

Supporting Information

Protonation activates anion binding and alters binding selectivity in new inherently fluorescent 2,6-bis(2-anilinoethynyl)pyridine bisureas

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Table of Contents

General Details	
Synthetic DetailsS2
NMR Titration ConditionsS8
Binding Constant Fits	
UV Titration ConditionsS15
Binding Constant Fits	
DFT DetailsS21

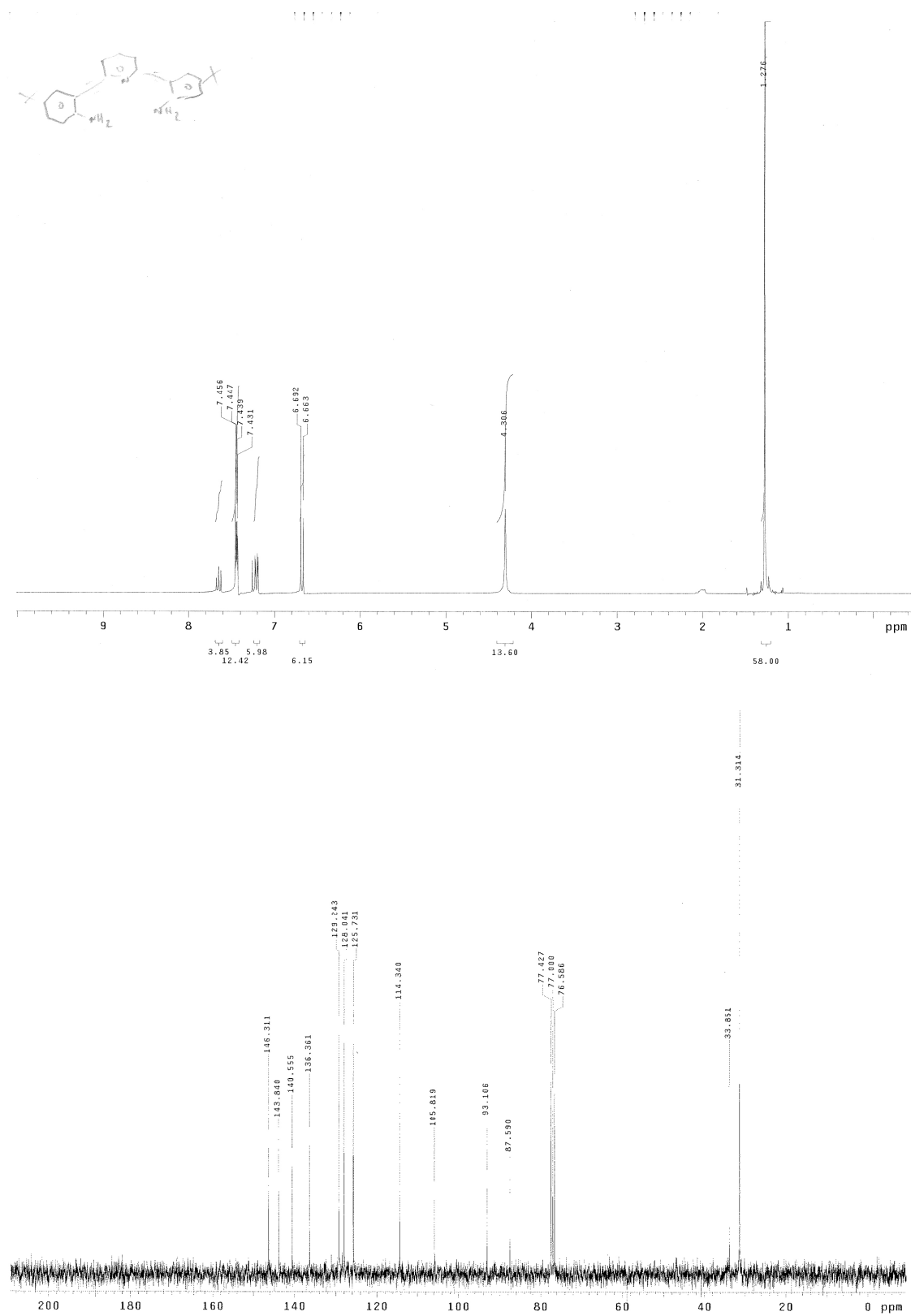
General Details

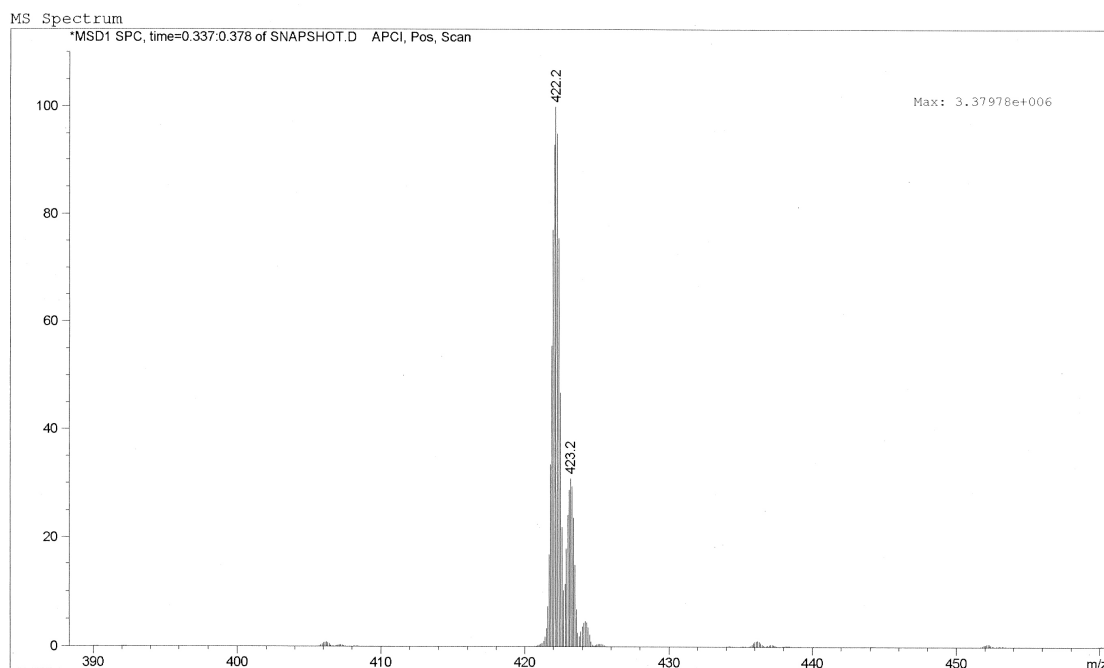
^1H and ^{13}C NMR spectra were obtained on a Varian 300 MHz spectrometer (^1H 299.95 Hz, ^{13}C 75.43 Hz) or Inova 500 MHz spectrometer (^1H 500.10 MHz, ^{13}C 125.75 MHz). Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS) using non-deuterated solvent present in the bulk deuterated solvent (CDCl_3 : ^1H 7.26 ppm, ^{13}C 77.0 ppm; THF-d_8 : ^1H 3.58 ppm, ^{13}C 67.57 ppm). Unless otherwise specified, solvents were obtained from distillation using published literature procedures directly before use. X-ray crystal data were obtained on a Bruker AXIS CCD diffractometer.

Synthesis

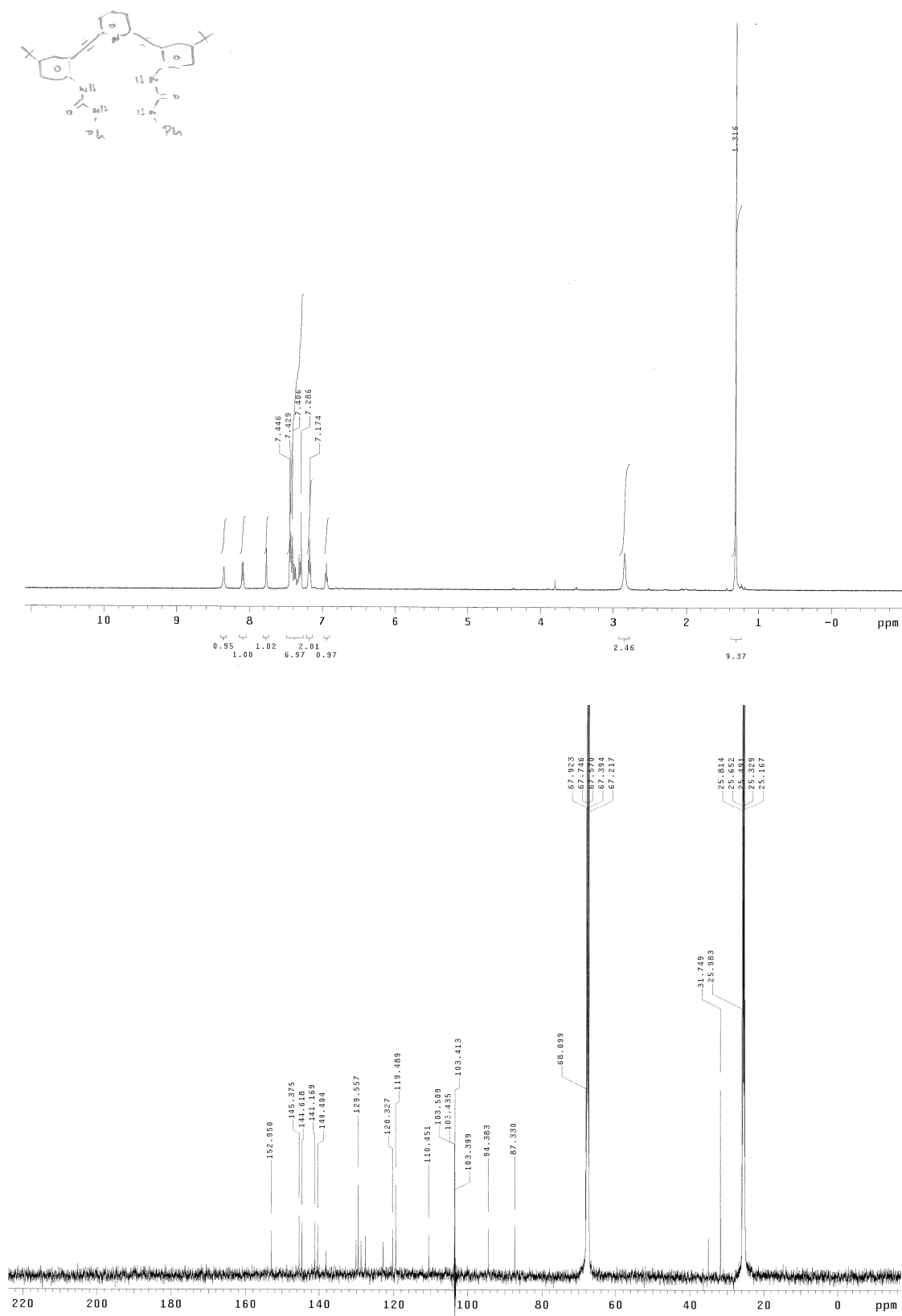
General Procedure for Sonogashira Cross-Coupling. To an Ar purged solution of haloarene in 1:1 freshly distilled THF/*i*-Pr₂NH (50 mM w.r.t. haloarene) was added CuI (0.05 equiv) and Pd(PPh₃)₄ (0.03 equiv). To this solution was added alkyne (1.05 equiv) in minimal THF dropwise with stirring over 4-8 h. Upon complete addition the reaction was stirred an additional 3-4 h and then concentrated *in vacuo*. The residue was taken up in Et₂O and filtered through a 2.5 cm Celite pad. The remaining salts were washed thrice with Et₂O and the organics combined, concentrated and purified by silica gel column chromatography.

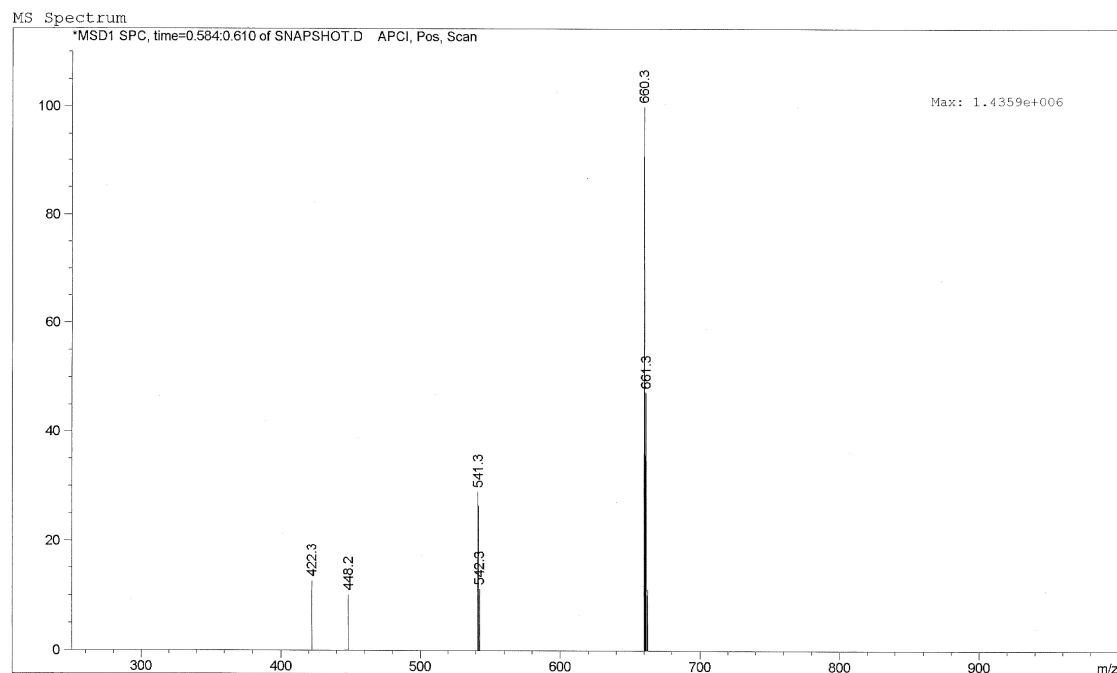
Dianiline 1. Iodoaniline **3** was reacted with TMSA according to the General Procedure for Sonogashira Cross-Coupling. After purification by column chromatography, a suspension of TMS-protected ethynylarene (227 mg, 0.92 mmol) and K₂CO₃ (6 equiv) in MeOH (30 mL) and Et₂O (15 mL) was stirred at rt and monitored by TLC until completion (15-20 min). The solution was diluted with Et₂O and washed thrice with water and brine. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Without further purification, the residue was taken up in THF (15 mL) and reacted with 2,6-dibromopyridine (96 mg, 0.40 mmol), Pd(PPh₃)₄ (4.7 mg, 0.04 mmol) and CuI (1.5 mg, 0.08 mmol) according to the General Procedure. The product was purified by column chromatography (3:2 hexanes/CH₂Cl₂) to afford **1** (135 mg, 81%) as a pale yellow crystalline solid. Mp: 226 °C. ^1H NMR (300 MHz, CDCl_3): δ 7.64 (t, J = 8.1 Hz, 1H), 7.45-7.43 (m, 4H), 7.20 (dd, J = 8.7, 1.8 Hz, 2H), 6.67 (d, J = 8.7 Hz, 2H), 4.35 (br s, 4H), 1.27 (s, 18H). ^{13}C NMR (CDCl_3): δ 146.31, 143.84, 140.56, 136.36, 129.24, 128.04, 125.73, 114.34, 105.82, 93.11, 87.59, 33.85, 31.31. MS (APCI) m/e 422.2 (M^+ , 100).



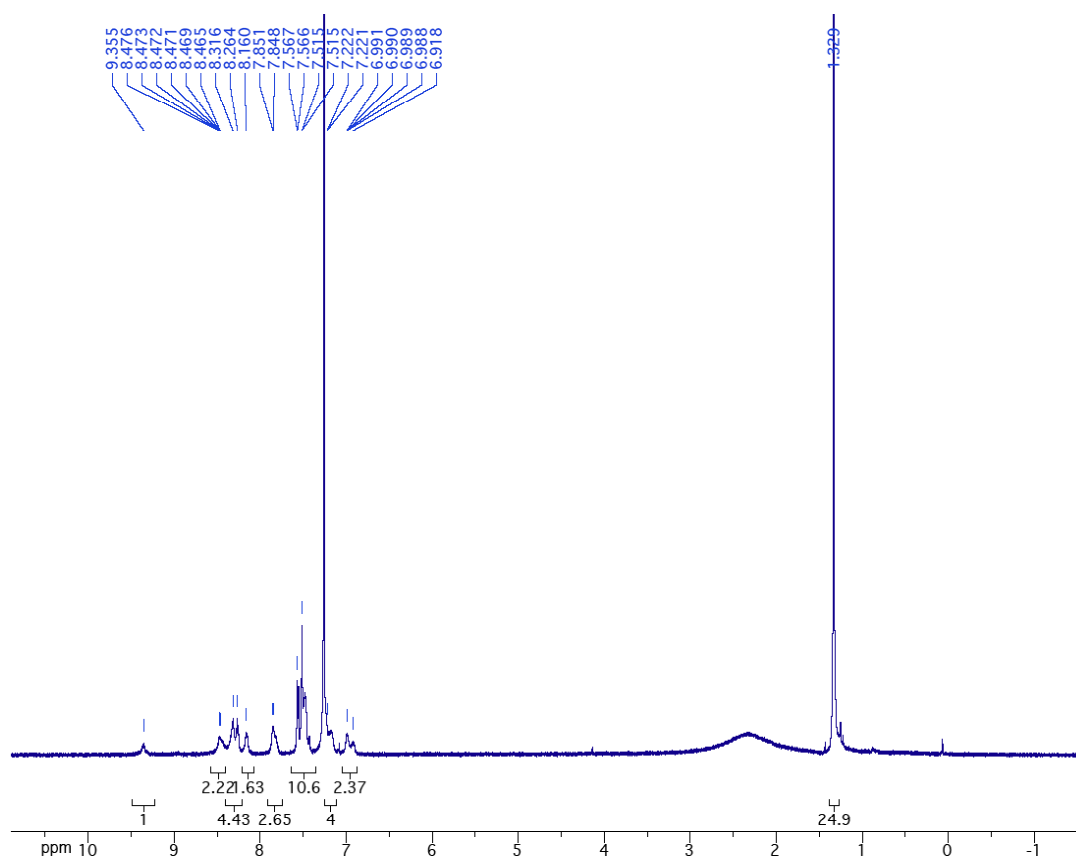


Phenylurea 2a. All glassware was flame-dried before use. Dianiline **1** (800 mg, 1.9 mmol) was reacted with phenyl isocyanate (3 equiv) in 10 mL toluene with stirring for 3 h under N₂. Following concentration *in vacuo*, the crude product was filtered through a short silica plug (2:1 hexanes/CHCl₃). Trituration with Et₂O afforded **2a** (1.15g, 92%) as a fluffy white powder. Mp: 212-215 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.32 (br s, 2H), 8.07 (d, *J* = 4.8 Hz, 2H), 7.74 (br s, 2H), 7.47-7.28 (m, 11H), 7.15 (t, *J* = 4.5 Hz, 4H), 6.92 (t, *J* = 4.5 Hz, 2H), 1.29 (s, 18H). ¹³C NMR (CDCl₃): δ 152.95, 145.38, 144.62, 141.17, 140.40, 138.15, 10.11, 129.56, 128.78, 127.65, 122.94, 120.33, 114.49, 110.45, 94.38, 87.33, 35.01, 31.75. MS (APCI) *m/e* 660.3 (M⁺, 100).



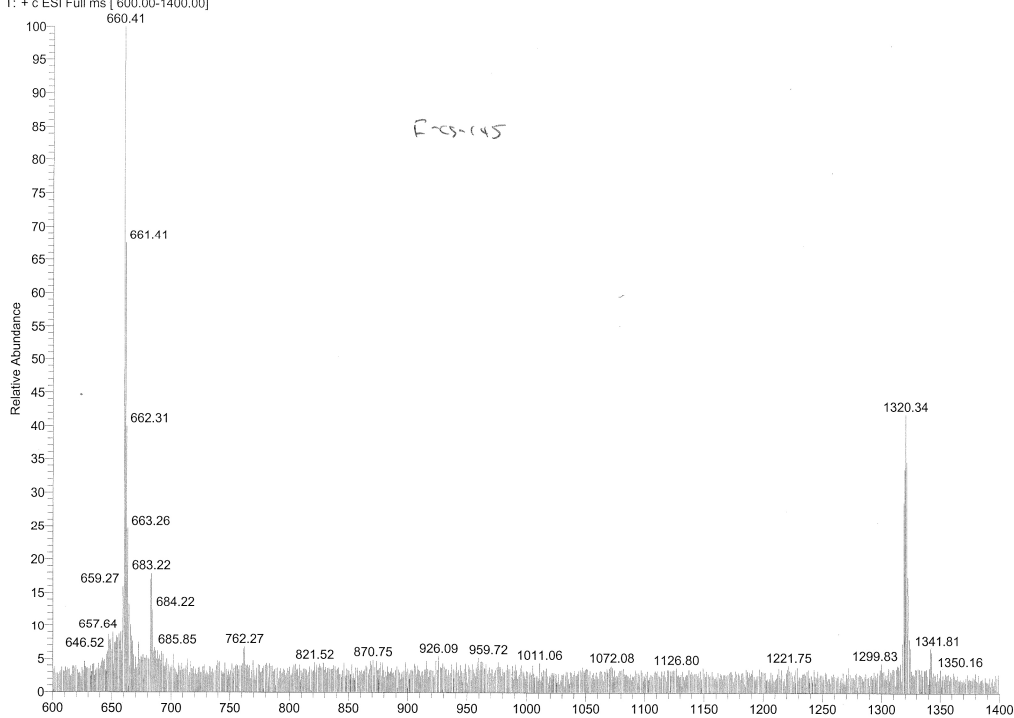


Phenylurea 2a•TFA Freebase phenylurea **2a** (200 mg, 30.3 mmol) was dissolved in CHCl_3 (15 mL) and a solution of trifluoroacetic acid in CHCl_3 (5 mL, 1.5 M) was added. The bright yellow solution was evaporated to ~10 mL, and pentane was added to precipitate the product as a bright orange, amorphous solid. Recrystallization from CHCl_3 /pentane yields an orange, crystalline solid. $^1\text{H-NMR}$ (600 MHz, CDCl_3): δ 9.35 (br s, 1H), 8.47 (br s, 2H), 8.31 (br s, 2H), 8.26 (t, J = 8.4 Hz, 1H), 8.15 (br s, 1H), 7.85 (br m, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.56-7.47 (m, 8H), 7.17 (br m, 2H), 6.98 (br s, 1H), 6.91 (br s, 1H), 1.32 (s, 18H).



C:\Xcalibur\data\Caldenwash
MMA(V)
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T: + c ESI Full ms [600.00-1400.00]

11/20/2008 2:38:42 PM Urea



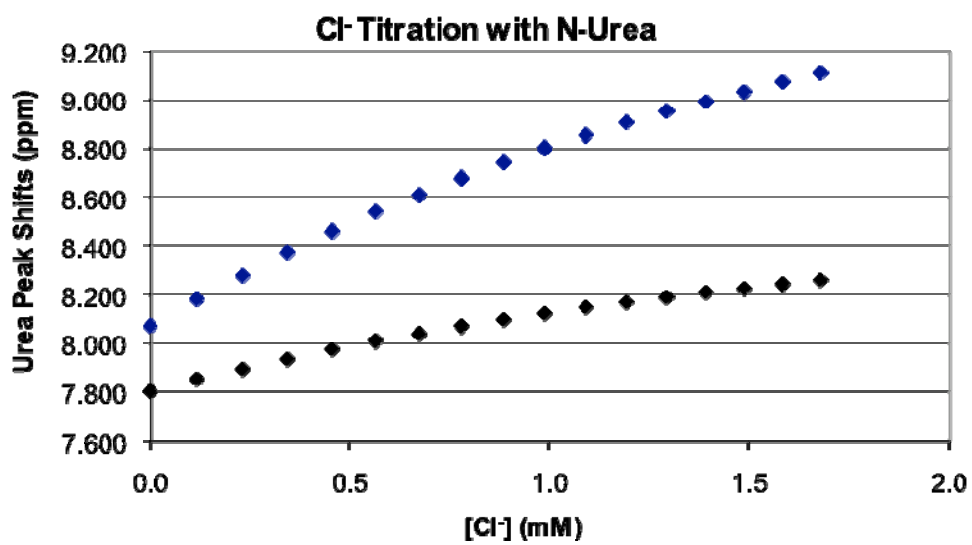
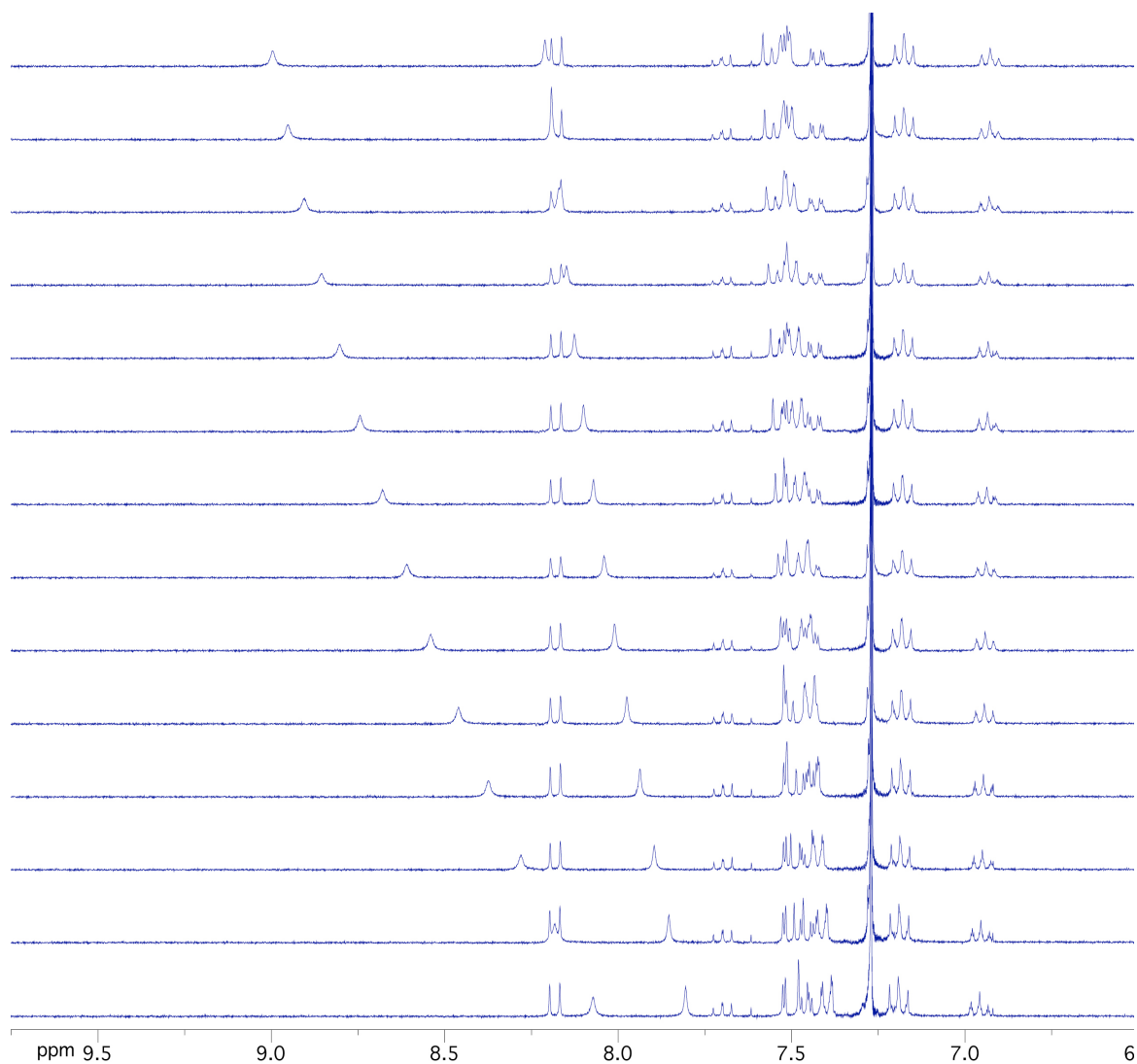
Titration Details

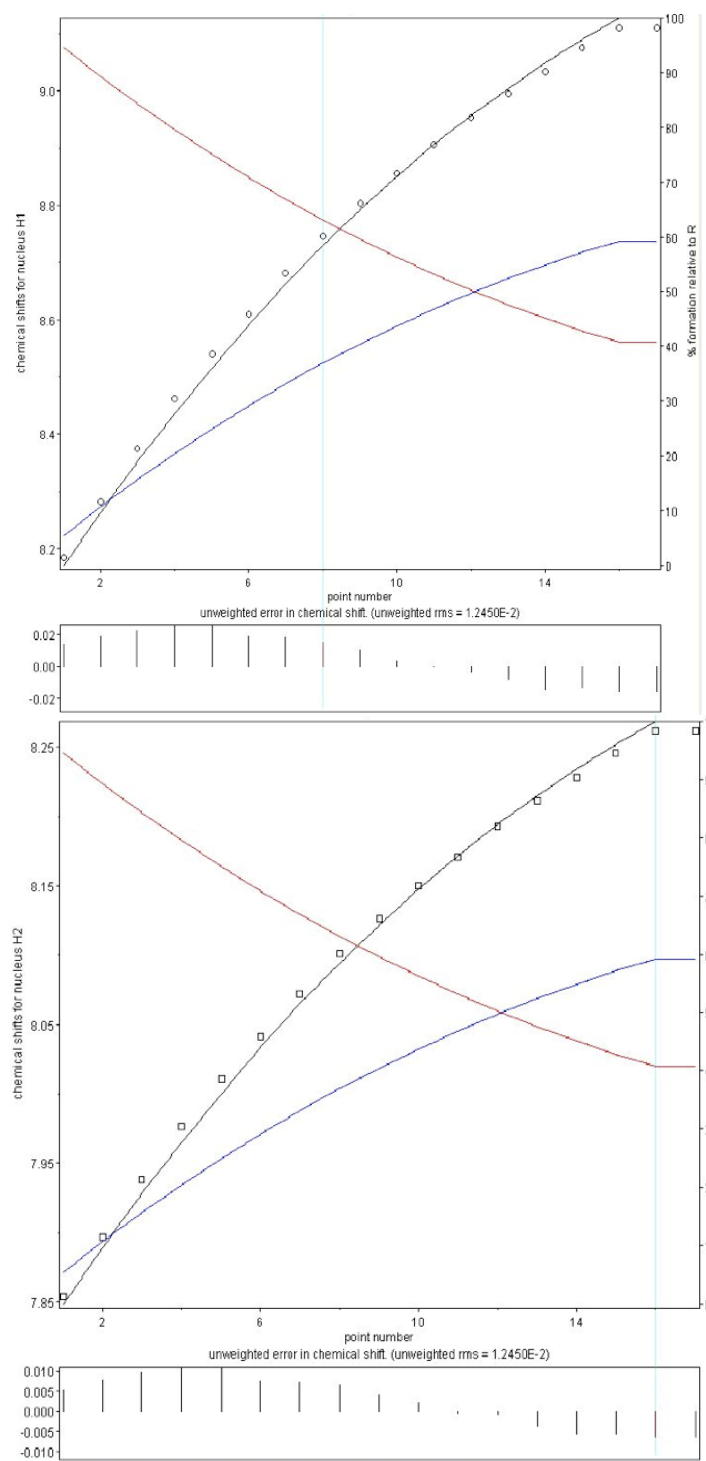
In all titrations care was taken to keep the receptor concentration constant during the titration. A stock solution of receptor was prepared and the guest serial dilution was prepared with the stock receptor solution. Receptor concentration was thus kept constant while titrating in the guest solution to avoid concentration effects on the urea N–H proton chemical shifts and provide clean isosbestic points in the UV spectra. All additions were done through septa with a Hamilton gas-tight syringe. Titrations were carried out in triplicate and the reported binding constants represent the average of the fits from three titrations. Representative data are provided for each set.

NMR Titration Conditions. ^1H NMR titrations were carried out on an Inova 500 MHz spectrometer (^1H 500.10 MHz). Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS) using non-deuterated solvent present in the bulk deuterated solvent (CDCl_3 ; ^1H 7.26 ppm). CDCl_3 was passed over activated alumina and saturated with deionized water (1:1 v/v CDCl_3 and water were mixed in a separatory funnel and the organic layer was collected). Tetrabutylammonium salts were used as purchased from TCI America.

Tetrabutylammonium chloride. A 10 mL stock solution of **2a** (12.60 mg, $[\text{R}]=1.60$ mM) in CDCl_3 was prepared and used in the dilution of TBACl guest solution (9.11 mg, $[\text{G}]=16.40$ mM). Starting volume of 700 μL .

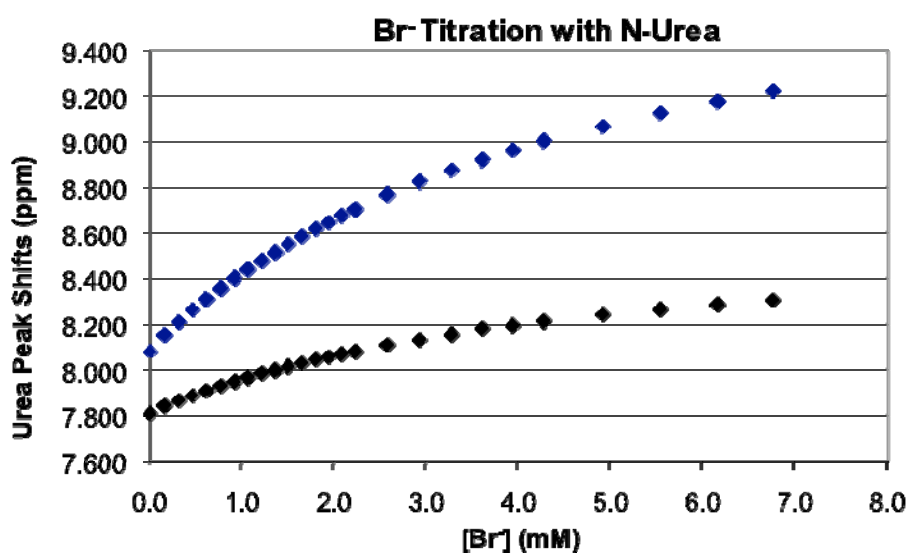
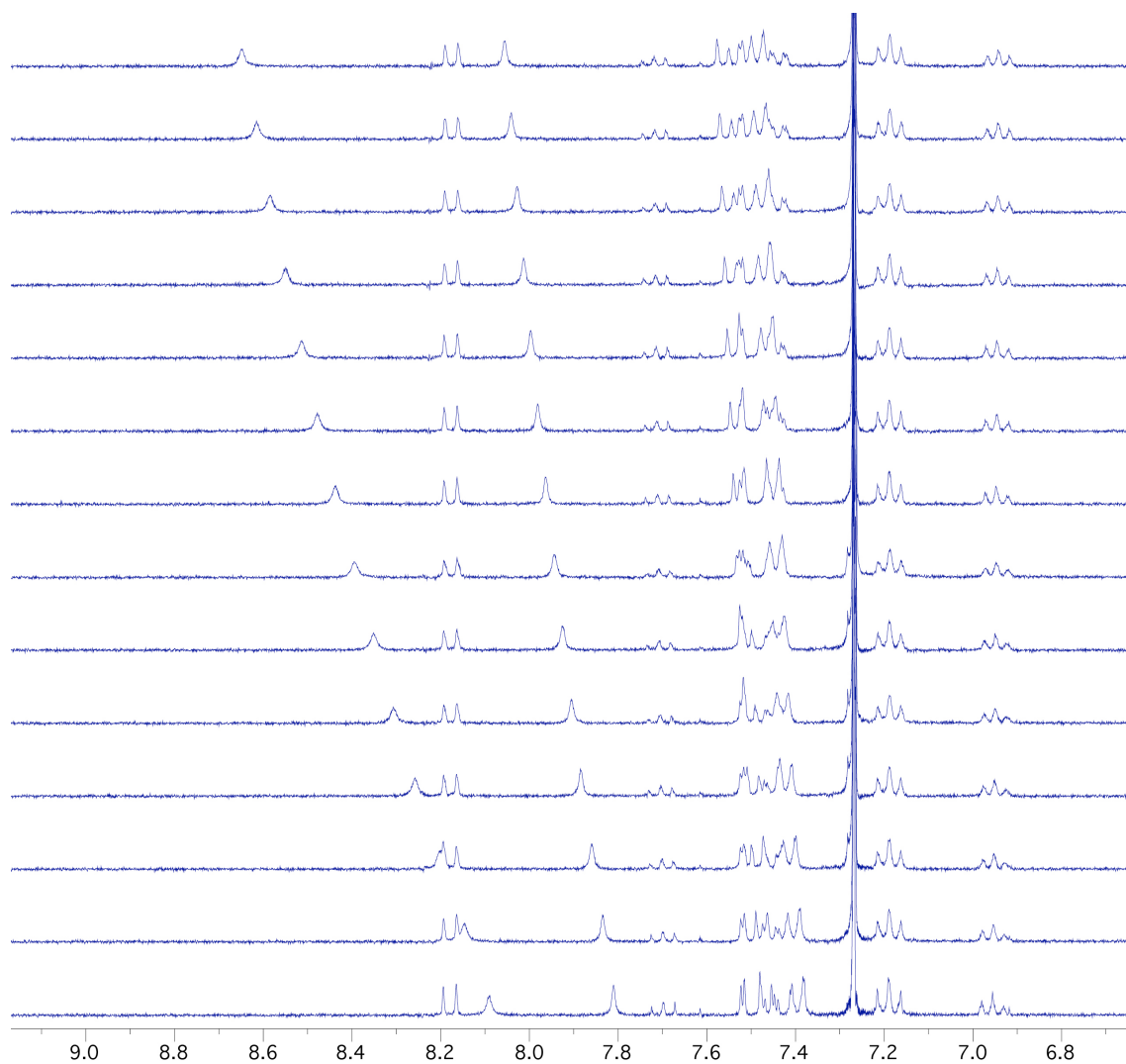
Addition (μL)	Volume of Anion (μL)	[TBACl]	Equivalents	s	s
0	0	0.00E+000	0	8.073	7.806
5	5	1.16E-004	0.07254619	8.184	7.854
5	10	2.31E-004	0.144070602	8.282	7.897
5	15	3.43E-004	0.214594673	8.374	7.938
5	20	4.55E-004	0.284139243	8.461	7.976
5	25	5.65E-004	0.352724577	8.541	8.011
5	30	6.73E-004	0.420370387	8.610	8.041
5	35	7.80E-004	0.487095845	8.681	8.072
5	40	8.85E-004	0.552919608	8.745	8.101
5	45	9.89E-004	0.61785983	8.803	8.126
5	50	1.09E-003	0.681934183	8.855	8.150
5	55	1.19E-003	0.745159869	8.907	8.171
5	60	1.29E-003	0.807553638	8.953	8.193
5	65	1.39E-003	0.869131802	8.996	8.211
5	70	1.49E-003	0.92991025	9.034	8.228
5	75	1.58E-003	0.989904459	9.075	8.246
5	80	1.68E-003	1.049129512	9.111	8.262

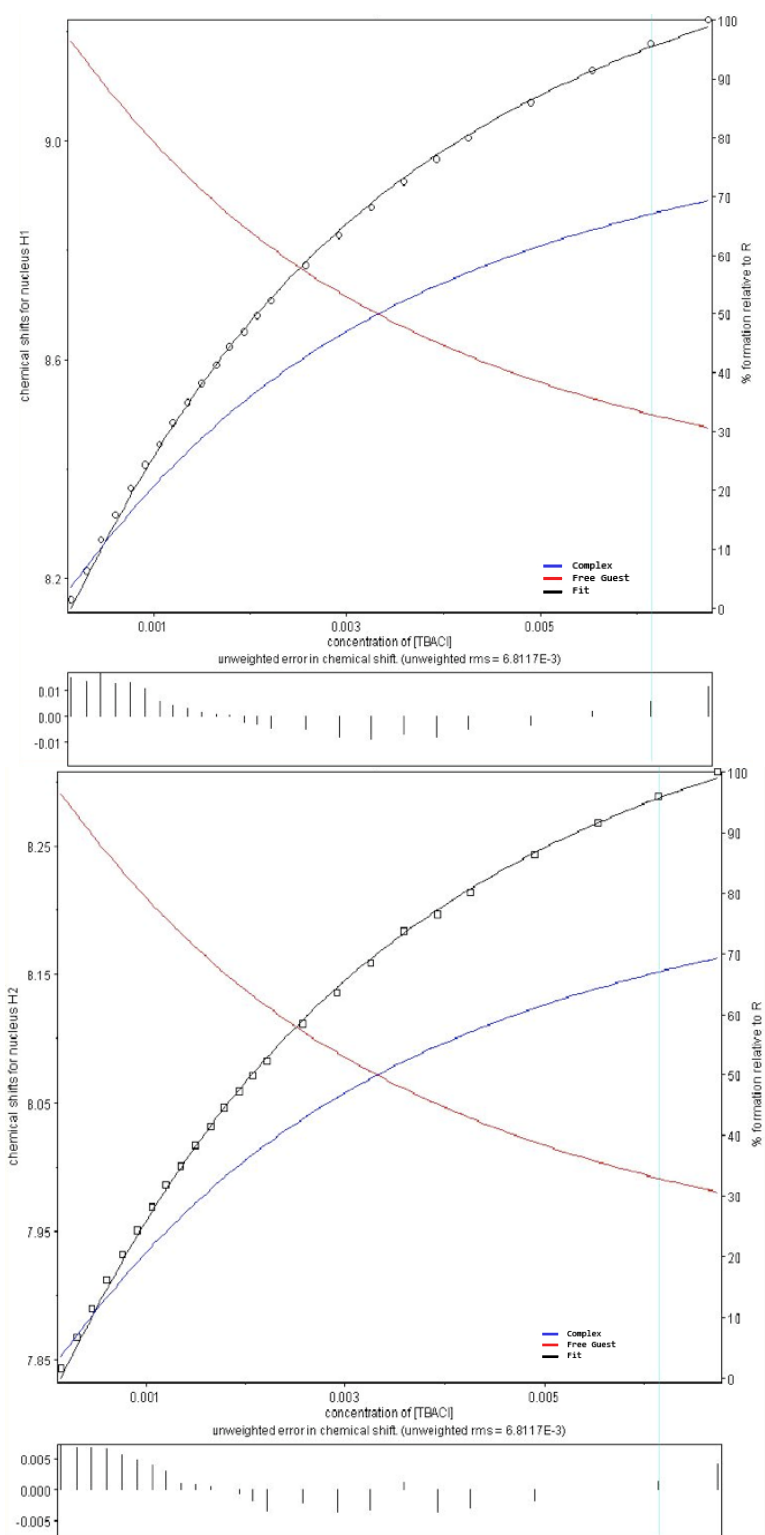




Tetrabutylammonium bromide. A 10 mL stock solution of **2a** (10.90 mg, [R]=1.38 mM) in CDCl₃ was prepared and used in the dilution of TBABr guest solution (34.8 mg, [G]=54.00 mM). Starting volume of 700 μL.

Addition (μL)	Volume of Anion (μL)	[TBABr]	Equivalents	s	s	s
0	0	0.00E+000	0.000	8.086	7.810	
2	2	1.54E-004	0.111	8.160	7.843	
2	4	3.07E-004	0.221	8.214	7.867	
2	6	4.59E-004	0.331	8.270	7.890	
2	8	6.10E-004	0.440	8.316	7.912	
2	10	7.60E-004	0.549	8.364	7.932	
2	12	9.10E-004	0.657	8.407	7.951	
2	14	1.06E-003	0.764	8.445	7.969	
2	16	1.21E-003	0.871	8.484	7.986	
2	18	1.35E-003	0.977	8.522	8.001	
2	20	1.50E-003	1.083	8.557	8.017	
2	22	1.64E-003	1.188	8.591	8.032	
2	24	1.79E-003	1.292	8.624	8.046	
2	26	1.93E-003	1.396	8.652	8.059	
2	28	2.08E-003	1.499	8.681	8.071	
2	30	2.22E-003	1.602	8.708	8.082	
5	35	2.57E-003	1.856	8.773	8.112	
5	40	2.92E-003	2.107	8.828	8.136	
5	45	3.26E-003	2.354	8.879	8.159	
5	50	3.60E-003	2.598	8.927	8.184	
5	55	3.93E-003	2.839	8.967	8.197	
5	60	4.26E-003	3.077	9.007	8.214	
10	70	4.91E-003	3.543	9.072	8.243	
10	80	5.53E-003	3.997	9.131	8.268	
10	90	6.15E-003	4.440	9.179	8.289	
10	100	6.75E-003	4.872	9.222	8.308	





Tetrabutylammonium iodide. A 10 mL stock solution of **2a** (11.60 mg, [R]=1.47 mM) in CDCl₃ was prepared and used in the dilution of TBAI guest solution (36.3 mg, [G]=54.00 mM). Starting volume of 700 μL. Titration to 2 equiv with 5 μL aliquots showed no shift in the N-H protons from the initial spectrum. Plotting N-H chemical shifts vs [TBAI] yielded flat isotherms. No binding was measurable.

UV-Vis Titration Conditions

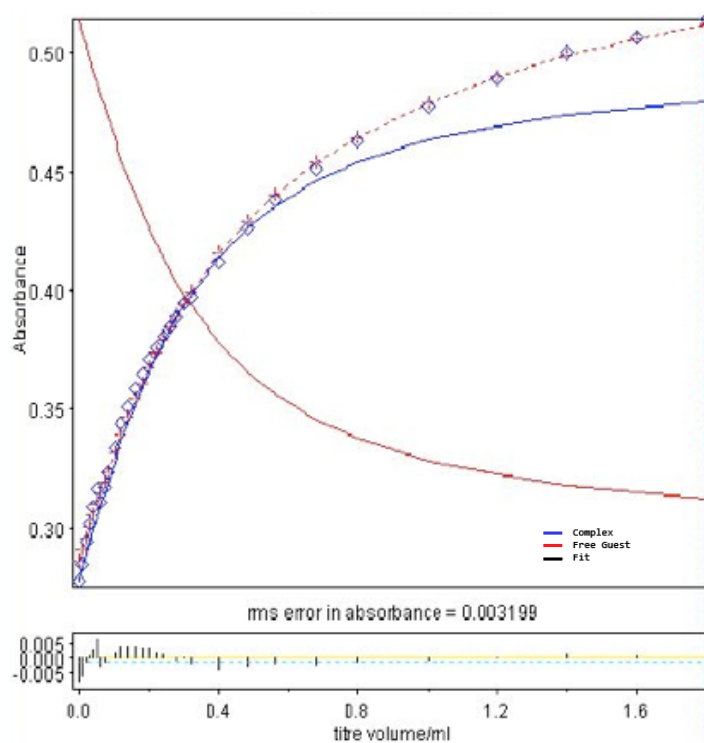
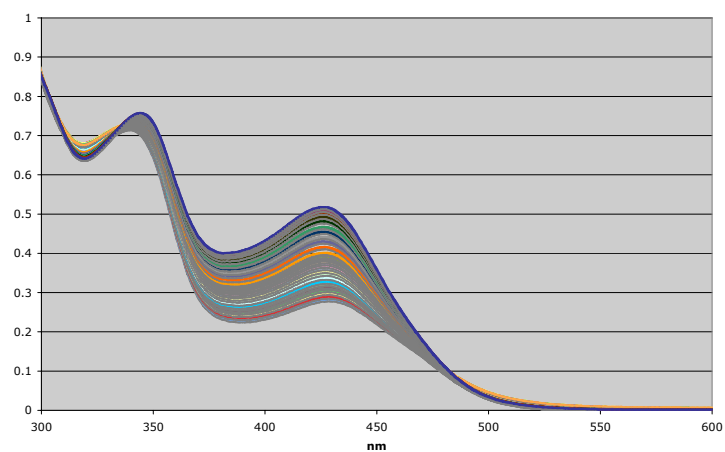
UV-Vis titrations held the receptor concentration constant as in the ¹H NMR titrations. Spectroscopically pure CH₃CN was passed over basic alumina, dried over 4 Å molecular sieves, and used immediately. Tetrabutylammonium salts were purchased from TCI America and purified by recrystallization and heating above the melting point *in vacuo* or by sublimation.

Tetrabutylammonium chloride. A 10 mL stock solution of **2a•TFA** (6.01 mg, 7.77 μmol) in CH₃CN was prepared and used in the dilution of TBACl guest solution (5.02 mg, 18.00 μmol). Serial dilution to [Host]= 6.00 x 10⁻⁵ ; [Guest]= 4.00 x 10⁻⁴. Host starting volume of 2 mL.

Additions	Total TBACl (mL)	mol Cl- Delivered	[Cl] Cuvett
0	0	0	0
1	0.01	4.00E-06	2.00E-06
2	0.02	8.00E-06	4.00E-06
3	0.03	1.20E-05	6.00E-06
4	0.04	1.60E-05	8.00E-06
5	0.05	2.00E-05	1.00E-05
6	0.06	2.40E-05	1.20E-05
7	0.07	2.80E-05	1.40E-05
8	0.08	3.20E-05	1.60E-05
9	0.09	3.60E-05	1.80E-05
10	0.1	4.00E-05	2.00E-05
11	0.11	4.40E-05	2.20E-05
12	0.12	4.80E-05	2.40E-05
13	0.13	5.20E-05	2.60E-05
14	0.14	5.60E-05	2.80E-05
15	0.15	6.00E-05	3.00E-05
16	0.3	1.20E-04	6.00E-05
17	0.45	1.80E-04	9.00E-05
18	0.6	2.40E-04	1.20E-04
19	0.75	3.00E-04	1.50E-04

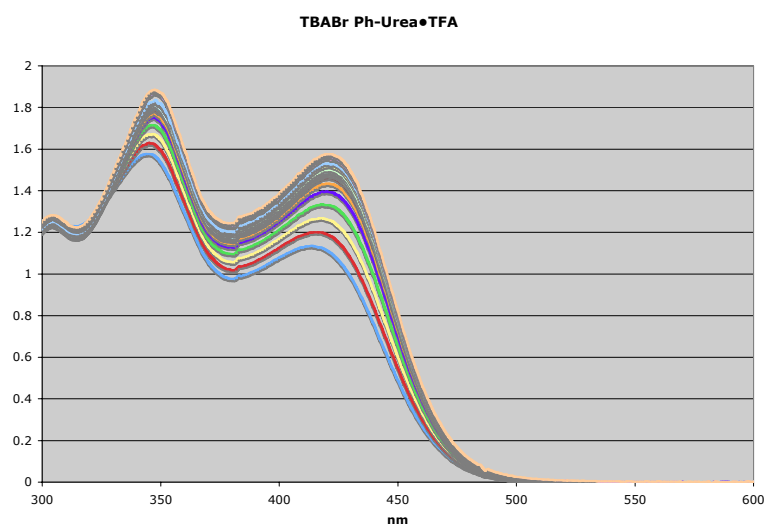
20	0.9	3.60E-04	1.80E-04
21	1.05	4.20E-04	2.10E-04
22	1.2	4.80E-04	2.40E-04
23	1.35	5.40E-04	2.70E-04
24	1.5	6.00E-04	3.00E-04
25	1.65	6.60E-04	3.30E-04

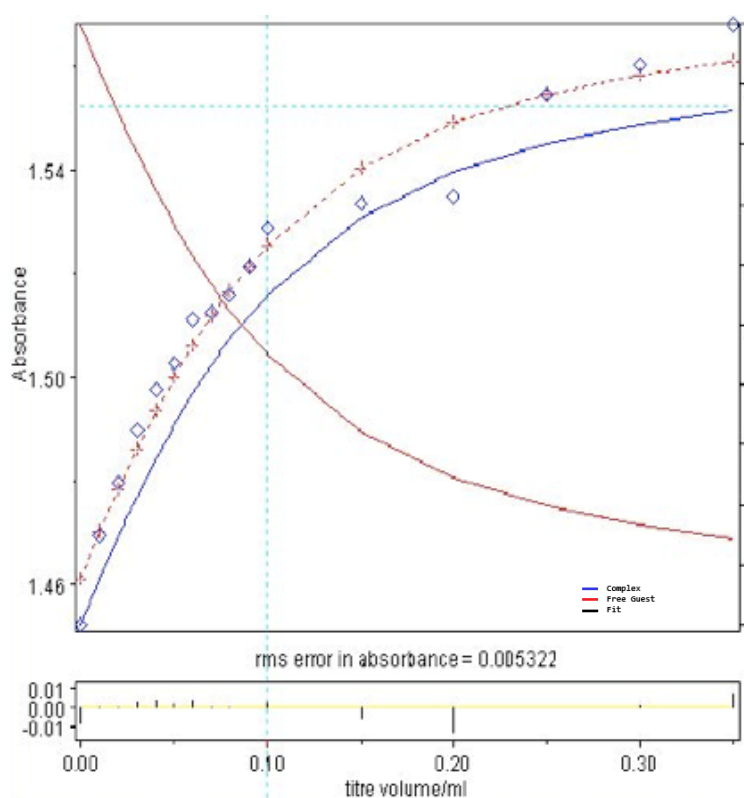
TBACl Ph-Urea•TFA



Tetrabutylammonium bromide A 10 mL stock solution of **2a**•TFA (6.01 mg, 7.77 μmol) in CH_3CN was prepared and used in the dilution of TBABr guest solution (12.26 mg, 38.03 μmol).
[Host]= 8.20×10^{-5} ; [Guest]= 1.82×10^{-3} . Host starting volume of 2.00 mL.

Additions	Total TBABr (mL)	mol Br- Delivered	[Br-] Cuvett
0	0	0	0
1	0.01	1.82E-05	9.10E-06
2	0.02	3.64E-05	1.82E-05
3	0.03	5.46E-05	2.73E-05
4	0.04	7.28E-05	3.64E-05
5	0.05	9.10E-05	4.55E-05
6	0.06	1.09E-04	5.46E-05
7	0.07	1.27E-04	6.37E-05
8	0.08	1.46E-04	7.28E-05
9	0.09	1.64E-04	8.19E-05
10	0.1	1.82E-04	9.10E-05
11	0.15	2.73E-04	1.37E-04
12	0.2	3.64E-04	1.82E-04
13	0.25	4.55E-04	2.28E-04
14	0.3	5.46E-04	2.73E-04
15	0.35	6.37E-04	3.19E-04



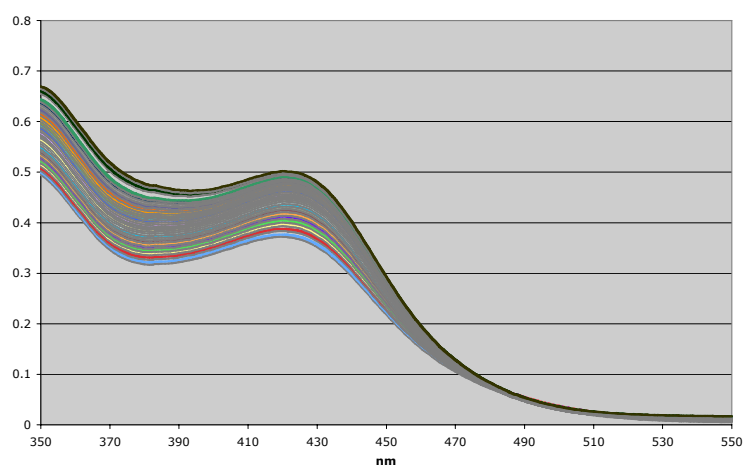


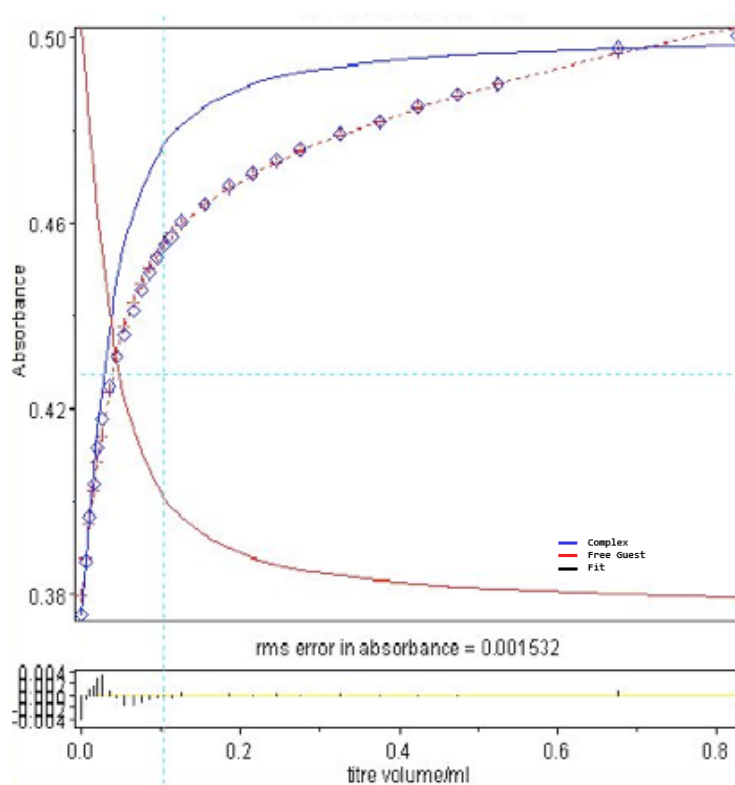
Tetrabutylammonium iodide A 10 mL stock solution of **2a**•TFA (3.95 mg, 5.11 μmol) was prepared and used in the dilution of TBAI guest solution (10.65 mg, 1.98 μmol). $[\text{Host}] = 2.60 \times 10^{-5}$; $[\text{Guest}] = 1.39 \times 10^{-3}$. Host starting volume of 2.00 mL.

Additions	Total X (mL)	mol X Delivered	[X] Cuvett
0	0	0	0
1	0.005	6.95E-06	3.48E-06
2	0.01	1.39E-05	6.95E-06
3	0.015	2.09E-05	1.04E-05
4	0.02	2.78E-05	1.39E-05
5	0.025	3.48E-05	1.74E-05
6	0.035	4.87E-05	2.43E-05
7	0.045	6.26E-05	3.13E-05
8	0.055	7.65E-05	3.82E-05
9	0.065	9.04E-05	4.52E-05
10	0.075	1.04E-04	5.21E-05
11	0.085	1.18E-04	5.91E-05
12	0.095	1.32E-04	6.60E-05
13	0.105	1.46E-04	7.30E-05
14	0.115	1.60E-04	7.99E-05

15	0.125	1.74E-04	8.69E-05
16	0.155	2.15E-04	1.08E-04
17	0.185	2.57E-04	1.29E-04
18	0.215	2.99E-04	1.49E-04
19	0.245	3.41E-04	1.70E-04
20	0.275	3.82E-04	1.91E-04
21	0.325	4.52E-04	2.26E-04
22	0.375	5.21E-04	2.61E-04
23	0.425	5.91E-04	2.95E-04
24	0.475	6.60E-04	3.30E-04
25	0.525	7.30E-04	3.65E-04
26	0.675	9.38E-04	4.69E-04
27	0.825	1.15E-03	5.73E-04

TBAI Ph Urea•TFA





DFT Details

B3LYP/6-31G = -2299.19660605 au				C	-2.71700800	2.57542600	-2.30684300
B3LYP/6-31G Zero Point Corrected Energy = -				O	5.75511200	-0.16802600	-0.62097700
2298.626895 au				N	3.83706300	-1.49226500	-0.68354800
NIMAG = 0				C	4.33310200	-2.66411700	-1.30434100
C	0.35263200	0.60728600	5.12140200	C	3.40521900	-3.69784300	-1.54216900
C	0.33553500	0.69053800	3.71895000	C	3.81438600	-4.89157200	-2.13642200
N	-0.71221500	0.11757500	3.04393000	C	5.15294300	-5.07932800	-2.50199800
C	-1.73773900	-0.55234600	3.66829700	C	6.07197700	-4.05157700	-2.26671200
C	-1.71217000	-0.66162900	5.07047700	C	5.67900700	-2.84702100	-1.67399100
C	-0.66972200	-0.07436400	5.78621900	C	-1.41817800	3.12071800	-2.30918800
C	1.35943000	1.32442700	2.98609000	C	-1.11210400	4.21388500	-3.11925900
C	-2.77565400	-1.06595700	2.87306100	C	-2.09368700	4.78222600	-3.94034800
C	2.31329700	1.86008000	2.43895000	C	-3.38204000	4.23751100	-3.93839600
C	-3.70952600	-1.45302000	2.18267500	C	-3.70635200	3.14188000	-3.13148400
C	3.43451600	2.48056000	1.83347800	H	-0.73893700	0.17049900	1.98455100
C	-4.79245400	-1.80392500	1.34758000	C	-0.27060800	-1.06002400	-1.64027800
C	3.83157600	3.76120100	2.28914100	F	0.68308000	-1.94645400	-2.10469400
C	4.94661400	4.39301400	1.75656200	F	-1.50486700	-1.68892000	-1.72433800
C	5.68876800	3.73792600	0.76070200	F	-0.29471100	0.02599500	-2.50996400
C	5.32520800	2.47739600	0.29396700	C	0.02228000	-0.58473900	-0.20661000
C	4.18834400	1.82090200	0.81198000	O	-0.95769700	0.03667700	0.36431500
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C	-5.75512500	-2.74441800	1.78802200	H	3.24175100	4.23945100	3.06374300
N	3.76966900	0.56361900	0.37209800	H	5.23951600	5.37589000	2.10807500
N	-3.96681800	-0.26255800	-0.33407500	H	6.56550600	4.21825000	0.33820900
C	4.54856000	-0.36386300	-0.34521400	H	5.91541700	1.97308200	-0.45531800
C	-4.09896800	0.73676100	-1.31238200	H	-6.24207200	-0.94282000	-1.62530300
N	-2.94553800	1.47734100	-1.44378100	H	-7.90356900	-2.59312700	-0.83110400
O	-5.15476900	0.92654100	-1.95755000				

H	-7.60029800	-3.75722300	1.35271700	H	5.47131500	-6.00797600	-2.96446300
H	-5.60703200	-3.22680100	2.74811200	H	7.11280700	-4.18100700	-2.54794900
H	2.83271400	0.26353500	0.63494700	H	6.39044600	-2.05399100	-1.50012300
H	-3.05672100	-0.33965200	0.11370600	H	-0.64939100	2.67395500	-1.68619900
H	-2.14979900	1.18875200	-0.87974900	H	-0.10455900	4.61783700	-3.11090900
H	2.83881400	-1.48644100	-0.47831100	H	-1.85620400	5.63174100	-4.57225000
H	2.36460600	-3.55440500	-1.27153100	H	-4.15203900	4.66648700	-4.57242400
H	3.08385400	-5.67466100	-2.31501800	H	-4.70075000	2.72127100	-3.13554500