Supporting Information

Multiwell Assay for the Analysis of Sugar Gut Permeability Markers: Discrimination of Sugar Alcohols with a Fluorescent Probe Array Based on Boronic Acid Appended Viologens

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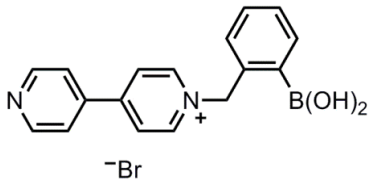
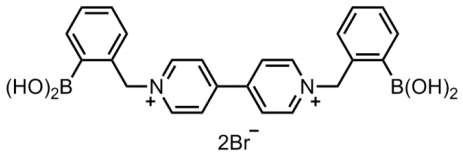
1. Synthesis

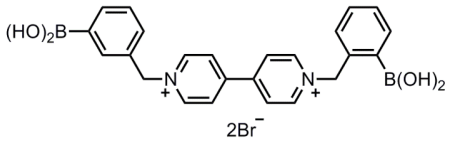
The syntheses of 4, 4'-*o*-BBV, 4, 4'-o,m-BBV, 3, 4'-o-BBV, 3, 3'-o-BBV, 4, 7-*o*-PBBV, and pB*o*B boronic acid receptors were synthesized according to previously published procedures with slight modifications.[1-6]



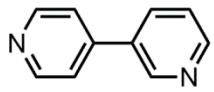
Preparation of monoalkylated bipyridyl boronic acid salt (1-(2-boronic acid-benzyl)-[4-4'] bipyridinyl bromide). To a solution of 2-bromomethyl phenyl boronic acid (0.405g, 1.86 mmol) in dry acetone (50 mL) was added 4, 4'-bipyridyl (1.16g, 7.4 mmol). This reaction was stirred at 25°C for 1 h. After the reaction went to completion, the solution was decanted onto a 100 mL round bottom flask and diethylether (100 mL) was added. The off-white precipitate was collected by centrifugation, washed with acetone several times, and dried under a stream of argon (0.5 g, 72% yield). ^{1}H-NMR (500 MHz, d_2o) δ 8.91 (d, J=5.0 Hz, 2H), 8.74 (d, J=5.0Hz, 2H), 8.60 (d, J=5.0Hz, 2H), 8.32 (d, J=5.0Hz, 2H), 7.86 (d, J=5.0Hz, 1H), 7.78 (d, J=5.0Hz, 1H), 7.72 (d, J=5.0Hz, 1H), 7.56 (m ,1H), 6.02 (s, 2H). ^{13}C-NMR (D_2O, 125MHz) δ 155.20, 151.45, 146.39, 143.74, 137.34, 136.46, 132.61, 123.49, 130.92, 127.02, 123.84, 65.69; ^{11}B-NMR (160MHz, D_2O) δ= +28.2



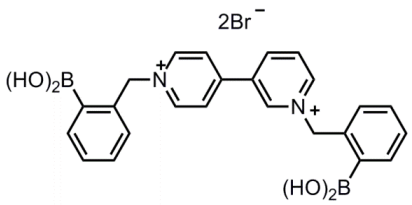
Preparation of 4,4'-*o*-BBV.[1] To a solution of 2-bromomethylphenyl boronic acid (0.77 g, 3.6 mmol) in dry MeCN (10 mL) was added MeOH (0.65 mL) drop wise , 4,4'-dipyridyl (0.23 g, 1.5 mmol), and the reaction was stirred at 55 °C for 48 hours. The reaction mixture was cooled to room temperature and acetone (10 mL) was added to induce further precipitation of a pale yellow solid. The precipitate was centrifuged, washed with acetone (2x 10mL) and dried under a stream of argon (0.85 g, 96%yield). ^{1}H NMR (500 MHz, D_2O) δ 9.06 (d, J=10 Hz, 4H), 8.47 (d, J=5 Hz, 4H), 7.79 (d, J=10Hz, 2H), 7.56 (m, 6H), 6.11 (s, 4H); ^{13}C NMR (126 MHz, D_2O) δ 146.72, 135.51, 132.80, 130.44, 128.35, 127.85, 66.13; ^{11}B NMR (160 MHz, D_2O) δ +29.83.



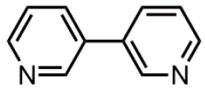
Preparation of 4,4'-*o,m*-BBV. To a solution of 4,4'-monoalkylated bipyridyl boronic acid salt (0.275 g, 0.74 mmol) in MeCN (10 mL) was added MeOH (0.65 mL) drop wise. The reaction stirred for 10 minutes, 3-bromomethyl phenyl boronic acid (0.191 g, 0.89 mmol) was added and the reaction stirred at 50 °C for 24 h. The resulting orange solution was cooled to room temperature and diluted with acetone (30 mL) to precipitate out the product as a pale yellow solid. The solution was stored at 4 ^{o}C for 1 h to induce further precipitation. The precipitate was collected by centrifugation, washed with acetone, and dried under a stream of argon to give a pale yellow solid (0.390 g, 90%). ^{1}H NMR (500 MHz, D_2O) δ 9.18 (d, J=5Hz, 2H), 9.08 (d, J=5Hz, 2H), 8.55 (d, J=5Hz, 2H), 8.50 (d, J=5Hz, 2H) , 7.88 (m, 2H), 7.82 (d, J=5 Hz, 1H), 7.59 (m, 5H), 6.12 (s, 2H), 5.99 (s, 2H); ^{13}C NMR (126 MHz, D_2O) δ 146.72, 135.51, 132.80, 130.44, 128.35, 127.85, 66.13; ^{11}B NMR (160 MHz, D_2O) δ +30.48.



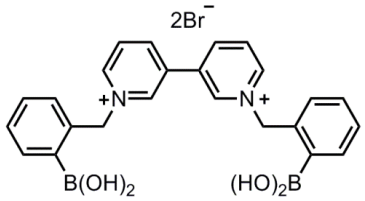
Preparation of 3,4' bipyridyl.[4] To a 25mL oven round bottom flask purged with argon, was added $Pd(OAc)_2$ (0.009 g, 0.04 mmol, 4 mol%), XPhos (0.023 g, 0.048 mmol, 4.8 mol%), 3-bromopyridine (0.096 mL, 1 mmol), 4-pyridinyl boronic acid (0.15 g, 1.2 mmol), and n-butanol (5.6 mL). After the mixture pre-stirred at 25 °C for 30 minutes, a degassed aqueous solution of NaOH (1.4 mL, 5.1 mmol, 1.2 M) was added to the mixture and vigorously stirred at 80 °C for 4 h. At the end of the reaction, the organics were extracted with ethyl acetate (10 mL x 2). The organic extracts were combined, dried with $MgSO_4$, and concentrated on a rotary evaporator. The resulting residue was purified by silica gel flash chromatography with MeOH:DCM (1:20) as eluent to give the title compound as a pale yellow oil (0.10 g, 70% yield). ^{1}H NMR (500 MHz, CDC_3) δ 8.89 (d, J=5Hz, 1H), 8.71 (d, J=5Hz, 2H), 8.69 (dd, J=5, 2Hz, 1H), 7.93 (dt, J=8, 2Hz, 1H), 7.54-7.52 (m, 2H), 7.43 (dd, J=5, 2Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.60, 150.46, 150.10, 148.06, 145.19, 134.36, 133.78, 123.84, 121.56, 121.41, 62.35.



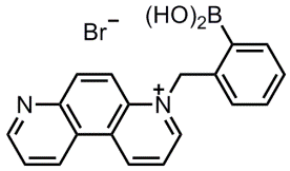
Preparation of 3,4'-o-BBV. [1] To a solution of 2-bromomethylphenyl boronic acid (0.3 g, 1.38 mmol) in DMF (10 mL) was added, 3, 4'-bipyridyl (0.095 g, 0.6 mmol), and the reaction was stirred at 70 °C for 48 hours. The reaction mixture was cooled to room temperature and acetone (10 mL) was added to induce further precipitation of a pale yellow solid. The precipitate was centrifuged, washed with acetone (2x 10 mL) and dried under a stream of argon (0.17 g, 45% yield). ^{1}H NMR (500 MHz, D_2O) δ 9.47 (s ,1H), 9.03 (m, 3H), 8.38 (d, J=5Hz, 1H), 8.25 (m, 1H), 7.81 (m, 1H), 7.60 (m ,5H), 6.14(s, 2H), 6.09(s, 2H); ^{13}C NMR (126 MHz, D_2O) δ 150.83, 146.77, 136.24, 132.30, 130.90, 127.31, 66.61. ^{11}B NMR (160 MHz, D_2O) δ +29.94.



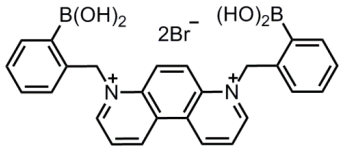
Preparation of 3,3' bipyridyl.[5] To an oven dried double neck round bottom flask (25 mL) fitted with a condenser and purged under argon, $[NiCl_2(PPh_3)_2]$ (0.327 g, 0.5 mmol), acid-washed zinc (0.164 g, 2.5 mmol), Et_4NI (0.435 g, 1.7 mmol), and dry THF (10 mL) was added. The mixture stirred under argon for 30 min at room temperature to give a brown-red solution to which 3-bromopyridine (0.16 mL, 1.7 mmol) was added and the mixture was brought to reflux and stirred for 24 h. The reaction mixture was then cooled to room temperature and poured into 2 M aqueous ammonia (50 mL) and filtered with ethyl acetate (25 mL). The aqueous layer was extracted with ethyl acetate (2x 25 mL) and the combined organic layers were extracted with 2 M hydrochloric acid (2x 25 mL). The acidic aqueous layers were combined and neutralized with Na_2CO_3. The neutralized solution was extracted with ethyl acetate (2x 25 mL) and organic layers were dried with anhydrous $MgSO_4$ and evaporated to give the product as a pale yellow oil (0.09 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.86 (s, 2H), 8.66 (d, J= 5 Hz, 2H), 7.89 (d, J=10 Hz, 2H), 7.41 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.31, 148.18, 134.48, 133.52, 123.80.



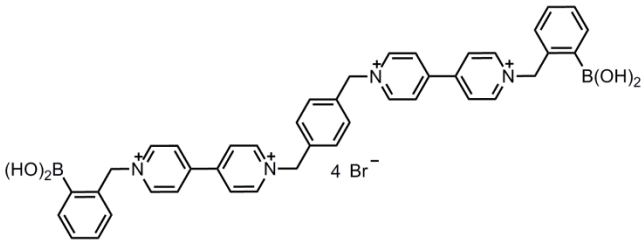
Preparation of 3,3'-o-BBV. To a solution of 2-bromomethylphenyl boronic acid (0.45 g, 2.85 mmol) in dry MeCN (10 mL) was added MeOH (0.65 mL) drop wise, 3,3'-bipyridyl (0.194 g, 1.2 mmol), and the reaction was stirred at 55 °C for 48 h. The reaction mixture was cooled to room temperature and acetone (10 mL) was added to induce further precipitation of a pale yellow solid. The precipitate was centrifuged, washed with acetone (2x 10 mL) and dried under a stream of argon (0.5 g, 70%yield). ^{1}H NMR (500 MHz, D_2O) δ 9.15 (s, 2H), 9.03 (d, J=10 Hz, 2H), 8.85 (d, J=5 Hz, 2H), 8.22 (t, J=5, 10 Hz, 2H), 7.80 (d, J=5 Hz, 2H), 7.56 (m, 6H), 6.10 (s, 4H); ^{13}C NMR (126 MHz, D_2O) δ 146.70, 145.84, 144.38, 136.53, 136.28, 135.41, 132.39, 130.95, 129.80, 66.48; ^{11}B NMR (160 MHz, D_2O) δ +29.94.



Preparation of *N*-(benzyl–2-boronic acid)-4,7-phenanthrolinium bromide (*o*-PBV).[6] An oven dried 100-mL round bottom flask equipped with a magnetic stir bar was cooled under argon and charged with 4,7-phenanthroline (5.20 mmol, 0.95 g) and 2-bromomethylphenyl boronic acid (5.10 mmol, 1.105 g). The flask was fit with a reflux condenser and purged with argon. CH_3CN (30 mL) was added via cannula and the reaction mixture was heated to reflux for 16 h. The solution was cooled under argon and the solvent removed under reduced pressure. The residue was triturated with acetone (2 x 50 mL), transferred to a fritted funnel, and washed with additional acetone (3 x 50 mL). The yellow-orange powder was dried under reduced pressure and isolated under a blanket of argon (1.70 g, 80%).^{1}H NMR (D_2O, 500 MHz) δ 9.88 (d, J=5 Hz, 1H), 9.23 (dd, J= 5, 20 Hz, 1H), 9.06 (d, J=5 Hz, 1H), 8.94 (d, J=5 Hz, 1H), 8.38 (d, J=10, 1H), 8.25 (d, J= 5 Hz, 1H), 7.88 (dd, J= 5, 20 Hz, 1H), 7.73 (d, J= 5 Hz, 2H), 7.45 (m, 2H), 7.08 (d, J= 5 Hz) , 6.49 (s, 2H). ^{13}C NMR (126 MHz, D_2O) δ 63.324, 121.541, 124.610, 126.038, 129.335, 129.882, 130.490, 131.690, 134.303, 134.440, 135.047, 137.660, 139.514, 140.851, 143.844, 147.915, 149.556, 154.676. ^{11}B NMR (160 MHz, D_2O) δ +29.023



Preparation of 4,7-*o*-PBBV.[6] An oven dried 100 mL side armed round bottom flask equipped with a magnetic stir bar, was cooled under argon and charged with ***o*-PBV** (2.5 mmol, 1.01 g) and 2-bromomethylphenyl boronic acid (25 mmol, 5.5 g). The flask was fit with a reflux condenser and purged with argon. DMF (45 mL) was added through the side arm and the reaction mixture was heated to 55 ° C for 3 h. The reaction mixture was cooled to room temperature and dripped into ice-cold acetone (150 mL) to induce precipitation. The material was allowed to sit at 4 °C overnight and then was washed with additional ice-cold acetone (2 × 225 mL). After removal of the solvent and drying under reduced pressure, a viscous red oil remained. The oil was dissolved in MeOH (4 mL) and added drop wise via cannula to a rapidly stirring ice-cold acetone (500 mL) solution to give a yellow-orange solid. The solid was isolated under argon and dried under reduced pressure. Further purification on a Biotage Flash 40+ C18 cartridge (20% methanol, 0.02% formic acid, in water) gave an orange powder (0.50 g, 35%).^{1}H NMR (D_2O, 500 MHz) δ 6.781 (s, 4H), 7.356 (d, *J* = 10 Hz, 2H), 7.680 (m, 4H), 7.972 (d, *J*= 10 Hz, 2H), 8.675 (dd, *J* = 5,10 Hz, 2H), 9.189 (s, 2H), 9.591 (dd, *J* = 5 Hz, 10 Hz, 2H), 10.370 (d, *J* = 5, 10 Hz, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 63.56, 126.85, 127.35, 129.17, 130.40, 130.55, 132.53, 136.40, 136.83, 140.24, 144.24, 151.63. ^{11}B NMR (106 MHz, D_2O) δ +30.97



Preparation of pB*o*B.[2] To a solution of 4,4'-monoalkylated bipyridyl boronic acid salt (0.5 g, 1.3 mmol) in DMF (15 mL) was added α, α'-dibromo-*p*-xylene (0.17 g, 0.67 mmol), and the reaction was stirred at 65 ^{o} C for 24 h. The resulting yellow precipitate was collected by centrifugation. The DMF supernatant was decanted, the yellow solid washed several times with acetone, then dried under a stream of argon to yield a yellow powder (0.50 g, 74% yield).^{1}H NMR (500 MHz, D_2O) δ 9.17 (d, J=10 Hz, 4H), 9.08 (d, J=10 Hz, 4H), 8.56 (d, J= 5 Hz, 4H), 8.50 (d, J= 5 Hz, 4H), 7.96 (d, J=5 Hz, 2H), 7.81 (d, J= 10 Hz, 2H), 7.60 (m, 10H), 6.12 (s, 4H), 6.00 (s, 4H); ^{13}C NMR (125 MHz, D_2O) δ 172.21, 146.85, 136.31, 132.37, 131.56, 128.46, 127.88, 66.21; ^{11}B NMR (160 MHz, D_2O) δ +30.07.

2. Construction of boronic acid array

The boronic acid array was pipetted onto a 96-well (Fisherbrand®, flat bottom, clear polystyrene, non-sterile) by preparing a stock "probe" solution of each boronic acid receptor at its respective quencher:dye ratio prepared as a twofold concentration in 0.1M NaH_2PO_4/Na_2HPO_4 pH 7.4 buffer. For 4, 4'-, 3, 4'-, 3, 3'-*o*-BBV and 4, 4'-*o, m*-BBV a ratio of 125:1 was used. For 4, 7-*o*-PBBV a ratio of 30:1 was used. For pBoB a ratio of 3:1 was used. To each receptor solution was added HPTS (8 μM) to complete the probe solution. Blank wells were given 40 μL of the sodium phosphate buffer. Baseline fluorescence ("probe") wells were given 20 μL of the probe and sodium phosphate buffer. The rest of the wells received 20 μL of probe and analyte.

	1	2	3	4	5	6	7	8	9	10	11	12
A		Sugar 1 →			Sugar 2 →			Sugar 3 →			Sugar 4 →	
B		Sugar 5 →			Sugar 6 →			Sugar 7 →			Sugar 8 →	
C		Sugar 9 →			Sugar 10 →			Sugar 11 →			Sugar 12 →	
D		Sugar 13 →			Sugar 14 →			Sugar 15 →			Sugar 16 →	
E		Sugar 17 →			Sugar 18 →			Sugar 19 →			Sugar 20 →	
F		Sugar 21 →			Sugar 22 →			Sugar 23 →			Sugar 24 →	
G		Sugar 25 →			Sugar 26 →			Sugar 27 →			Sugar 28 →	
H		Sugar 29 →			Sugar 30 →			"blank" →			"probe" →	

Figure S-1A. Map of multi-well plate for sugar alcohol and permeability marker study that outlines the location of each sugar and probe used.

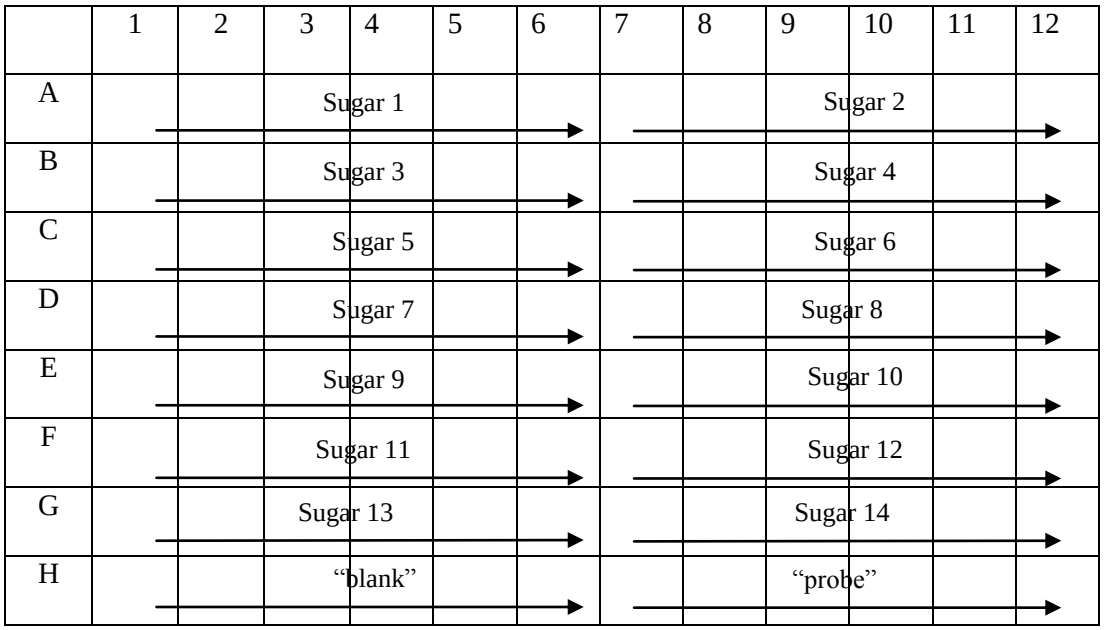


Figure S-1B. Map of multi-well plate for multivariate data collection for sugar alcohol, permeability marker and L:M ratios discrimination studies.

3. Analysis of sugar alcohols

Serial dilutions of each sugar alcohol or permeability marker were prepared as a twofold concentration in 0.1M NaH_2PO_4/Na_2HPO_4 pH 7.4 buffer to obtain eight data points for each analyte. Each data point was then measured with each receptor and the fluorescence recovery was obtained. Upon running assays, each well received 20 μL of probe solution and 20 μL of analyte solution. Blank wells received 40 μL of sodium phosphate buffer. The fluorescence recovery of HPTS was read using a 96-well plate on a 2103 Envision Multilabel Perkin Elmer reader (excitation filter: 405 nm, emission filter: 535 nm). After background (blank) subtraction, the relative fluorescence increase F/F_o for each receptor/analyte combination was calculated, resulting in an individual recognition pattern for each analyte and receptor.

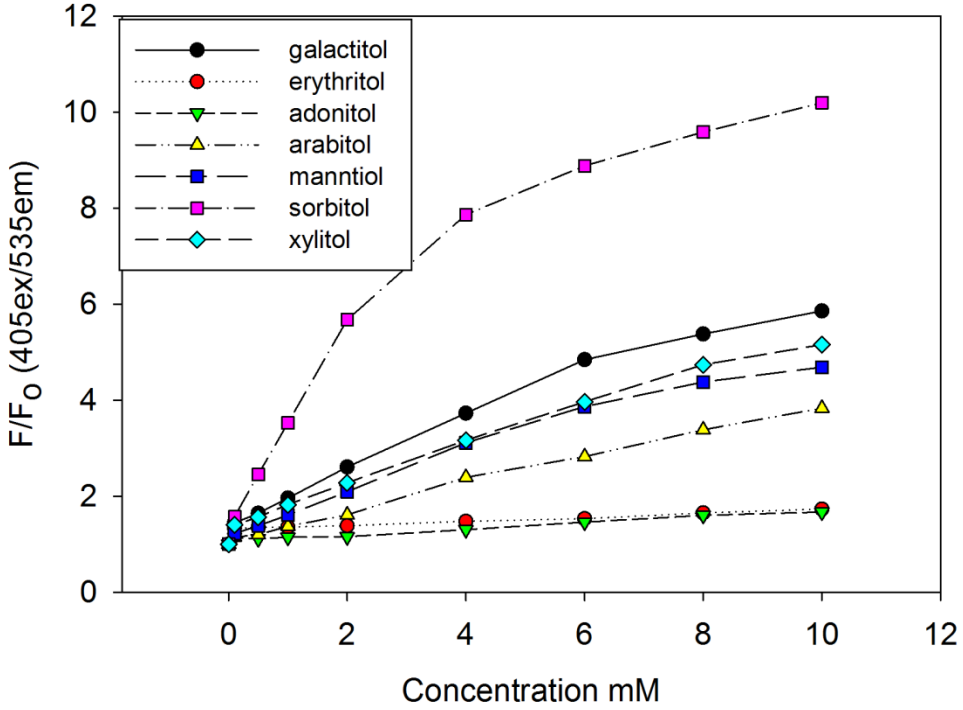


Figure S-2. Binding isotherms of six sugars and 4, 4'-*o*-BBV-HPTS combinations.

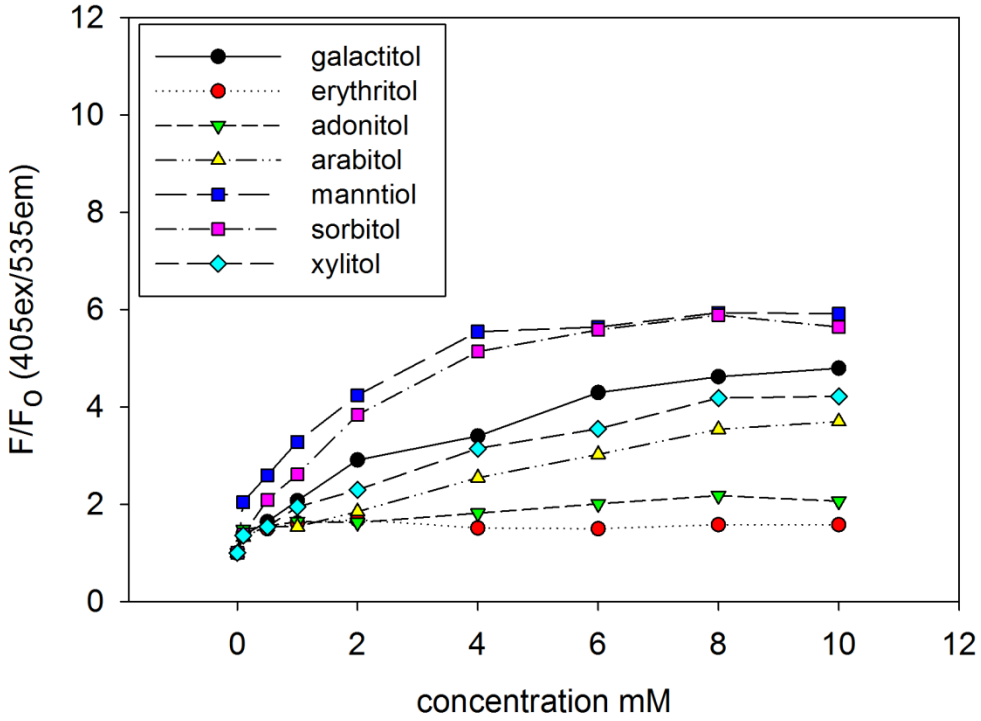


Figure S-3. Binding isotherms of six sugars and 3, 4'-*o*-BBV-HPTS combinations.

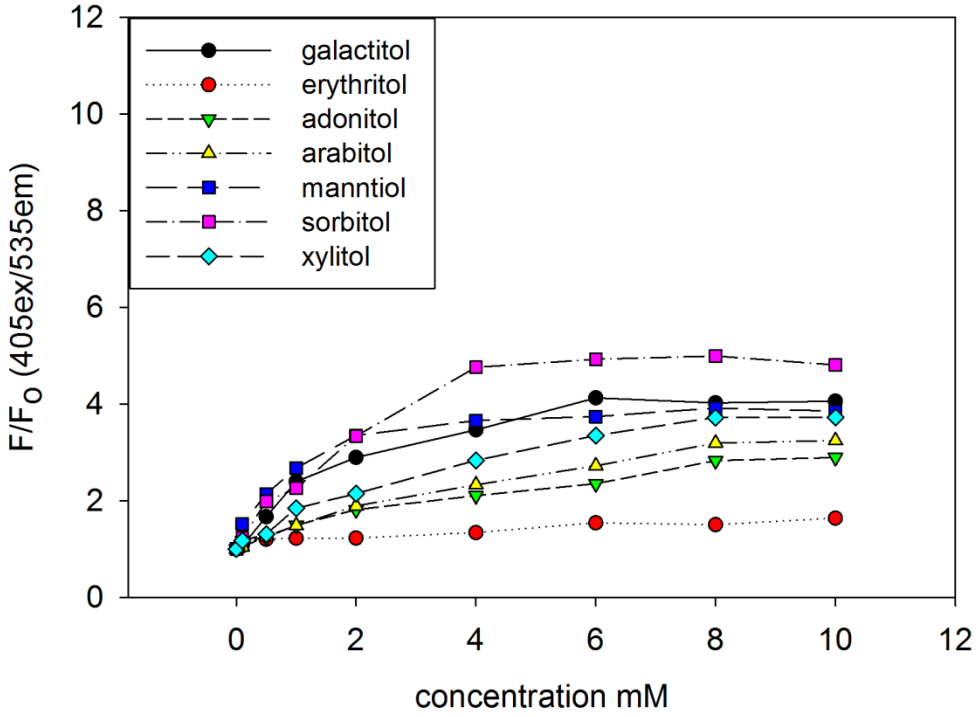


Figure S-4. Binding isotherms of six sugars and 3, 3'-*o*-BBV-HPTS combinations.

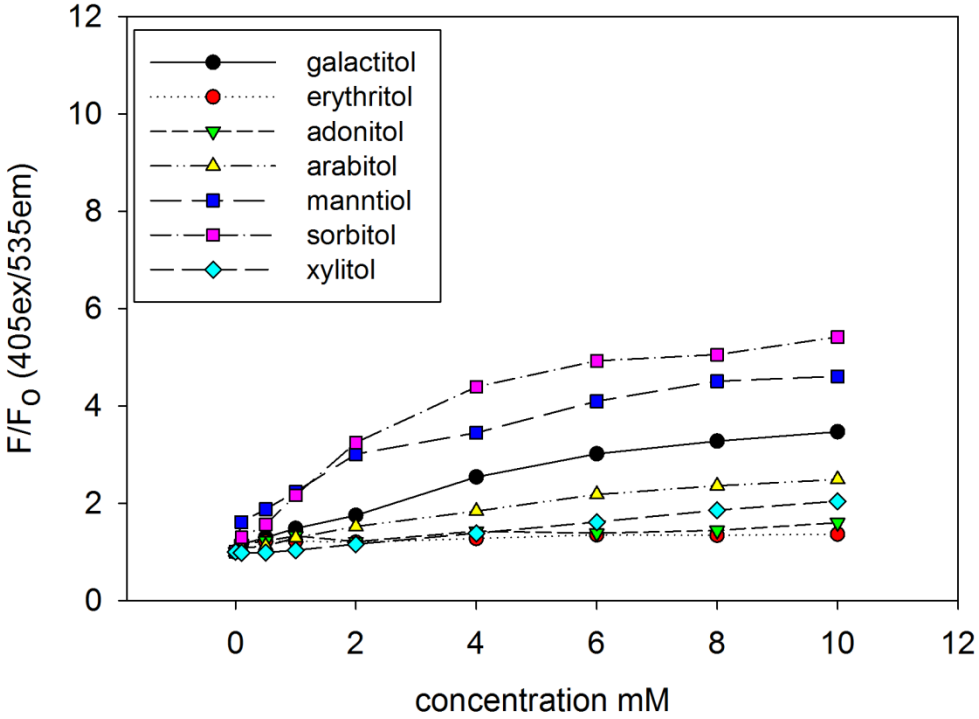


Figure S-5. Binding isotherms of six sugars and 4, 4'-*o,m*-BBV-HPTS combinations.

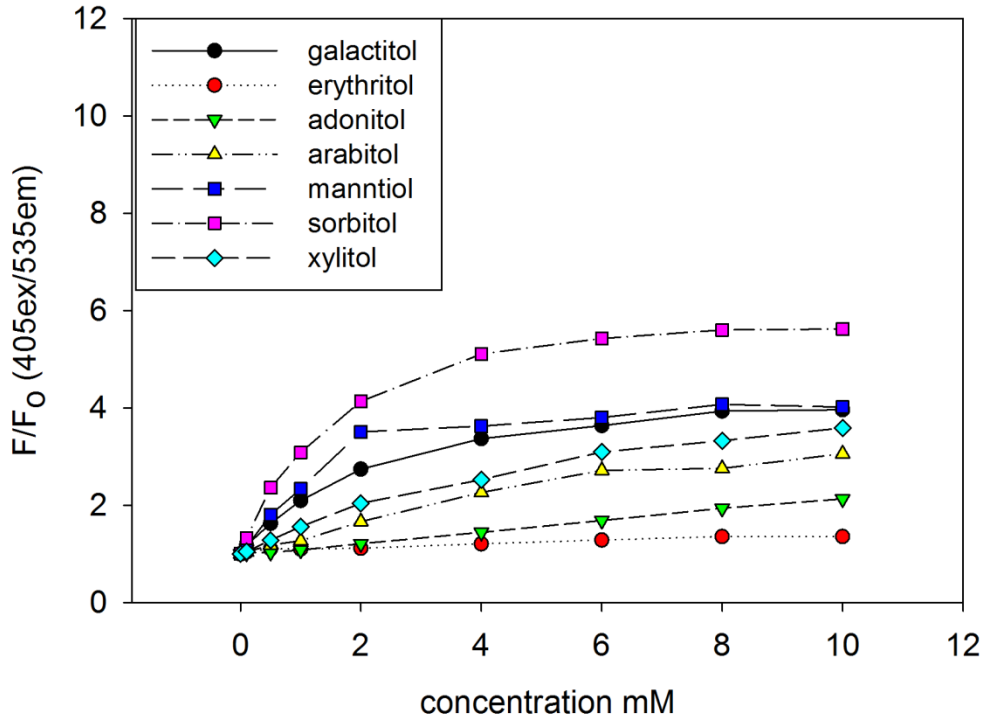


Figure S-6. Binding isotherms of six sugars and 4, 7-*o*-PBBV-HPTS combinations.

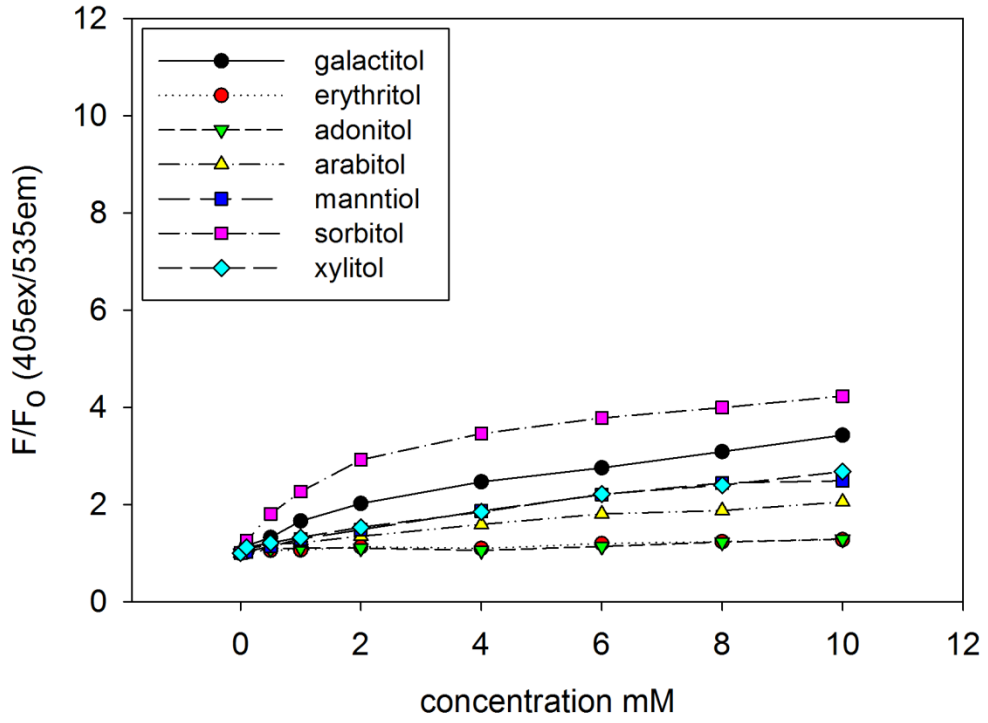


Figure S-7. Binding isotherms of six sugars and pB*o*B-HPTS combinations.

Sugar alcohol/ BBV	galactitol	erythritol	adonitol	arabitol	mannitol	sorbitol	xylitol
4,4'-*o*-BBV	111±21	15±7	19±8	29±9	88±13	290±19	82±22
3,4'-*o*-BBV	270±30	13±5	16±9	19±6	315±20	308±43	280±25
3,3'-*o*-BBV	305±45	19±7	106±32	122±19	129±25	370±30	105±23
4,4'-*o,m*-BBV	117±20	13±7	16±5	106±12	147±28	238±38	29±9
4, 7-*o*-PBBV	431±20	15±8	25±6	103±22	340±46	664±39	146±13
pB*o*B	192±33	11±6	14±8	120±28	101±16	512±32	84±19

Table S-1. Apparent stability constants (K_b, M^{-1}) for each BBV and sugar alcohol

Apparent stability constants were determined by non-linear curve fitting using Eq. 1.

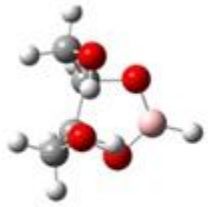
$$\frac{F}{F_o} = \frac{\left(1 + \frac{F_{max}}{F_o}\right) K_b[A]}{1 + K_b[A]}$$

where F_0 is the fluorescence intensity of the quenched dye, F is the fluorescence intensity after the addition of analyte, F_{max} is the fluorescence intensity at which no further signal is obtained with further analyte addition, K_b is the apparent stability constant, and [A] is analyte concentration. K_b was solved using OriginLab software (Originlab Corp, Northampton, MA, USA).

4. Energy minimization calculations

B3LYP/6-31+G* minimized structures of the lowest energy conformers described in the text

Trigonal Erythritol Borane **(*Erythro*-1-2-)**



Energy = -483.677523 hartree

C	-0.837779	0.243774	0.855190
C	0.281655	-0.842778	0.742574
H	-1.215614	0.274388	1.884532
H	0.201453	-1.519054	1.604801
O	-1.891157	-0.281671	0.015831
O	-0.070143	-1.601973	-0.438144
B	-1.363236	-1.260858	-0.772279
H	-1.957883	-1.791512	-1.651682
C	-0.525465	1.687537	0.442927
H	0.212862	2.102989	1.150215
H	-1.449074	2.263678	0.558178
C	1.750715	-0.428990	0.629292
H	2.008454	0.307934	1.396016
H	2.375438	-1.317406	0.790625
O	-0.115127	1.854122	-0.897470
H	0.777973	1.464859	-0.984290
O	2.075839	0.161308	-0.629316
H	1.852521	-0.493146	-1.314093

Trigonal Threitol Borane (*Threo*-1-2-)

Energy = -483.6805357 hartree

C	-0.473153	-0.239611	-0.613717
C	0.473142	-0.239648	0.613729
H	0.049678	-0.590882	-1.511336
O	-0.804147	1.158767	-0.818805
O	0.804208	1.158719	0.818840
B	0.000060	1.905705	0.000010
C	-1.762809	-1.032144	-0.430122
H	-1.524633	-2.082298	-0.227633
H	-2.345503	-0.990317	-1.361384
O	-2.532870	-0.577680	0.674071
H	-2.806838	0.334972	0.486548
H	-0.049684	-0.590892	1.511356
C	1.762791	-1.032208	0.430103
H	2.345494	-0.990431	1.361365
H	1.524626	-2.082353	0.227554
O	2.532806	-0.577706	-0.674087
H	2.806640	0.334991	-0.486598
H	0.000129	3.093543	-0.000026

Tetrahedral Erythritol Borate ester **(*Erythro*-1-2-)**

Energy = -559.5865407 hartree

C	-0.608907	-1.000068	0.573992
C	0.114234	0.194360	1.277833
H	-0.708971	-1.794887	1.346342
H	-0.128117	0.198013	2.356032
O	0.275984	-1.423313	-0.430429
O	1.482332	-0.115575	1.112474
B	1.632827	-0.856727	-0.158117
C	-2.041600	-0.796899	0.061262
H	-2.664815	-0.495269	0.926730
H	-2.411759	-1.775653	-0.276037
C	-0.200407	1.610393	0.767067
H	-1.232937	1.886959	1.025634
H	0.479007	2.304921	1.287325
O	-2.236739	0.111702	-1.011099
H	-1.456723	0.712790	-1.067881
O	-0.077890	1.773309	-0.644599
H	0.741963	1.276226	-0.944575
H	2.499314	-1.725401	-0.096982
O	1.970432	0.145849	-1.244765
H	1.826034	-0.286548	-2.099568

Tetrahedral Threitol Borate ester **(*Threo*-1-2-)**

Energy = -559.5901149 hartree

C	-0.786590	-0.378628	-0.288439
C	0.309563	-0.868041	0.701529
H	-0.650189	-0.820102	-1.288330
O	-0.651852	1.030312	-0.364776
O	0.739503	0.293963	1.378903
B	0.493645	1.459242	0.513640
C	-2.207001	-0.680674	0.207847
H	-2.270223	-0.452416	1.284839
H	-2.470224	-1.736282	0.056742
O	-3.159223	0.103302	-0.512444
H	-2.689596	0.954159	-0.631514
H	0.210520	2.473669	1.141946
O	1.720844	1.675180	-0.337834
H	1.469862	2.229566	-1.091040
H	-0.122112	-1.567638	1.443458
C	1.472420	-1.620485	0.032829
H	2.209696	-1.854148	0.822296
H	1.096394	-2.576689	-0.366177
O	2.090685	-0.939305	-1.043141
H	2.097646	0.033038	-0.828690

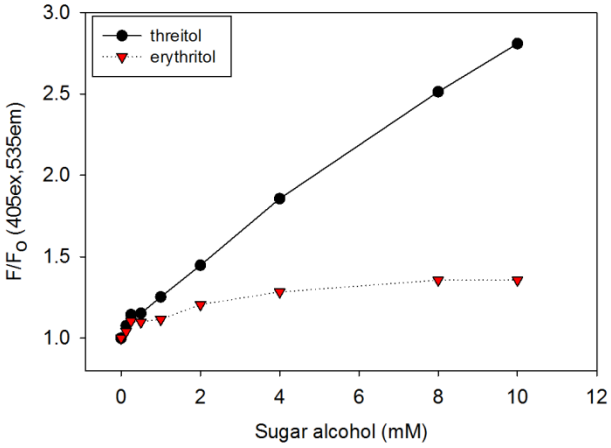
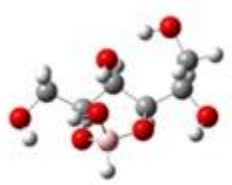


Figure. S-8. Binding isotherm of threitol and erythritol with 4,7-o-PBBV in buffer.

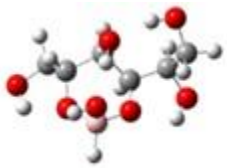
Sorbitol Boronate ester **(*Syn*-1-3-)**



Energy = -712.747835 hartree

C	0.656660	-0.772771	-0.428955
C	-0.653414	-0.054123	-0.769877
C	1.837604	0.140514	-0.803851
H	-0.686360	0.099973	-1.855082
H	0.717242	-1.678192	-1.056367
H	1.813544	0.299716	-1.891802
B	0.480139	1.908503	0.158575
O	-0.679096	1.257296	-0.148624
O	1.720786	1.419081	-0.154589
C	3.215126	-0.417914	-0.449037
H	3.989846	0.264027	-0.822556
H	3.357252	-1.395585	-0.920016
C	-1.933807	-0.836650	-0.403739
H	-1.705769	-1.903936	-0.516650
O	0.640011	-1.122966	0.947813
H	1.563032	-1.238693	1.247607
O	3.373727	-0.627873	0.957997
H	3.356387	0.243991	1.387801
O	-2.968165	-0.540609	-1.337887
H	-3.513839	0.155922	-0.925680
C	-2.449510	-0.583852	1.024675
H	-1.630251	-0.632956	1.747782
H	-3.194646	-1.344398	1.274026
O	-3.142468	0.667184	1.091638
H	-2.475708	1.362034	0.952022
H	0.414248	2.957453	0.719949

D-mannitol Boronate ester **(*Anti*-1-3-)**



Energy = -712.7421689 hartree

C	0.654279	0.723500	0.834816
C	-0.801644	0.207077	0.804946
C	1.513762	0.003566	-0.213836
H	-1.332906	0.624202	1.667984
H	1.052786	0.463942	1.825755
O	-0.801109	-1.225565	0.972293
O	1.360464	-1.430749	-0.068944
C	-1.618403	0.533494	-0.461659
H	-1.123039	0.104828	-1.344615
O	0.759363	2.130735	0.721785
H	0.041056	2.447444	0.141476
O	-1.644252	1.969970	-0.555281
H	-2.074956	2.233488	-1.384007
C	-3.058362	-0.010222	-0.394950
H	-3.643470	0.457902	-1.195643
H	-3.514453	0.283895	0.563319
O	-3.149161	-1.406086	-0.615868
H	-2.628229	-1.846920	0.076577
H	1.193204	0.266436	-1.230214
C	3.003117	0.304765	-0.082872
H	3.173567	1.373611	-0.227828
H	3.342412	0.030158	0.928600
O	3.761177	-0.371682	-1.075099
H	3.571174	-1.320933	-0.992578
C	0.259487	-1.973639	0.515491
H	0.197060	-3.155910	0.643963

5. Analysis of permeability markers in urine

Baseline urine was obtained from healthy volunteers who had consumed 0.5-1.0 L of water 3 h prior to urine collection. From the urine that was collected, 2 mL was subjected to a C18 solid phase extraction twice to remove majority of the hydrophobic components found in urine. This purified fraction is considered as the baseline urine without endogenous permeability markers which was then spiked with lactulose or mannitol to obtain lower limits of quantification (LOQ) and detection (LOD) for 4,4'-*o*-BBV, 4,7-*o*-PBBV, and pB*o*B. Lactulose or mannitol were prepared in the baseline urine fraction and serially diluted to obtain five data points. Probe solutions were prepared as a fourfold initial concentration (1.6 mM for 4,4'-*o*-BBV, 800 µM for 4,7-*o*-PBBV, 64 µM for pB*o*B, and 16 µM for HPTS) in 0.1 M sodium phosphate, 0.1 M HEPES, 0.04 % Triton X-100, pH 7.4. Upon running assays, wells for permeability marker (i.e. analyte) analysis received 10 µL of probe (i.e. BBV + HPTS) and 30 µL of analyte solution. Baseline fluorescence ("probe") wells were given 10 µL of the probe and 30 µL of baseline urine. Blank wells received 10 µL of buffer and 30 µL of baseline urine. Completed plates were put on a shaker for 30 minutes and centrifuged at 2500 RCF for 10 min and the fluorescence was read on a plate reader (Tecan M-200 Infinite, gain 70, Exc 405 nm, Em 535 nm).

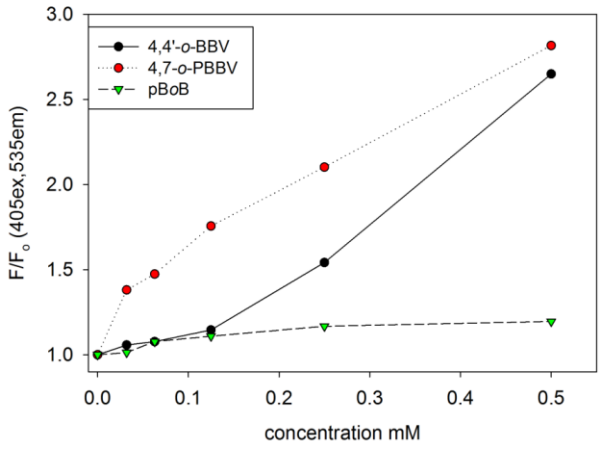


Figure S-9. Binding isotherm of lactulose with 4,4'-*o*-BBV, 4,7-*o*-PBBV, and pB*o*B in human baseline urine.

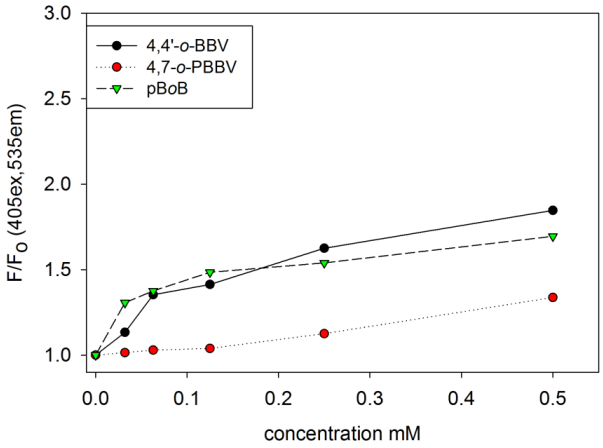


Figure S-10. Binding isotherm of mannitol with 4,4'-o-BBV, 4,7-o-PBBV, and pBoB in human baseline urine .

Table S-2. Measurement of lower limits of detection (LOD) and quantification (LOQ) of lactulose and mannitol.

Receptor	LOD μM (urine, buffer)	LOQ μM (urine, buffer)
Lactulose:		
4,4'-*o*-BBV [a]	113, 88	450 , 350
4,7-*o*-PBBV	40[b], 24 [c]	72, 57
pBoB	275, 52	950,225
Mannitol:		
4,4'-*o*-BBV [a]	560 , 125	750 , 300
4,7-*o*-PBBV	272 [b], 190[c]	650, 400
pB*o*B	900, 650	1400, 1250

Each data point was obtained by generating a standard curve of lactulose in urine or buffer. All data points were measured on two separate runs.[a] Final 4,4'-o-BBV concentration is 400 μM (reproduced as in ref. 20.[b] Final 4,7-o-PBBV concentration is 200 μM, spiked into human urine after C18 SPE.[c] Final 4,7-o-PBBV concentration is 160 μM, spiked into phosphate/HEPES buffer.

6. Analysis of low and increased permeability

Low and increased permeability ratios of lactulose:mannitol (L:M) were prepared in 0.025 M sodium phosphate-HEPES buffer pH 7.4 containing 0.01 % Triton X-100 for the buffer studies and were prepared in human baseline urine for urine studies. The human baseline urine was processed as mentioned above. Each permeability ratio was obtained by preparing a stock solution of each subgroup mixture and serially diluting to the desired concentration in buffer or human baseline urine. Probe solutions were prepared as fourfold concentration (1.6 mM for 4,4'-*o*-BBV, 3,4'-*o*-BBV, 3,3'-*o*-BBV, and 4,4'-*o,m*-BBV. 800 μM for 4,7-*o*-PBBV, 64 μM for pBoB, and 16 μM for HPTS) in 0.1 M sodium phosphate-HEPES buffer pH 7.4 containing 0.04% Triton X-100. Upon running assay, each well received 10 μL of each probe solution and 30 μL of each L:M mixture. Each concentration data point was repeated six times for reproducibility. Completed plates were put on a shaker for 30 minutes and centrifuged at 2500 RCF for 10 minutes and the fluorescence was read on a plate reader (Tecan M-200 Infinite, gain 70, excitation: 405nm, emission: 535nm).

Table S-3. Concentrations of L:M ratios representing low (L) or increased (I) gut permeability. Mixtures were prepared in buffer or urine

L:M Ratio	Subgroup	Concentration (Lactulose/Mannitol) μM
0.1	L1	100/100
	L2	200/2000
	L* (circled in Fig.S11A)	500/5000*
0.2	L3	100/500
	L4	200/1000
	L5	500/2500
0.3	I1	700/2300
	I2	1000/3333
	I3	2000/6000
0.4	I4	700/1750
	I5	1000/2500
	I6	2000/5000
0.5	I7	700/1400
	I8	1000/2000
	I9	2000/4000

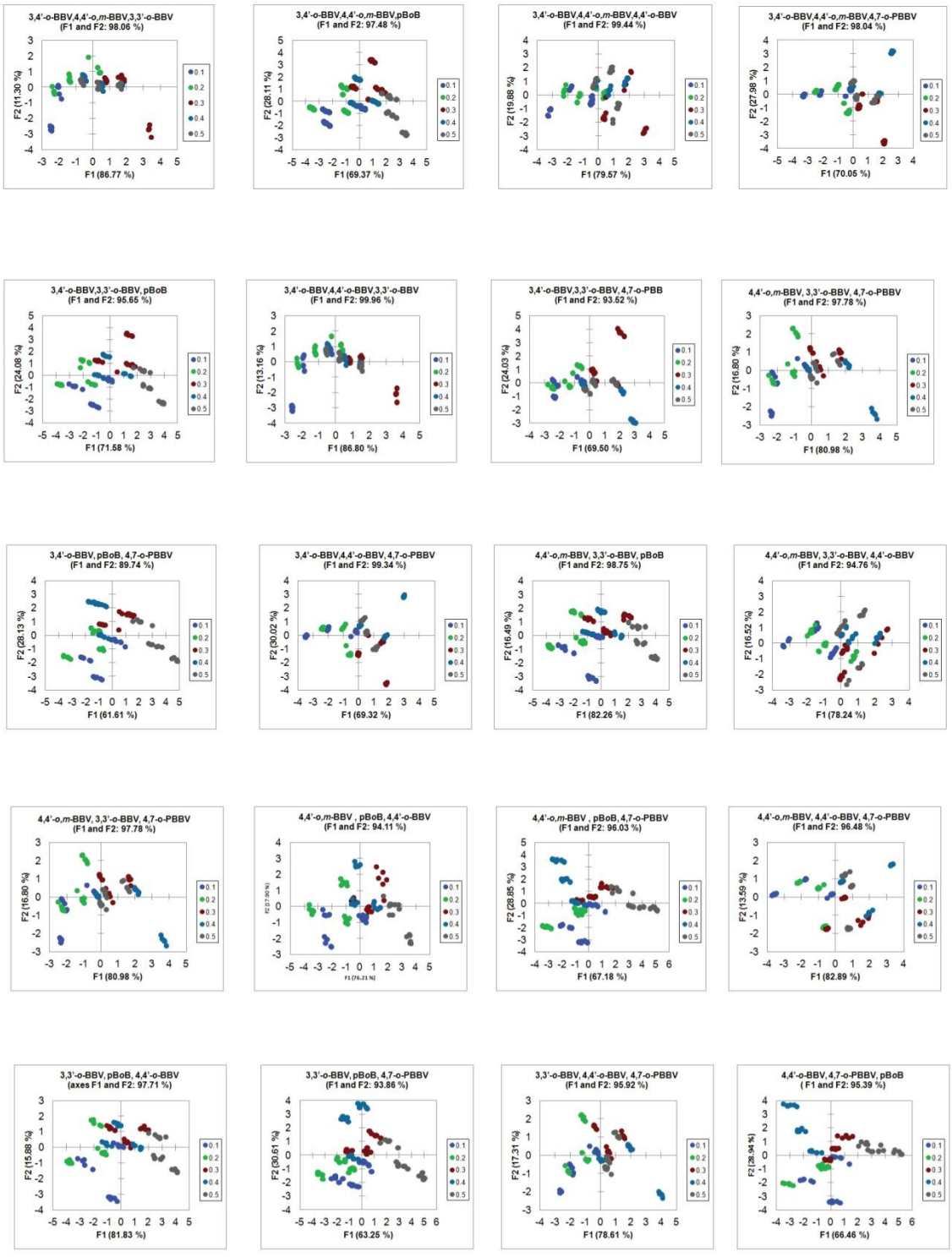


Figure S-11. Linear discriminant analysis of L:M mixtures by 20 boronic acid receptor triads in 0.025 M sodium phosphate-HEPES buffer pH 7.4 containing 0.01 % Triton X-100 buffer.

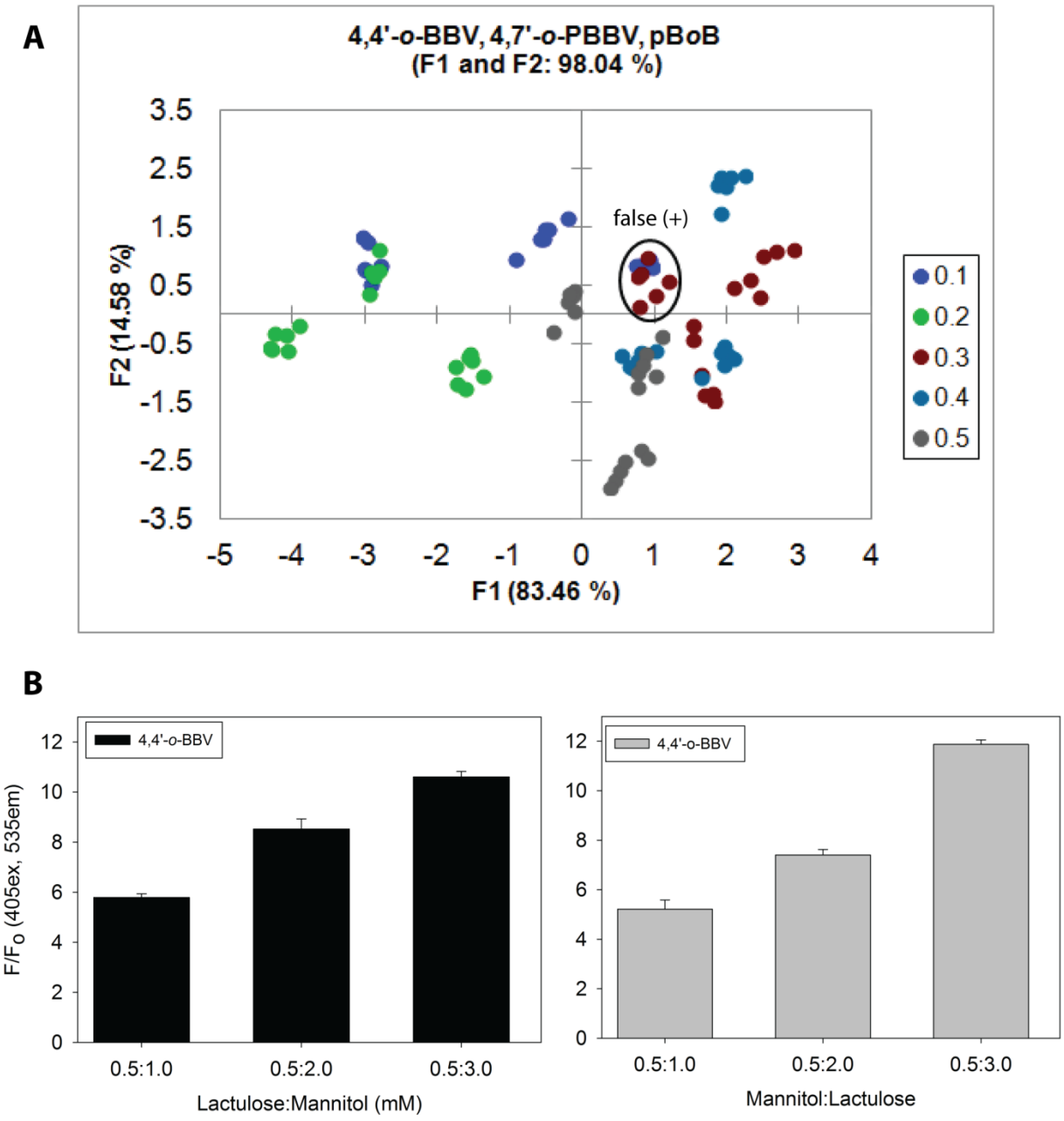


Figure S-12. **A.** Linear discriminant analysis (LDA) of mixtures of lactulose and mannitol with low (L1-L5) and increased (I1-I9) L:M ratios by boronic acid receptor triads (4,4'-o-BBV, 4,7-o-PBBV, and pBoB) in 0.025 M sodium phosphate-HEPES buffer pH 7.4 containing 0.01 % Triton X-100 buffer. **B.** Normalized fluorescence intensity using 4,4'-o-BBV and HPTS with different L:M ratio using lactulose (0.5 mM) with increasing mannitol (1 mM, 2 mM, and 3 mM) on left. Mannitol (0.5 mM) with increasing lactulose (1mM, 2 mM, and 3mM) on right.

7. ^{1}H and ^{13}C NMR Spectra

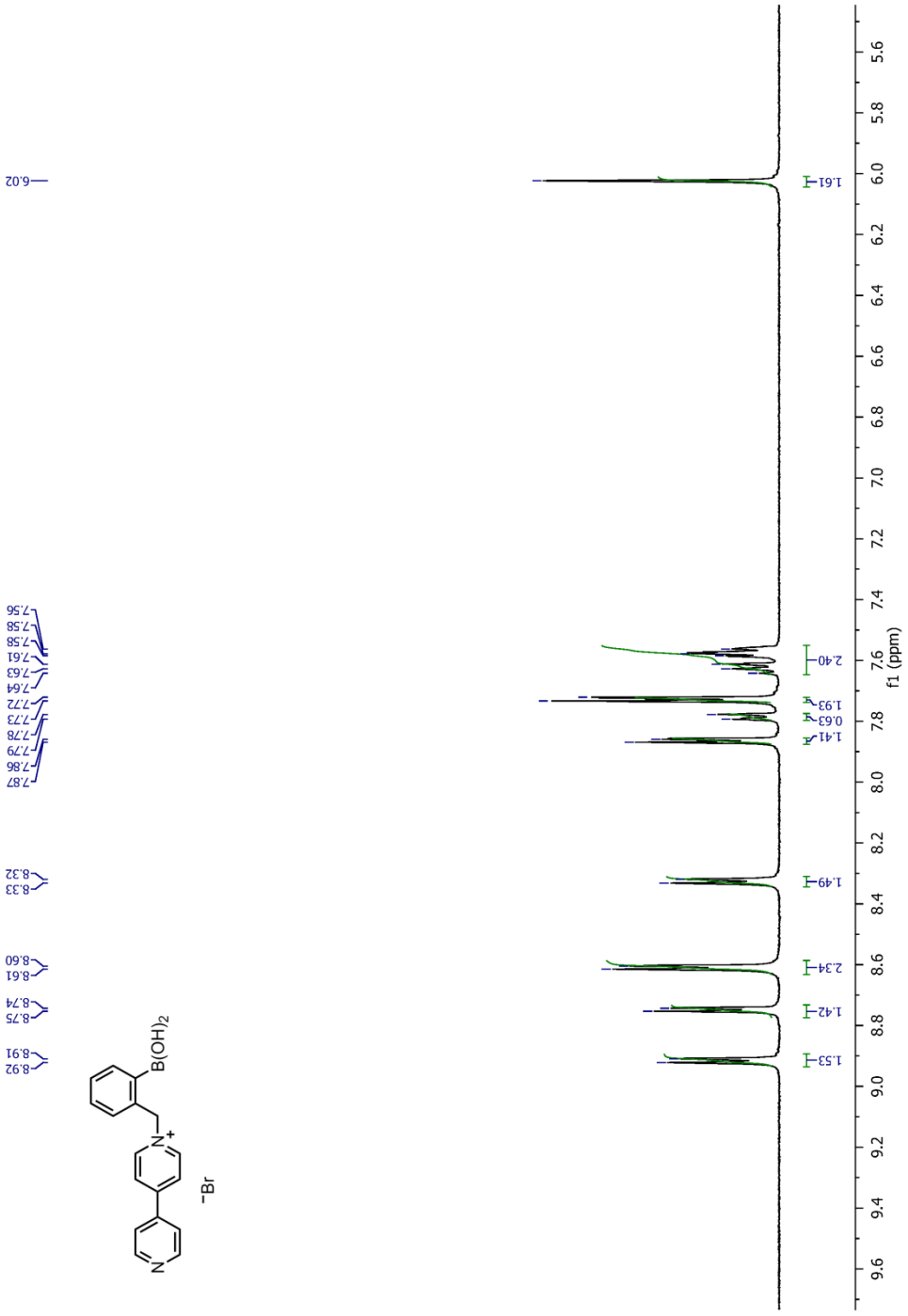


Figure S-13. ^{1}H NMR of monoalkylated bipyridyl boronic acid salt

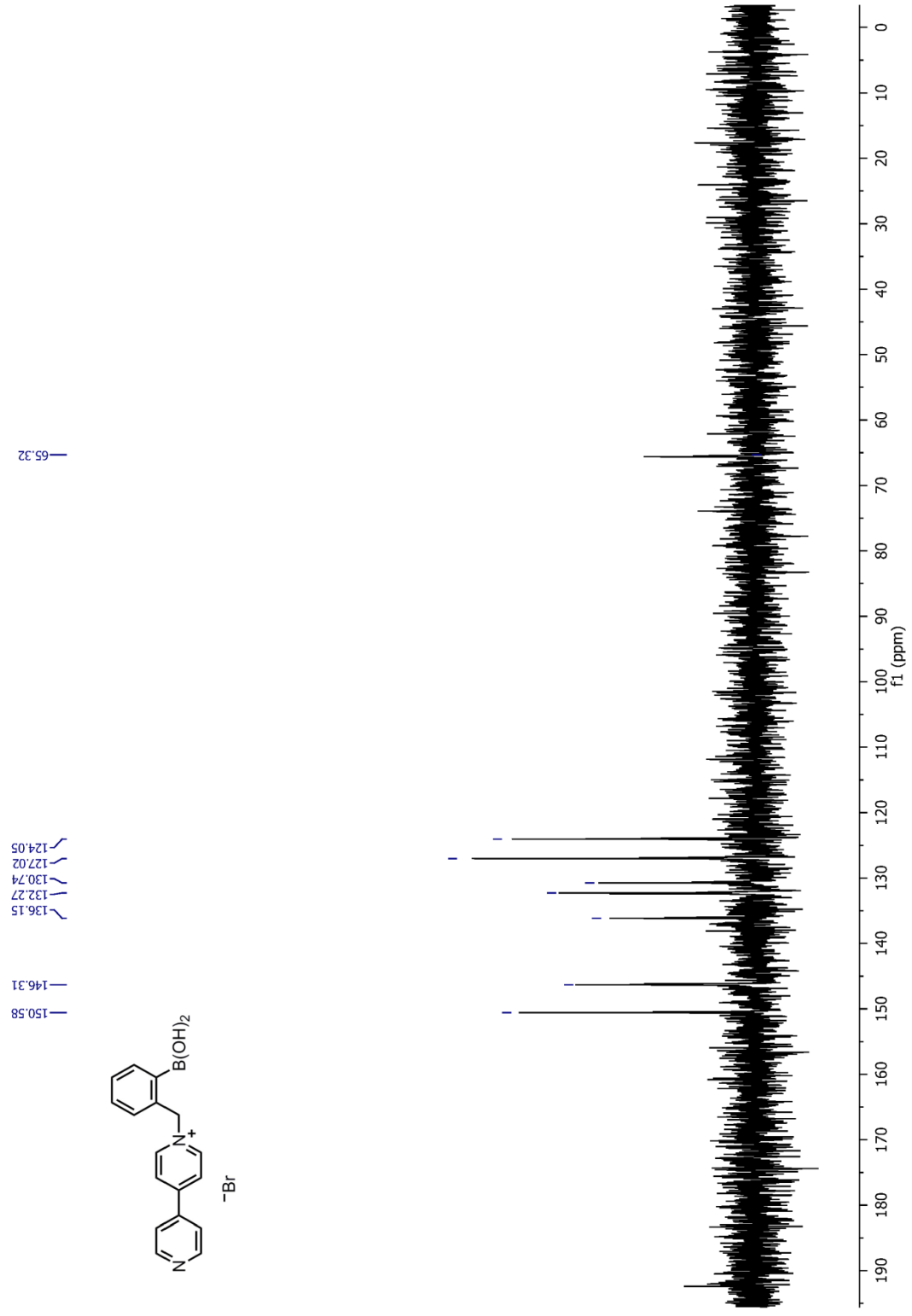


Figure S-14. ^{13}C NMR of monoalkylated bipyridyl boronic acid salt

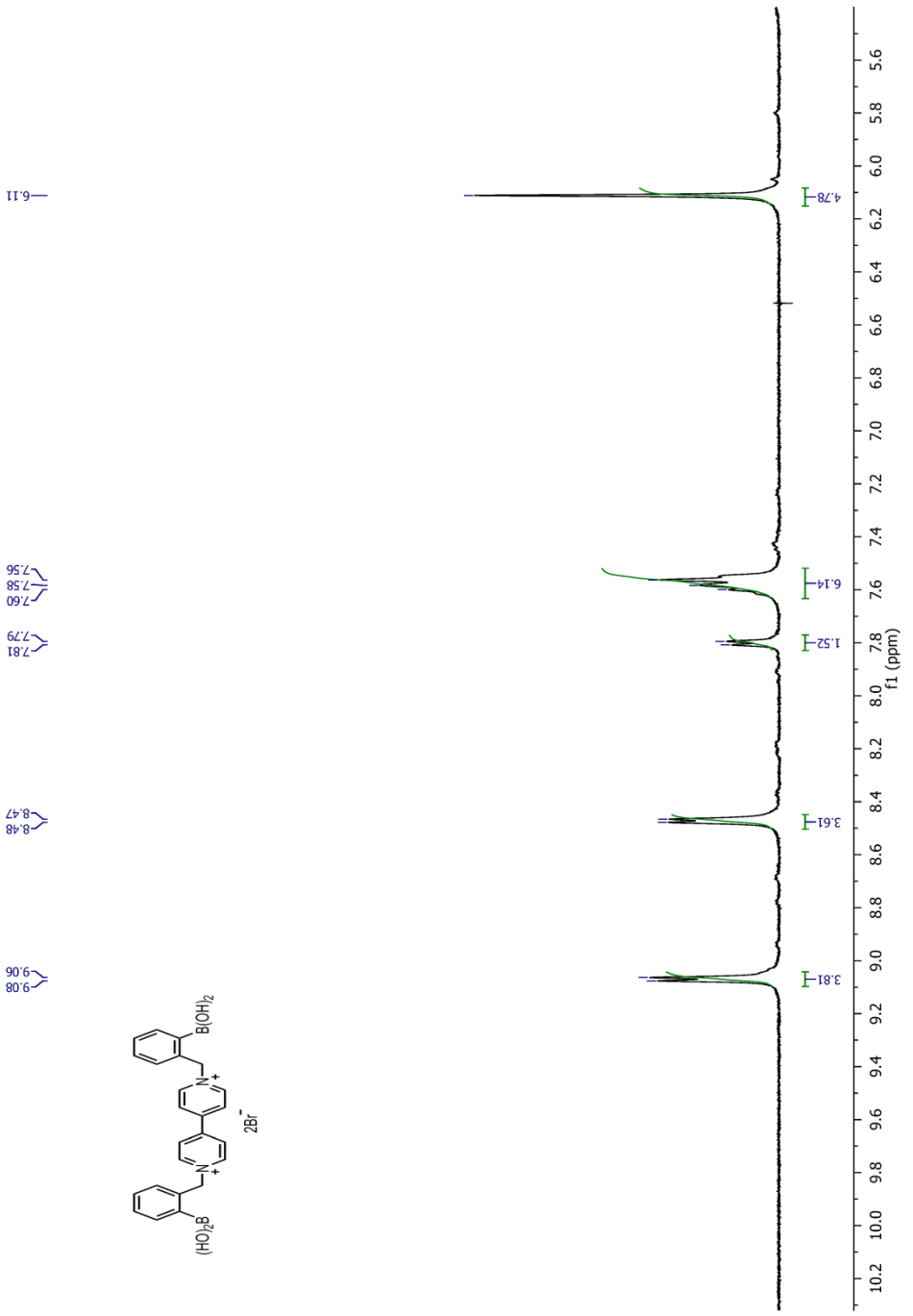


Figure S-15. ^{1}H NMR of 4,4'-*o*-BBV

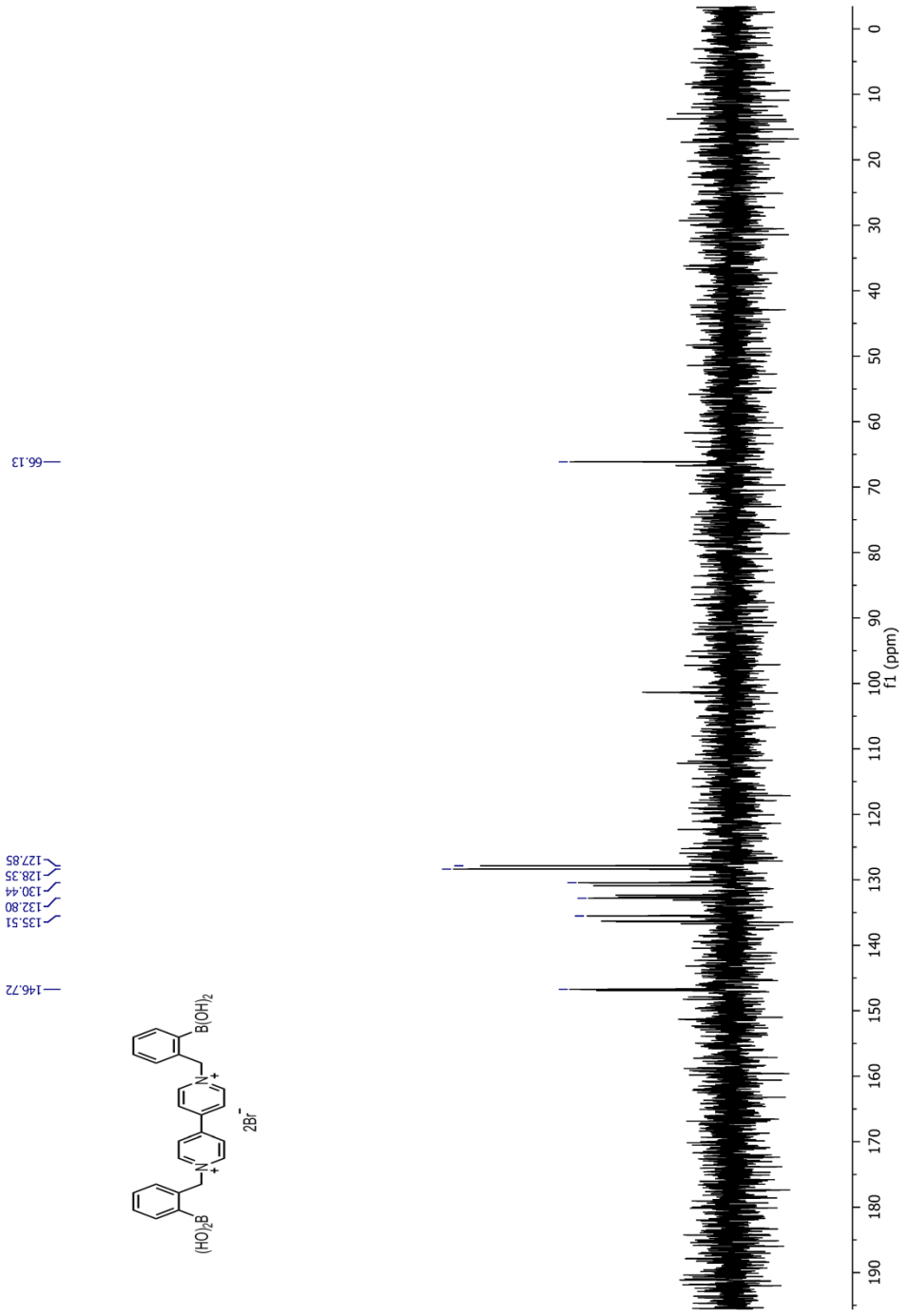


Figure S-16. ^{13}C NMR of 4,4'-*o*-BBV

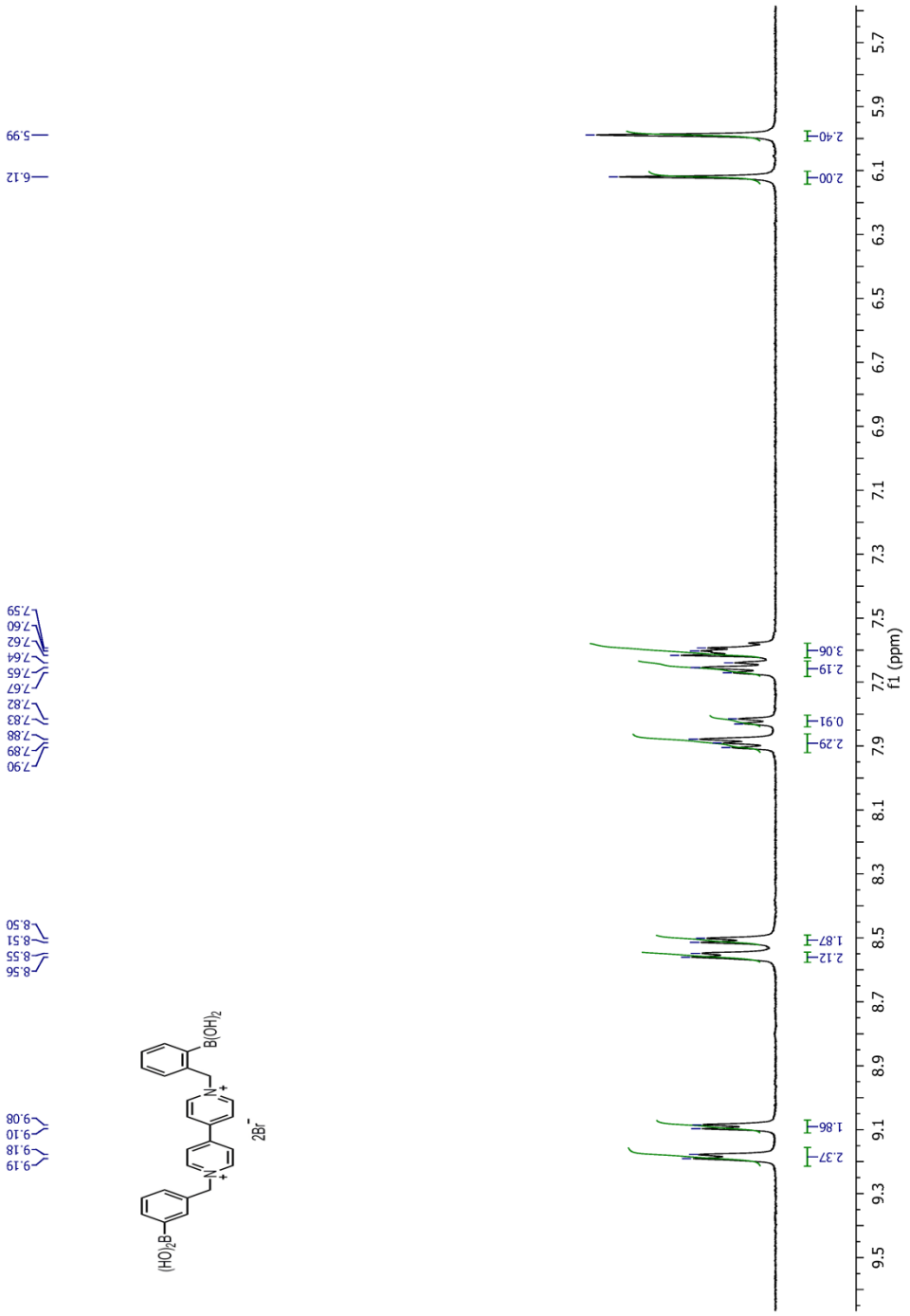


Figure S-17. ^{1}H NMR of 4,4'-*o,m*-BBV

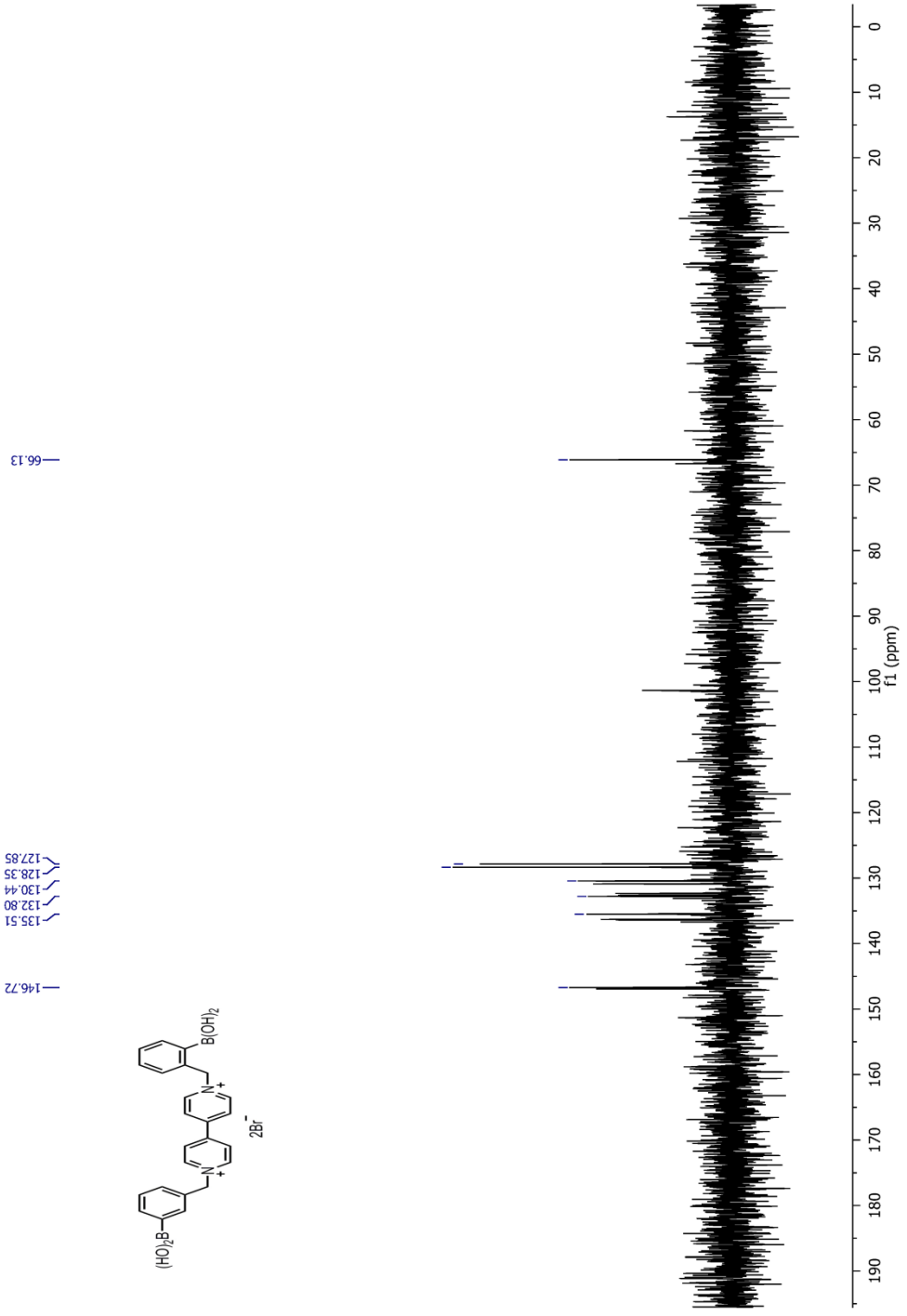


Figure S-18. ^{13}C NMR of 4,4'-*o,m*-BBV

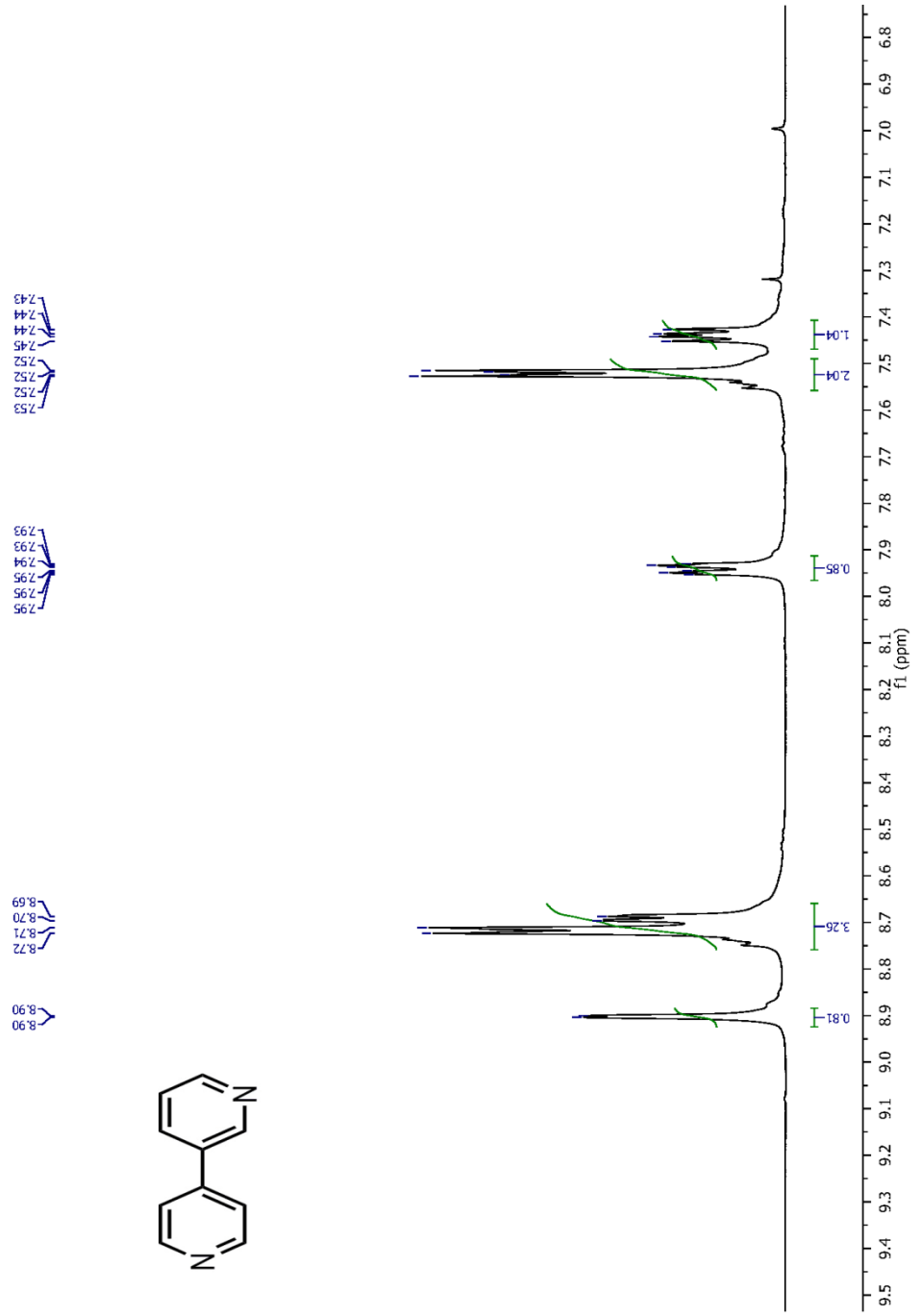


Figure S-19. ^{1}H NMR of 3,4'- bipyridyl

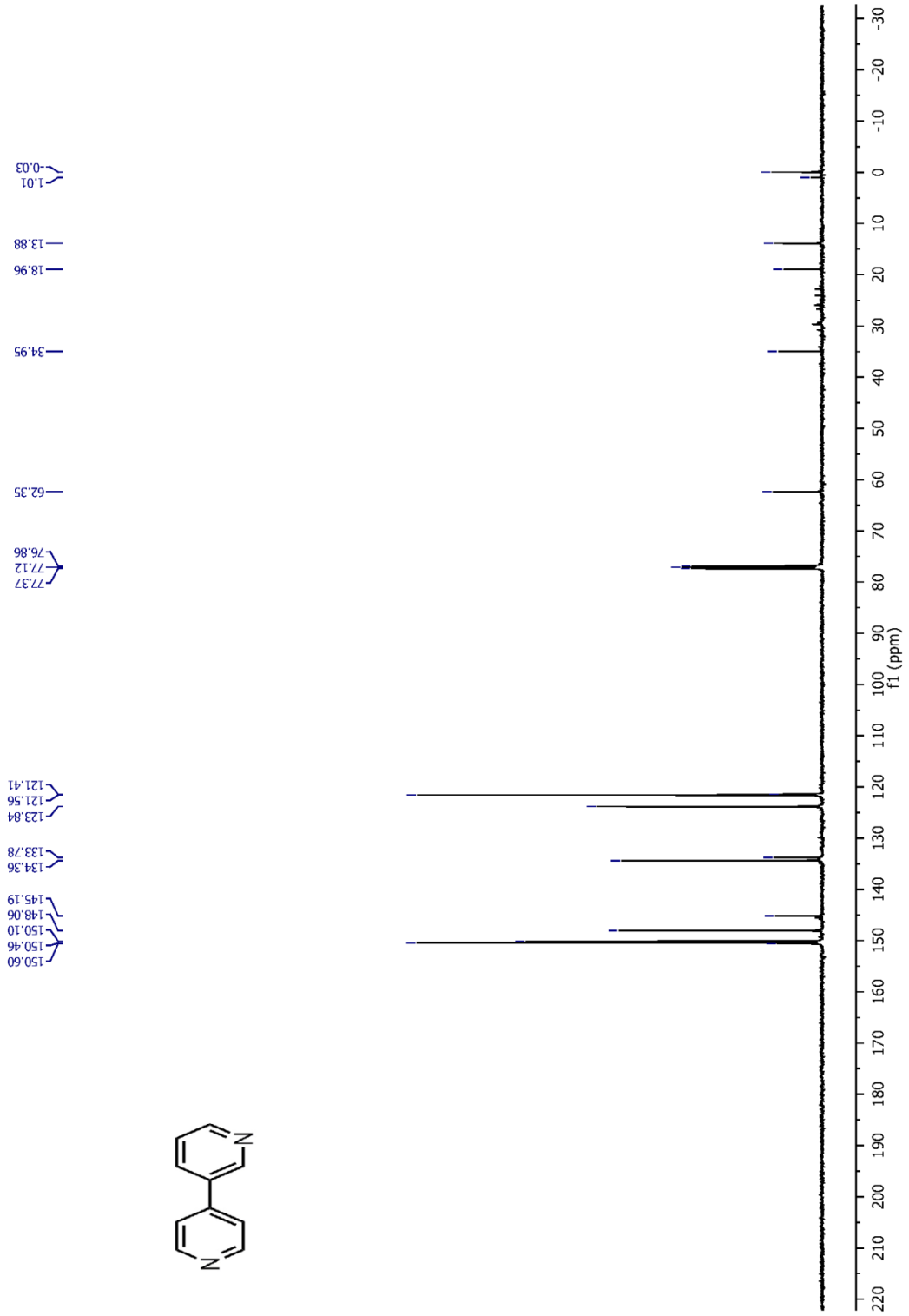


Figure S-20. ^{13}C NMR of 3,4'- bipyridyl

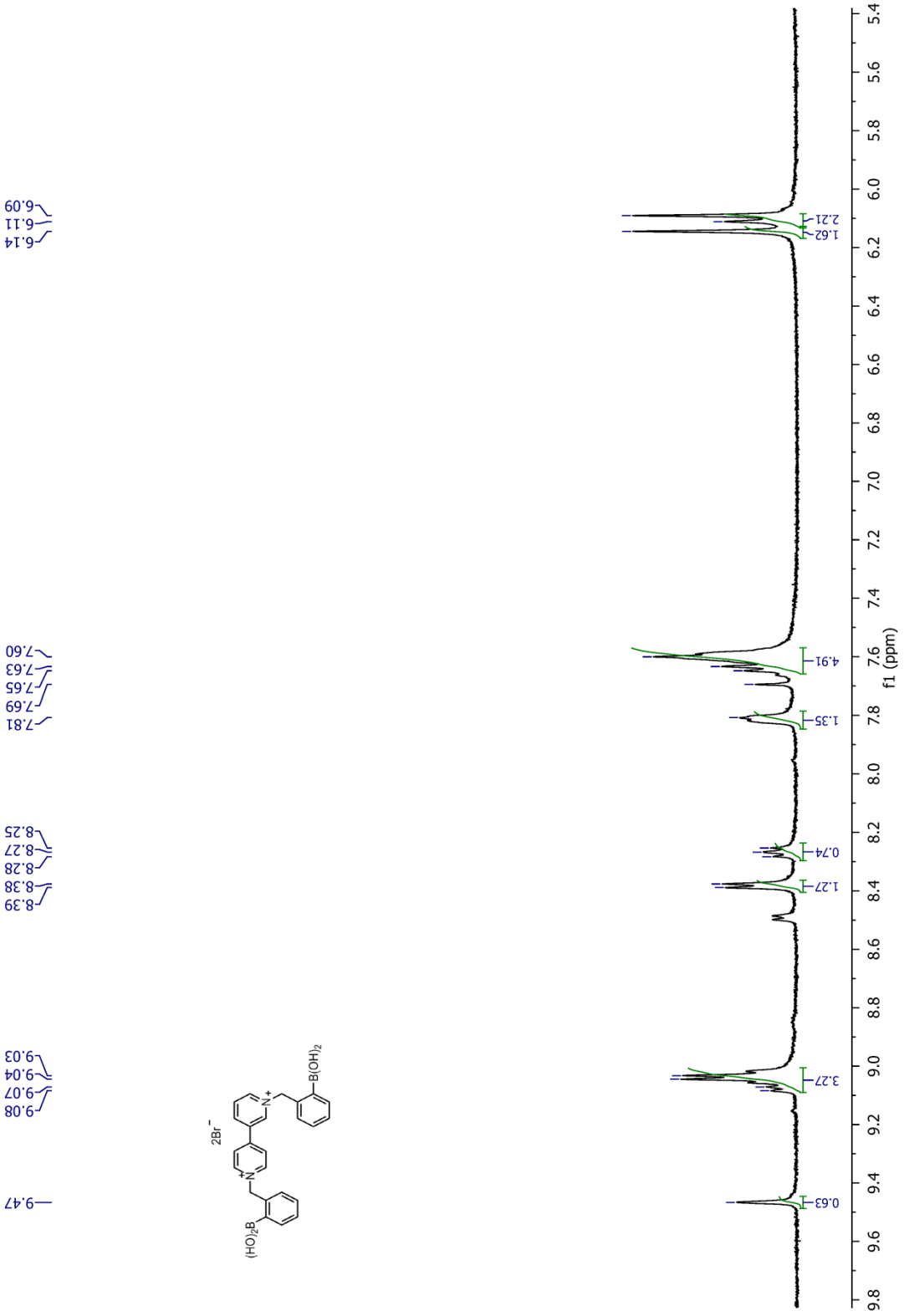


Figure S-21. ^{1}H NMR of 3,4'-*o*-BBV

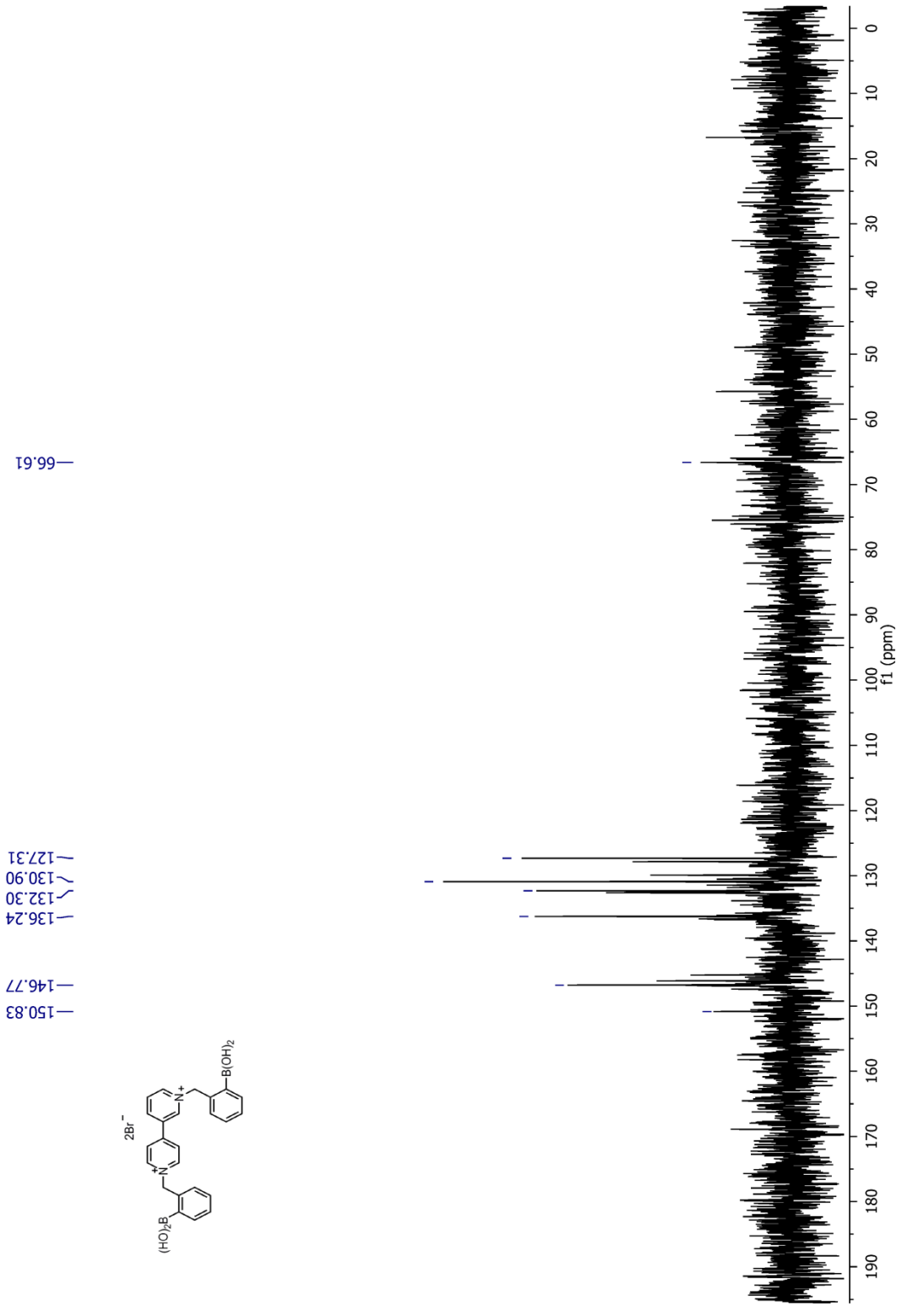


Figure S-22. ^{13}C NMR of 3,4'-*o*-BBV

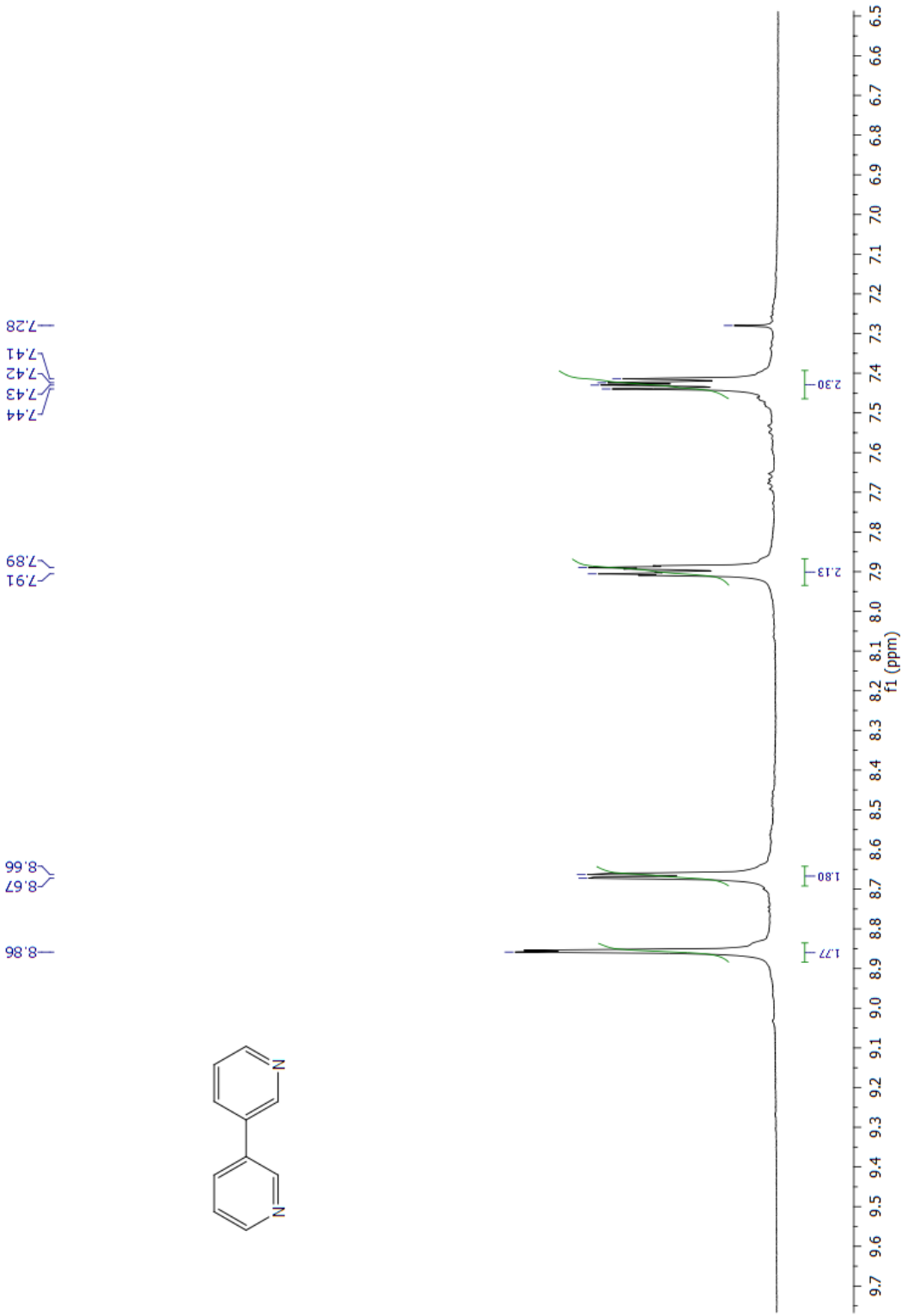


Figure S-23. ^{1}H NMR of 3,3'-bipyridyl

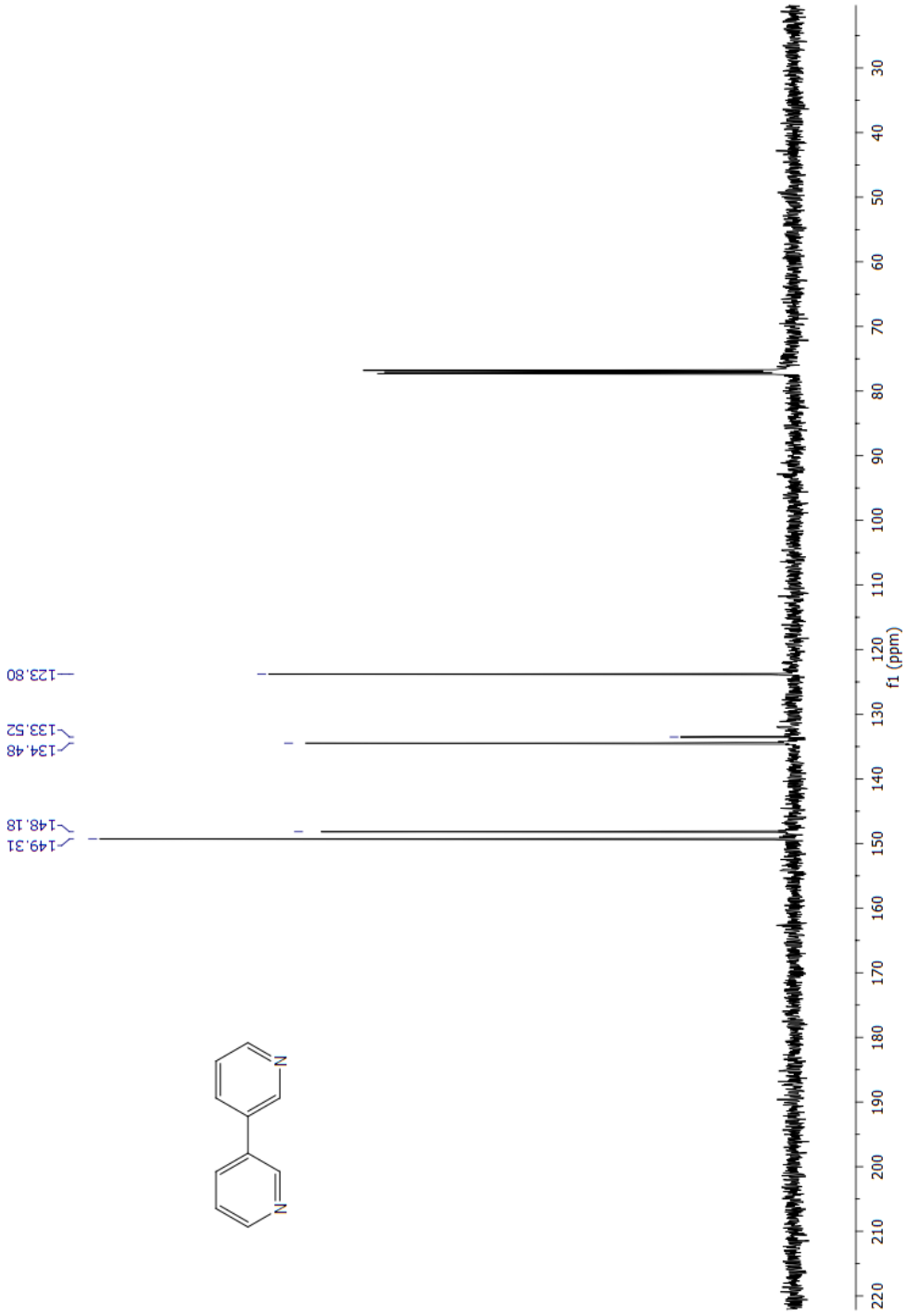


Figure S-24. ^{13}C NMR of 3,3'-bipyridyl

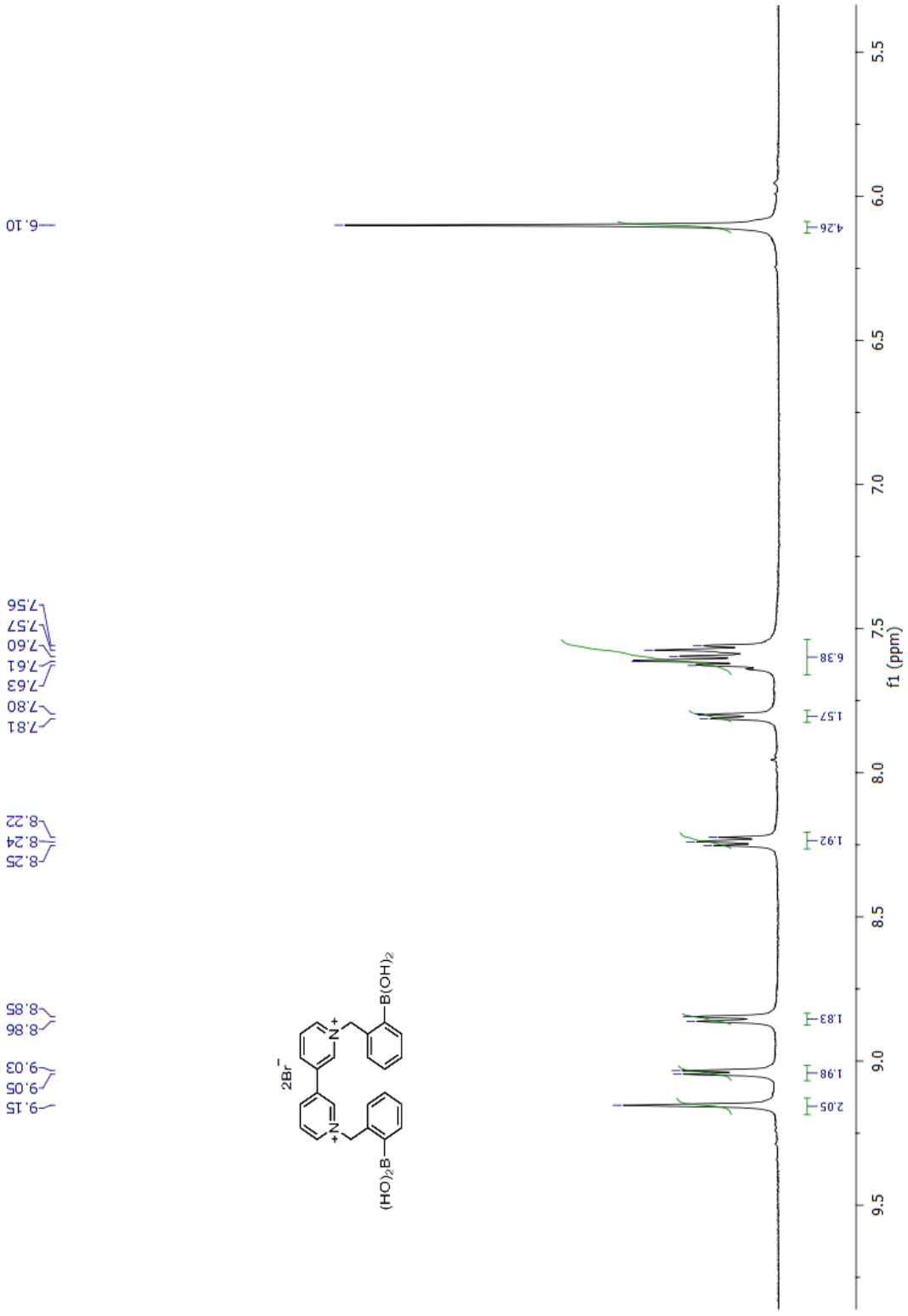


Figure S-25. ^{1}H NMR of 3,3'-*o*-BBV

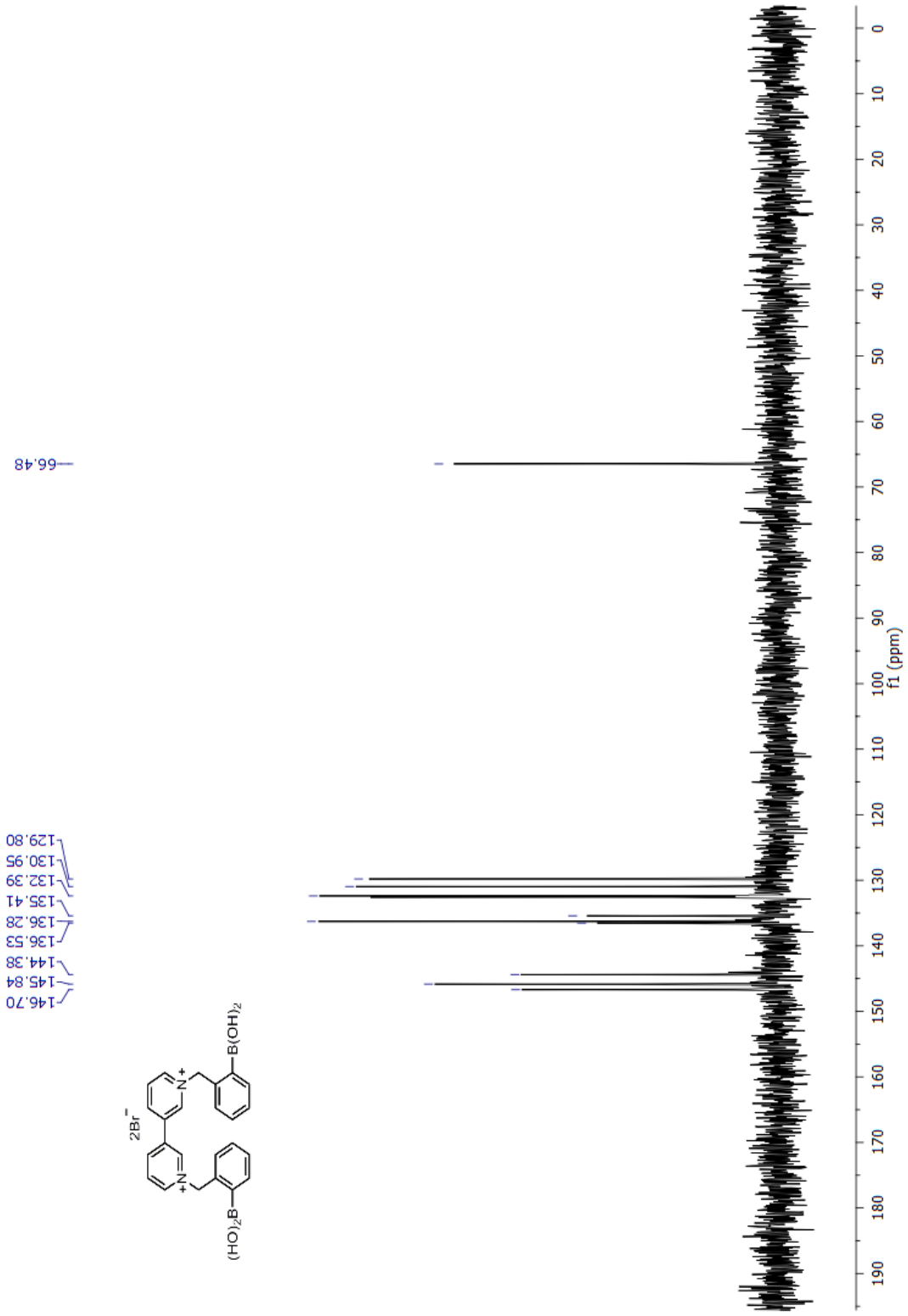


Figure S-26. ^{13}C NMR of 3,3'-*o*-BBV

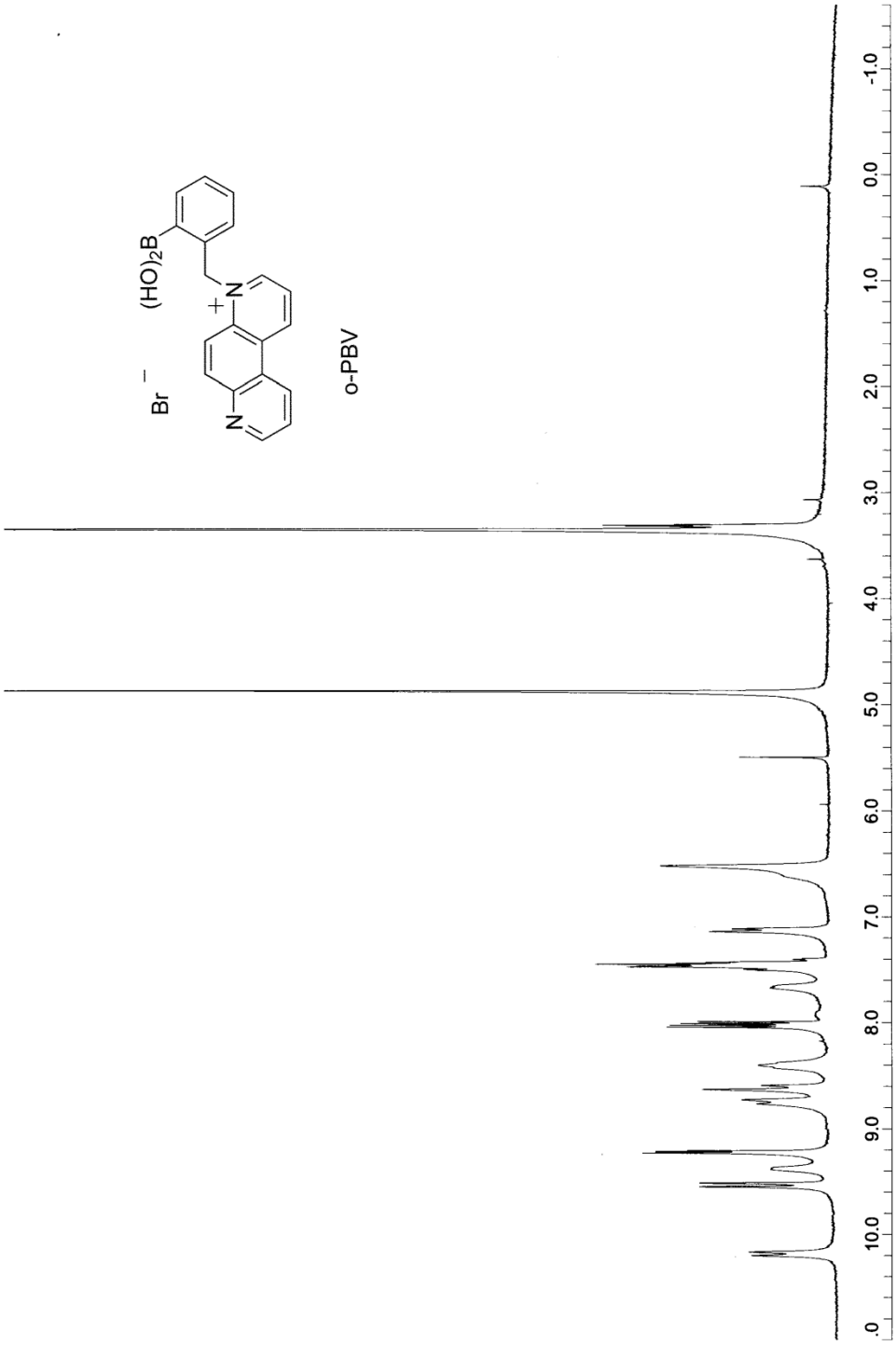


Figure S-27. ^{1}H NMR of *o*-PBV

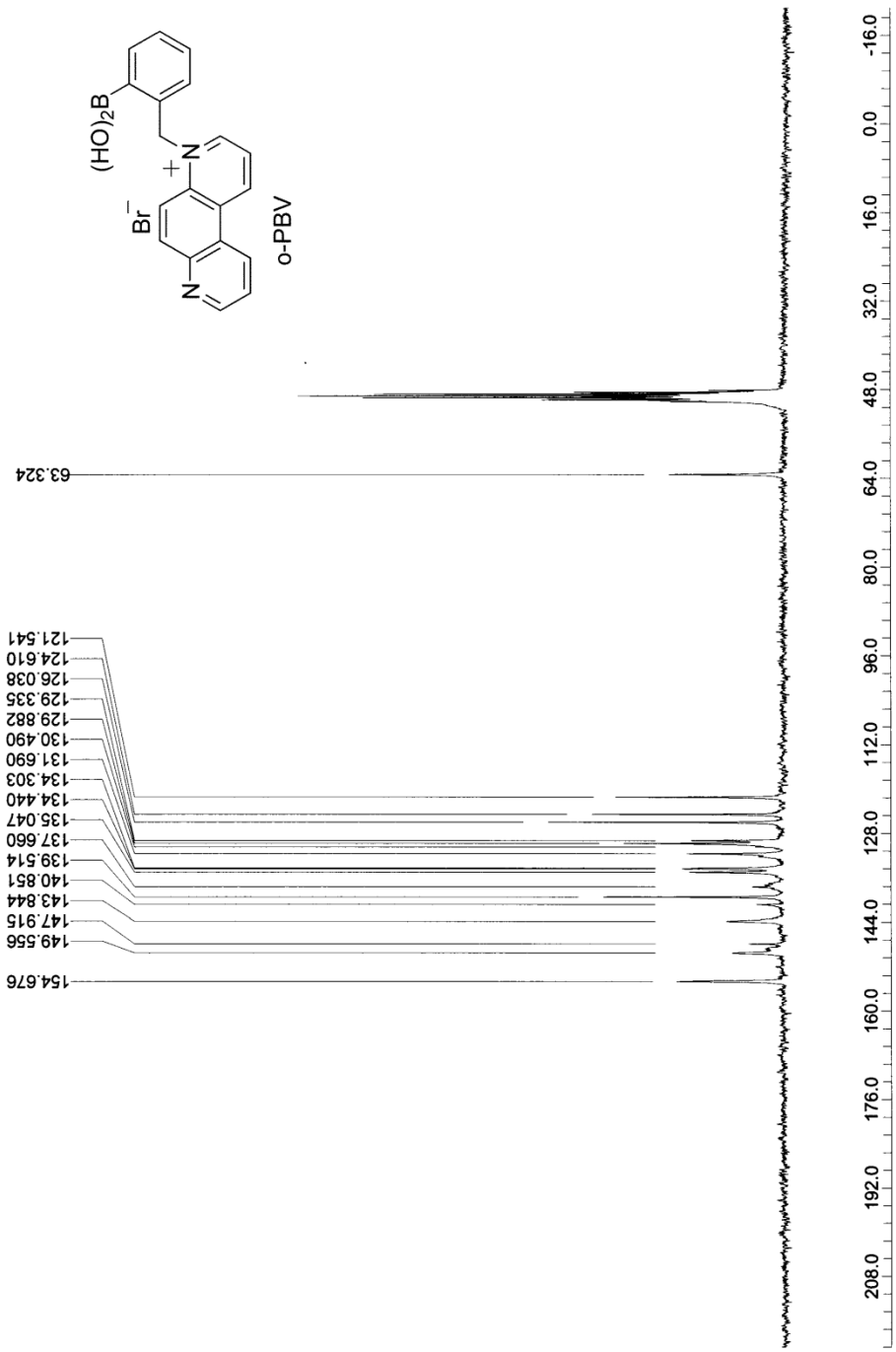


Figure S-28. ^{13}C NMR of *o*-PBV

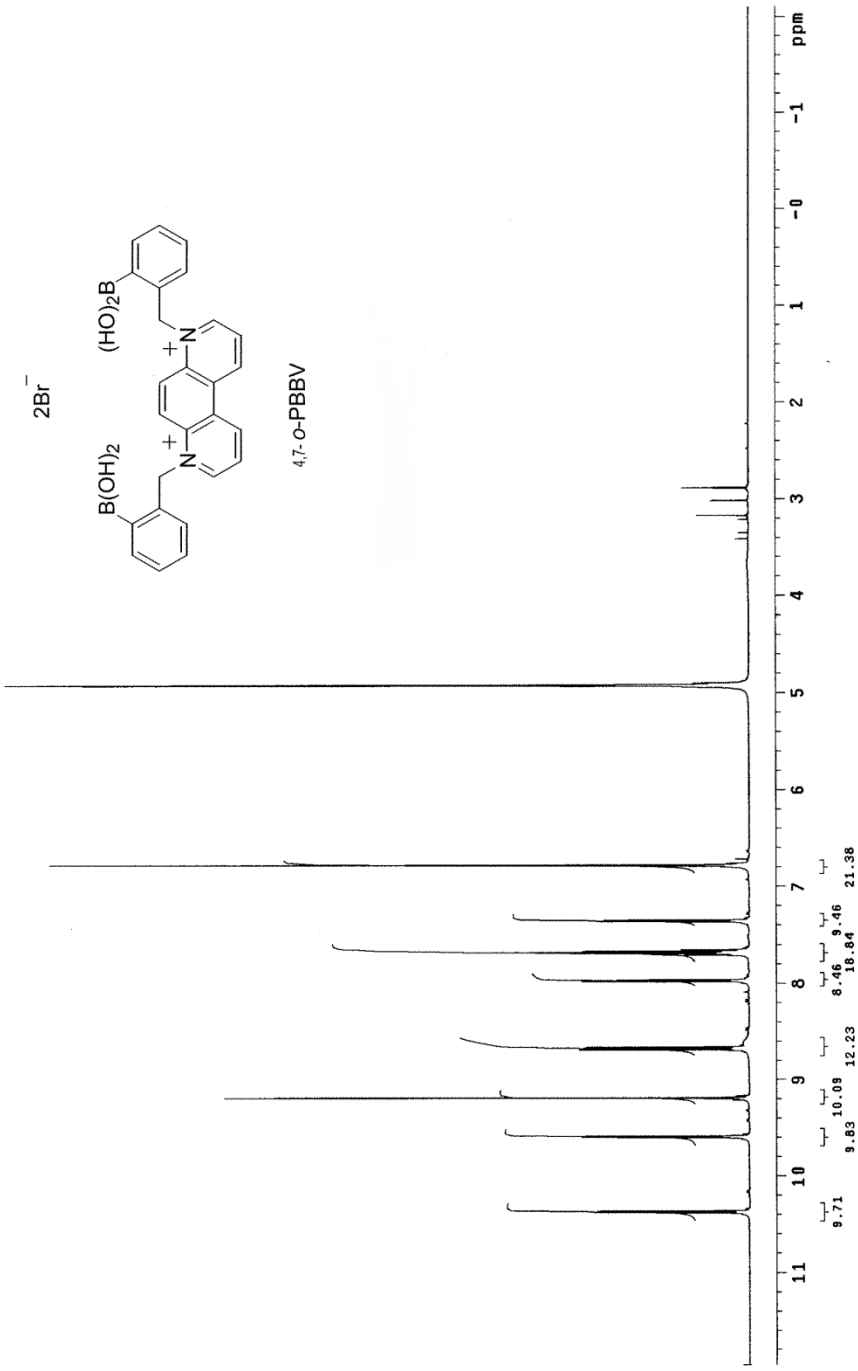


Figure S-29. ^{1}H NMR of 4,7-*o*-PBBV

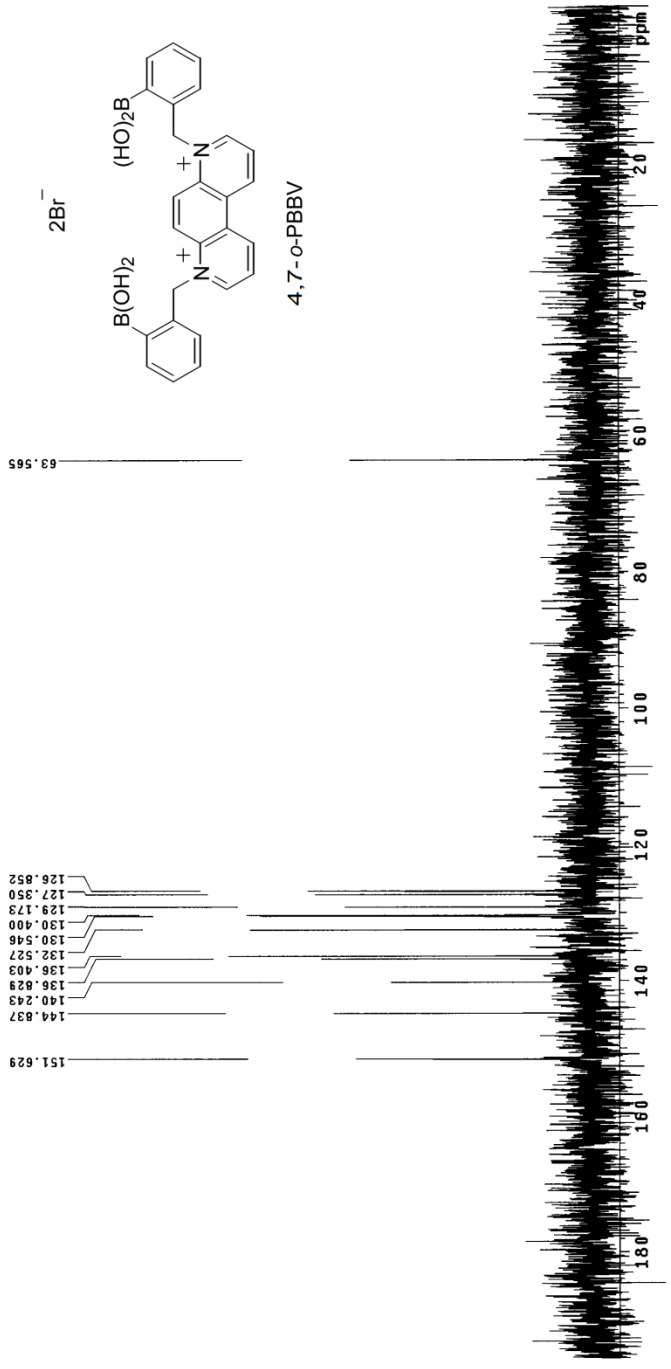


Figure S-30. ^{13}C NMR of 4,7-*o*-PBBV

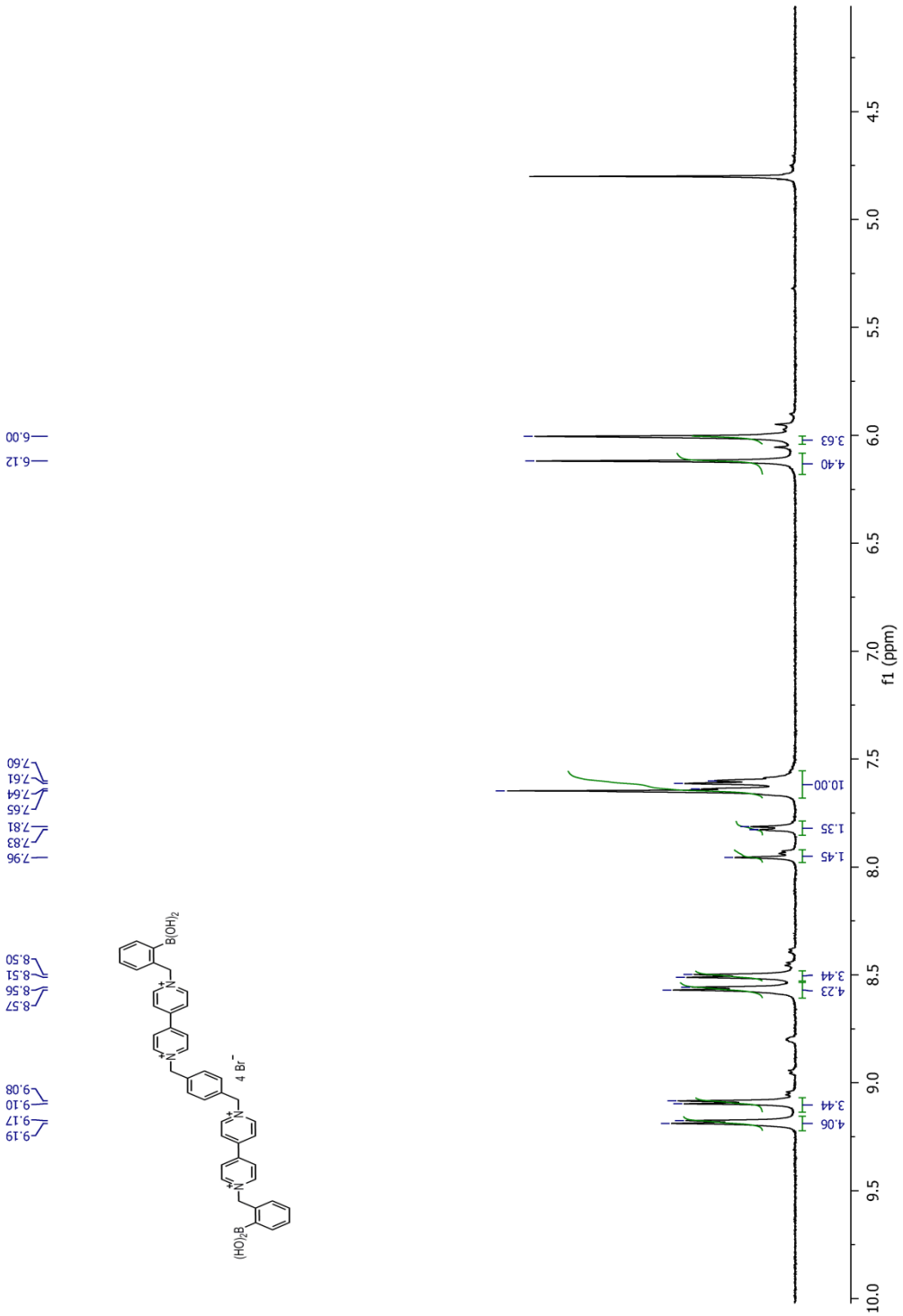


Figure S-31. ^{1}H NMR of pBoB

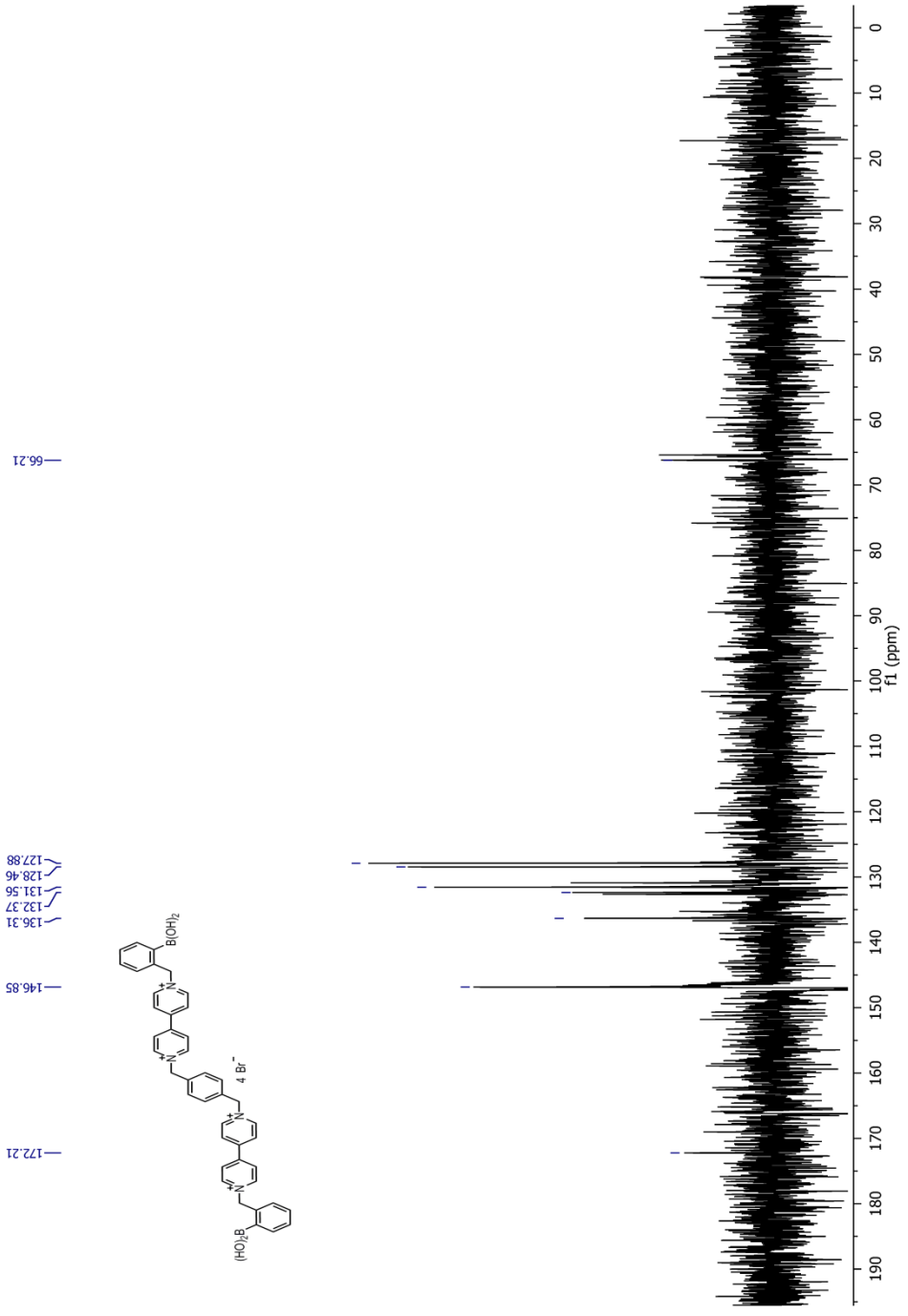


Figure S-32. ^{13}C NMR of pB*o*B

8. References

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