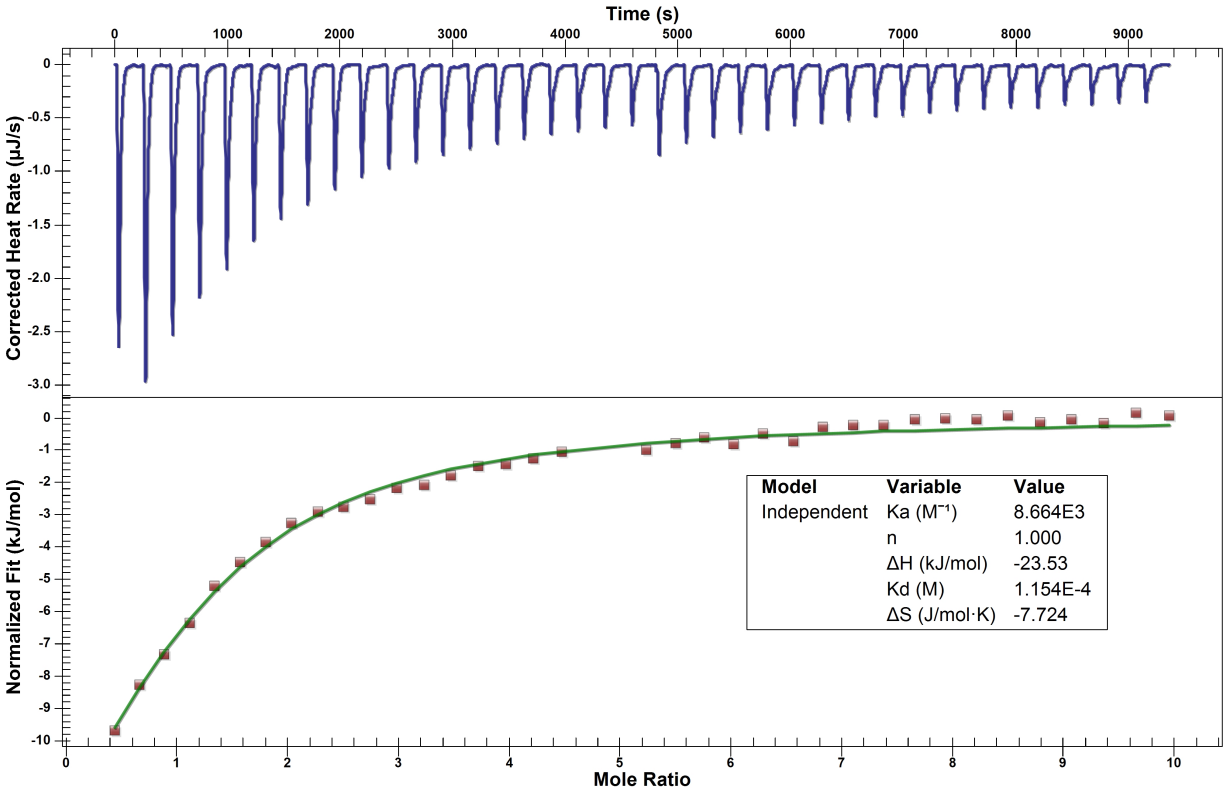
Toluene to L99A



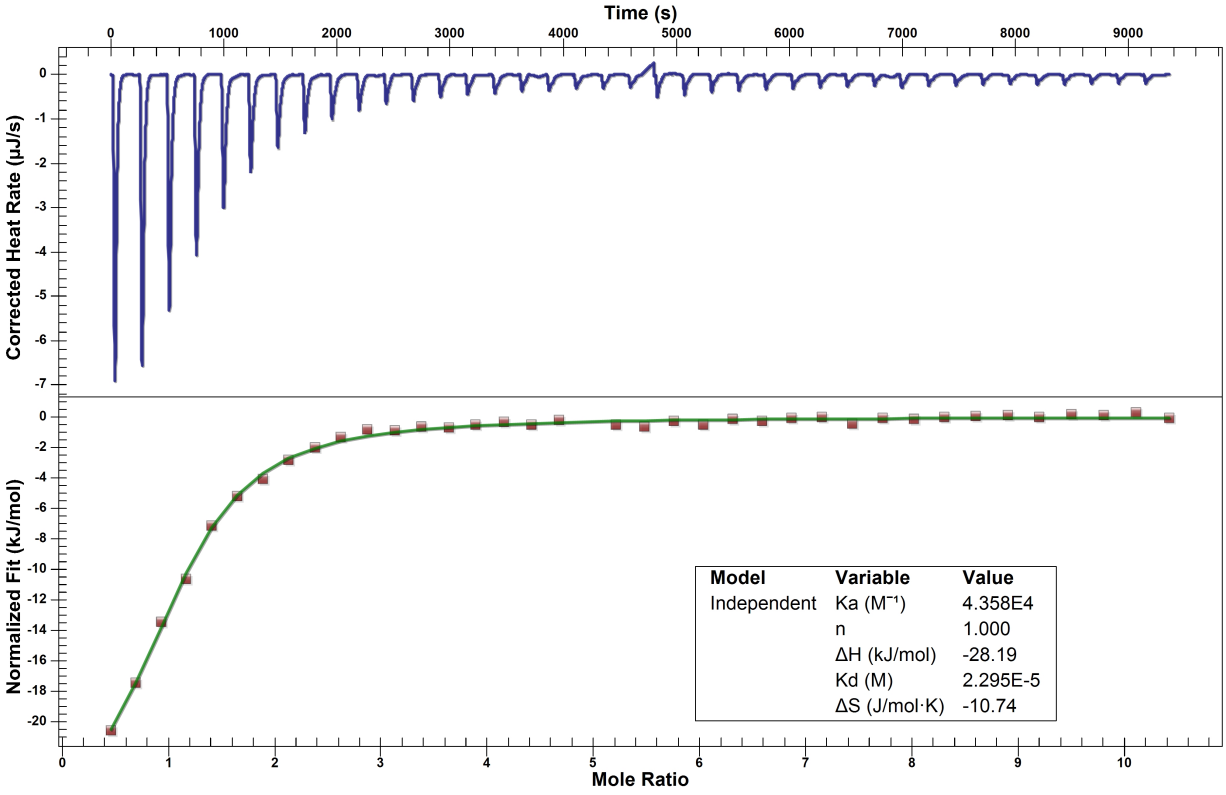

Toluene to M102Q



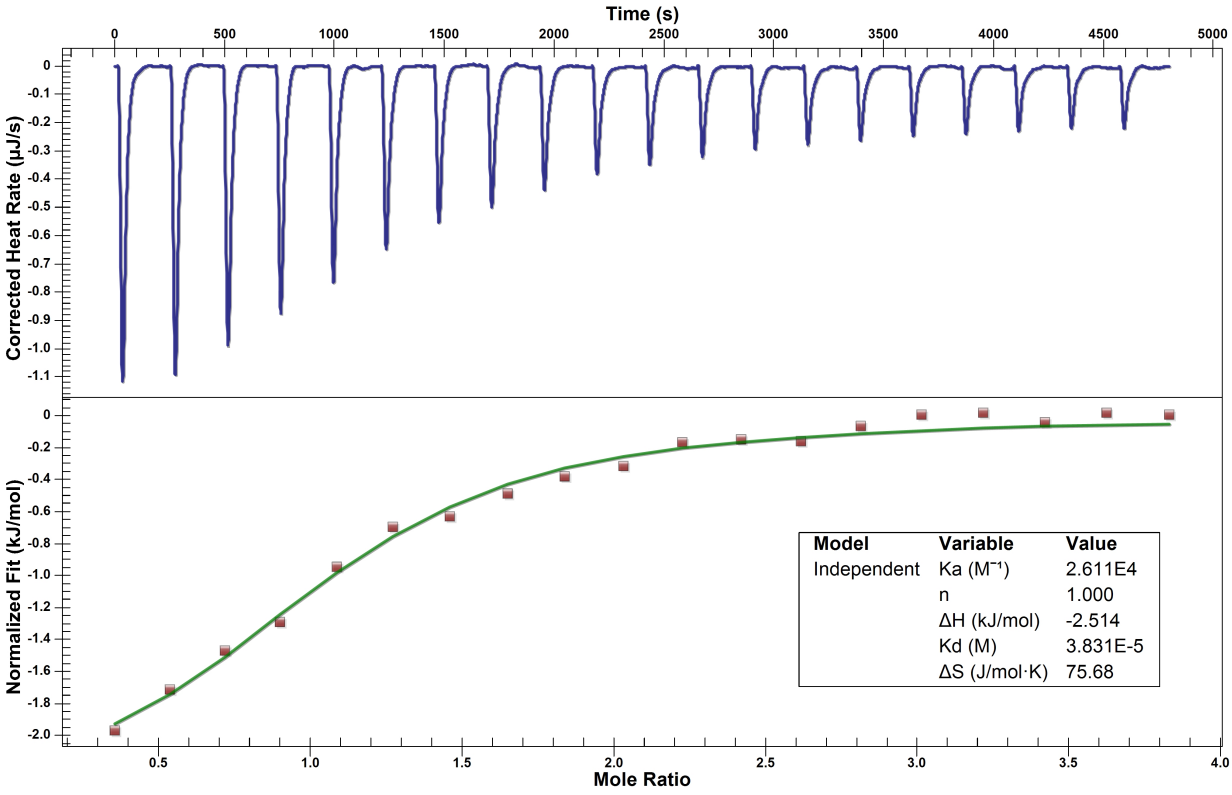
NHBMe to L99A



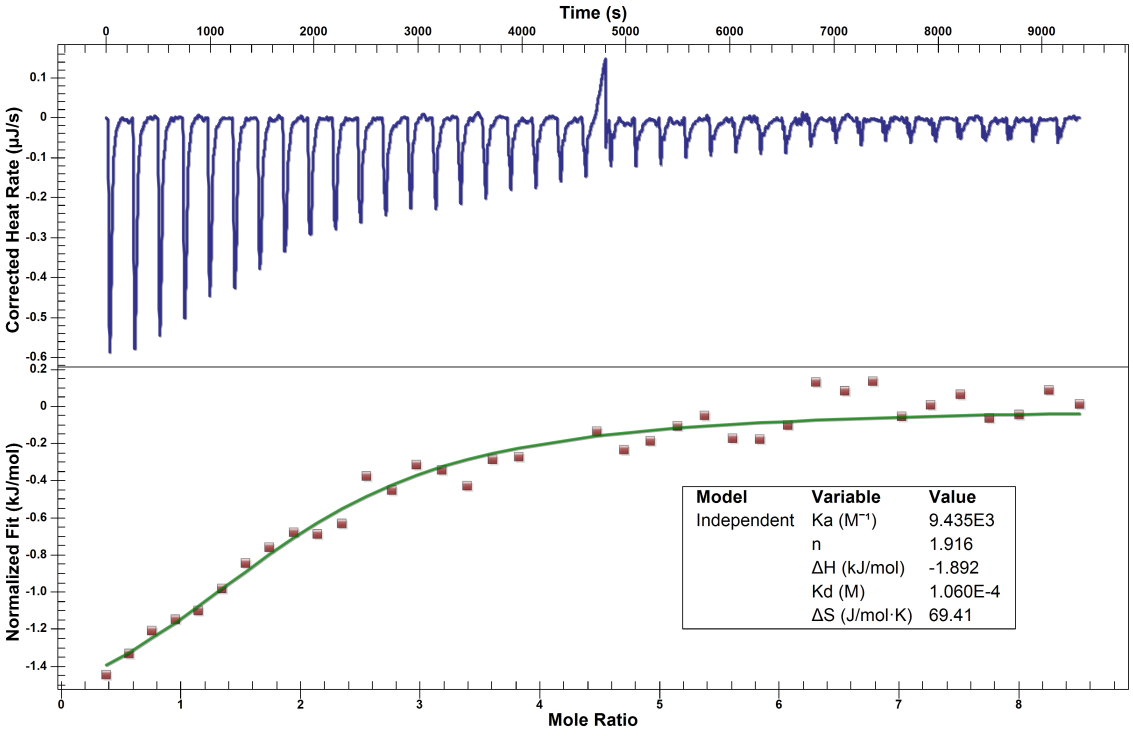

NHBMe to M102Q



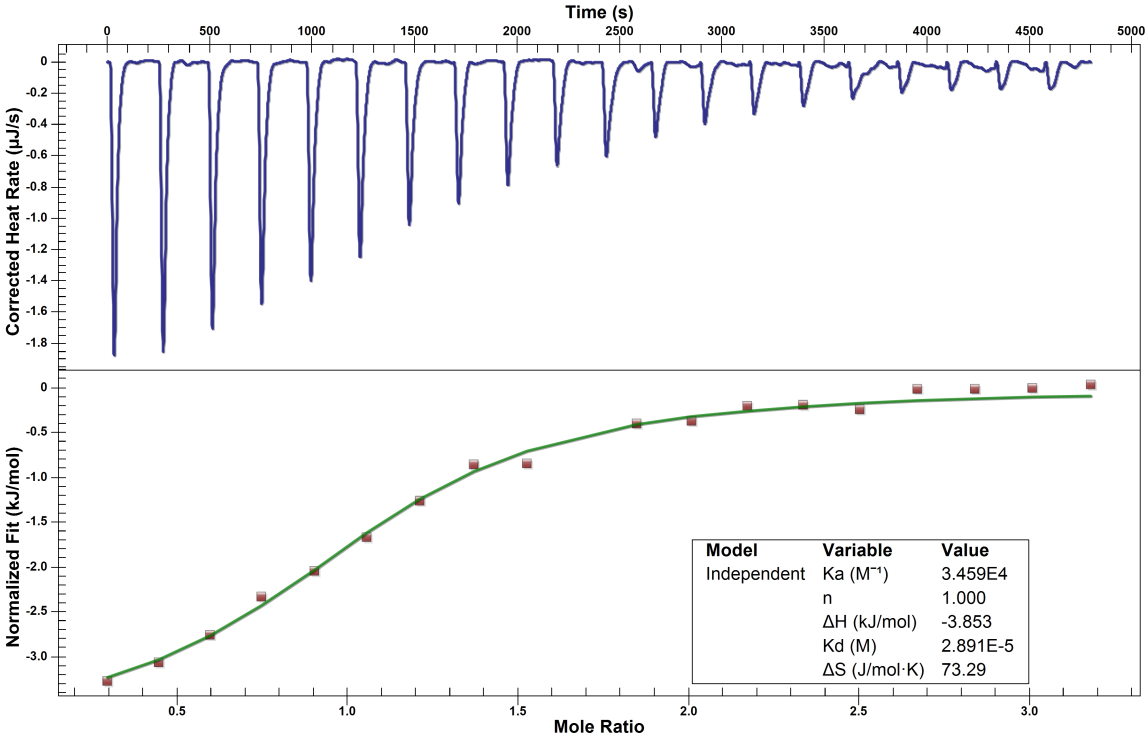
n-Propylbenzene to L99A



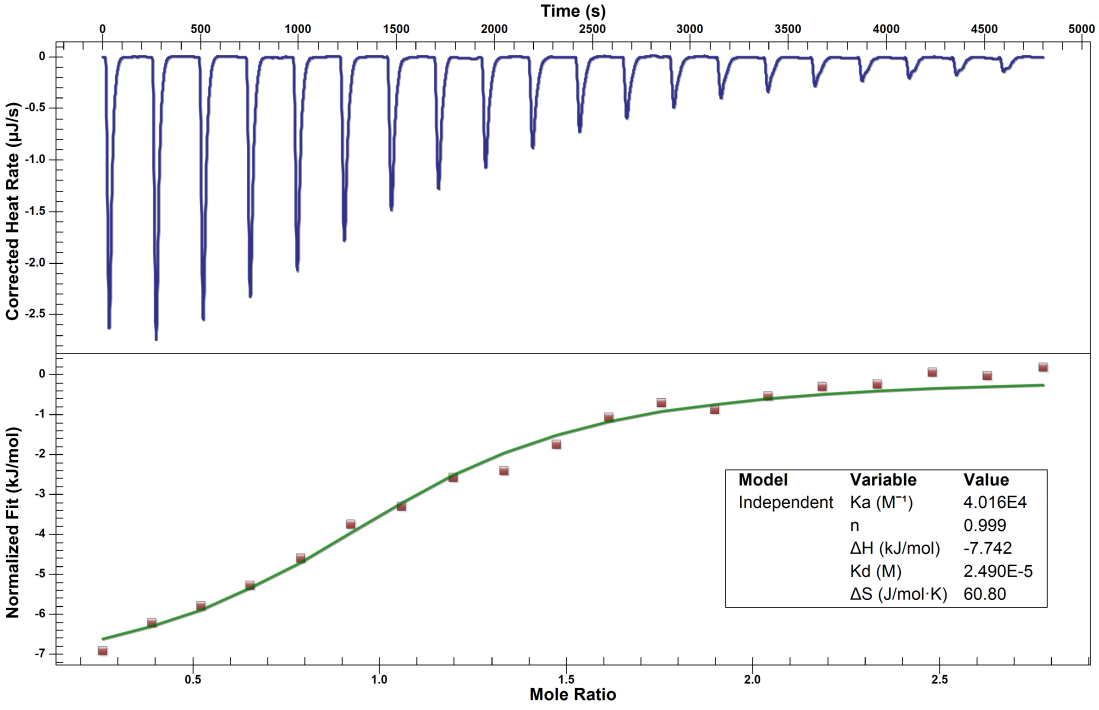

n-Propylbenzene to M102Q



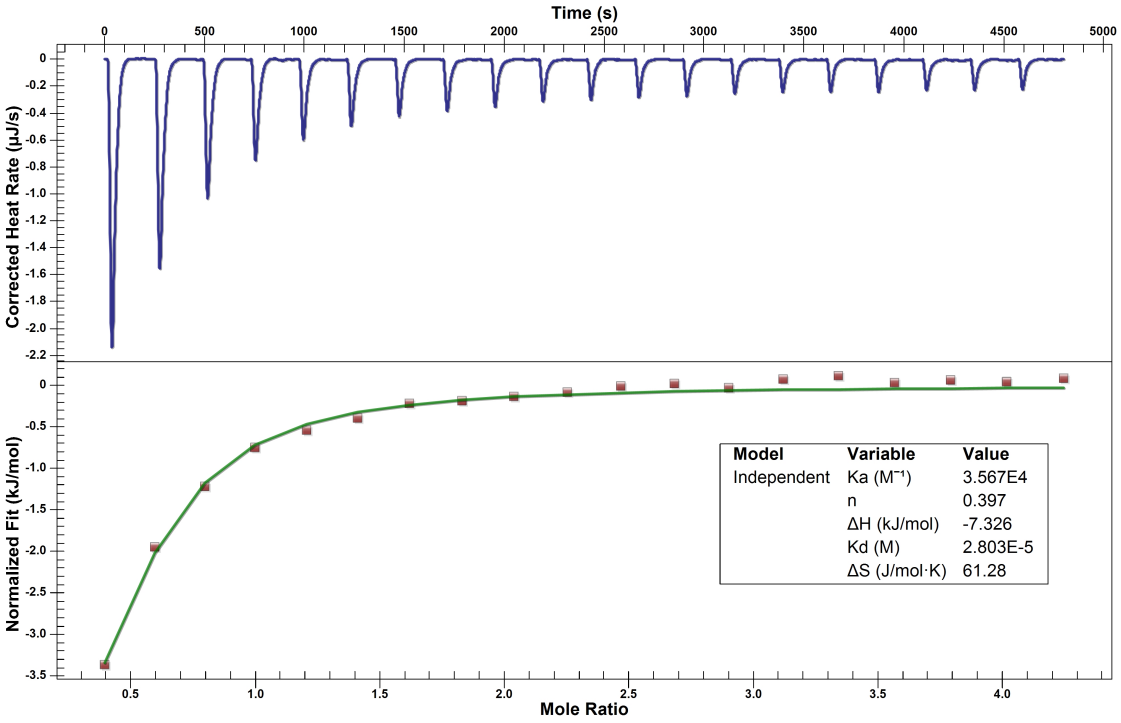
NHBnPr to L99A



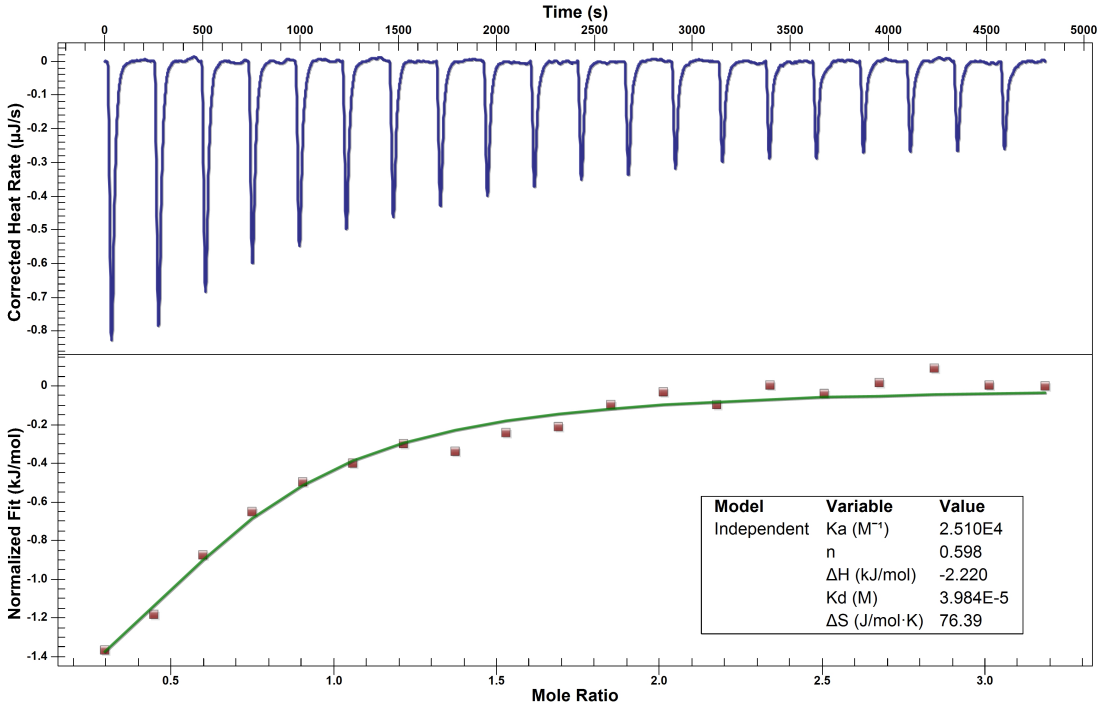

NHBnPr to M102Q



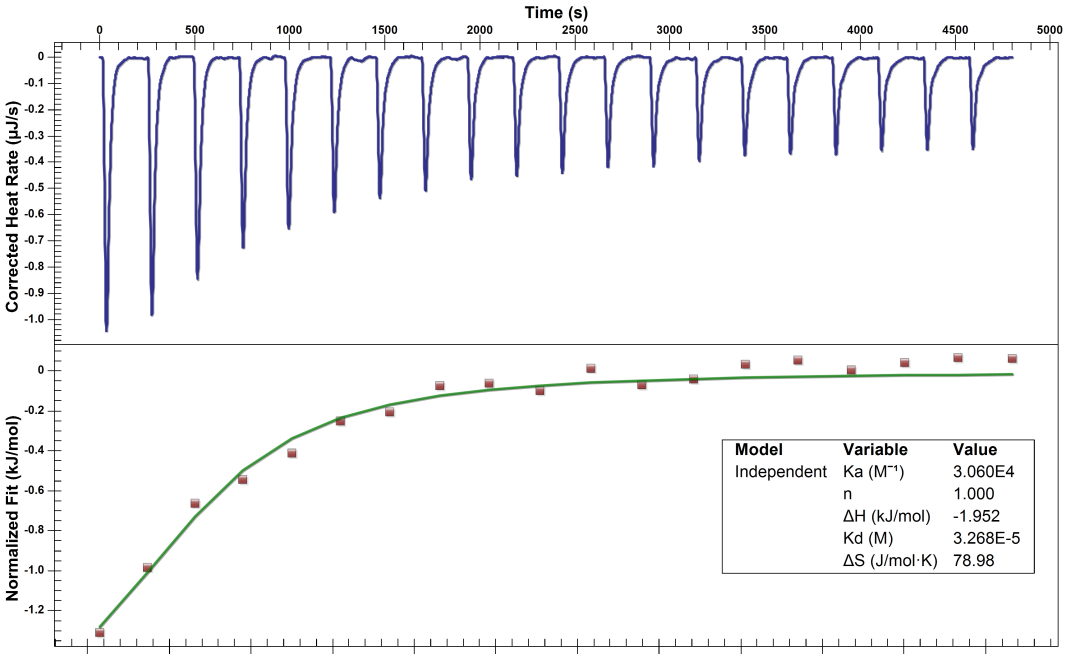
Toluene to L99A for displacement reference



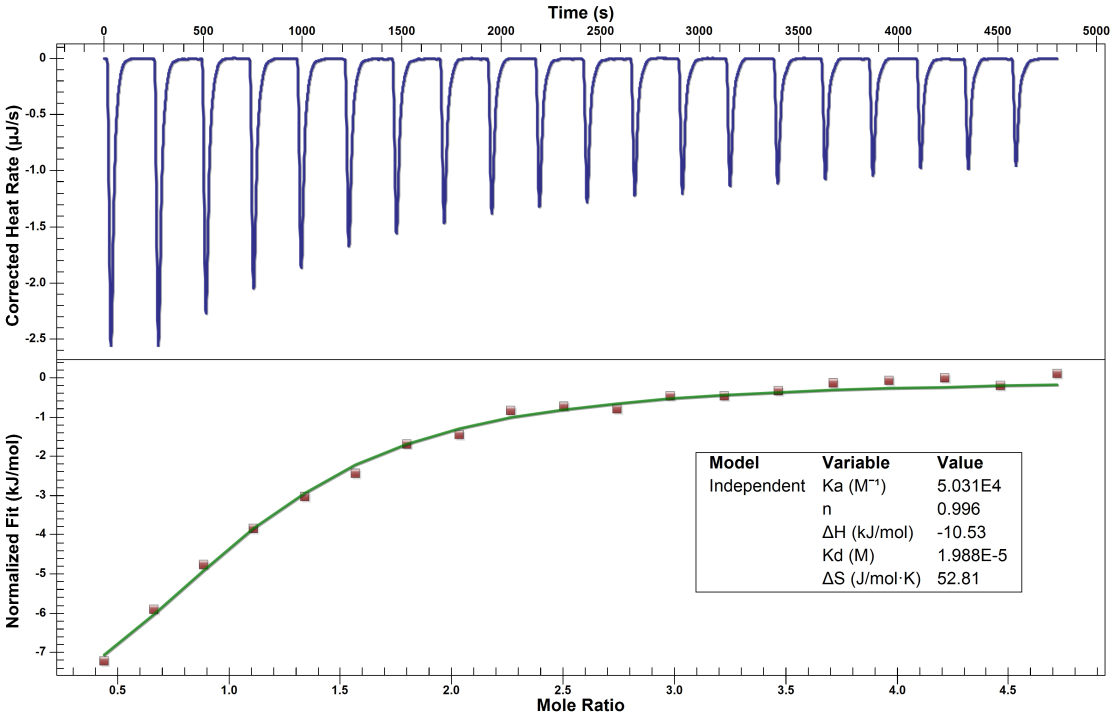

Toluene to L99A with i-Propylbenzene in cell



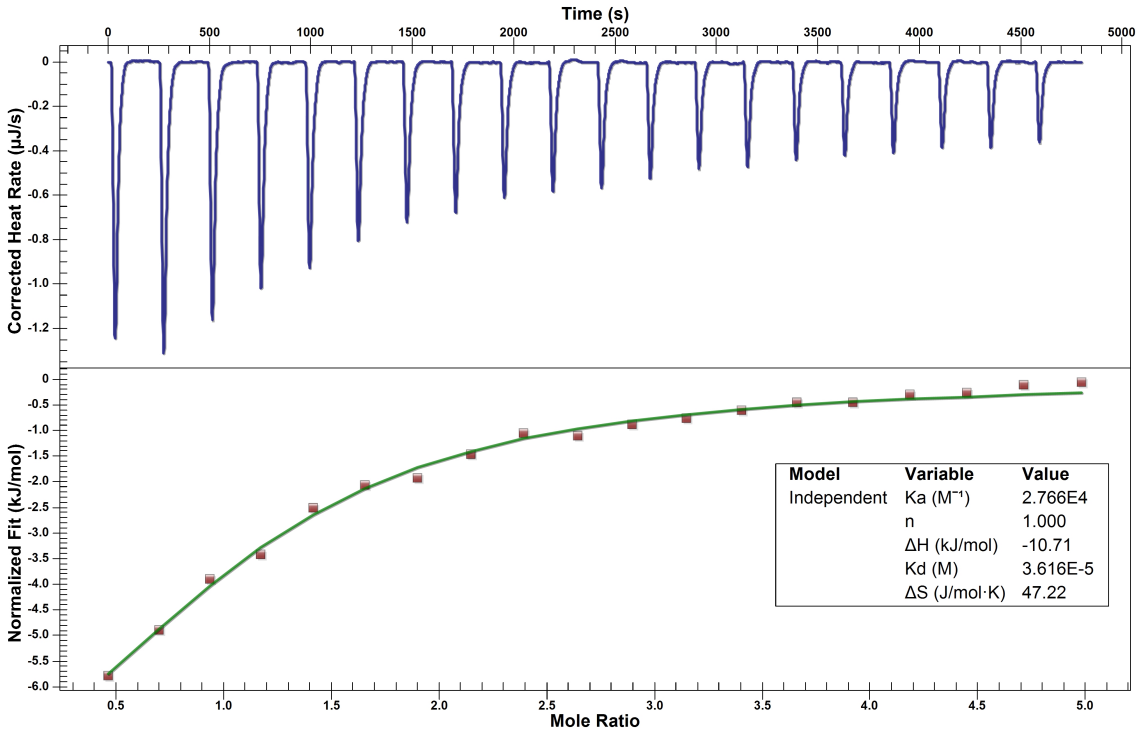
Toluene to L99A with NHBiPr in cell



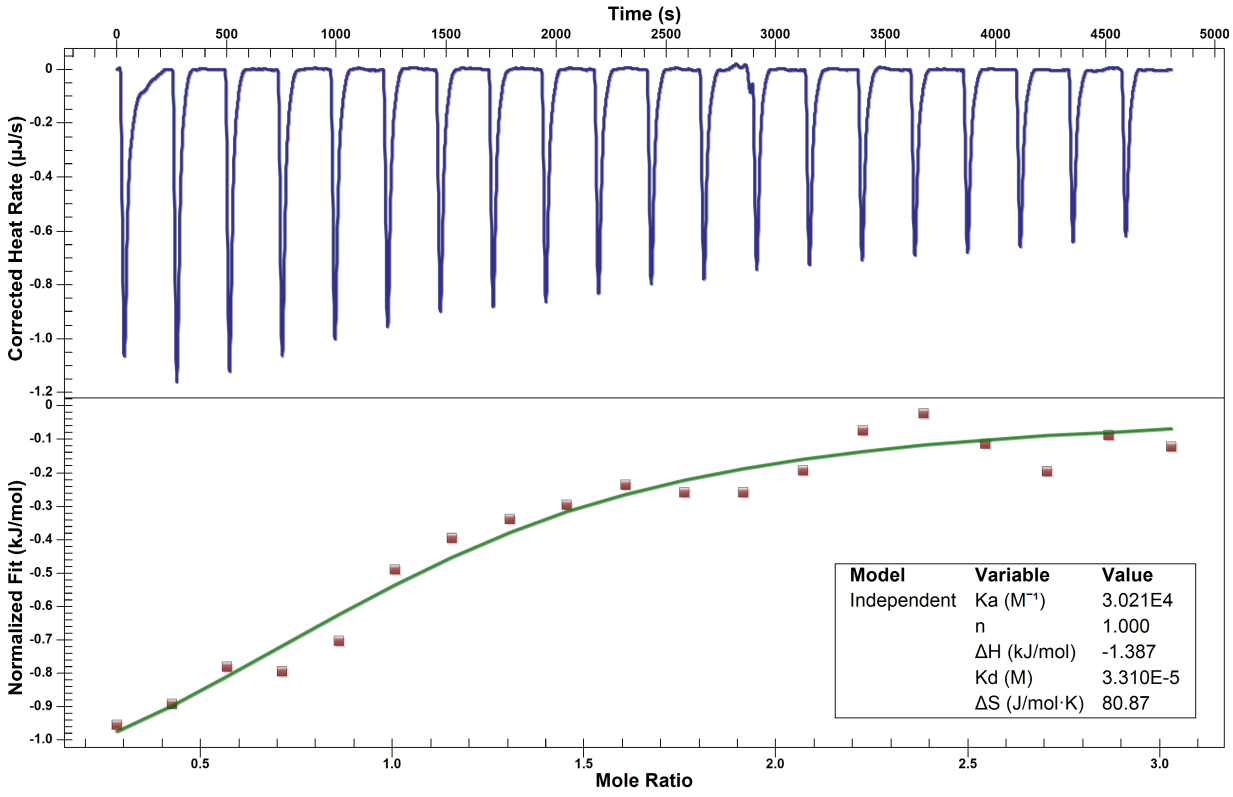

Phenol to M102Q for displacement reference



Phenol to M102Q with i-Propylbenzene in cell



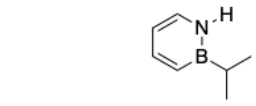

Phenol to M102Q with NHBiPr in cell



YL-02-NHBisopropyl_pumped

Sample Name YL-02-NHBisopropyl_pumped Pulse sequence PROTON Temperature 25 Study owner Liu
Date collected 2018-01-05 Solvent cd2cl2 Spectrometer nmr18-vnmrs500 Operator Liu

4



2-isopropyl-1,2-dihydro-1,2-azaborinine

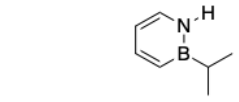



Data file /home/Liu/vnmrsys/data/Julia/YL-02-NHBisopropyl_pumpe.fid

Plot date 2019-05-01

YL-01-NHBisopropyl-13C

Sample Name YL-01-NHBisopropyl-13C Pulse sequence CARBON Temperature 25 Study owner Liu
Date collected 2018-09-11 Solvent cd2cl2 Spectrometer nmr18-vnmrs500 Operator Liu



2-isopropyl-1,2-dihydro-1,2-azaborinine

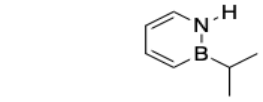



Data file exc

Plot date 2018-09-11

YI-01-005-step2-11B

Sample Name YI-01-005-step2-11B | Pulse sequence s2pul | Temperature 25 | Study owner Liu
Date collected 2017-01-11 | Solvent cd2cl2 | Spectrometer nmr11-inova500 | Operator Liu



2-isopropyl-1,2-dihydro-1,2-azaborinine

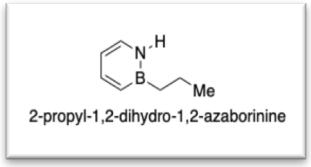



Data file exp

Plot date 2017-01-11

YL-01-003-step2-1H

Sample Name YL-01-003-step2-1H | Pulse sequence PROTON | Temperature 25 | Study owner Liu
Date collected 2017-01-27 | Solvent cd2cl2 | Spectrometer nmr11-inova500 | Operator Liu

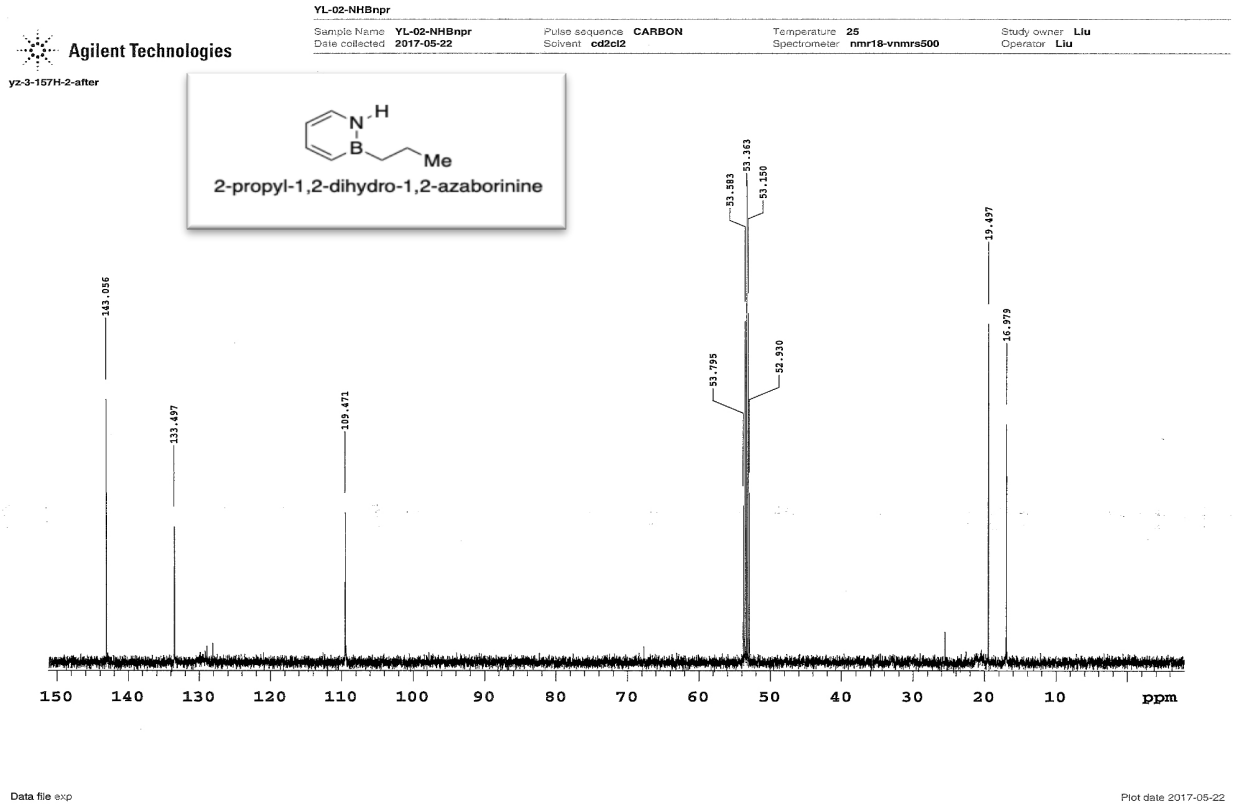


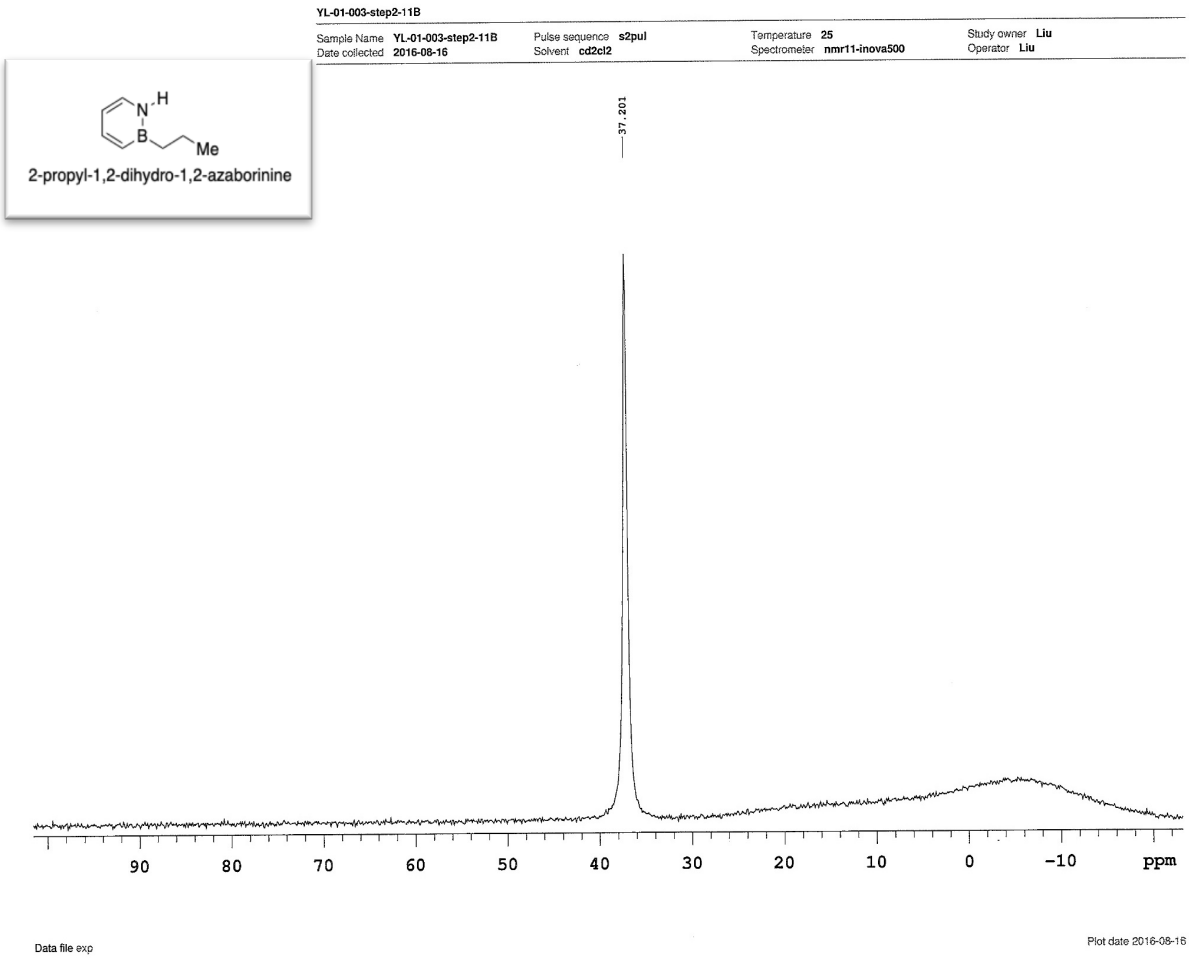
2-propyl-1,2-dihydro-1,2-azaborinine




Data file exp

Plot date 2017-01-27


YL-02-NHBnpr
Agilent Technologies
Sample Name YL-02-NHBnpr
Date collected 2017-05-22
Pulse sequence CARBON
Solvent cd2cl2
Temperature 25
Spectrometer nmr18-vnmrs500
Study owner Liu
Operator Liu
yz-3-157H-2-after
2-propyl-1,2-dihydro-1,2-azaborinine
143.056
133.497
109.471
53.583
53.363
53.150
53.795
52.930
19.497
16.979
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
Data file exp
Plot date 2017-05-22


YL-01-003-step2-11B
Sample Name YL-01-003-step2-11B
Date collected 2016-08-16
Pulse sequence s2pul
Solvent cd2cl2
Temperature 25
Spectrometer nmr11-inova500
Study owner Liu
Operator Liu
2-propyl-1,2-dihydro-1,2-azaborinine
37.201
90 80 70 60 50 40 30 20 10 0 −10 ppm
Data file exp
Plot date 2016-08-16

***References***

1. M. R. Duff, J. Vis. Exp, 2011, **55**, 2796.

2. Y L. Zhang and Z. Y. Zhang, *Anal. Biochem.*,1998, **261**, 139-148.

3. B. W. Sigurskjold, *Anal. Biochem.*, 2000, **277**, 260-266.