Fall 12-14-2011

Arylboronic Acids With Strong Fluorescence Intensity Changes Upon Sugar Binding

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ARYLBORONIC ACIDS WITH STRONG FLUORESCENCE INTENSITY CHANGES UPON SUGAR BINDING

by

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Under the Direction of Dr. Binghe Wang

ABSTRACT

Boronic acids play an important role in the design and synthesis of chemosensors for carbohydrates due to their ability to reversibly bind with diol-containing compounds. Along this line, the availability of boronic acids that change fluorescence upon sugar binding is critical to a successful sensor design effort. Here, two boronic acids that show strong fluorescent intensity changes upon sugar binding are reported: isoquinoline-7-boronic acid (7-IQBA) and phenoxathiin-4-boronic acid (4-POBA).

INDEX WORDS: Chemosensor, Boronic acids, Saccharide recognition, Isoquinoline-7-boronic acid, Phenoxathiin-4-boronic acid, Fluorescence spectroscopy

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

in the College of Arts and Science

Georgia State University

2011

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Electronic Version Approved:

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December 2011

DEDICATION

To my mother and sister, both of whom have supported me throughout my academic endeavors

and

In loving memory of my father, Darrell Lafain Laughlin, who passed away on May 4, 2011. I only hope I made you as proud of me, as I am of you. For a degree that meant so much to you, this is our achievement.

ACKNOWLEDGEMENTS

A great deal of admiration goes to Dr. Binghe Wang for his exceptional leadership during the pursuit of this degree. I would especially like to thank my mentor, Dr. Nanting Ni, for her constant guidance and kind words. A large portion of this would not have been possible without the assistance of Mr. Yingji Wang and a special thank you to Mr. Weixuan Chen for his help with the molecular modeling. I would also to acknowledge the rest of my fellow group members, whom have helped me mature both academically and personally.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS **v**

LIST OF FIGURES **viii**

LIST OF SCHEMES **ix**

LIST OF TABLES **x**

LIST OF EQUATIONS **xi**

1 INTRODUCTION **1**

1.1 CURRENT DRUG DESIGN **1**

1.2 BORONIC ACIDS **1**

1.3 SACCHARIDE DETECTION USING BORONIC ACIDS **2**

1.4 PURPOSE OF THIS STUDY **2**

2 RESULTS & DISCUSSION **5**

2.1 APPARENT ASSOCIATION CONSTANTS (K_a) **5**

2.2 FLUORESCENCE INTENSITY CHANGES **9**

2.2.1 Isoquinoline-7-Boronic Acid **9**

2.2.2 Phenoxathiin-4-Boronic Acid **12**

2.3 IONIZATION STATES **17**

2.3.1 Isoquinoline-7-Boronic Acid **17**

2.3.2 Phenoxathiin-4-Boronic Acid **24**

2.4 FLUORESCENCE QUANTUM YIELD (Φ_F) **28**

3 CONCLUSIONS **33**

4 EXPERIMENTAL **33**

5 NOTES AND REFERENCES **35**

6 APPENDICES **37**

APPENDIX A Apparent Association Constants (K_a) **37**

APPENDIX B Fluorescence Quantum Yield (Φ_F) **47**

APPENDIX C pH Profiles & Identification of pK_a Values **58**

APPENDIX D Titration Reports for Identification of pK_a Values **69**

LIST OF FIGURES

Figure 1. Structures of 7-IQBA and 4-POBA **4**

Figure 2. D-Saccharides used **4**

Figure 3. Absorbance spectra of boronic acids **6**

Figure 4. Fluorescence spectra of 7-IQBA upon addition of sugar **10**

Figure 5. Fluorescence intensity changes of 7-IQBA upon addition of sugar **11**

Figure 6. Fluroescence spectra of 4-POBA upon addition of sugar **13**

Figure 7. Fluorescence intensity changes of 4-POBA upon addition of sugar **14**

Figure 8. Molecular models for intramolecular hydrogen bond formation of 4-POBA **16**

Figure 9. pH profile of 7-IQBA using λ_{em} = 344 nm **22**

Figure 10. pH profile of 7-IQBA using λ_{em} = 378 nm **22**

Figure 11. pH profile of 4-POBA using λ_{em} = 430 nm **26**

Figure 12. Fluroescence quantum yield slopes of 7-IQBA and reference **29**

Figure 13. Evidence of species aggregation **32**

LIST OF SCHEMES

Scheme 1.	Proposed ionization of 7-IQBA in the absence of sugar	**19**
Scheme 2.	Proposed ionizations of 7-IQBA in the presence of a sugar	**19**
Scheme 3.	Proposed ionization of 4-POBA in the absence of sugar	**25**
Scheme 4.	Proposed ionization of 4-POBA in the presence of sugar	**25**

LIST OF TABLES

Table 1. Apparent association constants (K_a) of 7-IQBA and 4-POBA **8**

Table 2. Apparent pK_a values of 7-IQBA acid and 4-POBA **18**

Table 3. Presumed structures of 7-IQBA and its sugar esters at pH 7.4 **23**

Table 4. Presumed structures of 4-POBA and its sugar esters at pH 7.4 **27**

Table 5. Fluorescence quantum yields (Φ_F) of 7-IQBA and 4-POBA **30**

LIST OF EQUATIONS

Equation 1. Apparent association constant (K_a) **7**

Equation 2. (a) pK_a value curve fitting calculation from Sigma Plot 10.0 **17**

(b) pK_a value curve fitting calculation from Sigma Plot 12.0 **17**

Equation 3. Fluorescence quantum yield (Φ_F) **28**

Equation 4. pH 7.4Fluorescence quantum yield ratio used for this study **28**

1 INTRODUCTION

1.1 CURRENT DRUG DESIGN

Health issues such as monitoring blood glucose for people with diabetes mellitus[1] and cancer diagnosis are current areas of interest.[2] In drug discovery, screening and computational methods are often employed to identify potential drug molecules from libraries of enzyme inhibitors.[3] Other prospects in drug development focus on gene therapy to recognize specific sequences of nucleic acids to treat metabolic disorders, cancers, and human immunodeficiency virus.[4] Designing receptors for the specific purpose of diagnostic and therapeutic effects remains a challenge. Structure, stereochemistry, and polarity are only a few factors that influence the reactivity of a receptor. The development and success of marketed drugs is based on their selective recognition for target molecules.

1.2 BORONIC ACIDS

Boronic acids are often used in the syntheses of heterocyclic and aryl-substituted compounds.[5] Aside from their use in organic syntheses, they have also been used to recognize adenosine triphosphate (ATP) and nucleic acids,[6] serine protein motifs,[7] α-hydroxy carboxylic acids,[8] and fluoride ions.[9] They are often found in a trigonal planar neutral form, but can easily convert to a tetrahedral anion at pH above the pK_a for a particular species. This is due to the fact that boron behaves as a Lewis acid and accepts lone pair electrons from donating atoms to form covalent bonds. The boronic acid group can form tight complexes with compounds that contain two adjacent nucleophiles, especially *cis*-diols. With a high intrinsic affinity for sugars and a rapid, reversible equilibrium, boronic acids continue to gain more interests in sensor scaffold design for the recognition of carbohydrates.[7c, 10]

1.3 SACCHARIDE DETECTION USING BORONIC ACIDS

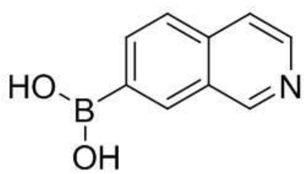
Carbohydrates are known to be extremely important in many biochemical processes and are endogenous in even the simplest of cells.[11] Carbohydrates are often linked to cell surfaces and proteins which can affect activity.[12] For example, the carbohydrate chain of a red blood cell is what determines blood type. Modified saccharide chains have been found in many degenerative diseases,[12a, 13] and glycosolated proteins have been shown to play roles in cell-cell communication and cancer metastasis.[14]

A large number of boronic acids have focused on sensing glucose and fructose because of their abundance in metabolic processes. However, the detection of other carbohydrates remains equally important. Therefore, the design and synthesis of boronic acids is important for specificity to sugars. Boronic acids are useful for sensing many types of saccharides and several boronic acids have been developed to recognize cell-surface carbohydrates.

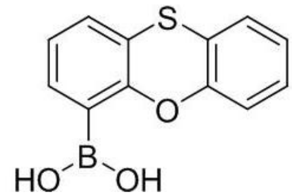
1.4 PURPOSE OF THIS STUDY

Boronic acids are good fluorescence sensors because they have intrinsic affinity for carbohydrates, fluorescence intensity changes upon binding with sugars, and a rapid and reversible equilibrium. These studies can be performed in aqueous solution under physiological conditions. A long-term goal in our group is to create a library of boronic acid scaffolds with known values that can be later used for the design and synthesis of chemosensors to identify specific biomarkers. This work examines two commercially available aryl-boronic acids: isoquinoline-7-boronic acid and phenoxathiin-4-boronic acid. Our group recently reported a new class of issoquinoinyl boronic acids (4, 5, 6, 8-IQBA) that were used for binding with sugars and six-member ring diols. 7-IQBA was not included in this earlier report, which therefore encouraged us to include it in this class of IQBAs. The structure of 4-POBA is comparatively different from IQBAs which enabled us to compare and contrast the two compounds. Binding

affinities of boronic acid with saccharides were determined from calculated K_a values to show how tightly the boronic acids bind to sugars. The pK_a values for each boronic acid in both the absence and presence of saccharides were determined to better understand the mechanism by which changes in fluorescence occurred. Additionally, fluorescence quantum yields (Φ_F) of both boronic acids with and without sugar were used to illustrate the fluorescence efficiency associated for boronic acid and their saccharide esters.

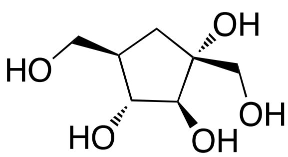


Isoquinoline-7-boronic acid (**1**)

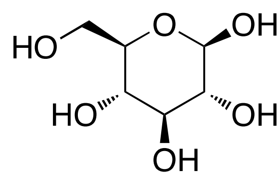


Phenoxathiin-4-boronic acid (**2**)

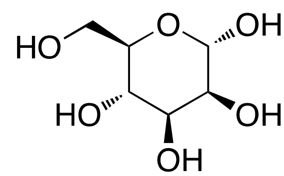
Figure 1. Structures of 7-IQBA and 4-POBA.



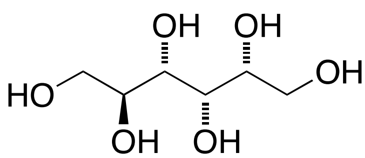
D-fructose



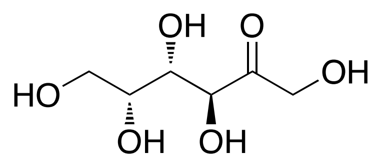
D-glucose



D-mannose



D-sorbitol



D-tagatose

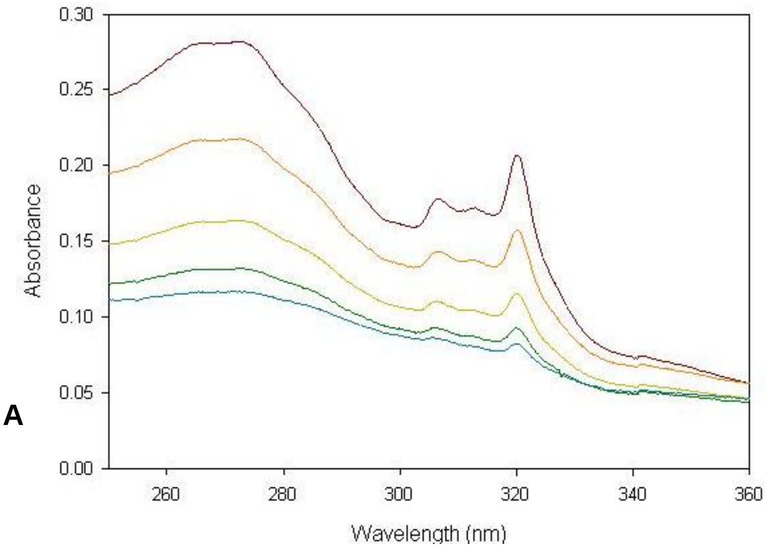
Figure 2. D-Saccharides used for this study.

2 RESULTS & DISCUSSION

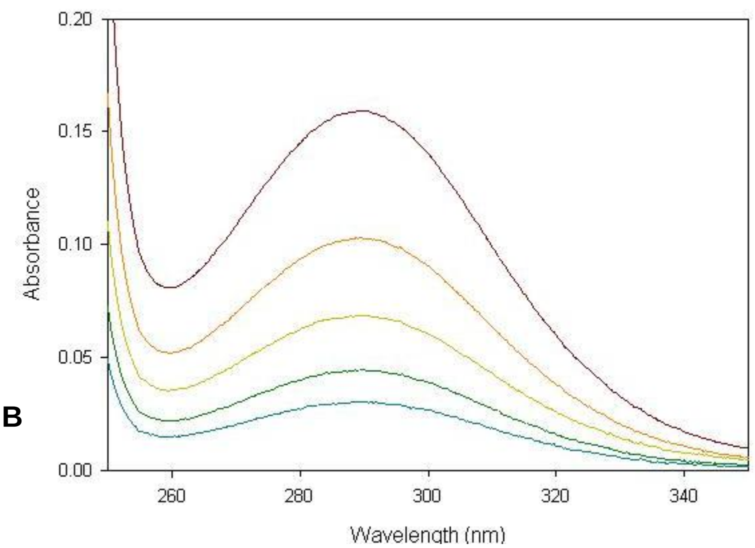
2.1 APPARENT ASSOCIATION CONSTANTS (K_a)

Binding affinities were determined to illustrate how tightly boronic acids bind with sugars. To do this, binding affinities were determined from K_a values. The K_a values were calculated using a derived equation that correlates the fluorescence intensity changes to the concentration of sugar. Excitation wavelengths used for fluorescence emission were chosen based on the maximum intensity observed at a specific wavelength (Figure 3). Binding constants for boronic acids were determined by measuring the fluorescence intensity at the emission wavelength maximum upon addition of sugar.

The K_a value was calculated using a derived equation that correlates the fluorescence intensity changes to the concentration of sugar. The K_a value for boronic acid-sugar ester can be calculated from equation (1). At equilibrium, boronic acid-sugar complex concentration is [BS], boronic acid concentration is [B]-[BS], and sugar concentration is [S]-[BS]. Assume that the concentration of sugar is much larger than both the concentrations of boronic acid and boronic acid-sugar complex. Also assume that [BS] = $n\Delta I$ and [B] = mI_0. Following the derivation, the linear equation used to calculate K_a value is shown.



A



B

Figure 3. Absorbance spectra for boronic acid. Excitation wavelengths for fluorescence emission of boronic acids were chosen based on maximum absorbance intensity. Experiments were performed in phosphate buffer (0.1 M), pH 7.4: 7-IQBA, λ_{max} = 271 nm; 4-POBA λ_{max} = 299 nm. A) 7-IQBA only, B) 4-POBA only.

(*Equation 1*)

$$\text{Boronic acid} + \text{sugar} \rightleftharpoons \text{B-S Complex}$$

Boronic acid	sugar	B-S Complex
$[B]$	[S]	[BS]
[B]-[BS]	[S]-[BS]	[BS]

$$[B] = mI_0 \ , [BS] = n\Delta I$$

$$K_a = \frac{[BS]}{[B][S]} = \frac{[BS]}{([B]\ [BS]) \cdot ([S] - [\mathrm{BS}])}$$

$$\therefore\ [S] \gg [B]\ \ \therefore [S] \gg [BS]$$

$$K_a = \frac{[BS]}{([B] - [BS]) \cdot [S]}$$

$$K_a = \frac{n\Delta I}{(mI_0 - n\Delta I) \cdot [S]}$$

$$K_a[S] \cdot (mI_0 - n\Delta I) = n\Delta I$$

$$K_a[S](mI_0) - K_a[S](n\Delta I) = n\Delta I$$

$$K_a[S](mI_0) = K_a[S](n\Delta I) + n\Delta I$$

$$K_a[S](mI_0) = (K_a[S] + 1) \cdot n\Delta I$$

$$\frac{K_a[S](mI_0)}{\Delta I} = (K_a[S] + 1) \cdot n$$

$$\frac{I_0}{\Delta I} = \frac{(K_a[S] + 1) \cdot n}{m \cdot K_a[S]}$$

$$\frac{I_0}{\Delta I} = \frac{n}{mK_a} \cdot \frac{1}{[S]} + \frac{n}{m}$$

$$y = a \cdot x + b$$

$$K_a = \frac{b}{a} = \frac{{}^{n}/_{m}}{{}^{n}/_{mK_a}} = K_a$$

The binding affinities of 7-IQBA with D-sorbitol, D-tagatose, D-mannose, and D-fructose are listed in Table 1.The trend for the binding affinities of 7-IQBA began with D-sorbitol (1600 M^{-1}), followed by D-fructose (1100 M^{-1}), D-tagatose (620 M^{-1}), and D-mannose (58 M^{-1}).The fluorescence intensity of 7-IQBA with D-glucose was low, even as sugar concentration increased. The K_a value between 7-IQBA with D-glucose was not detected. The binding affinities of 4-POBA with saccharides varied slightly in order of binding and is also listed in Table 1. Binding between 4-POBA with D-sorbitol had the highest K_a value (740 M^{-1}). The trend followed with D-tagatose (520M^{-1}), D-fructose (370M^{-1}), D-mannose (31M^{-1}), and finally D-glucose (14M^{-1}).

Table 1. Apparent association constants (K_a) of 7-IQBA and 4-POBA.

	7-IQBA		4-POBA	
	K_a [M^{-1}]	$\Delta I/I_0$	K_a [M^{-1}]	$\Delta I/I_0$
D-fructose	1100±10	20.0	370±82	-0.72
D-glucose	Not Detected		14±2	-0.90
D-mannose	58±23	5.1	31±7	-0.82
D-sorbitol	1600±588	8.0	740±68	-0.81
D-tagatose	620±38	6.9	520±78	-0.78

2.2 FLUORESCENCE INTENSITY CHANGES

2.2.1 Isoquinoline-7-Boronic Acid

Fluorescence intensity changes of 7-IQBA upon sugar binding are listed in Table 1. The changes ranged from the intensity in the absence of sugar (initial intensity, I_0) to the intensity in the highest concentration of sugar (final intensity, I_F).The intensities of 7-IQBA upon binding with saccharides increased, except for 7-IQBA with D-glucose. In particular, 7-IQBA showed a 20-fold increase in fluorescence intensity upon binding with D-fructose. Changes in fluorescence intensities of 7-IQBA experienced a 5 to 8-fold increase with the remaining sugars (Figures 4 and 5).The maximum fluorescence intensity red-shifted from around 344 nm to 378 nm as saccharide concentrations increased (Figure 4).

The fluorescence intensity of 7-IQBA only is lower than its sugar esters. At pH 7.4, 7-IQBA is predominantly found as a neutral trigonal planar species and its ester is the tetrahedral anionic form at pH 7.4. The ester form is the fluorescent species while 7-IQBA is non-fluorescent at pH 7.4. Therefore, the fluorescence intensities increase upon addition of sugar. These results are further explained with apparent pK_a values.

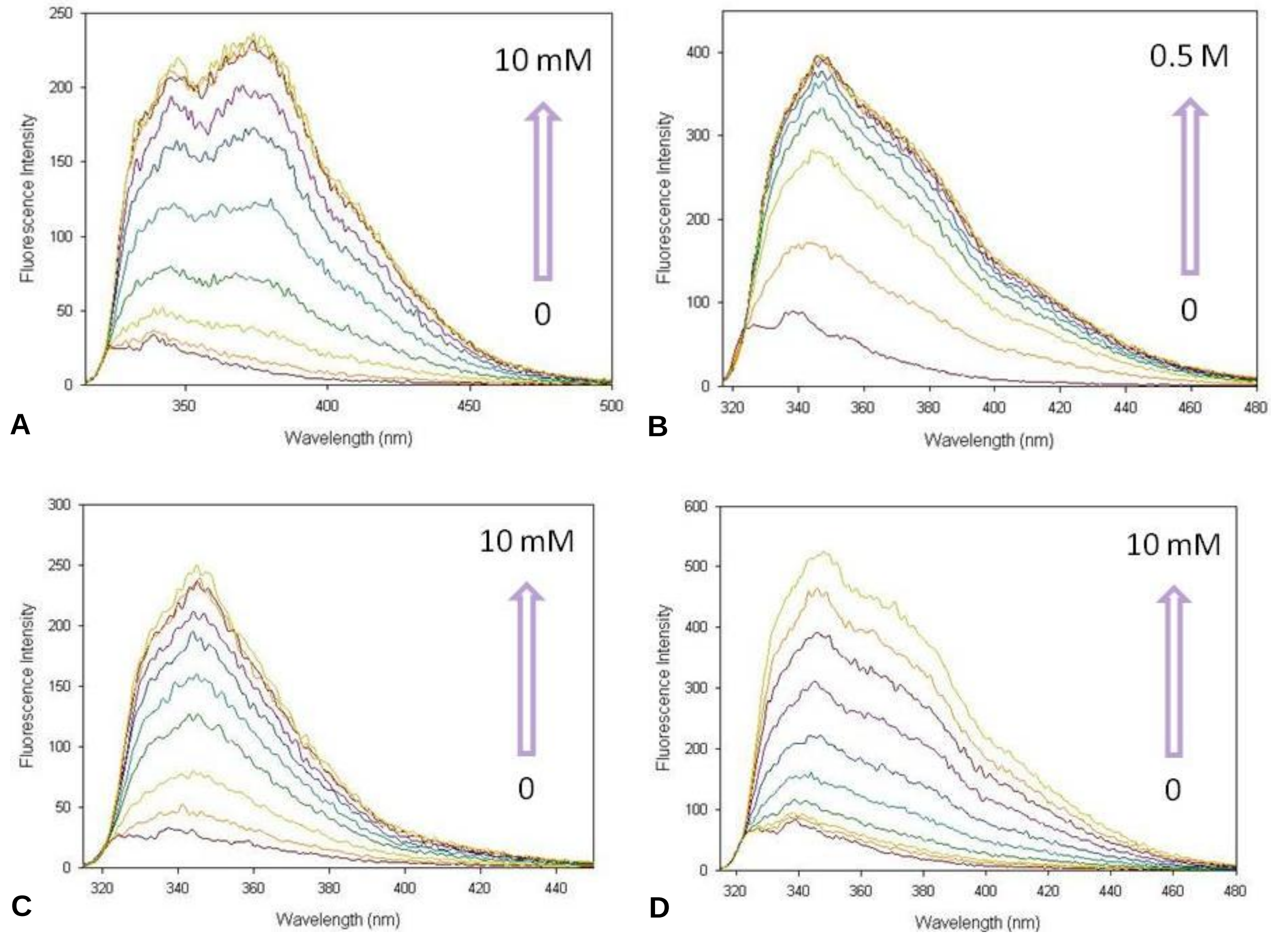


Figure 4. Fluorescence spectra of 7-IQBA (10 μM) upon addition of increasing sugar concentrations in phosphate buffer (0.1 M) at pH 7.4: λ_{ex}= 280 nm, D-fructose and D-sorbitol; λ_{ex}= 272 nm, D-mannose and D-tagatose. All experiments were performed in triplicate. A) D-fructose, B) D-mannose, C) D-sorbitol, D) D-tagatose.

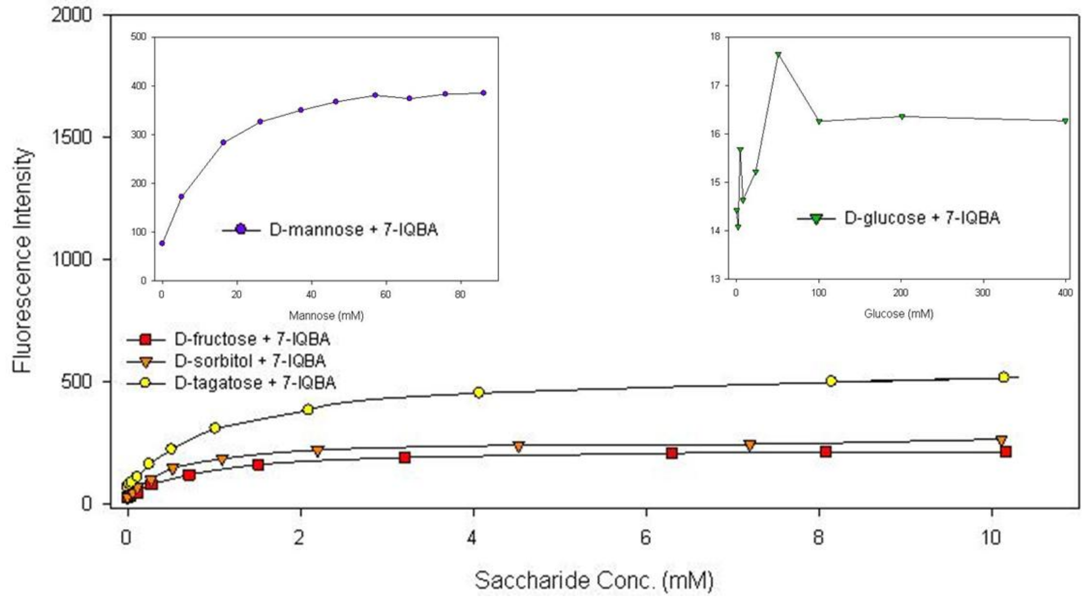


Figure 5. Fluorescence intensity changes of 7-IQBA (10 μM) upon increasing sugar concentration in phosphate buffer (0.1 M) at pH 7.4: λ_{ex}= 280 nm, λ_{em} = 370 nm (D-fructose) and λ_{em}= 344 nm (D-sorbitol); λ_{ex}= 272 nm, λ_{em} = 347 nm (D-mannose) and λ_{em}= 346 nm (D-tagatose). All experiments were performed in triplicate.

2.2.2 Phenoxathiin-4-Boronic Acid

The fluorescence intensity of 4-POBA upon addition of saccharides decreased. The changes are listed in Table 1. Addition of D-glucose with 4-POBA produced the largest intensity decrease by 90%. In the presence of D-mannose, D-sorbitol, or D-tagatose, the intensities decreased 80%. A decrease of 72% showed that binding between 4-POBA and D-fructose experienced the smallest change in fluorescence.

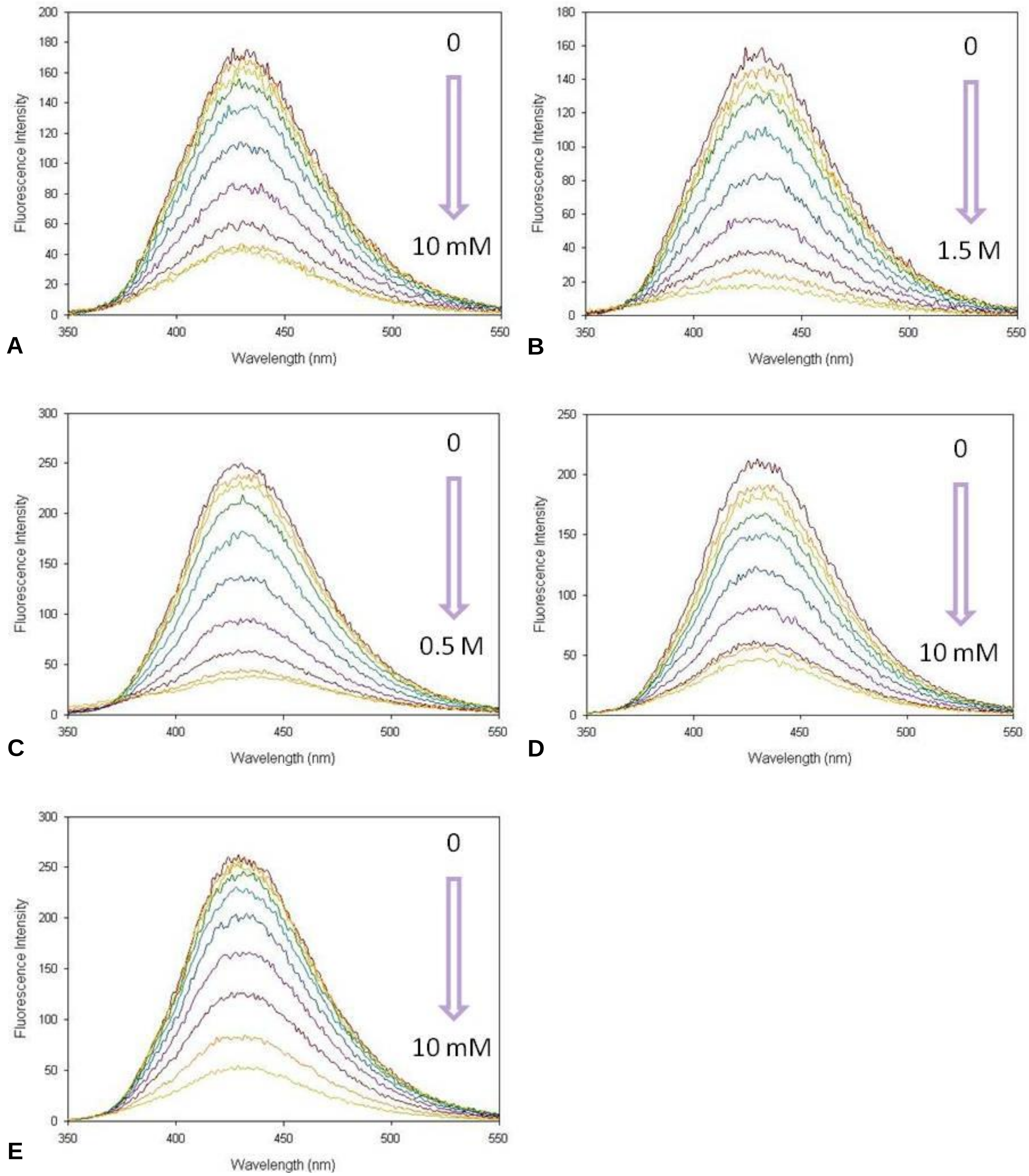


Figure 6. Fluorescence spectra of 4-POBA (10 μM) upon addition of increasing sugar concentration in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, all sugars. All experiments were performed in triplicate. A) D-fructose, B) D-glucose, C) D-mannose, D) D-sorbitol, E) D-tagatose.

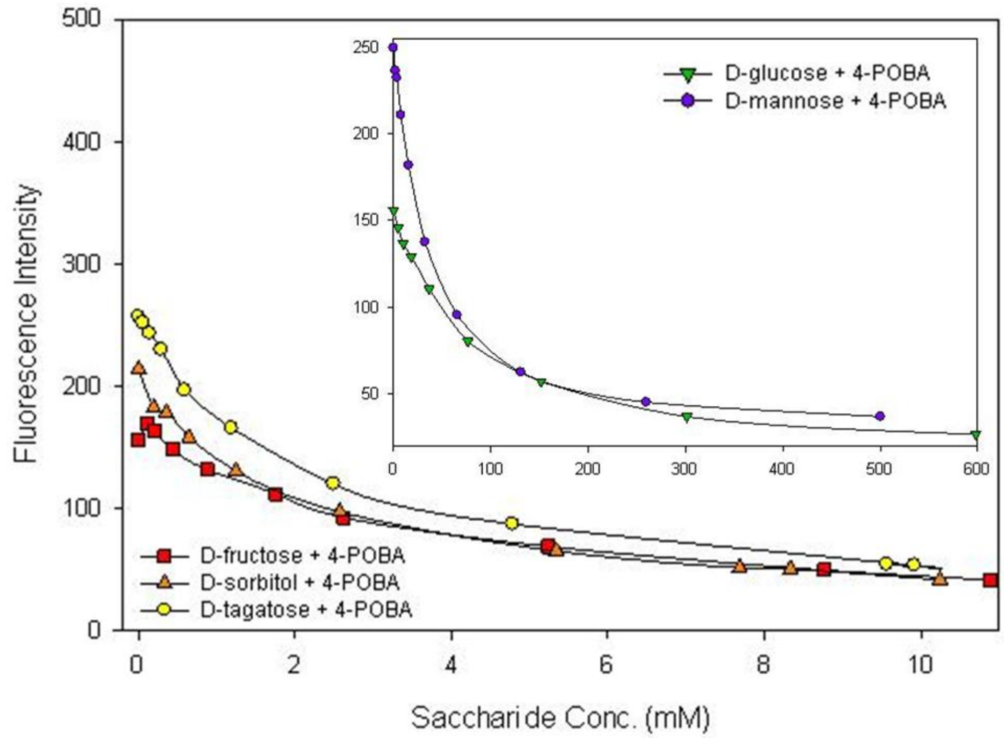


Figure 7. Fluorescence intensity changes of 4-POBA (10 μM) upon increasing sugar in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, λ_{em} = 430 nm (D-fructose, D-glucose, D-mannose, D-sorbitol, and D-tagatose). All experiments were performed in triplicate.

For 4-POBA, the fluorescence intensity decreased upon increasing pH values of free boronic acid. The measured distance between the oxygen of the phenoxathiin moiety and hydrogen of boronic acid was 2.04 Å. Intrinsic distance for hydrogen bonding is roughly 2 Å. We can predict that there is hydrogen bond interaction between these two groups. In the ester form, pH of the solution is higher than the pK_a value of 4-POBA-sugar esters and the hybridization changes from trigonal planar to tetrahedral anionic form. There is no hydrogen on the boronic ester, thus no hydrogen bonding will exist. Hydrogen bonding between the free boronic acid and oxygen may contribute to the stability of the boronic acid and to the fluorescence intensity. We can speculate that breaking this hydrogen bond may play a role in lowering the fluorescence intensity upon sugar addition or with increasing pH.

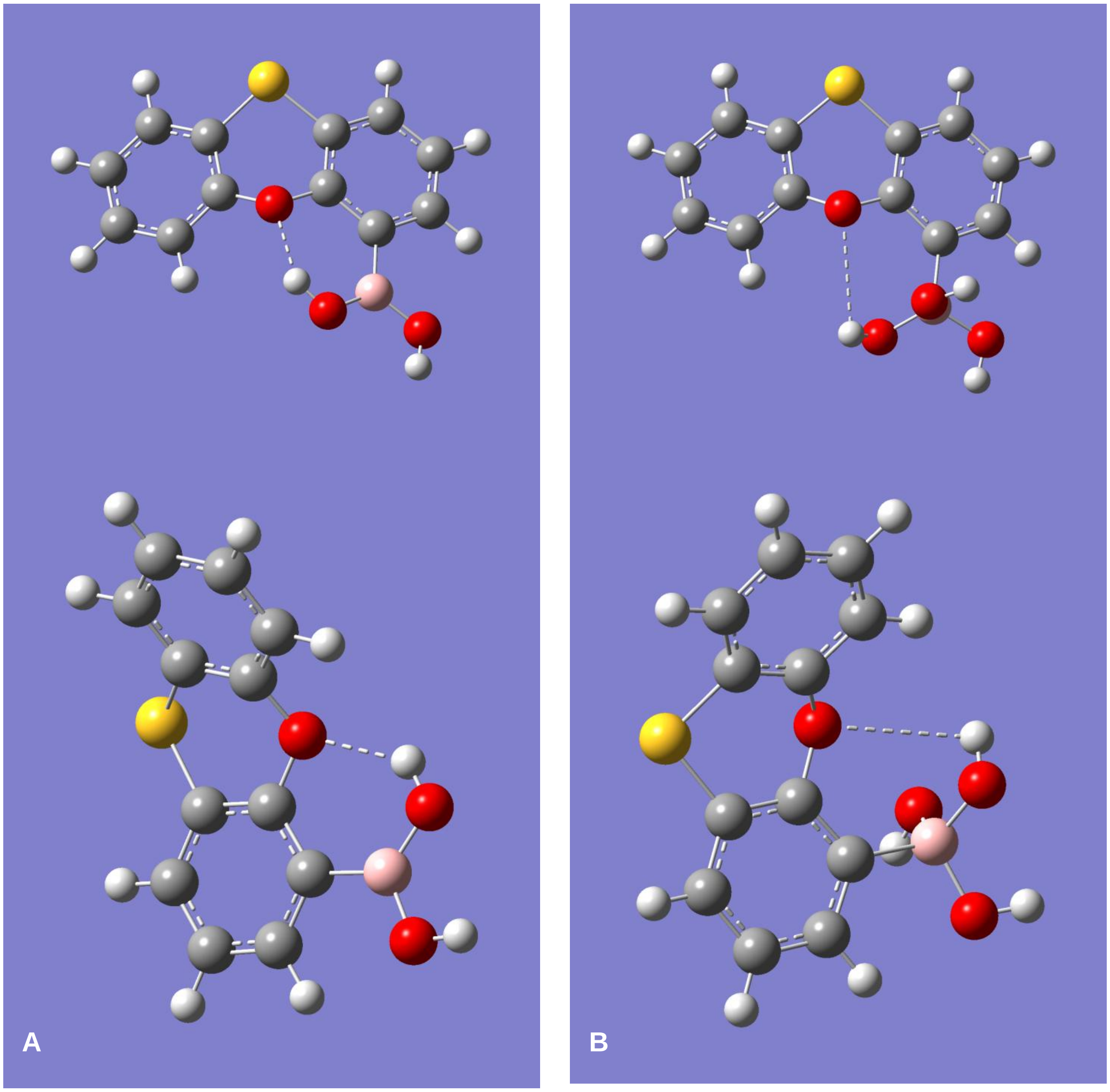


Figure 8. Molecular models for intramolecular hydrogen bond formation of 4-POBA. Distance between the donor species (hydroxyl group of boronic acid) and acceptor species (oxygen of phenoxathiin) is 2.04 Å and 2.84 Å for trigonal planar (A) and tetrahedral (B) hybridizations, respectively.

2.3 Ionization States

2.3.1 Isoquinoline-7-Boronic Acid

To better understand the basic mechanism by which the fluorescence changes occurred, the pK_a values of 7-IQBA in the absence and presence of various sugars were determined. The fluorescence intensities of boronic acids and their esters were measured at varied pH values. Titration curves were created from the intensities for each measured pH value using the emission maximum (maxima). Statistical software was used to fit titration curves. Equation (2a) or (2b) was used and pK_a values were assigned to *coefficient x0* or *EC50*, respectively. Equation (2a) was used with Sigma Plot 10.0 and (2b) with Sigma Plot 12.0.

(*Equation 2a*)

```
f1=min + (max-min)/(1 + abs(x/EC50)^Hillslope)
f2=min + (max-min)*(abs(x/EC50)^abs(Hillslope))/(1 + abs(x/EC50)^abs(Hillslope)))
f = if(x<=0, if(Hillslope>0,max,min), if(Hillslope>0, f1, f2))
```

(*Equation 2b*)

```
f = y0+a/(1+exp(-(x-x0)/b))
```

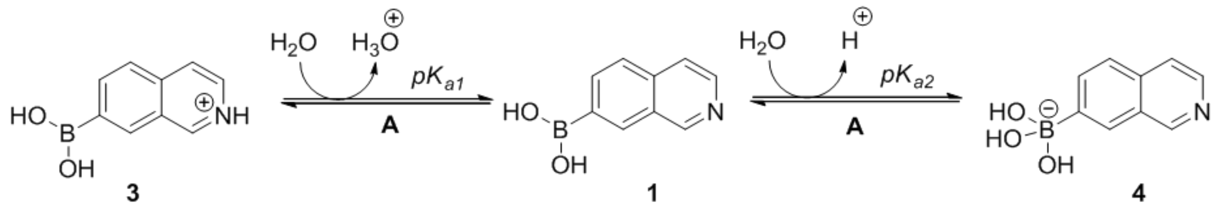
For 7-IQBA, there are two ionizable functional groups: the boronic acid group and the isoquinolinium nitrogen. Therefore, two pK_a values were expected for 7-IQBA. As shown in Figure 4**,** the fluorescence intensities of the boronic acids and their presumed esters changed with pH. Such results demonstrate that fluorescent properties are associated with the ionization state of the boronic acids and their corresponding esters. For 7-IQBA, there are two ionizable functional groups: the boronic acid group and the isoquinolinium nitrogen. Therefore, there should be two pK_a values.

Based on literature precedents,[15] it was reasonable to assume that the ionization steps of 7-IQBA followed route **A** in which deprotonation of the isoquinolinium nitrogen precedes the hybridization state change of the boronic acid seen in Scheme 1.[15] On the other hand, the ionization of esters of 7-IQBA most likely follow route **B**. Thus, the first ionization step for the esters is the hybridization state change of the boronic ester group, followed by deprotonation of the isoquinolinium nitrogen.

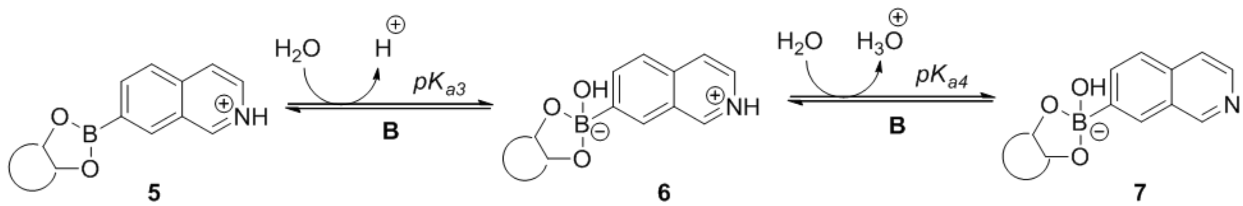
Table 2. Apparent pK_a values of 7-IQBA and 4-POBA.

	D-fructose	D-glucose	D-mannose	D-sorbitol	D-tagatose	No Sugar
7-IQBA	4.9, 6.9	ND[a]	5.2, 7.4	4.1, 6.8	5.1, 6.9	5.5, 7.8
4-POBA	6.3	6.3	6.4	6.6	6.6	8.0

[a] Not determined.



Scheme 1. Proposed ionizations of 7-IQBA in the absence of sugar.



Scheme 2. Proposed ionizations of 7-IQBA in the presence of a sugar.

It is interesting to note that 7-IQBA and all of its esters have two emission maxima. Therefore, the pH profiles of the fluorescence intensities were constructed at different wavelengths to obtain the pK_a values listed in Table 2. For 7-IQBA only, it was surprising to find that increasing the pH induced an 80% fluorescence intensity decrease at 378 nm with an apparent pK_a value of around 5.5, which was assigned as pK_{a1} based on comparison with an earlier report.[15] However, when the pH profiles of the fluorescence intensities were drawn at 344 nm, two approximate pK_a values could be obtained: 5.5 with pK_{a1} and 7.8 with pK_{a2}. As seen in Figure 4, 7-IQBA itself did not have strong fluorescence (378 nm) at low pH. At pH 7.4, 7-IQBA existed predominantly in the deprotonated form, which was nearly non-fluorescent.

In the presence of various sugars, the fluorescence intensities of 7-IQBA increased 17-, 13-, 37- and 17-fold at 344 nm, corresponding to the addition of D-fructose, D-mannose, D-sorbitol, and D-tagatose. The apparent pK_a values were observed as 6.9, 7.4, 6.8 and 6.9, respectively. Based on the data obtained from earlier studies, all of these pK_a values were assigned as pK_{a4}.[15] For the data collected at 378 nm, the second set of pK_a values were observed as 4.9, 5.2, 4.1 and 5.1 for the esters of D-fructose, D-mannose, D-sorbitol, and D-tagatose, which could be assigned as pK_{a3} based on the previous studies.[15] Such results indicate that the pK_a value of the boronic acid group is higher in the absence of a sugar and lower in the presence of a sugar.

The switching of pK_a also correlated with the strong fluorescence changes at pH 7.4. Thus, it is reasonable to assume that the zwitterionic species (**6**) is more fluorescent than the boronic ester (**5**). At pH 7.4, 7-IQBA itself existed predominantly in the neutral trigonal-coordinated boron form (**1**) since the pK_a of 7-IQBA is 7.8. The D-fructose, D-sorbitol, and D-tagatose esters existed predominantly in the boronate form (**7**). Since the pK_a value of the D-mannose ester of 7-IQBA is 7.4, one would expect to find a mixture of the zwitterionic isoquinolinium boronate (**6)** and isoquinolinyl boronate forms (**7**) at pH 7.4. Overall, **1** and **4** are

almost non-fluorescent while the boronate ester forms **6** and **7** are fluorescent. Therefore, the binding between 7-IQBA with sugars leads to the fluorescence increase at pH 7.4.

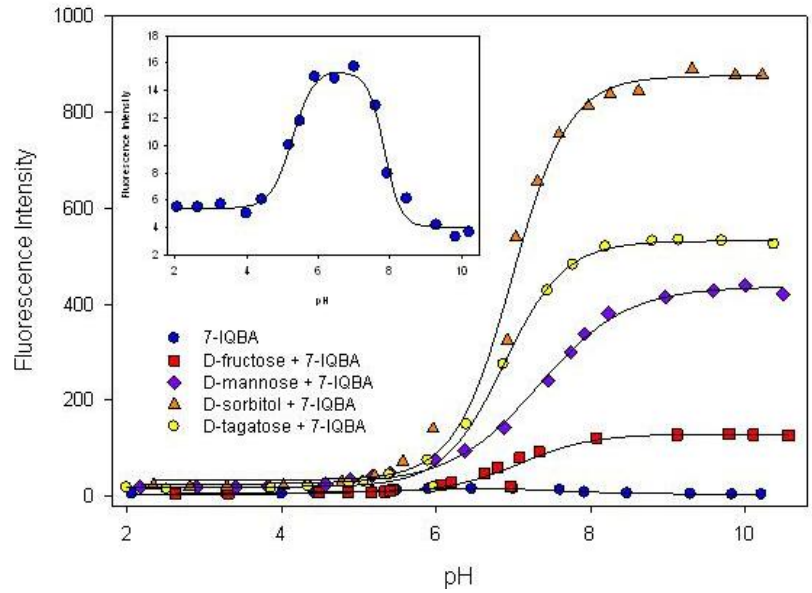


Figure 9. pH profile of 7-IQBA (10 μM) in the absence and presence of various sugars (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 272 nm (7-IQBA only, D-fructose, D-glucose, D-sorbitol, and D-tagatose); λ_{ex} = 271 nm (D-mannose), $\boldsymbol{\lambda_{em}}$ = 344 nm. All experiments were performed in duplicate.

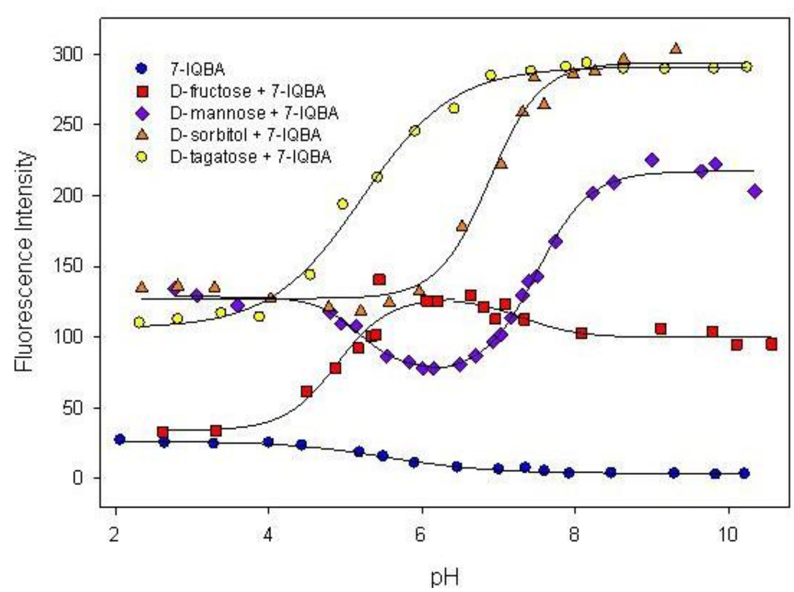
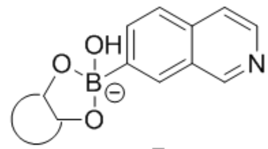
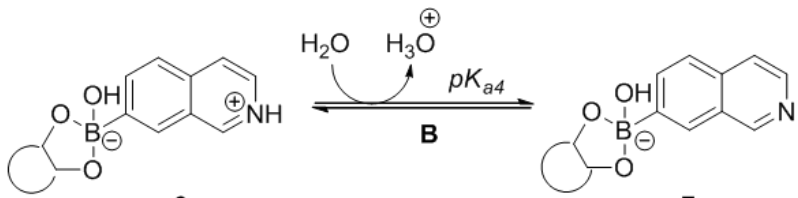
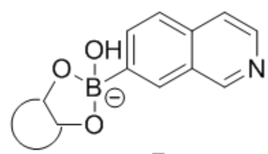
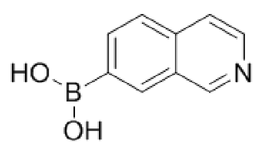


Figure 10. pH profiles of 7-IQBA (10 μM) in the absence and presence of various sugars (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 272 nm (7-IQBA only, D-fructose, D-glucose, D-sorbitol, and D-tagatose); λ_{ex} = 271 nm (D-mannose), $\boldsymbol{\lambda_{em}}$ = 378 nm. All experiments were performed in duplicate.

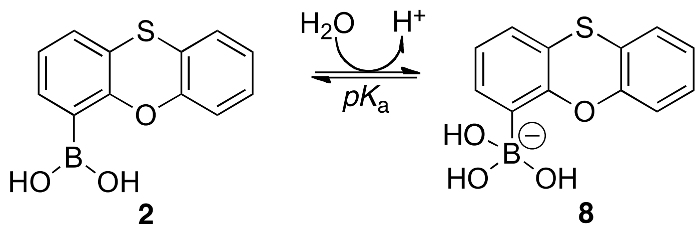
Table 3. Presumed structures of 7-IQBA and its sugar esters at pH 7.4.

	pK_a	Structure
D-fructose	4.9, 6.9	 **7**
D-glucose	ND[a]	ND[a]
D-mannose	5.2, 7.4	 **6** **7**
D-sorbitol	4.1, 6.8	
D-tagatose	5.1, 6.9	 **7**
No Sugar	5.5, 7.8	 **1**

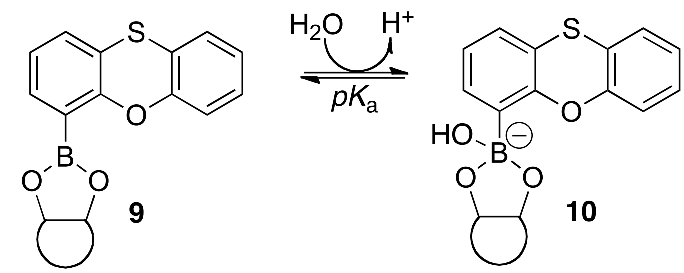
[a] Not determined.

2.3.2 Phenoxathiin-4-Boronic Acid

In the case of 4-POBA, there is only one ionizable group, the boronic acid. It was found that the fluorescence intensity increased 30-fold when the pH decreased with an apparent pK_a of 8.0. Such results indicate that the neutral trigonal form (**2**) is the more fluorescent species. In the presence of sugars, the apparent *p*K_a values were 6.3 (D-fructose ester and D-glucose ester), 6.4 (D-mannose ester), and 6.6 (D-sorbitol ester and D-tagatose ester). Similar to 4-POBA alone, the trigonal neutral ester form (**9**) is the more fluorescent species, while the tetrahedral ester form (**10**) is mostly non-fluorescent (Scheme 2). At pH 7.4, 4-POBA exists in the trigonal form (**2**) and 4-POBA ester exists predominantly in the tetrahedral form (**10**). The boronic acid form of 4-POBA (**2**) is fluorescent and the boronate form (**10**) is non-fluorescent. Therefore, the binding between 4-POBA with sugars leads to a decrease in fluorescence intensity.



Scheme 3. Proposed ionization of 4-POBA in the absence of sugar.



Scheme 4. Proposed ionization of 4-POBA in the presence of sugar.

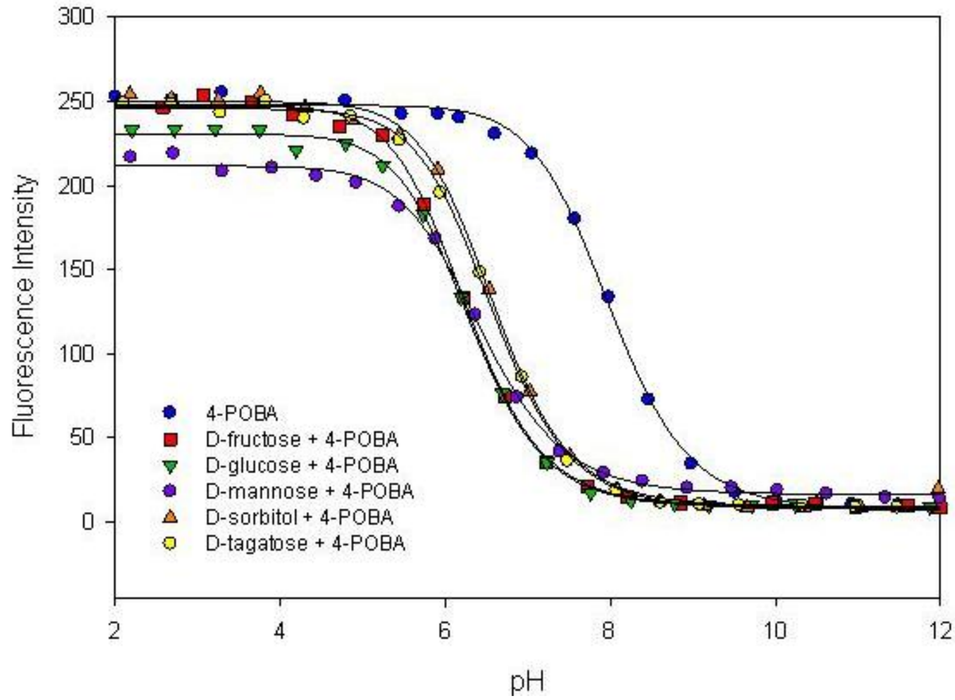
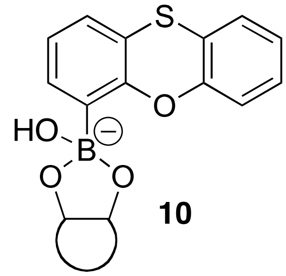
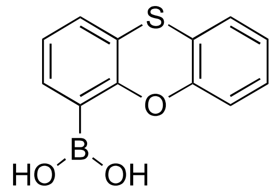


Figure 11. pH profiles of 4-POBA (10 μM) in the absence and presence of various sugars (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 299 nm, λ_{em} = 430 nm. All experiments were performed in duplicate.

Table 4. Presumed structures of 4-POBA and its sugar esters at pH 7.4.

	pK_a	Structure
D-fructose	6.3	 **10**
D-glucose	6.3	
D-mannose	6.4	
D-sorbitol	6.6	
D-tagatose	6.6	
No Sugar	8.0	 **2**

2.4 FLUORESCENCE QUANTUM YIELD (Φ_F)

A fluorescence quantum yield test is a measure of how efficient the fluorescence from a species is. By definition, the fluorescence quantum yield is the number of photons emitted over the number of photons absorbed. Quantum yield can be calculated from a photon counter using equation (3). Our laboratory does not have an instrument of this nature so relative fluorescence quantum yields were determined using equation (4). We compared the unknown boronic acids and their esters to a reference. We used 8-IQBA as the reference because it has a known quantum yield (2.2%) and an excitation wavelength maximum (271 nm) near those of 7-IQBA and 4-POBA. From the known quantum yield of 8-IQBA, we varied the concentrations of 8-IQBA and measured the absorbance and fluorescence. A curve of integrated fluorescence peak area over absorbance intensity (Figure 12) was created to obtain the slope. We repeated this for 7-IQBA by obtaining the absorbance intensities and the fluorescence peak areas.

(*Equation 3*)

$$\Phi_F = photons\ emitted/photons\ absorbed$$

(*Equation 4*)

$$\Phi_F = Q = Q_R \left(\frac{I}{I_R}\right) \cdot \left(\frac{OD_R}{OD}\right)$$

Using the slopes and the known quantum yield of 8-IQBA, we used equation (4) to set up a ratio of the known values to determine the quantum yield of 7-IQBA. This same procedure was duplicated for free boronic acids and their sugar-esters.

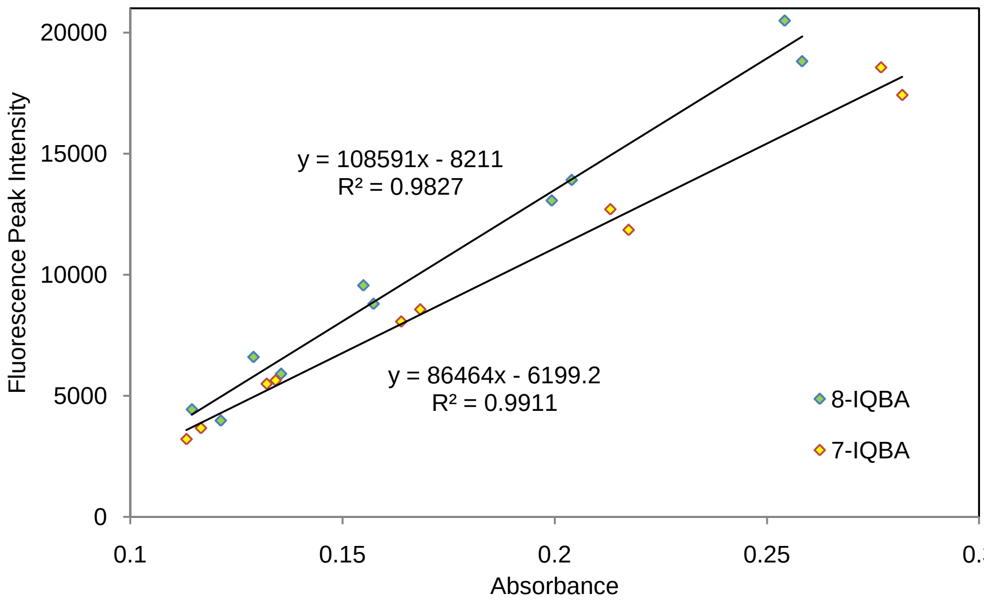


Figure 12. Fluorescence quantum yield slope determination of 8-IQBA and 7-IQBA. Fluorescence quantum yield slopes of 8-IQBA and 7-IQBA ($^1/_3$ dilutions of 10 μM) in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 271 nm, λ_{em} range = 315 – 421 nm. Experiments were duplicated.

The results for the fluorescence quantum yields are shown in Table 2. The quantum yields for 7-IQBA in the absence and presence of saccharide followed the trend D-fructose ester > D-tagatose ester > D-sorbitol ester > D-mannose ester > D-glucose ester > 7-IQBA only. Sugar complexes had higher quantum yields than 7-IQBA itself. For example, the quantum yield for the D-fructose ester of 7-IQBA was 48% while 7-IQBA alone was only 2%, a 24-fold difference. D-Tagatose ester and D-sorbitol ester also had high quantum yields of 34% and 28%, respectively. D-Mannose ester showed a lower quantum yield of 8%, but still over 4-fold higher than 7-IQBA itself.

Table 5. Fluorescence quantum yields (Φ_F) of 7-IQBA and 4-POBA.

	Φ_F (%)					
	D-fructose	D-glucose	D-mannose	D-sorbitol	D-tagatose	No Sugar
7-IQBA	48±0.55	ND[a]	7.8±0.06	28±0.24	34±1.2	1.7±0.13
4-POBA	0.72±0.05	0.72±0.05	0.55±0.05	0.86±0.06	0.99±0.07	8.0±0.16

[a] Not determined.

The quantum yield for 4-POBA displayed a very different tendency compared to 7-IQBA. The boronic acid itself had a much higher quantum yield than any of the sugar ester forms. For instance, the quantum yield for 4-POBA was 8%, while quantum yields for boronates fell below 1%, which is consistent with the binding study as shown in Figure 6 and 7 where the fluorescence intensities decreased upon binding to sugar. For instance, D-tagatose ester (0.99%) showed the smallest fluorescence intensity decrease of 8-fold and 14 to 20-fold decreases from D-fructose ester (0.72%), D-glucose ester (0.72%), D-mannose ester (0.55%), and D-sorbitol ester (0.86%). The dominant fluorescent species was 4-POBA alone.

Environmental factors (e.g. solvent, buffer, ions) may affect fluorescence and factors such as species aggregation may influence the quantum yield. For instance, at higher concentrations of 8-IQBA, we observed the fluorescence intensity to reach a maximum, which was likely due to species aggregation in solution. As seen in Figure 13, the maximum fluorescence intensity decreased upon reaching a certain concentration (around 12 μM). Therefore, low concentrations of boronic acids (10 μM) were used. In buffered solutions, there are often issues with buffer interactions. The presence of ions may have a quenching effect on fluorescence. However, phosphate ions with boronic acid have no effect on fluorescence intensity which is why phosphate buffer is commonly used.[7d]

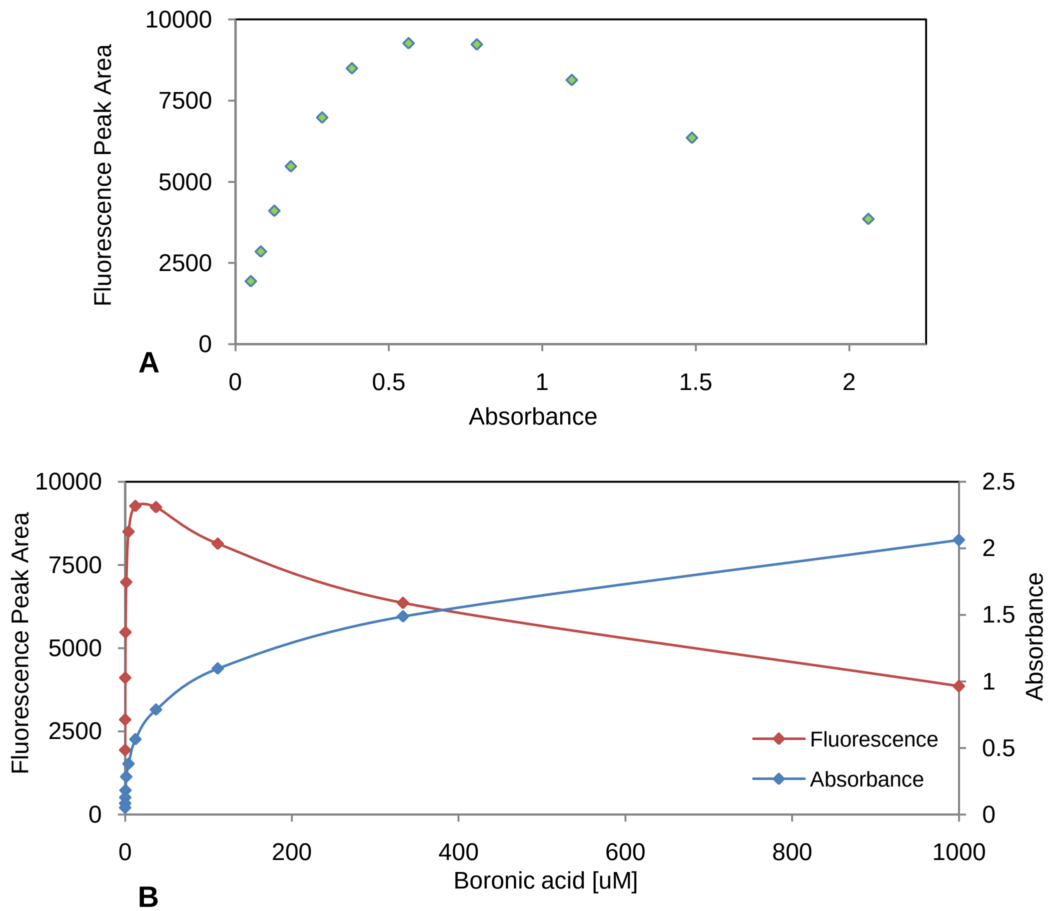


Figure 13. Evidence of species aggregation for 8-IQBA. Integrated fluorescence area versus absorbance intensities of 8-IQBA. B) Fluorescence peak area and absorbance intensity versus 8-IQBA concentrations (16 pM – 1 mM). λ_{ex} = 271 nm, λ_{em} range = 315 – 421 nm.

3 CONCLUSIONS

Isoquinoline-7-boronic acid and phenoxathiin-4-boronic acid were examined to determine their potential use as scaffolds in chemosensor design. Several properties of these two arylboronic acids were studied under physiological conditions, which included the apparent binding affinity, proposed ionization schemes, and fluorescence quantum yields. Binding of 7-IQBA and 4-POBA with several monosaccharides was reported using micromolar concentrations of boronic acid. Both boronic acids showed large changes in fluorescence intensity upon the addition of sugar. Using this data, sensors can be designed accordingly, depending on desired characteristics for the recognition of a particular biomarker. These results indicate that 7-IQBA may be more useful than 4-POBA in the design and synthesis of boronic acid scaffolds for sensing carbohydrates in biological systems.

4 EXPERIMENTAL

Absorbance spectra were obtained using Shimadzu PharmaSpec UV-1700 UV-Visible Spectrophotometer and UVProbe Software. Fluorescence emission spectra were obtained using Shimadzu RF-5301PC Spectrofluorophotometer and RF530XPC Software. Quartz crystal cuvettes were used for absorbance and fluorescence experiments. All pH values were determined using Fisher Scientific Accumet Research AR10 pH Meter. Water used for buffer solutions was purified using Thermo Scientific Barnstead Nanopure filtration system. All pK_a values were calculated using Systat Software Inc. SigmaPlot 12.

Buffer was prepared with Na_2HPO_4 (0.077 mol) and NaH_2PO_4 (0.023 mol) in dH_2O (1 L), and adjusted to pH 7.4. Isoquinoline-7-boronic acid and phenoxathiin-4-boronic acid were commercially available from Frontier Scientific. Boronic acids were dissolved in MeOH to 0.01 M and diluted to 10 μM using 0.1 M phosphate buffer at pH 7.4. Sugars were available though

Fisher Scientific and Sigma-Aldrich and prepared in 0.1 M phosphate buffer, pH 7.4. Excitation wavelengths were determined by absorbance spectra.

For binding affinities, standard solutions of D-glucose (1.5 M), D-mannose (0.5 M), and D-fructose, D-sorbitol and D-tagatose (10 mM) were prepared with boronic acid (10 µM) in phosphate buffer (0.1 M), pH 7.4. Experiments were performed in triplicate. To determine pK_a values, saccharide solutions (0.01 M) were prepared in boronic acid (10 µM) in phosphate buffer (0.01 M), pH 7.4. The pH was adjusted from 2 to 12 using concentrated HCl and saturated NaOH, in keeping with consistent volumes (40 mL) and concentrations. Experiments were duplicated. For quantum yield studies, solutions of boronic acid (10 µM) with and without sugars were prepared (0.03 M for D-mannose, 0.01 M for all others) in phosphate buffer (0.01 M), pH 7.4. One-third, serial dilutions of boronic acid (10 µM) were made to obtain absorbance and fluorescence emission spectral data. All experiments were duplicated.

5 NOTES & REFERENCES

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16. Experiments performed by Mr. Yingji Wang.

APPENDICES

APPENDIX A – Apparent Association Constants (K_a)

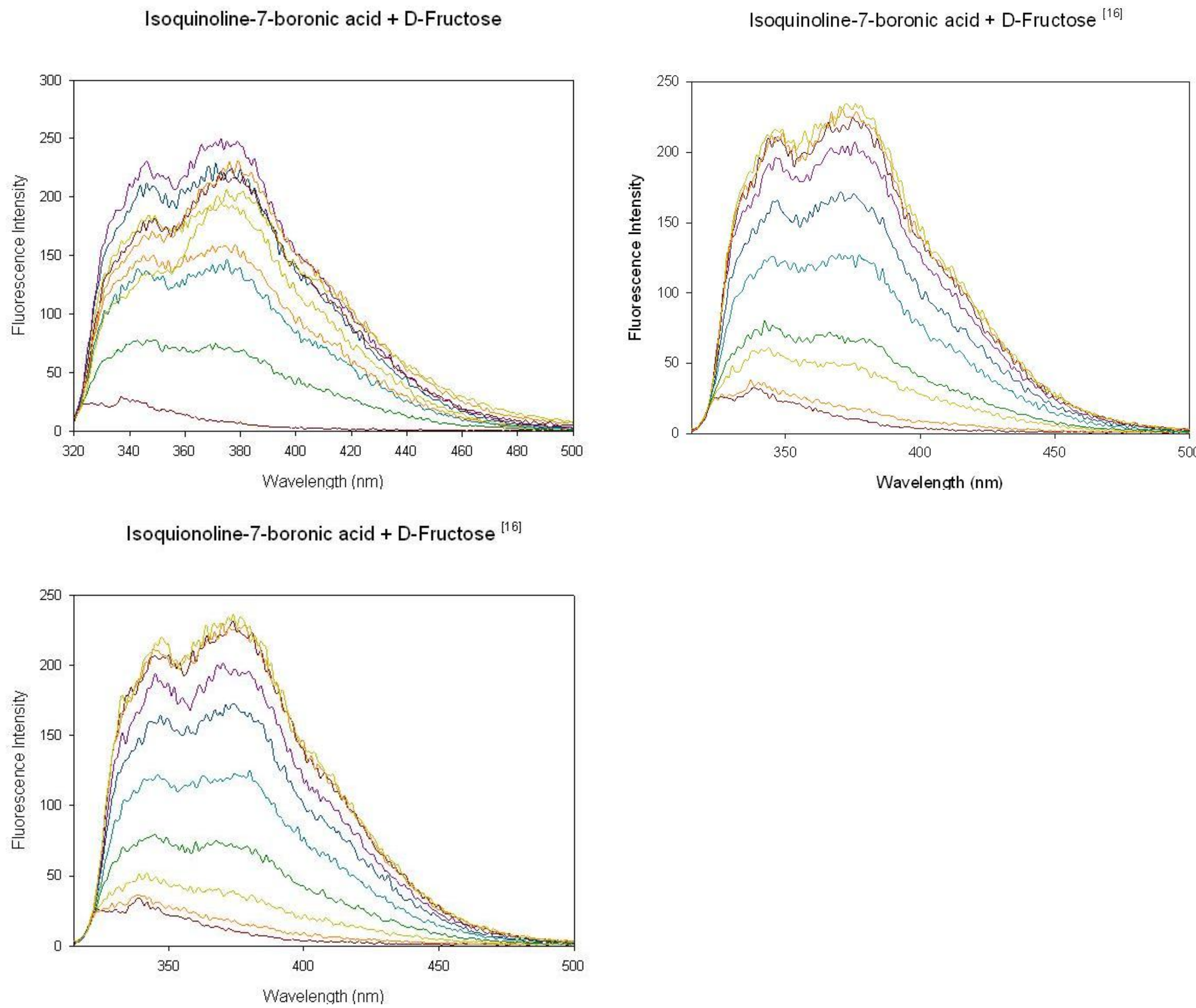

Figure A1. Fluorescent spectral changes of 7-IQBA (1×10^{-5} M) upon addition of D-fructose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 280 nm, λ_{em} = 370 nm.

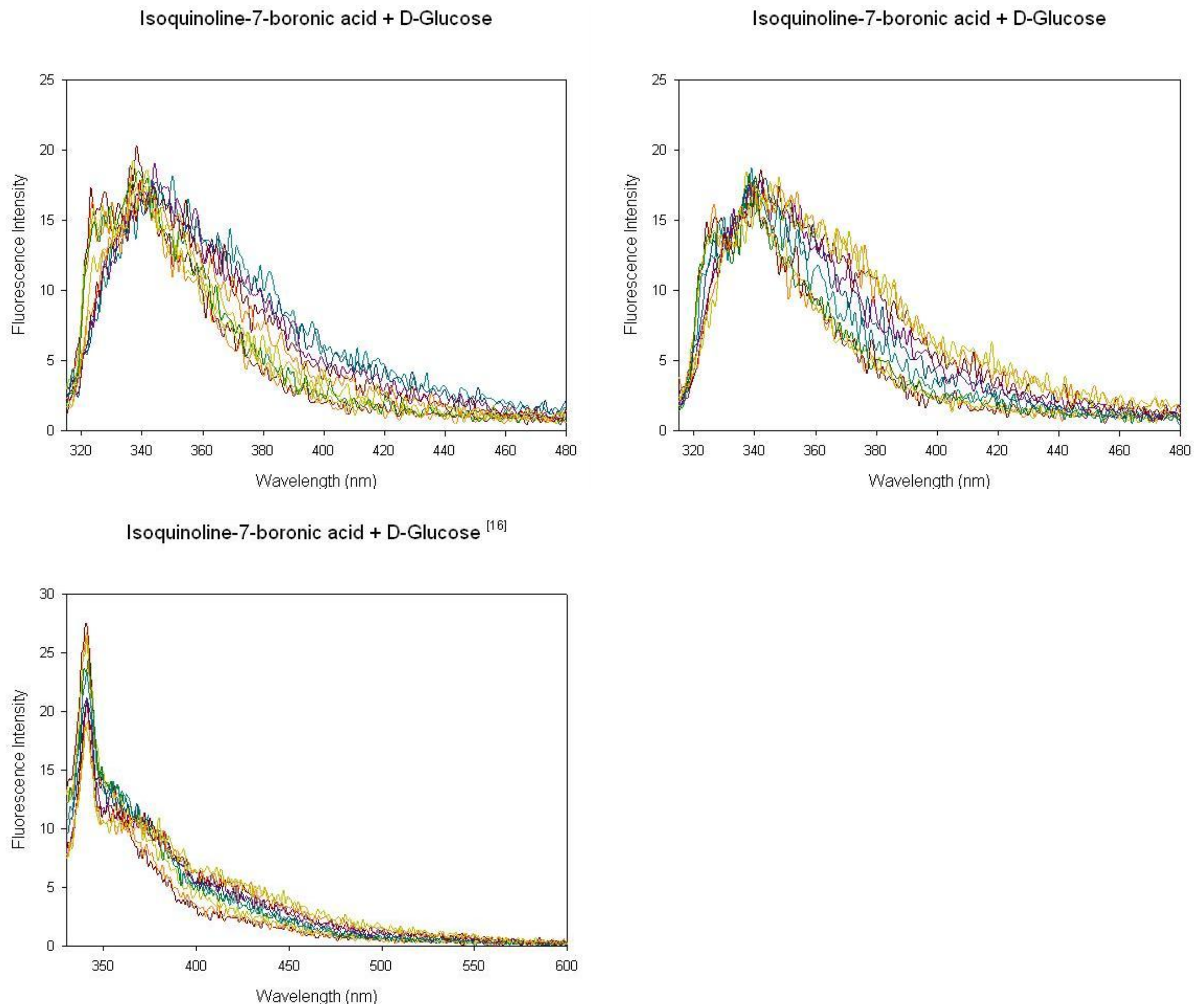

Figure A2. Fluorescent spectral changes of 7-IQBA (1 × 10^{-5} M) upon addition of D-glucose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = nm, λ_{em} 344 = nm.

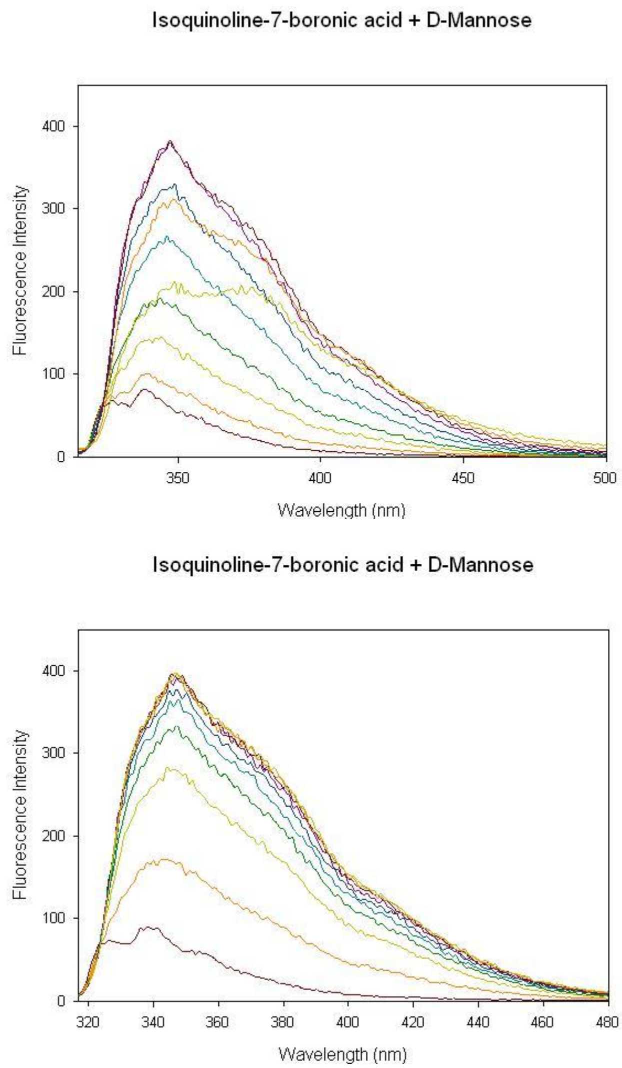

Figure A3. Fluorescent spectral changes of 7-IQBA (1×10^{-5} M) upon addition of D-mannose in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex} = 272$ nm, $\lambda_{em} = 347$ nm.

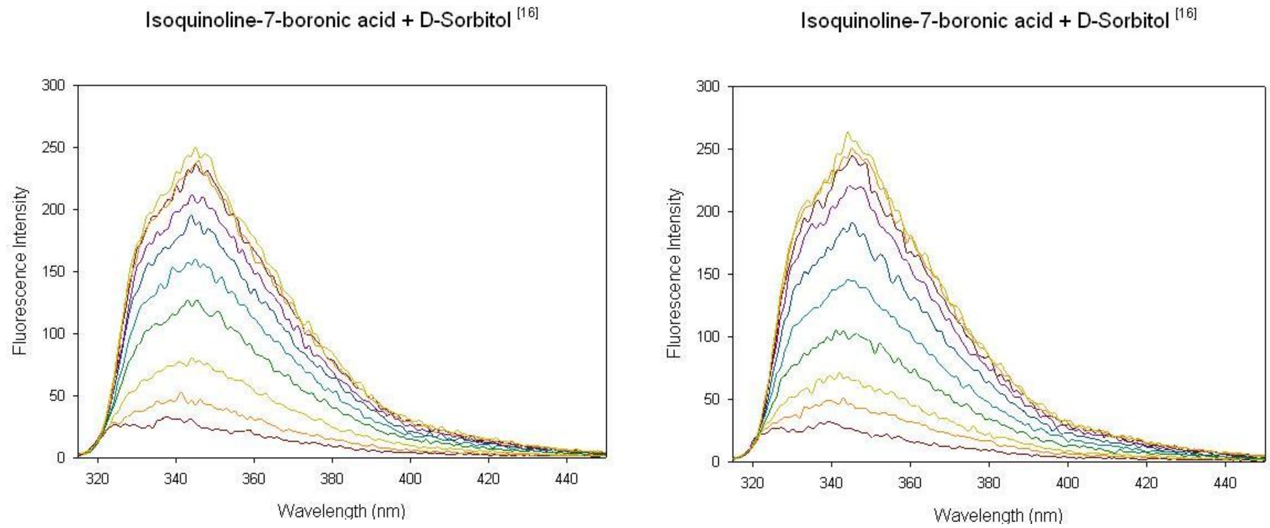

Figure A4. Fluorescent spectral changes of 7-IQBA (1×10^{-5} M) upon addition of D-sorbitol in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 280 nm, λ_{em} = 344 nm.

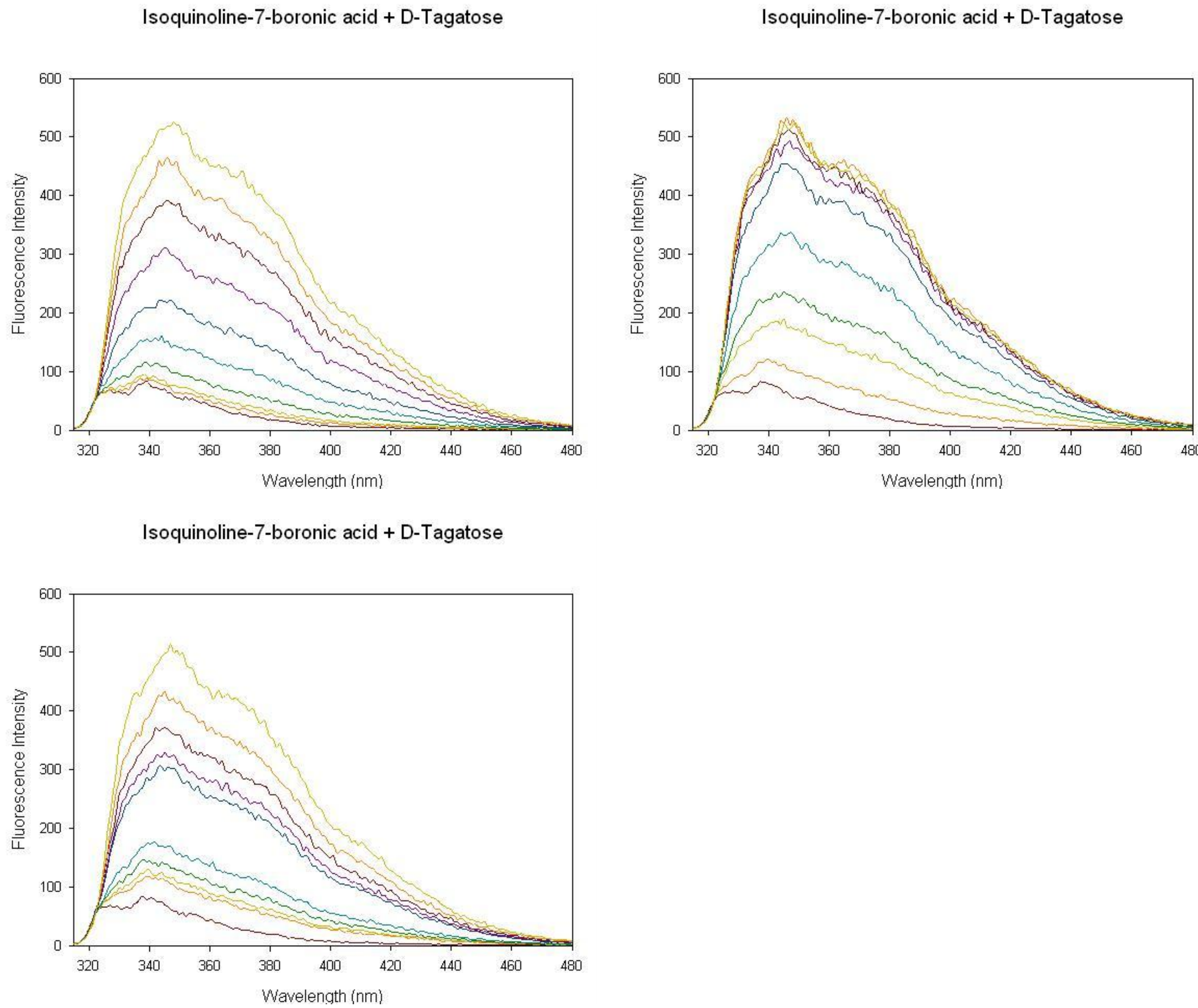

Figure A5. Fluorescent spectral changes of 7-IQBA (1×10^{-5} M) upon addition of D-tagatose in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex} = 272$ nm, $\lambda_{em} = 346$ nm.

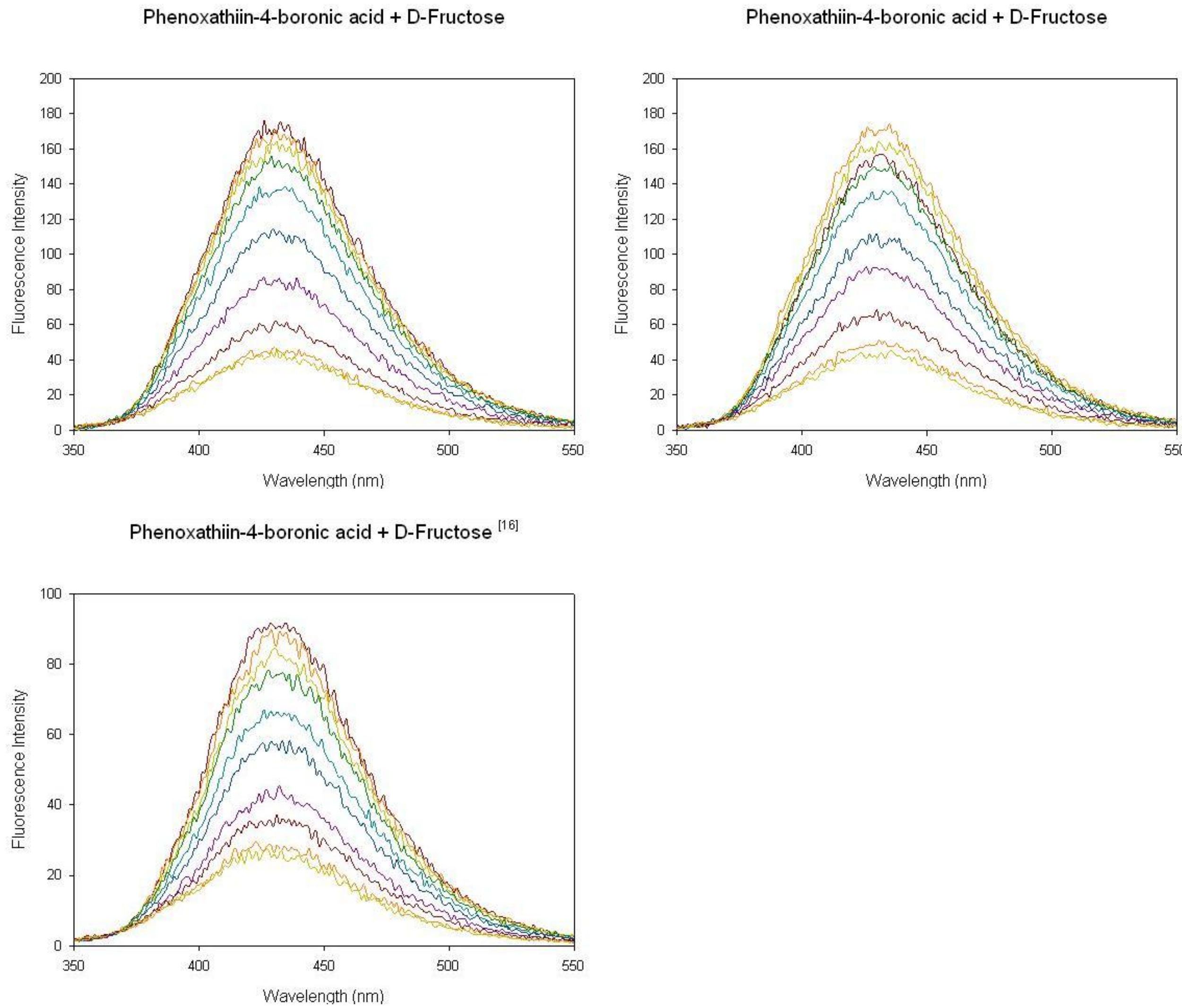

Figure A6. Fluorescent spectral changes of 4-POBA (1×10^{-5} M) upon addition of D-fructose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, λ_{em} = 430 nm.

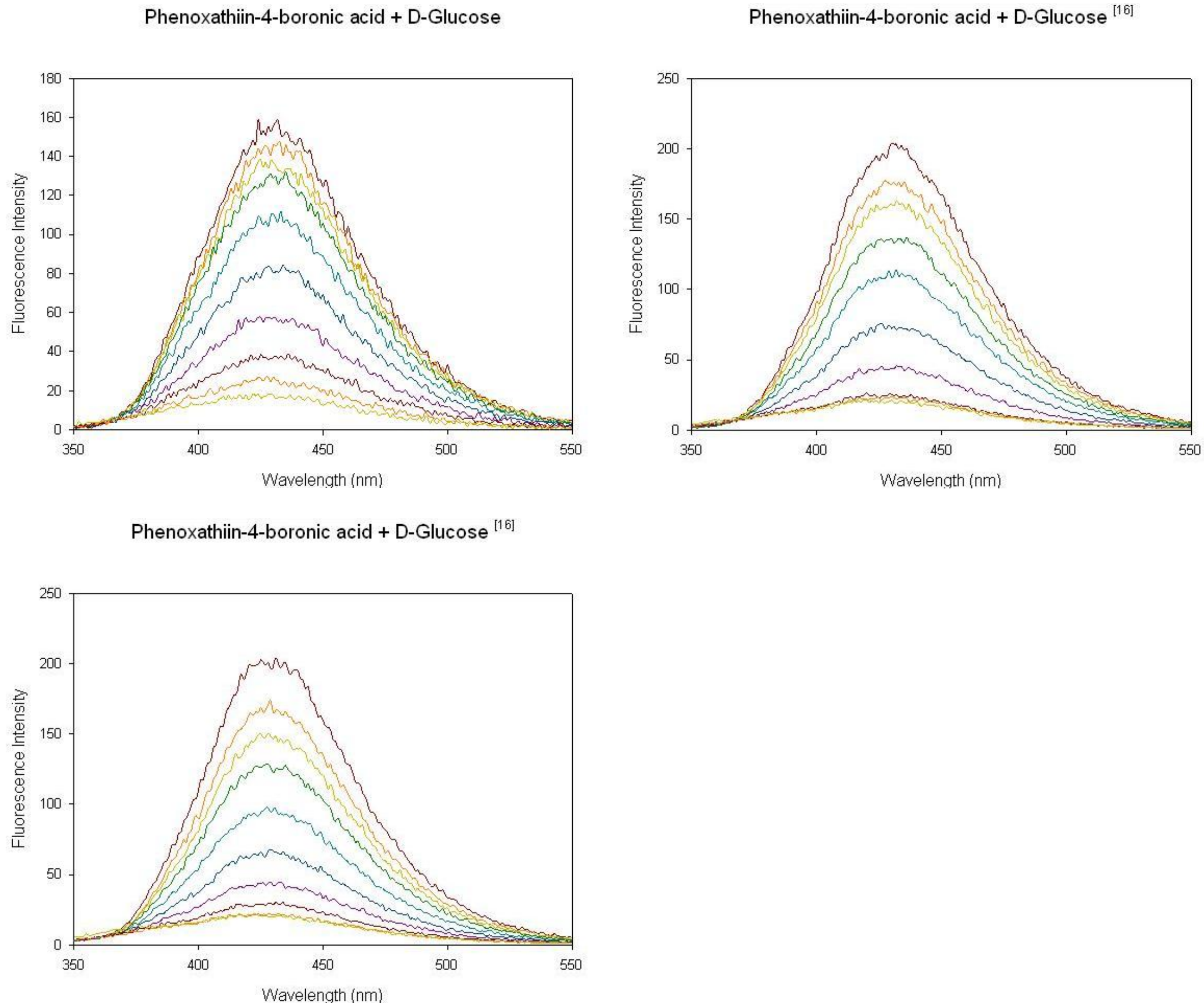

Figure A7. Fluorescent spectral changes of 4-POBA (1×10^{-5} M) upon addition of D-glucose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, λ_{em} = 430 nm.

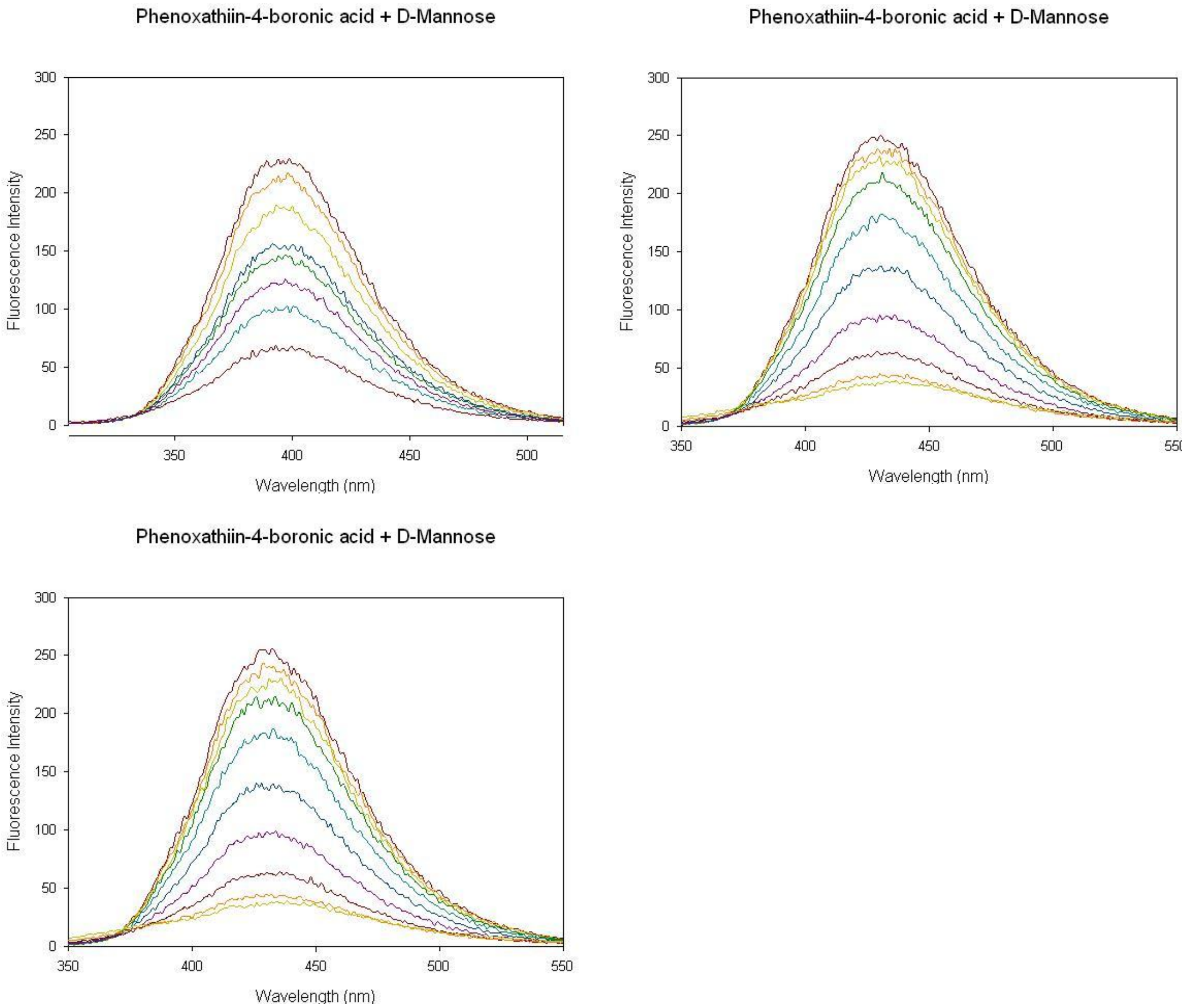

Figure A8. Fluorescent spectral changes of 4-POBA (1×10^{-5} M) upon addition of D-mannose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, λ_{em} = 430 nm.

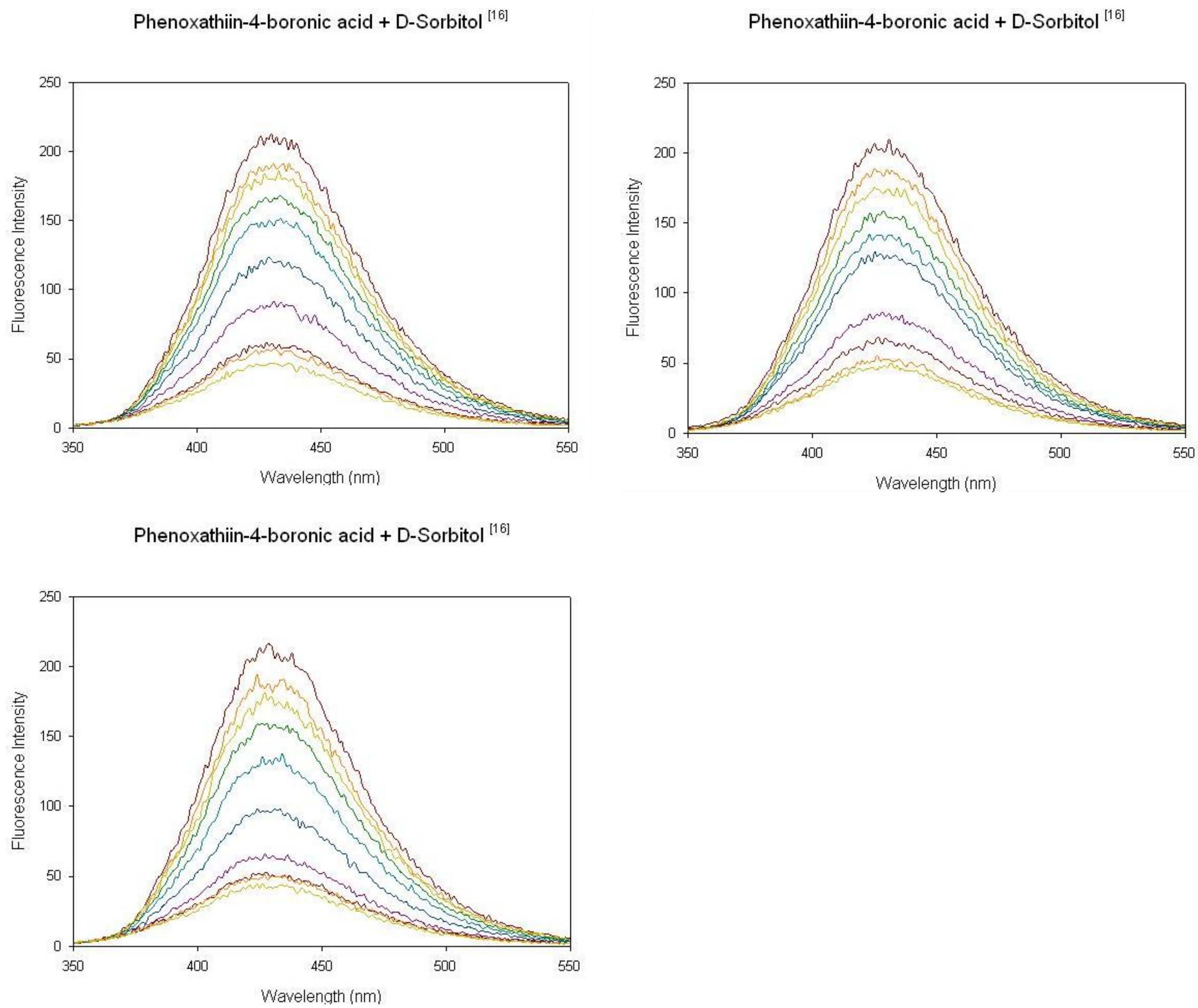

Figure A9. Fluorescent spectral changes of 4-POBA (1×10^{-5} M) upon addition of D-sorbitol in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, λ_{em} = 430 nm.

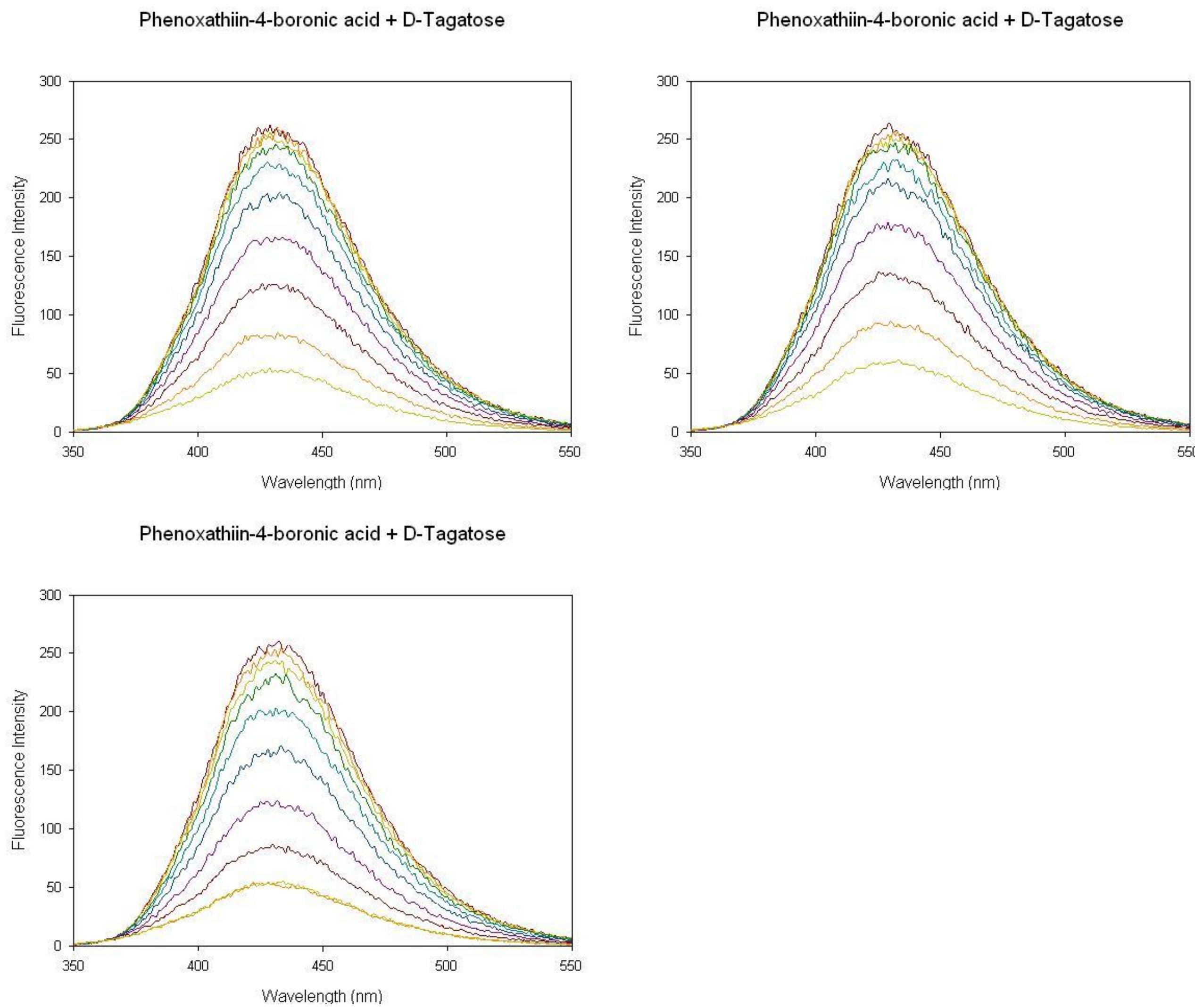

Figure A10. Fluorescent spectral changes of 4-POBA (1×10^{-5} M) upon addition of D-tagatose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, λ_{em} = 430 nm.

APPENDIX B – Fluorescence Quantum Yield (Φ_F)

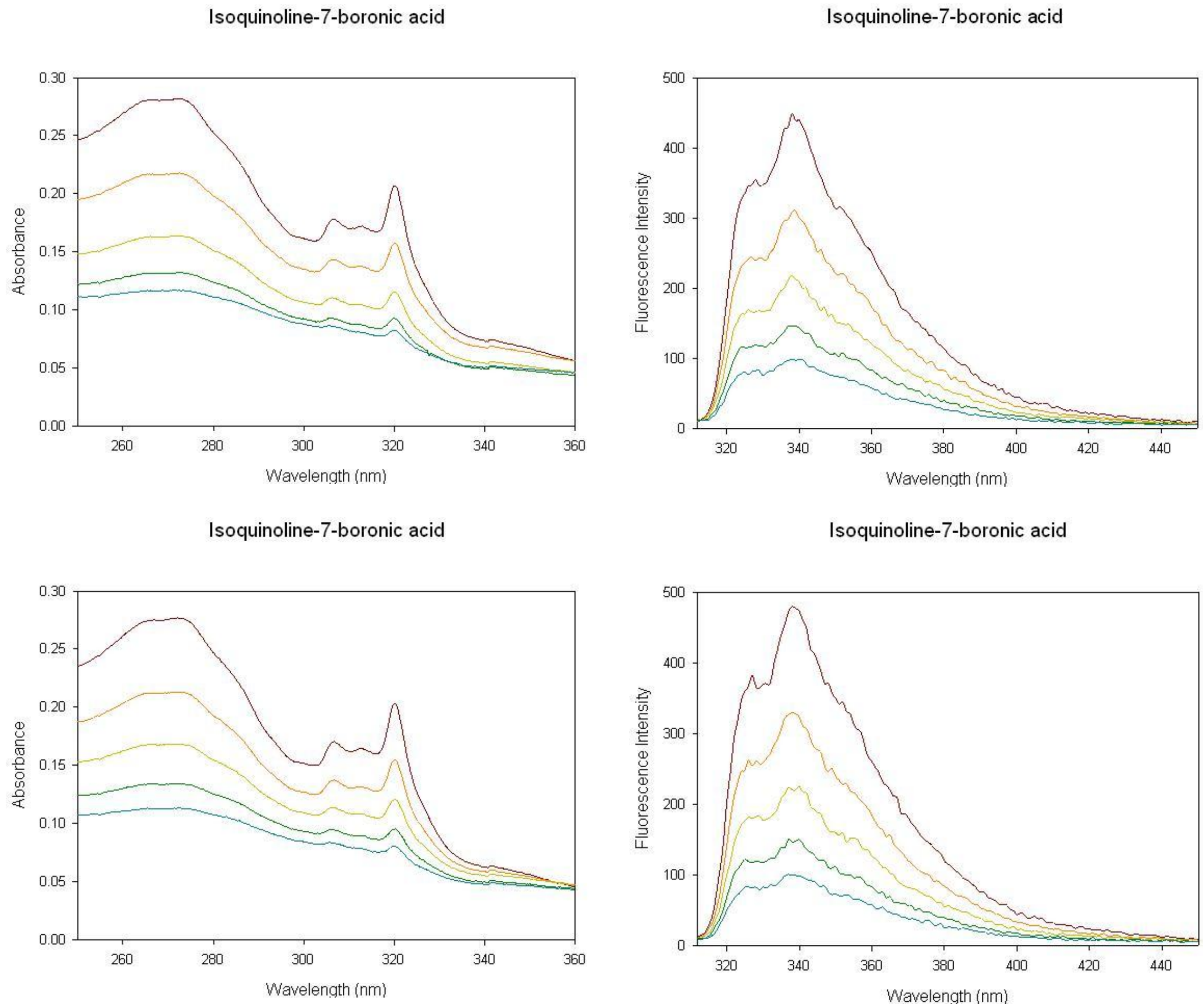

Figure B1. Absorbance and fluorescence spectra of decreasing 7-IQBA concentrations (initial: 1×10^{-5} M with 1/3 serial dilutions) in the absence of sugar in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 272 nm, area range λ_{em} = 275 – 500 nm.

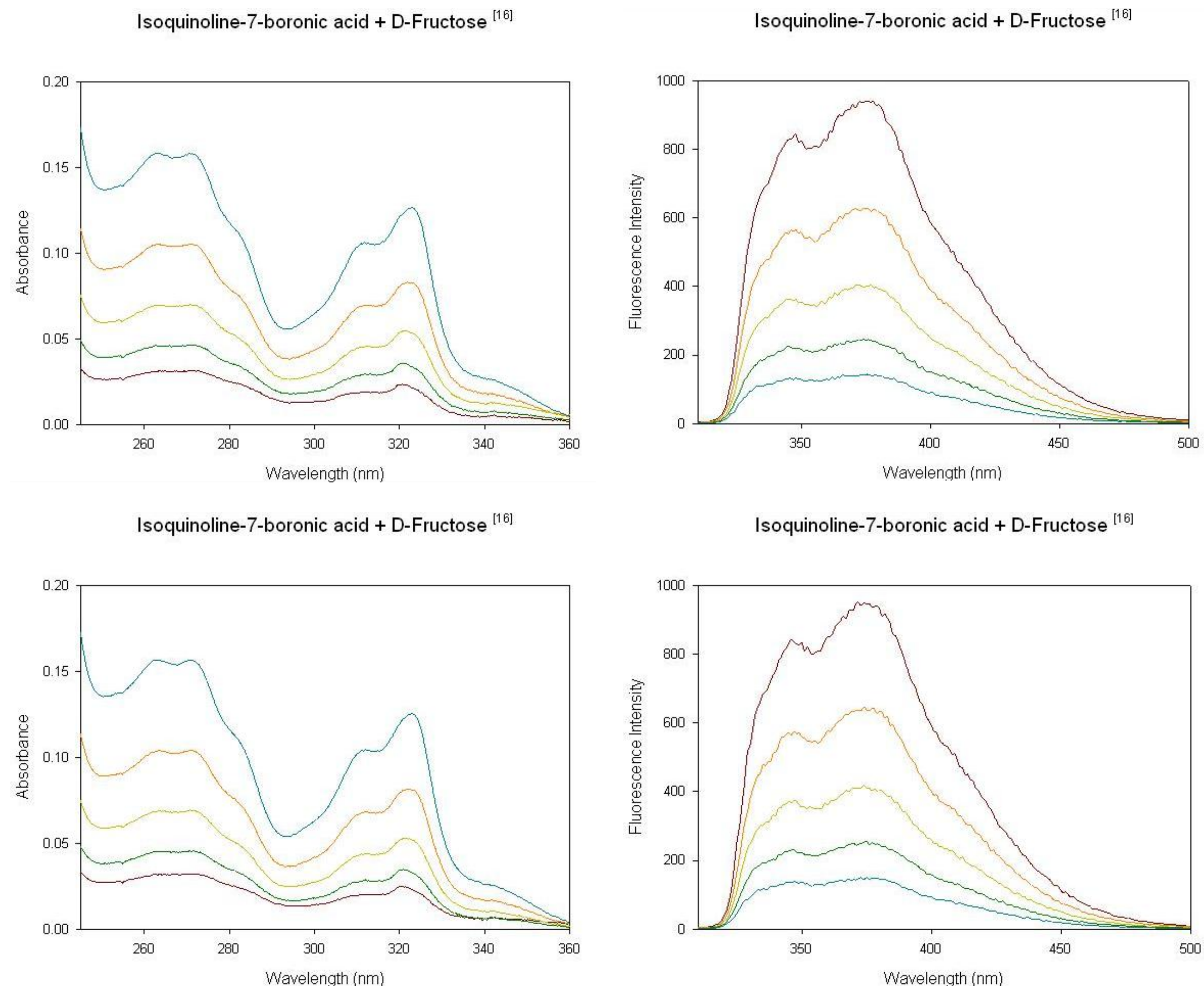

Figure B2. Absorbance and fluorescence spectra of decreasing 7-IQBA concentrations (initial: 1 × 10^{-5} M with 1/3 serial dilutions) in the presence of D-fructose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 272 nm, area range λ_{em} = 310 – 500 nm.

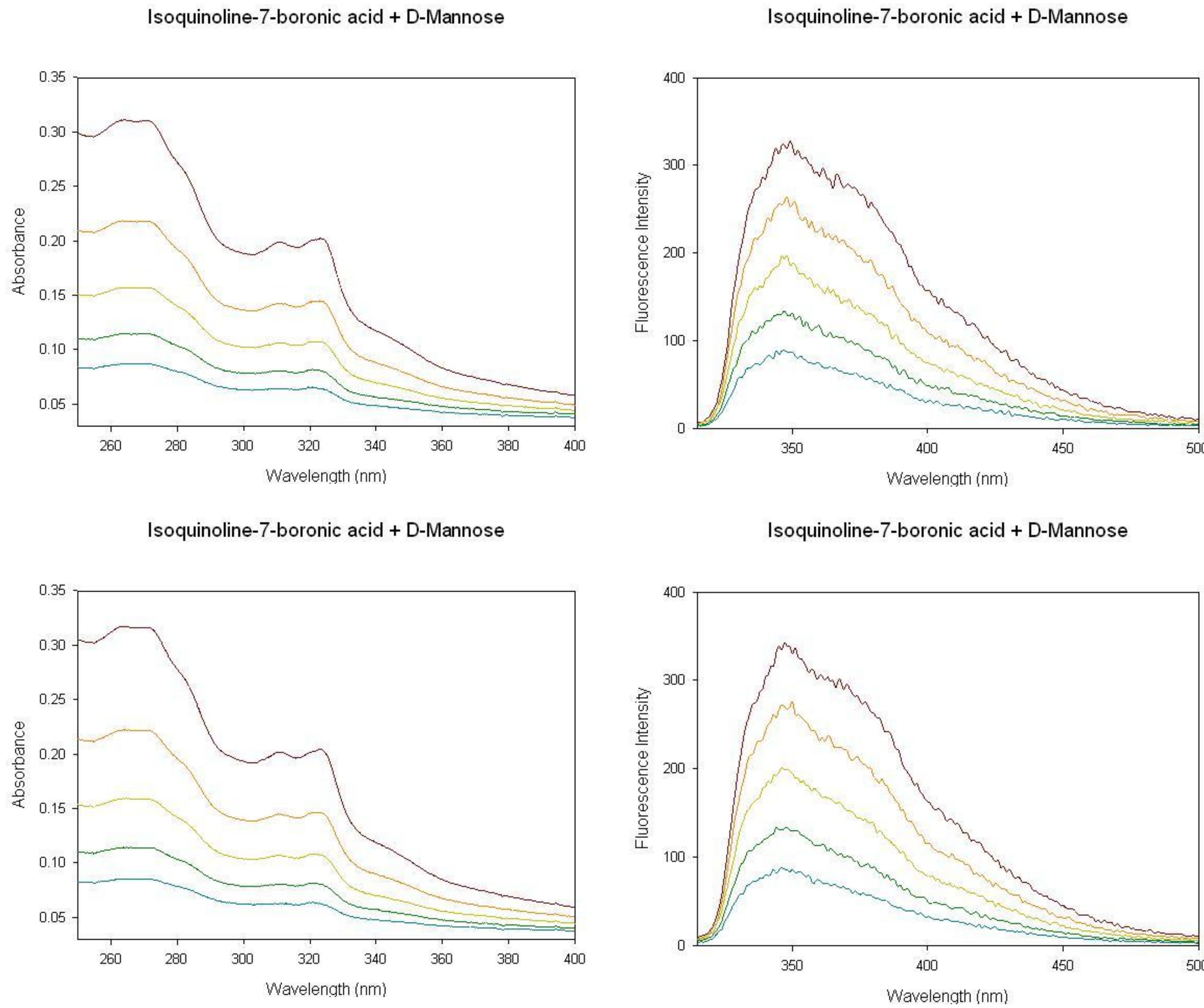

Figure B3. Absorbance and fluorescence spectra of decreasing 7-IQBA concentrations (initial: 1×10^{-5} M with 1/3 serial dilutions) in the presence of D-mannose in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex} = 272$ nm, area range $\lambda_{em} = 315 - 500$ nm.

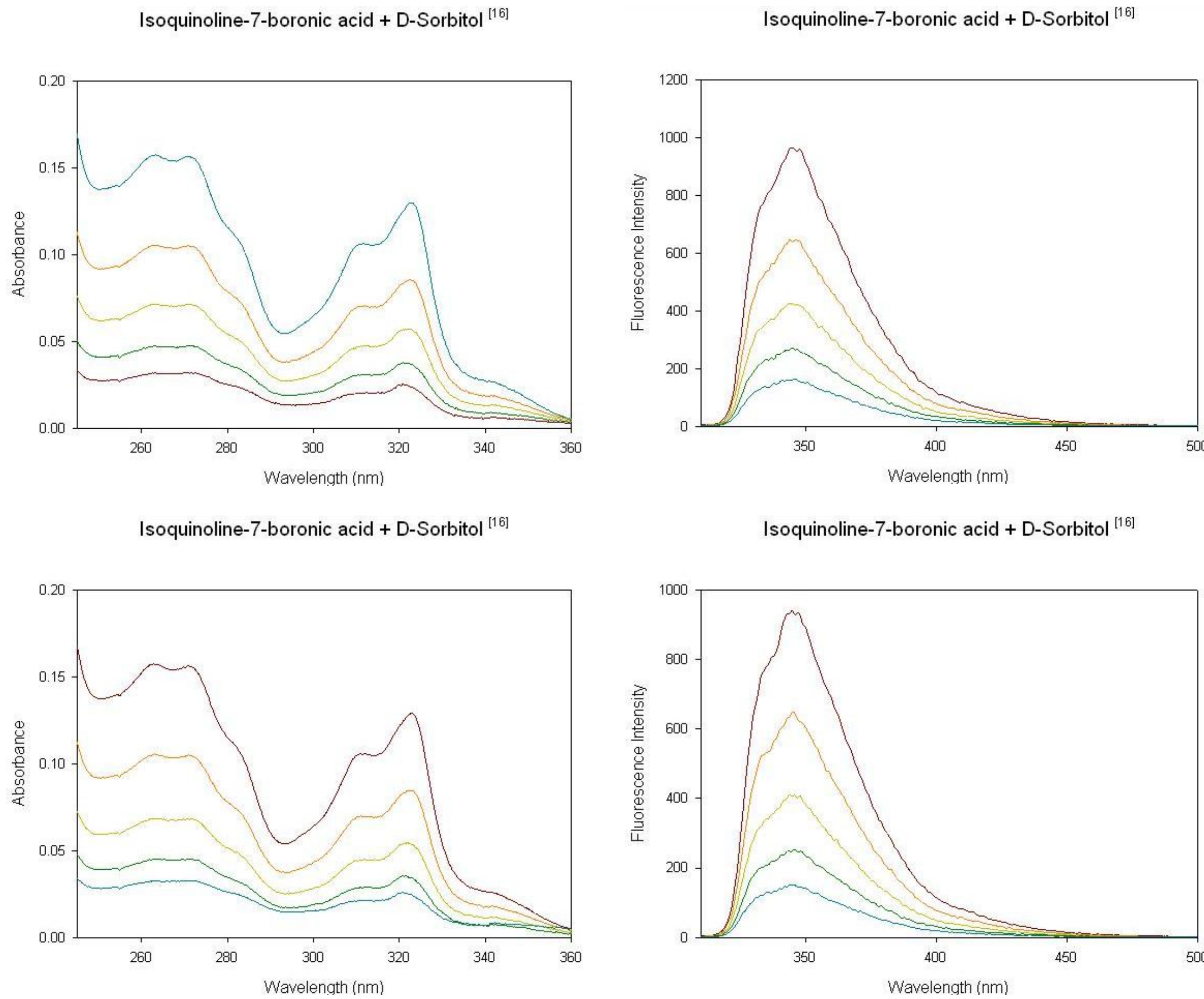

Figure B4. Absorbance and fluorescence spectra of decreasing 7-IQBA concentrations (initial: 1 × 10^{-5} M with 1/3 serial dilutions) in the presence of D-sorbitol in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 272 nm, area range λ_{em} = 310 – 500 nm.

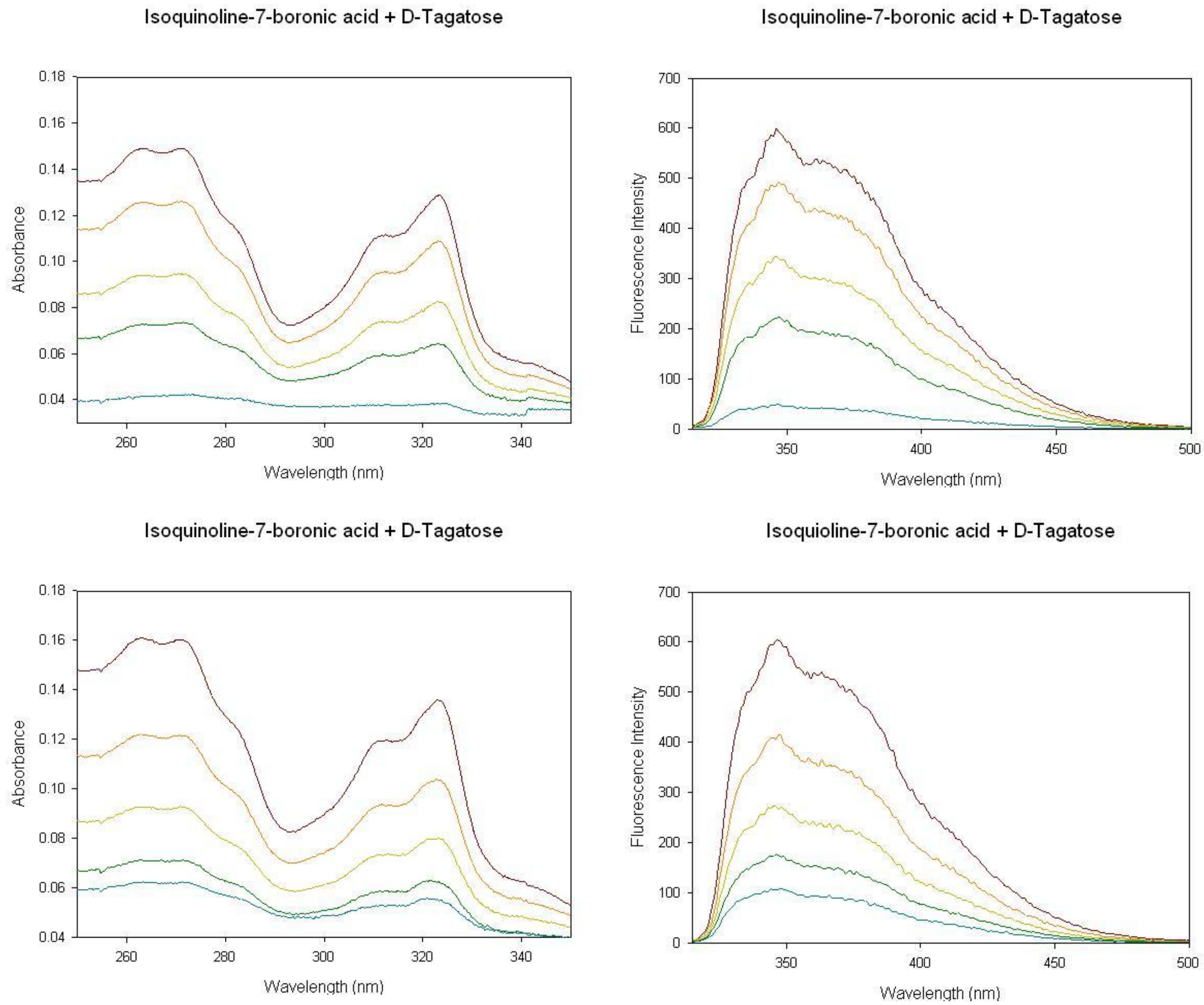

Figure B5. Absorbance and fluorescence spectra of decreasing 7-IQBA concentrations (initial: 1×10^{-5} M with 1/3 serial dilutions) in the presence of D-tagatose in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex} = 272$ nm, area range $\lambda_{em} = 315 – 500$ nm.

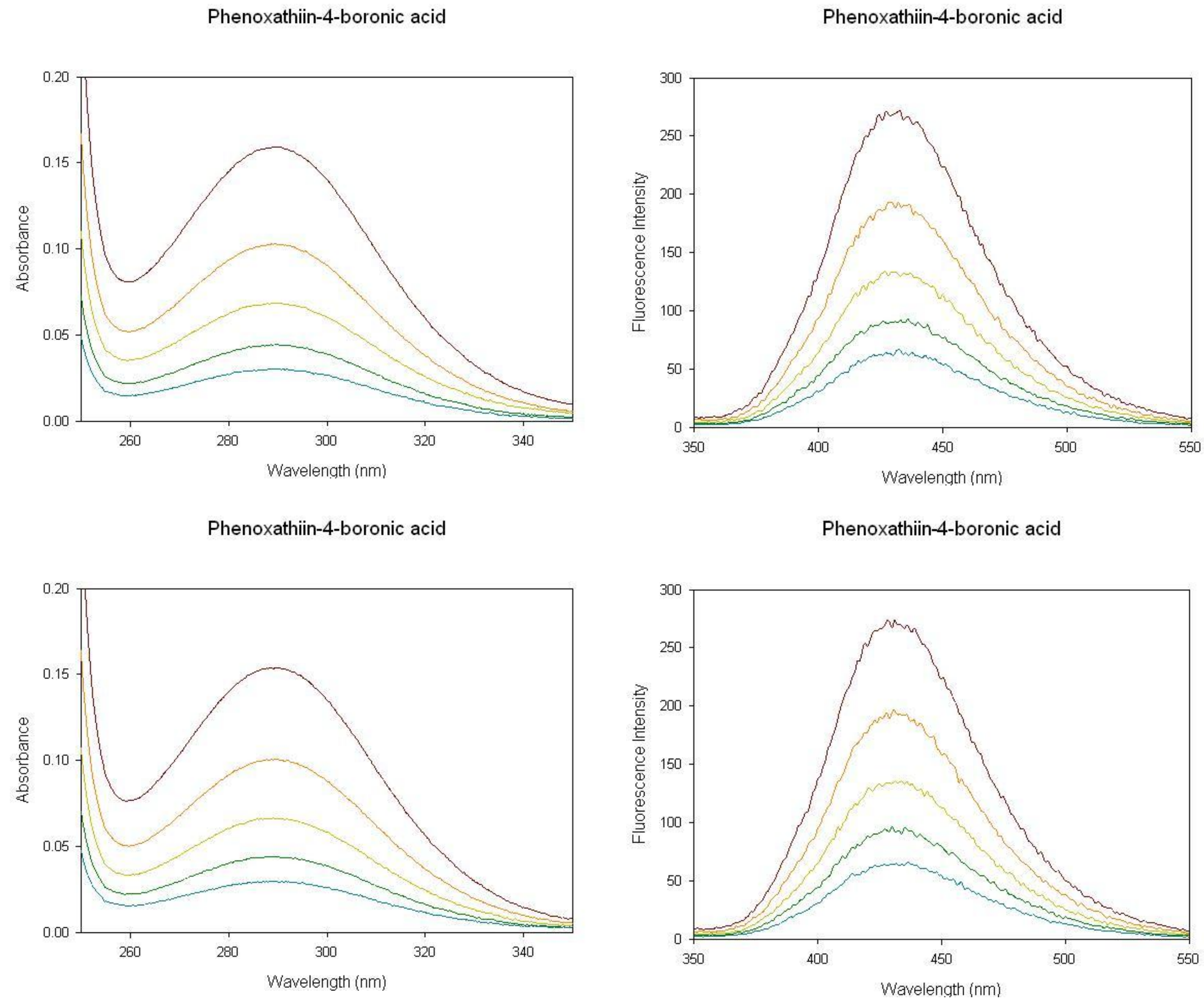

Figure B6. Absorbance and fluorescence spectra of decreasing 4-POBA concentrations (initial: 1 × 10^{-5} M with 1/3 serial dilutions) in the absence of sugar in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, area range λ_{em} = 350 – 550 nm.

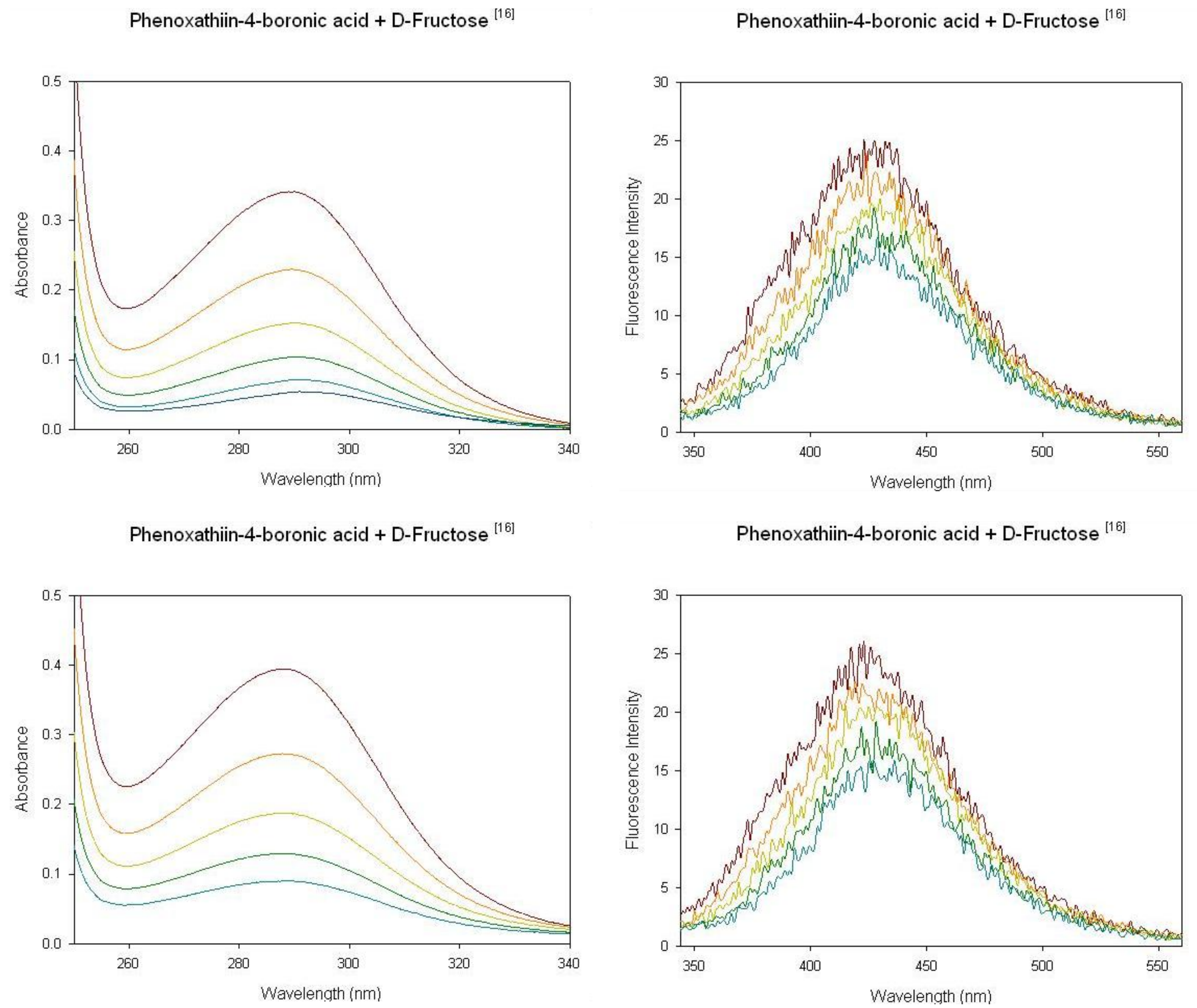

Figure B7. Absorbance and fluorescence spectra of decreasing 4-POBA concentrations (initial: 1×10^{-5} M with 1/3 serial dilutions) in the presence of D-fructose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm**,** area range λ_{em} = 344 – 560 nm.

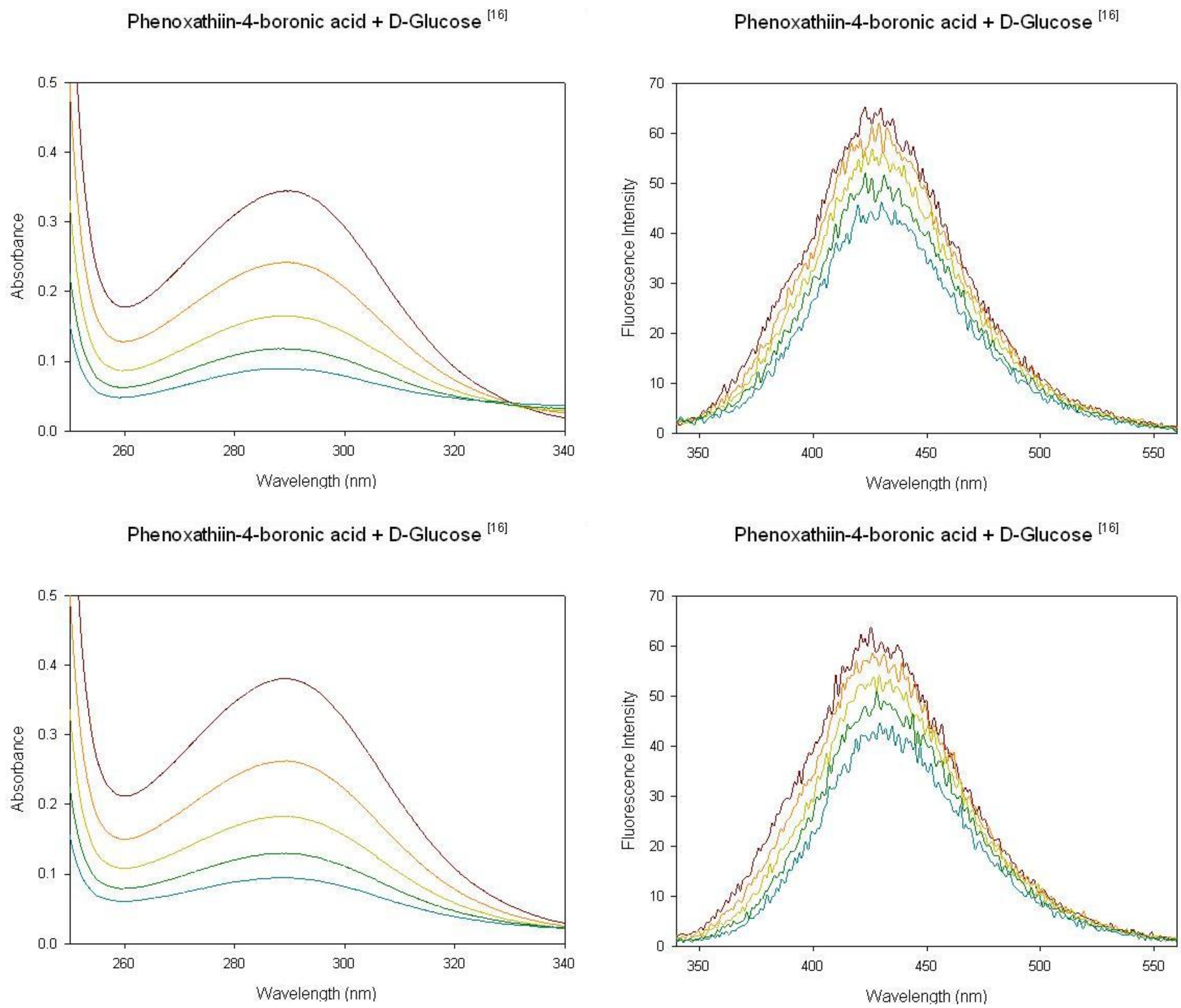

Figure B8. Absorbance and fluorescence spectra of decreasing 4-POBA concentrations (initial: 1×10^{-5} M with 1/3 serial dilutions) in the presence of D-glucose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm**,** area range λ_{em} = 340 – 560 nm.

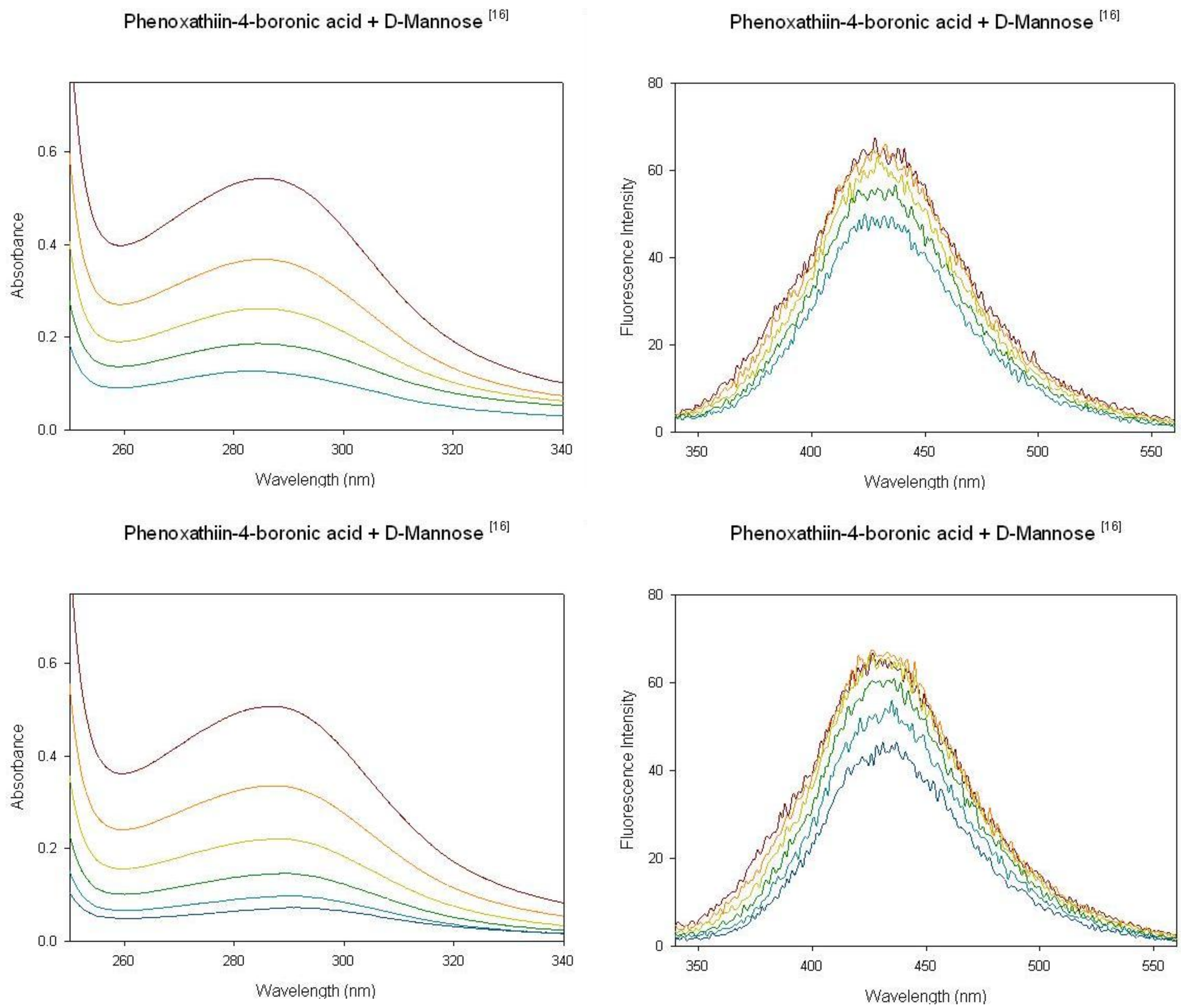

Figure B9. Absorbance and fluorescence spectra of decreasing 4-POBA concentrations (initial: 1 × 10^{-5} M with 1/3 serial dilutions) in the presence of D-mannose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, area range λ_{em} = 340 – 560 nm.

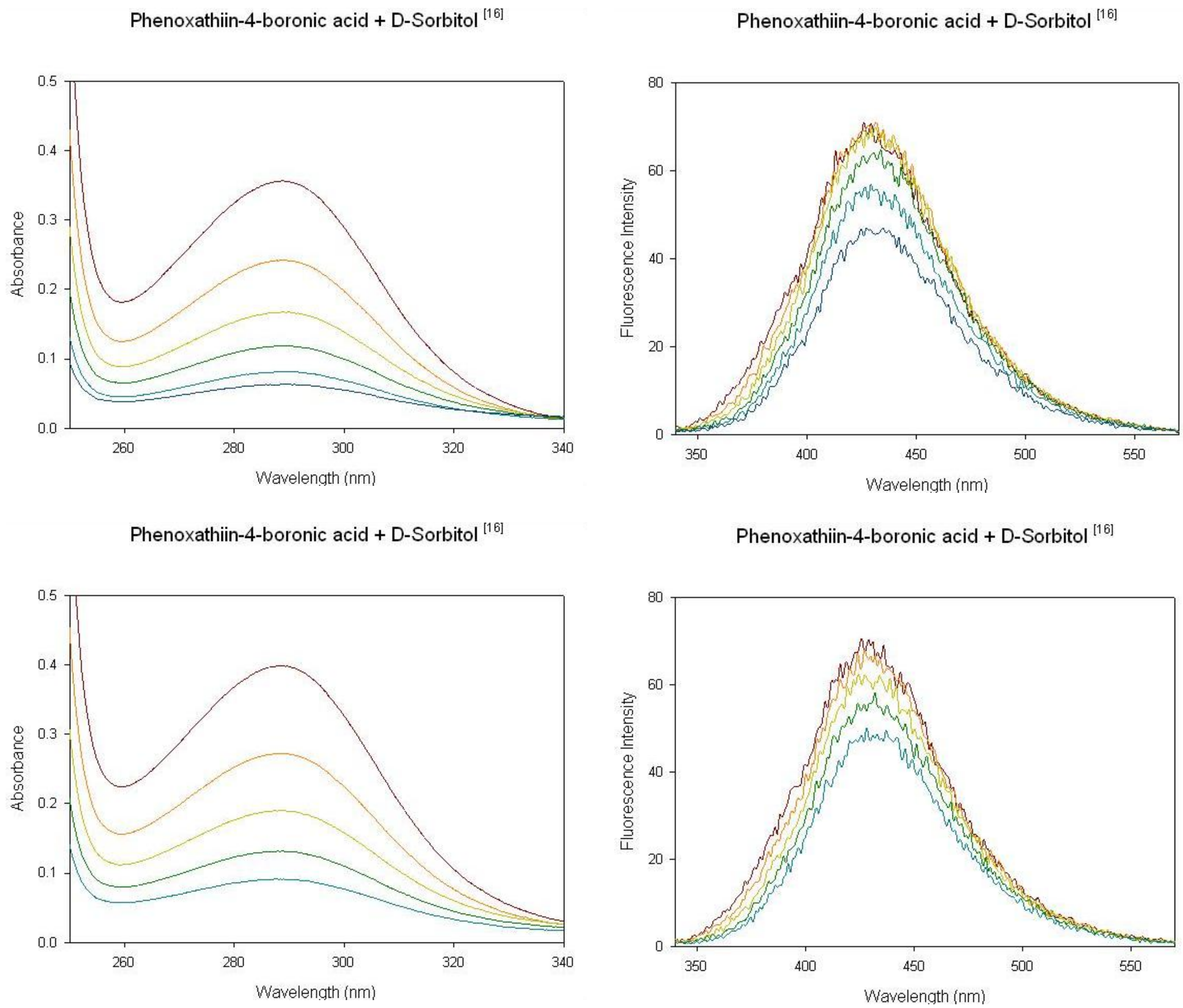

Figure B10. Absorbance and fluorescence spectra of decreasing 4-POBA concentrations (initial: 1 × 10^{-5} M with 1/3 serial dilutions) in the presence of D-sorbitol in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, area range λ_{em} = 340 – 570 nm.

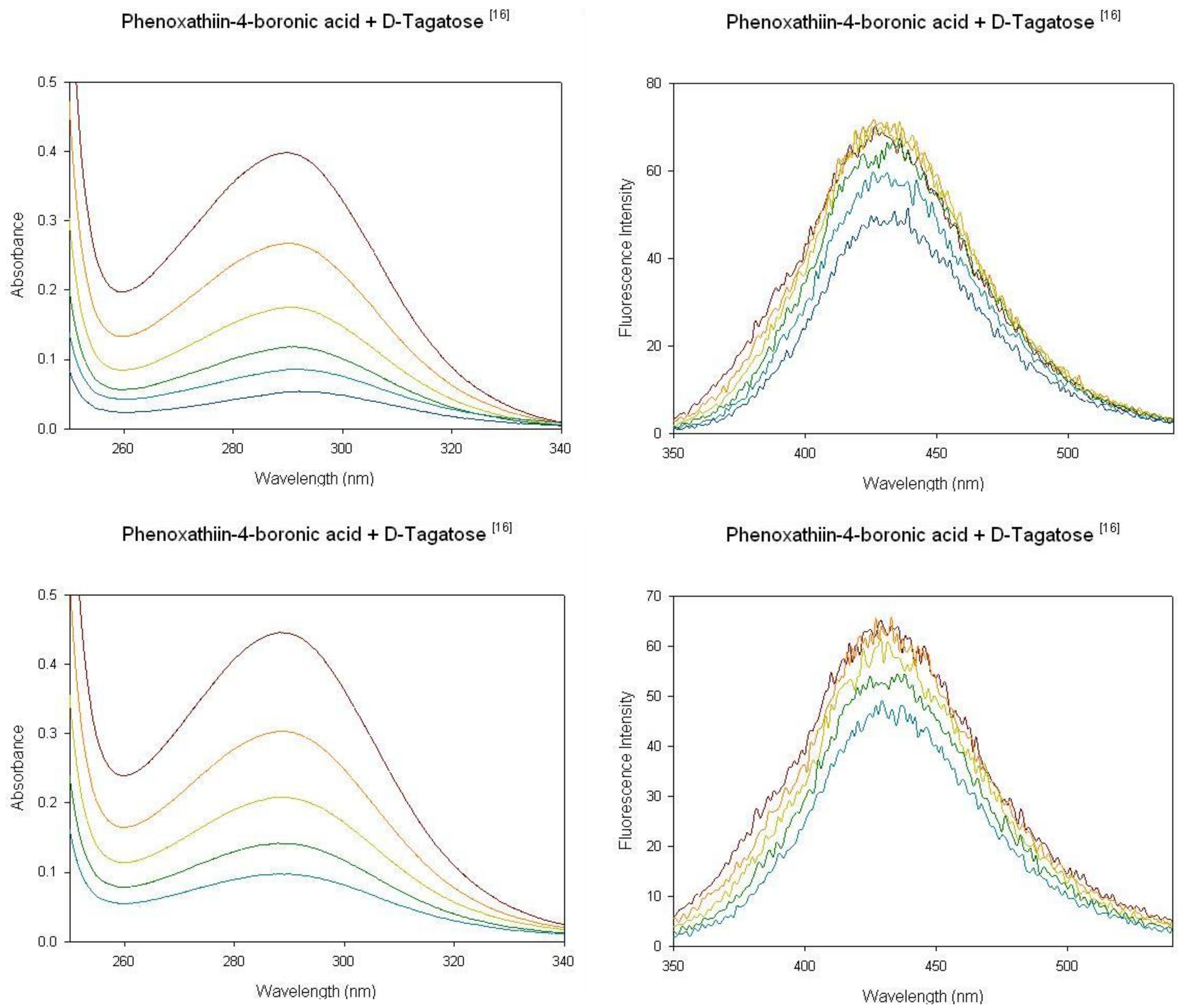

Figure B11. Absorbance and fluorescence spectra of decreasing 4-POBA concentrations (initial: 1 × 10^{-5} M with 1/3 serial dilutions) in the presence of D-tagatose in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 299 nm, area range λ_{em} = 350 – 540 nm.

APPENDIX C – pH Profiles & Identification of pK_a Values

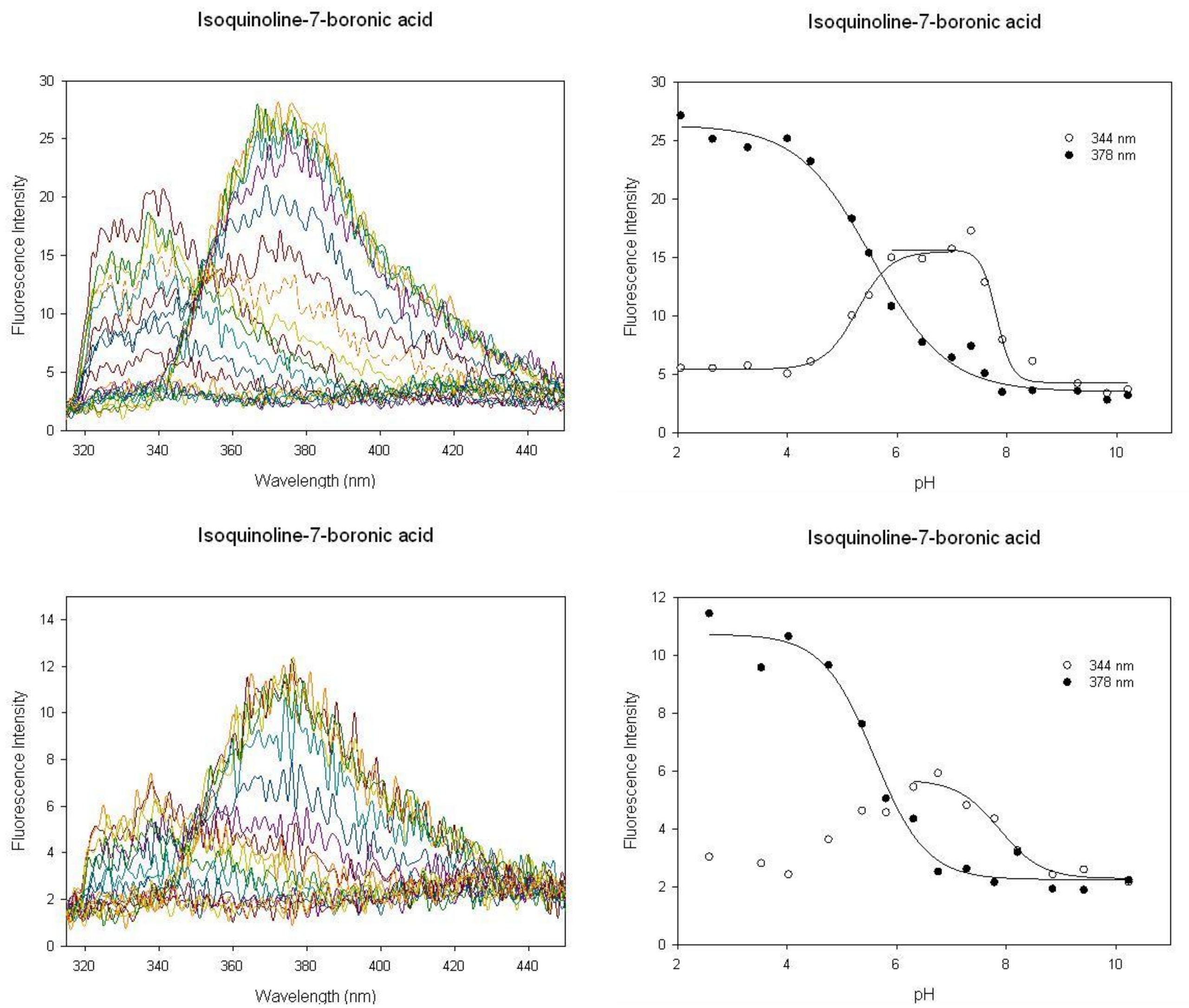

Figure C1. pH profiles of fluorescent intensities for 7-IQBA (1×10^{-5} M) in the absence of sugar in phosphate buffer (0.1 M): λ_{ex} = 272 nm, λ_{em} = 344 nm and 378 nm.

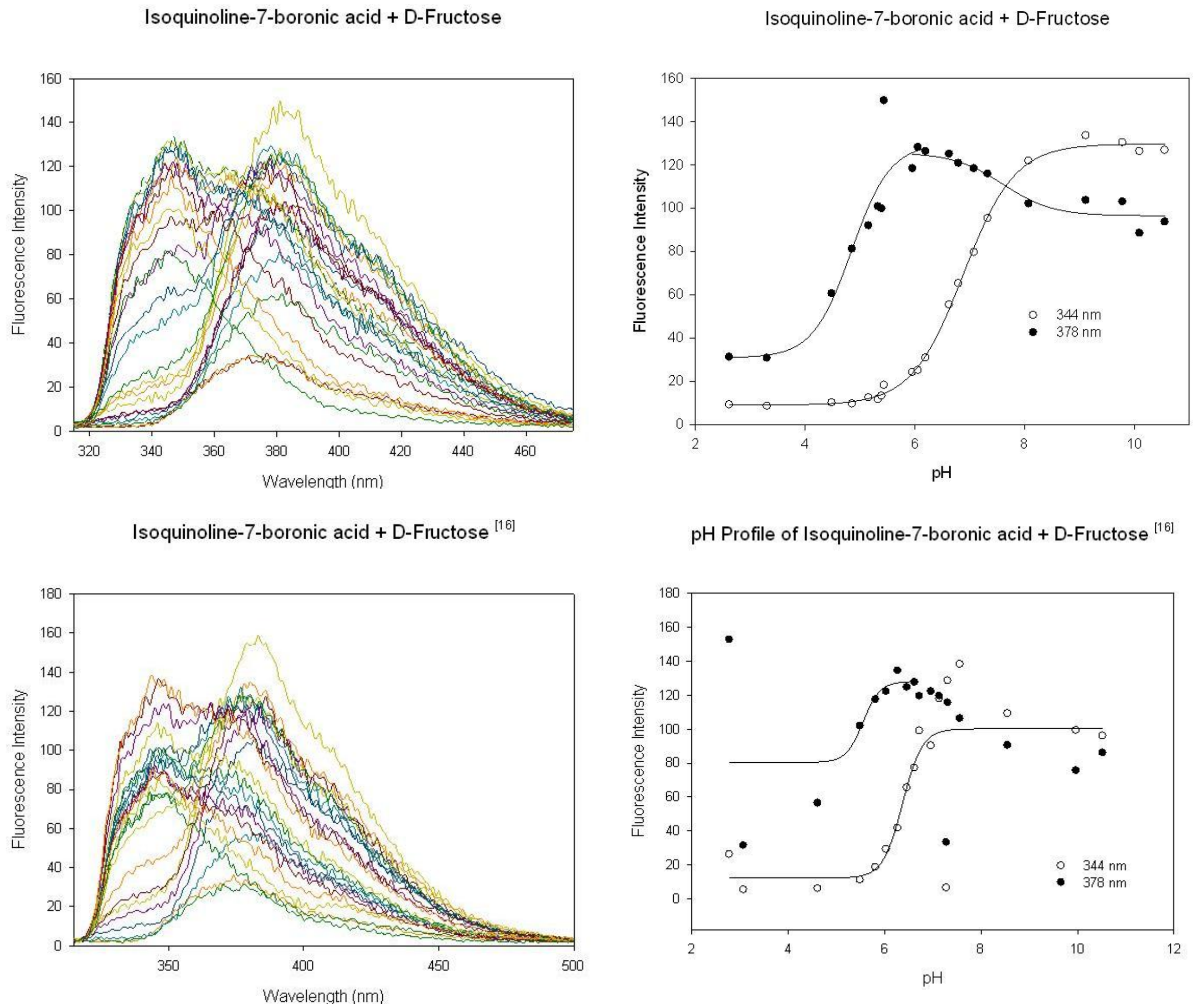

Figure C2. pH profiles of fluorescent intensities for 7-IQBA (1×10^{-5} M) in the presence of D-fructose (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 272 nm, λ_{em} = 344 nm and 378 nm.

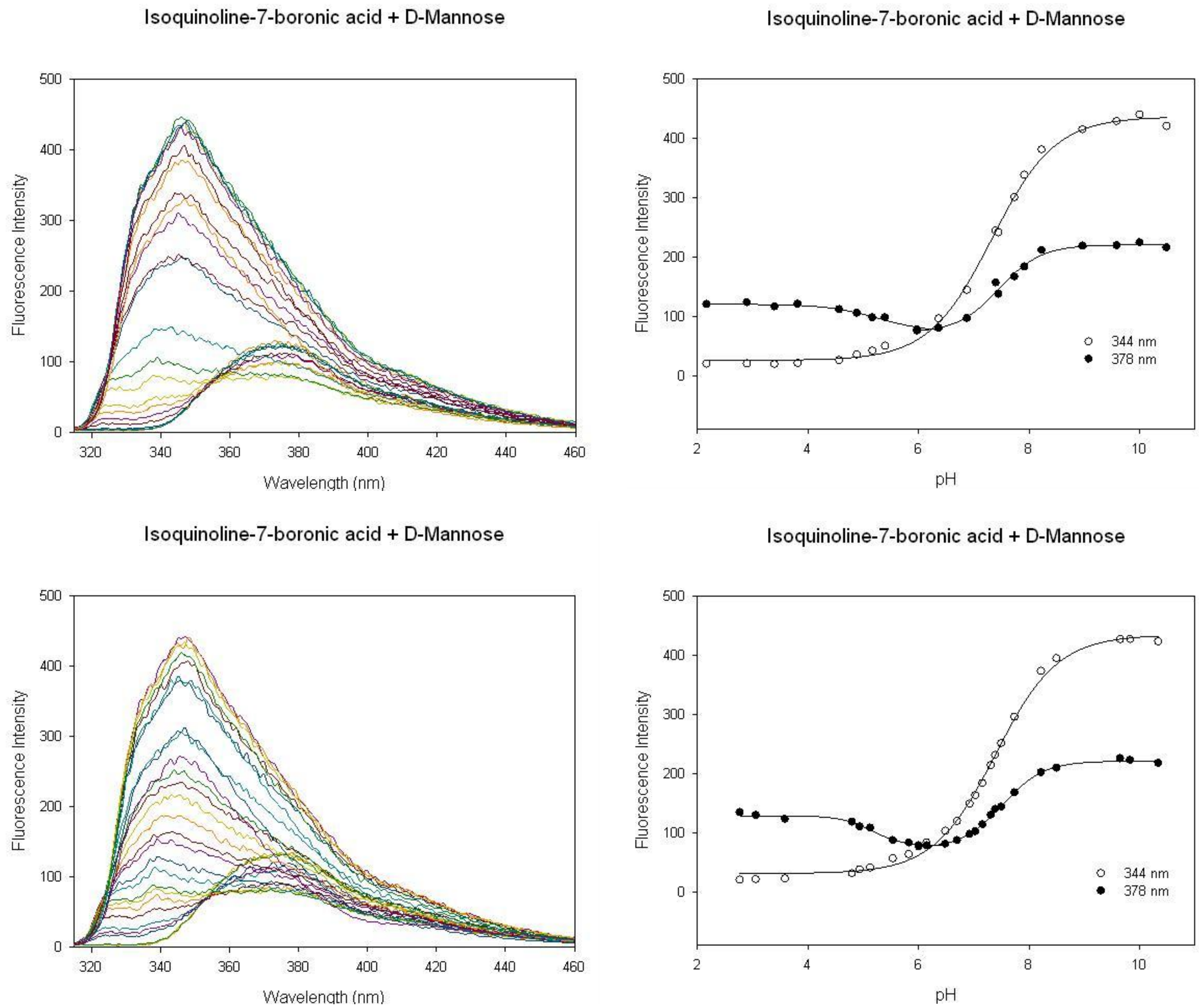

Figure C4. pH profiles of fluorescent intensities for 7-IQBA (1×10^{-5} M) in the presence of D-mannose (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 271 nm, λ_{em} = 344 nm and 378 nm.

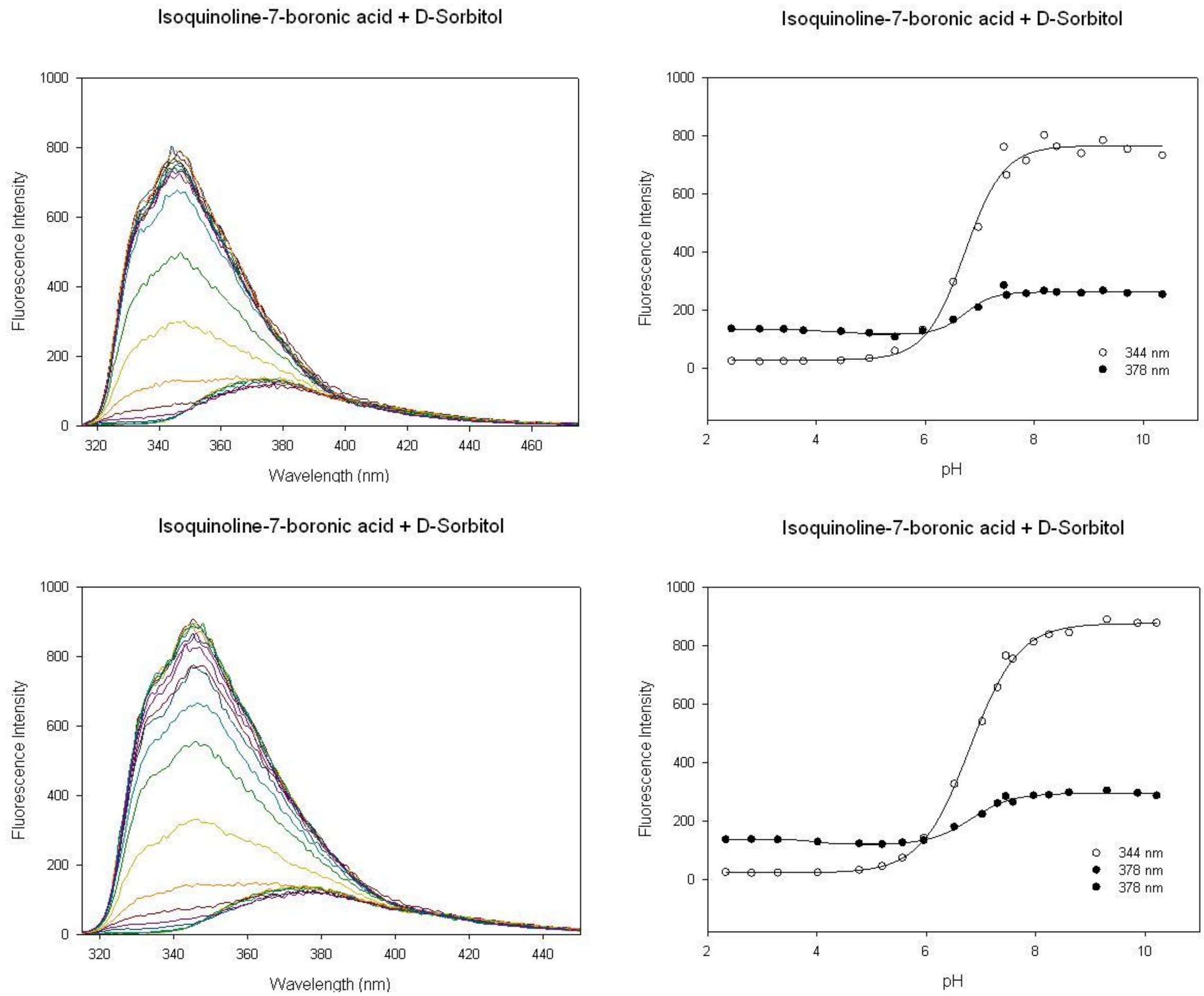

Figure C5. pH profiles of fluorescent intensities for 7-IQBA (1×10^{-5} M) in the presence of D-sorbitol (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 272 nm, λ_{em} = 344 nm and 378 nm.

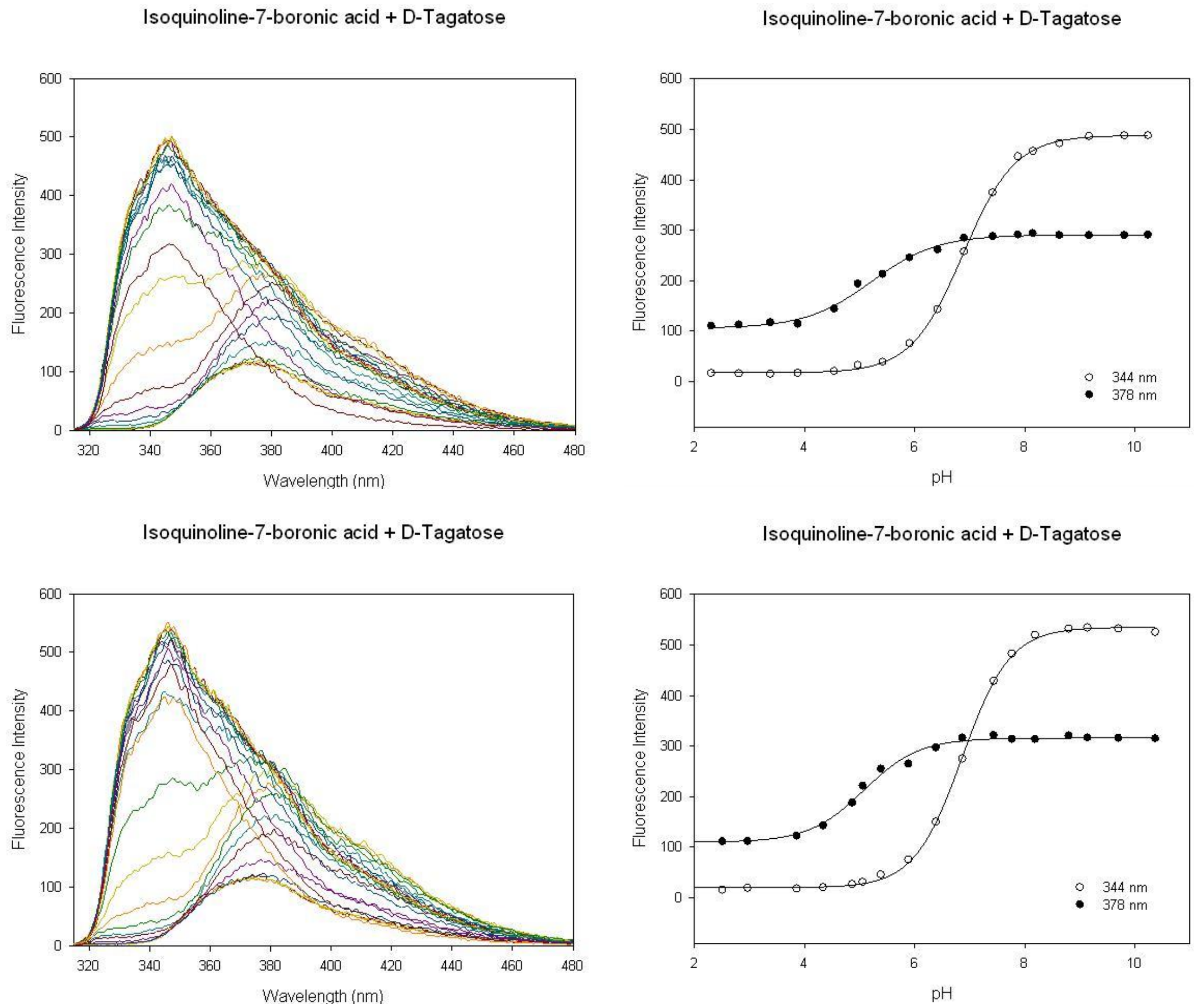

Figure C6. pH profiles of fluorescent intensities for 7-IQBA (1×10^{-5} M) in the presence of D-tagatose (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 272 nm, λ_{em} = 344 nm and 378 nm.

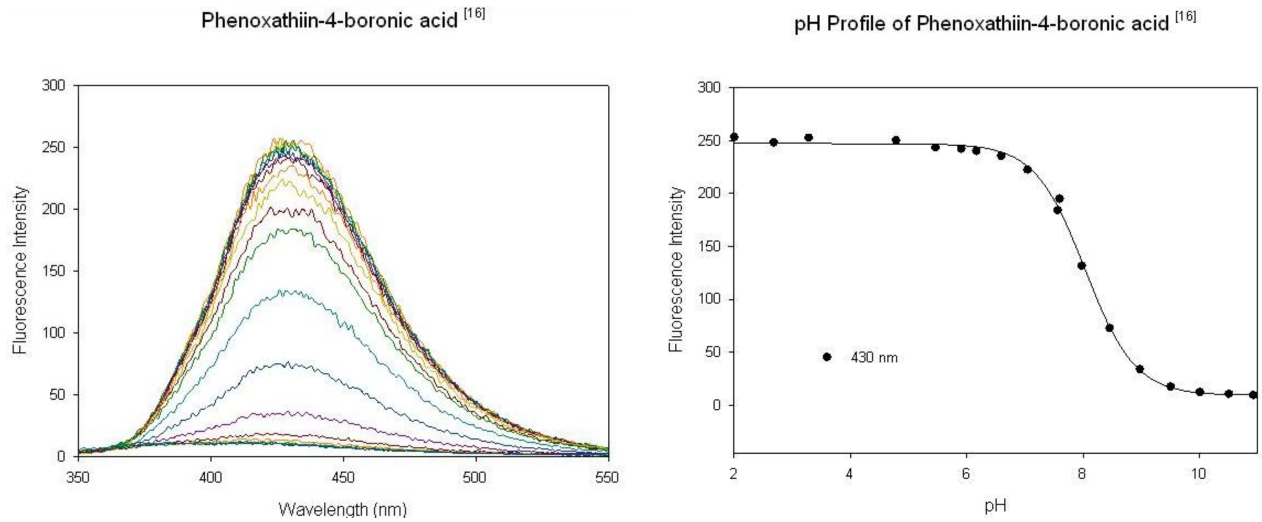

Figure C7. pH profiles of fluorescent intensities for 4-POBA (1×10^{-5} M) in the absence of sugar in phosphate buffer (0.1 M): λ_{ex} = 299 nm, λ_{em} = 430 nm.

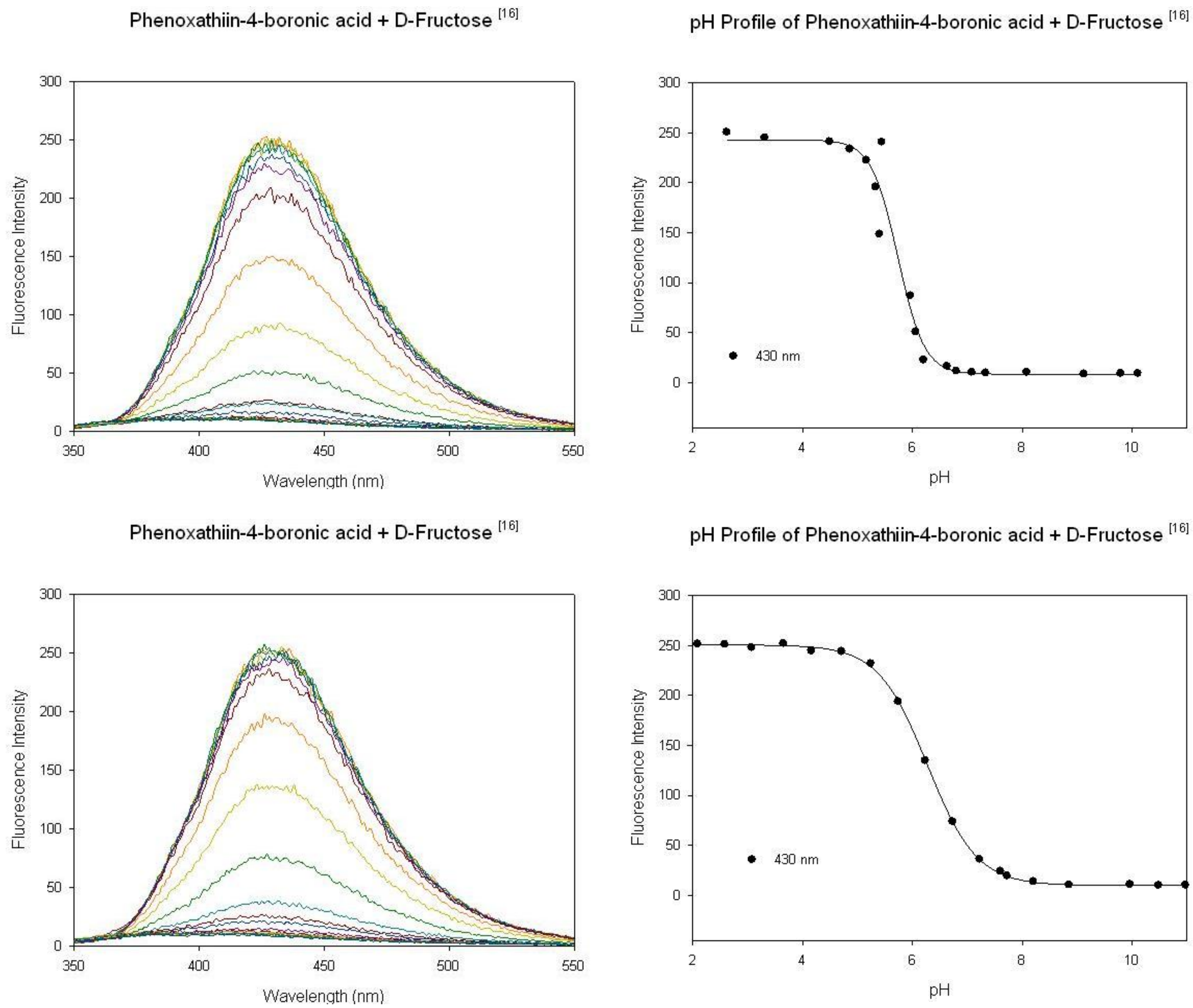

Figure C8. pH profiles of fluorescent intensities for 4-POBA (1×10^{-5} M) in the presence of D-fructose (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 299 nm, λ_{em} = 430 nm.

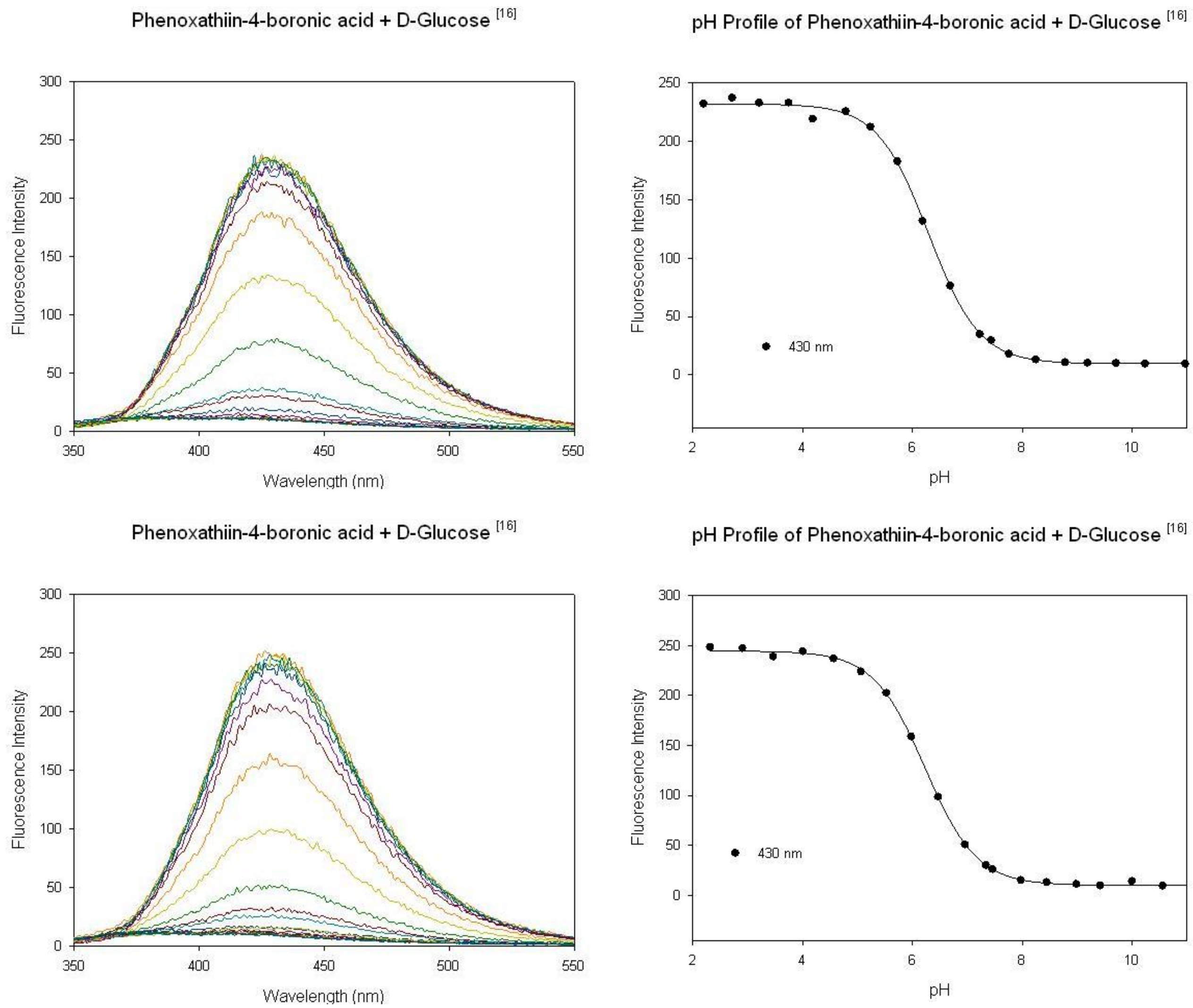

Figure C9. pH profiles of fluorescent intensities for 4-POBA (1×10^{-5} M) in the presence of D-glucose (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 299 nm, λ_{em} = 430 nm.

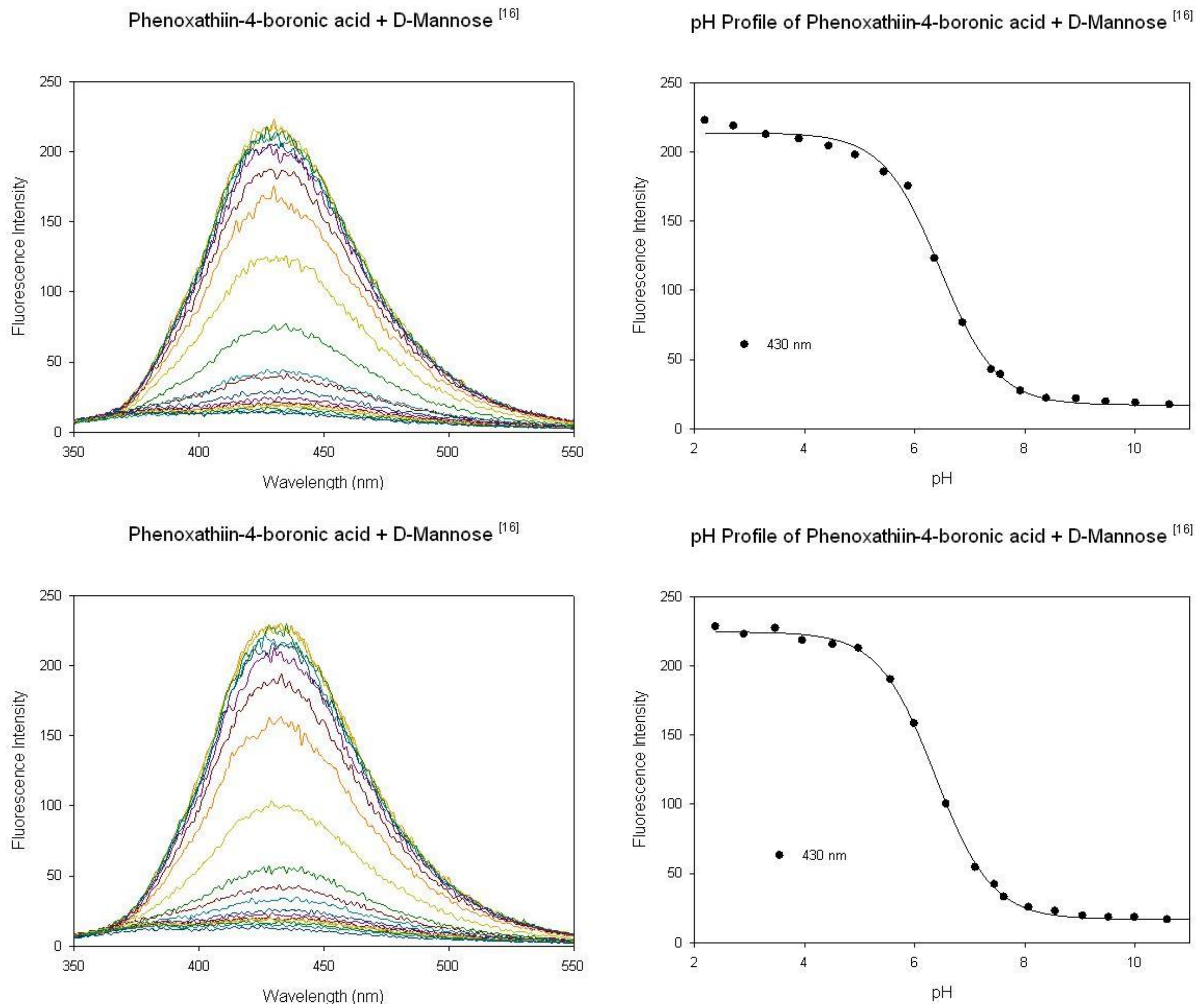

Figure C10. pH profiles of fluorescent intensities for 4-POBA (1×10^{-5} M) in the presence of D-mannose (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 299 nm, λ_{em} = 430 nm.

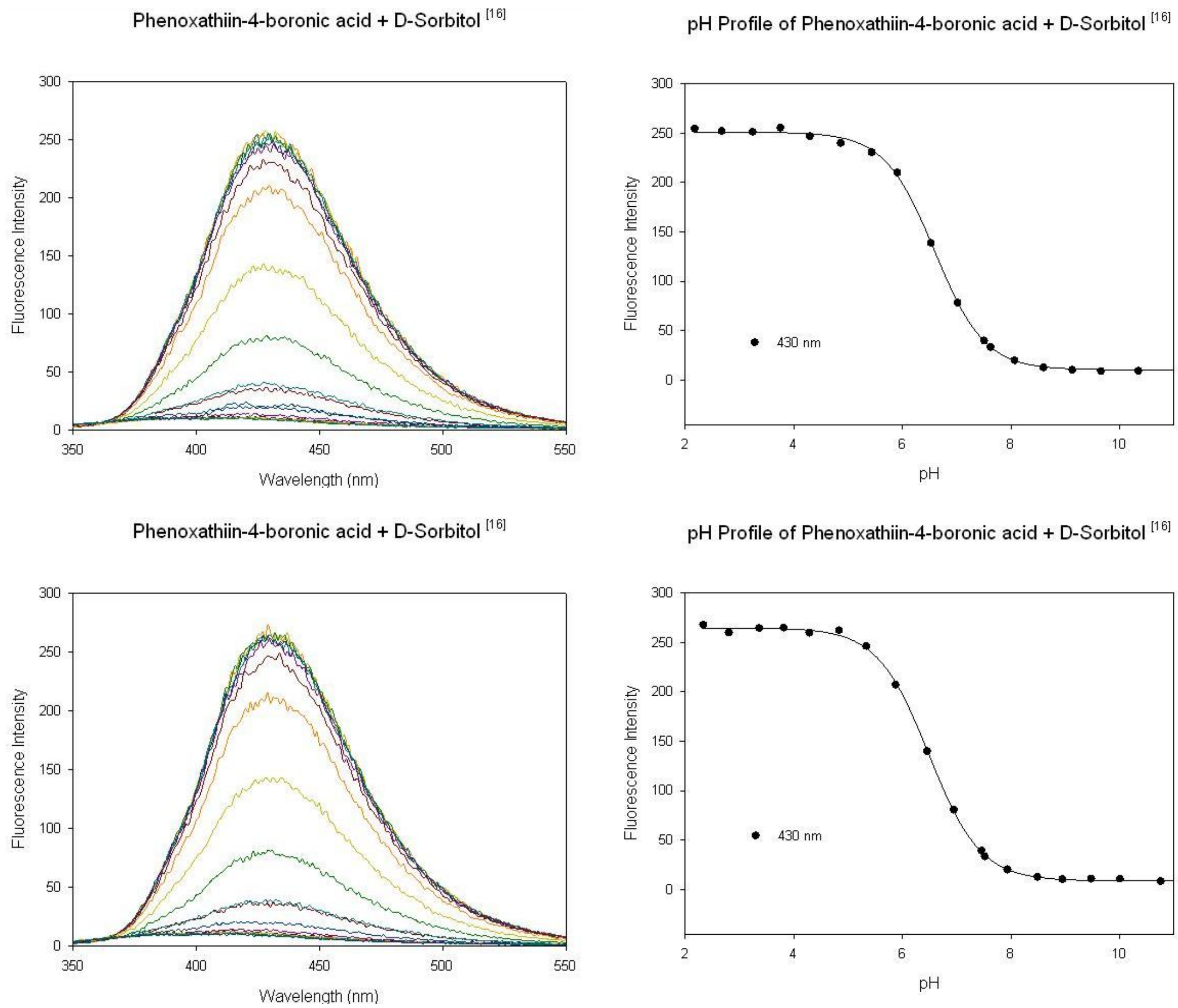

Figure C11. pH profiles of fluorescent intensities for 4-POBA (1×10^{-5} M) in the presence of D-sorbitol (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 299 nm, λ_{em} = 430 nm.

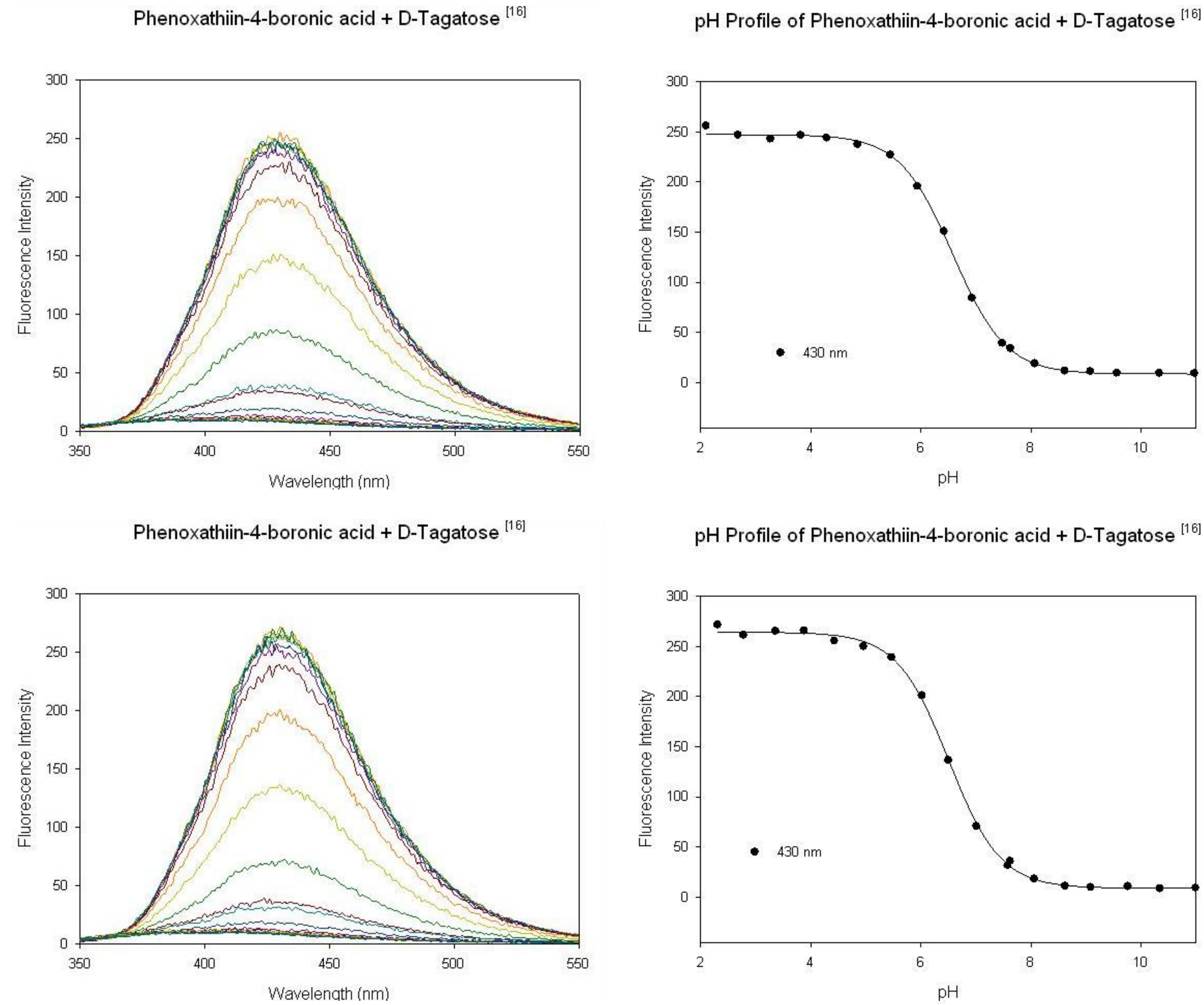

Figure C12. pH profiles of fluorescent intensities for 4-POBA (1×10^{-5} M) in the presence of D-tagatose (0.01 M) in phosphate buffer (0.1 M): λ_{ex} = 299 nm, λ_{em} = 430 nm.

APPENDIX D – Titration Reports for Identification of pK_a Values

7-IQBA_344nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 11:06:50 AM**

Data Source: Data 2 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9964	0.9929	0.9894	0.4629

	Coefficient	Std. Error	t	P
a	10.1206	0.4645	21.7865	<0.0001
b	0.3003	0.0563	5.3380	0.0018
x0	5.2692	0.0511	103.1944	<0.0001
y0	5.4157	0.2353	23.0206	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	1084.2156	271.0539
Residual	6	1.2856	0.2143
Total	10	1085.5012	108.5501

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	179.9288	59.9763	279.9163	<0.0001
Residual	6	1.2856	0.2143		
Total	9	181.2143	20.1349		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.1196)

W Statistic= 0.8767 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.1988)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 10.1206}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.300289}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 5.26924}}
y0 = min(y) "Auto {{previous: 5.41571}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_344nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 11:08:14 AM**

Data Source: Data 2 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9821	0.9645	0.9467	1.2880

	Coefficient	Std. Error	t	P
a	11.3878	0.9894	11.5100	<0.0001
b	-0.1421	0.0587	-2.4204	0.0518
x0	7.8091	0.0699	111.7759	<0.0001
y0	4.2861	0.6568	6.5257	0.0006

Analysis of Variance:

	DF	SS	MS
Regression	4	1287.8817	321.9704
Residual	6	9.9533	1.6589
Total	10	1297.8350	129.7835

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	270.0429	90.0143	54.2622	<0.0001
Residual	6	9.9533	1.6589		
Total	9	279.9961	31.1107		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0145)

W Statistic= 0.8000 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.8113)

Fit Equation Description:
[Variables]
x = col(3)
y = col(4)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 11.3878}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.142105}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 7.8091}}
y0 = min(y) "Auto {{previous: 4.28608}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_344nm_3

Nonlinear Regression **Wednesday, September 21, 2011, 11:26:08 AM**

Data Source: Data 3 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9865	0.9731	0.9530	0.3163

	Coefficient	Std. Error	t	P
a	3.4254	0.4560	7.5124	0.0017
b	-0.3843	0.1478	-2.6009	0.0600
x0	7.8743	0.1467	53.6845	<0.0001
y0	2.2831	0.2400	9.5133	0.0007

Analysis of Variance:

	DF	SS	MS
Regression	4	134.0835	33.5209
Residual	4	0.4003	0.1001
Total	8	134.4838	16.8105

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	14.5004	4.8335	48.3032	0.0013
Residual	4	0.4003	0.1001		
Total	7	14.9006	2.1287		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.5424)

W Statistic= 0.9329 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.1823)

Fit Equation Description:

```
[Variables]
x = col(3)
y = col(4)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 3.4254}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.384297}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 7.87428}}
y0 = min(y) "Auto {{previous: 2.2831}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_378nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 11:14:29 AM**

Data Source: Data 2 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	**Rsqr**	**Adj Rsqr**	**Standard Error of Estimate**
0.9952	0.9903	0.9881	1.0215

	Coefficient	**Std. Error**	**t**	**P**
a	22.8653	0.9344	24.4717	<0.0001
b	-0.6784	0.0845	-8.0285	<0.0001
x0	5.5446	0.0883	62.8233	<0.0001
y0	3.5108	0.4874	7.2033	<0.0001

Analysis of Variance:

	DF	**SS**	**MS**
Regression	4	4046.8964	1011.7241
Residual	13	13.5660	1.0435
Total	17	4060.4624	238.8507

Corrected for the mean of the observations:

	DF	**SS**	**MS**	**F**	**P**
Regression	3	1389.2712	463.0904	443.7700	<0.0001
Residual	13	13.5660	1.0435		
Total	16	1402.8372	87.6773		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.2549)

W Statistic= 0.9341 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.0934)

Fit Equation Description:
[Variables]
x = col(6)
y = col(7)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 22.8653}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.678392}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 5.54456}}
y0 = min(y) "Auto {{previous: 3.51075}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_378nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 11:27:48**

Data Source: Data 3 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9896	0.9792	0.9730	0.5983

	Coefficient	Std. Error	t	P
a	8.4982	0.5113	16.6205	<0.0001
b	-0.4714	0.1006	-4.6879	0.0009
x0	5.5897	0.1103	50.6602	<0.0001
y0	2.2486	0.2611	8.6108	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	568.6705	142.1676
Residual	10	3.5797	0.3580
Total	14	572.2502	40.8750

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	168.7683	56.2561	157.1539	<0.0001
Residual	10	3.5797	0.3580		
Total	13	172.3480	13.2575		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.8881)

W Statistic= 0.9709 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.2311)

Fit Equation Description:
[Variables]
x = col(5)
y = col(6)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 8.49823}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.471437}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 5.58972}}
y0 = min(y) "Auto {{previous: 2.24859}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Fructose_344nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 12:00:46 PM**

Data Source: Data 2 in Fructose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9998	0.9995	0.9994	1.2161

	Coefficient	Std. Error	t	P
a	122.5452	0.8558	143.1989	<0.0001
b	0.4593	0.0115	39.9326	<0.0001
x0	6.9139	0.0117	591.8407	<0.0001
y0	6.3341	0.5320	11.9059	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	103723.8963	25930.9741
Residual	16	23.6641	1.4790
Total	20	103747.5604	5187.3780

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	48142.7817	16047.5939	10850.2696	<0.0001
Residual	16	23.6641	1.4790		
Total	19	48166.4458	2535.0761		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.2891)

W Statistic= 0.9443 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0320)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 122.545}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.459337}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.91393}}
y0 = min(y) "Auto {{previous: 6.3341}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Fructose_344nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 12:18:53 PM**

Data Source: Data 3 in Fructose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9837	0.9677	0.9602	9.4984

	Coefficient	Std. Error	t	P
a	111.7730	7.1488	15.6352	<0.0001
b	0.3985	0.0801	4.9724	0.0003
x0	6.9083	0.0928	74.4381	<0.0001
y0	9.4282	4.7674	1.9777	0.0696

Analysis of Variance:

	DF	SS	MS
Regression	4	103964.1648	25991.0412
Residual	13	1172.8495	90.2192
Total	17	105137.0143	6184.5303

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	35131.9032	11710.6344	129.8020	<0.0001
Residual	13	1172.8495	90.2192		
Total	16	36304.7527	2269.0470		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0491)

W Statistic= 0.8915 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0328)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 111.773}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.398512}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.90831}}
y0 = min(y) "Auto {{previous: 9.42824}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Fructose_378nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 12:00:56 PM**

Data Source: Data 2 in Fructose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9622	0.9258	0.9036	10.8224

	Coefficient	Std. Error	t	P
a	91.5319	10.0518	9.1060	<0.0001
b	0.3356	0.1109	3.0266	0.0128
x0	4.8578	0.1296	37.4773	<0.0001
y0	33.8199	7.9944	4.2304	0.0017

Analysis of Variance:

	DF	SS	MS
Regression	4	150054.1496	37513.5374
Residual	10	1171.2531	117.1253
Total	14	151225.4027	10801.8145

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	14618.5278	4872.8426	41.6037	<0.0001
Residual	10	1171.2531	117.1253		
Total	13	15789.7808	1214.5985		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0016)

W Statistic= 0.7578 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.6482)

Fit Equation Description:
[Variables]
x = col(4)
y = col(5)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) ''Auto {{previous: 91.5319}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) ''Auto {{previous: 0.335641}}
x0 = x50(x,y-min(y),.5) ''Auto {{previous: 4.85782}}
y0 = min(y) ''Auto {{previous: 33.8199}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Fructose_378nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 12:19:18 PM**

Data Source: Data 3 in Fructose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9678	0.9367	0.9096	12.7260

	Coefficient	Std. Error	t	P
a	97.2885	11.2153	8.6746	<0.0001
b	0.3222	0.1465	2.1996	0.0638
x0	4.8528	0.2367	20.5008	<0.0001
y0	30.3212	9.1963	3.2971	0.0132

Analysis of Variance:

	DF	SS	MS
Regression	4	129600.9988	32400.2497
Residual	7	1133.6513	161.9502
Total	11	130734.6501	11884.9682

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	16780.1424	5593.3808	34.5377	0.0001
Residual	7	1133.6513	161.9502		
Total	10	17913.7937	1791.3794		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0267)

W Statistic= 0.8344 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.6731)

Fit Equation Description:
[Variables]
x = col(4)
y = col(5)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 97.2885}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.322204}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 4.85279}}
y0 = min(y) "Auto {{previous: 30.3212}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Mannose_344nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 12:27:46 PM**

Data Source: Data 2 in Mannose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9987	0.9974	0.9969	9.3090

	Coefficient	Std. Error	t	P
a	411.2727	7.1456	57.5558	<0.0001
b	0.5683	0.0322	17.6508	<0.0001
x0	7.3277	0.0326	224.7619	<0.0001
y0	25.5070	3.6672	6.9555	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	1234828.8085	308707.2021
Residual	16	1386.5061	86.6566
Total	20	1236215.3146	61810.7657

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	531524.9221	177174.9740	2044.5634	<0.0001
Residual	16	1386.5061	86.6566		
Total	19	532911.4282	28047.9699		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.7093)

W Statistic= 0.9679 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.6718)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 411.273}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.568275}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 7.32768}}
y0 = min(y) "Auto {{previous: 25.507}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Mannose_344nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 12:36:34 PM**

Data Source: Data 1 in Mannose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9985	0.9970	0.9966	8.4797

	Coefficient	Std. Error	t	P
a	404.3340	6.9148	58.4735	<0.0001
b	0.5604	0.0267	21.0144	<0.0001
x0	7.3929	0.0266	278.2704	<0.0001
y0	30.6869	3.4728	8.8363	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	1218819.2134	304704.8034
Residual	20	1438.1142	71.9057
Total	24	1220257.3277	50844.0553

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	484104.7857	161368.2619	2244.1647	<0.0001
Residual	20	1438.1142	71.9057		
Total	23	485542.8999	21110.5609		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.1043)

W Statistic= 0.9313 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.2058)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 404.334}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.560362}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 7.39293}}
y0 = min(y) "Auto {{previous: 30.6869}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_Mannose_378nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 12:27:58 PM**

Data Source: Data 2 in Mannose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9851	0.9705	0.9557	3.4942

	Coefficient	Std. Error	t	P
a	47.3771	8.3675	5.6620	0.0013
b	-0.4651	0.1673	-2.7794	0.0320
x0	5.2664	0.1907	27.6140	<0.0001
y0	72.9391	7.2637	10.0416	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	111831.8009	27957.9502
Residual	6	73.2582	12.2097
Total	10	111905.0591	11190.5059

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	2406.1841	802.0614	65.6905	<0.0001
Residual	6	73.2582	12.2097		
Total	9	2479.4422	275.4936		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.7453)

W Statistic= 0.9565 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.2274)

Fit Equation Description:
[Variables]
x = col(4)
y = col(5)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 47.3771}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.465073}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 5.26638}}
y0 = min(y) "Auto {{previous: 72.9391}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Mannose_378nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 12:28:10 PM**

Data Source: Data 2 in Mannose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9931	0.9862	0.9810	7.7687

	Coefficient	Std. Error	t	P
a	148.2224	9.4948	15.6110	<0.0001
b	0.3808	0.0672	5.6639	0.0005
x0	7.4585	0.0696	107.1389	<0.0001
y0	72.4580	7.7414	9.3598	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	362409.5753	90602.3938
Residual	8	482.8203	60.3525
Total	12	362892.3955	30241.0330

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	34476.8546	11492.2849	190.4193	<0.0001
Residual	8	482.8203	60.3525		
Total	11	34959.6749	3178.1523		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.3635)

W Statistic= 0.9284 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.8690)

Fit Equation Description:
[Variables]
x = col(6)
y = col(7)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) ''Auto {{previous: 148.222}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) ''Auto {{previous: 0.380833}}
x0 = x50(x,y-min(y),.5) ''Auto {{previous: 7.45846}}
y0 = min(y) ''Auto {{previous: 72.458}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Mannose_378nm_3

Nonlinear Regression **Wednesday, September 21, 2011, 12:36:46 PM**

Data Source: Data 1 in Mannose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9895	0.9791	0.9702	3.7770

	Coefficient	Std. Error	t	P
a	51.2683	3.9593	12.9489	<0.0001
b	-0.2836	0.0719	-3.9440	0.0056
x0	5.1598	0.0783	65.9234	<0.0001
y0	77.2766	3.0584	25.2673	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	119771.1654	29942.7914
Residual	7	99.8579	14.2654
Total	11	119871.0233	10897.3658

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	4681.6177	1560.5392	109.3932	<0.0001
Residual	7	99.8579	14.2654		
Total	10	4781.4756	478.1476		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.9941)

W Statistic= 0.9877 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.5567)

Fit Equation Description:

```
[Variables]
x = col(4)
y = col(5)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 51.2683}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.283592}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 5.15981}}
y0 = min(y) "Auto {{previous: 77.2766}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_Mannose_378nm_4

Nonlinear Regression **Wednesday, September 21, 2011, 12:36:55 PM**

Data Source: Data 1 in Mannose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9990	0.9980	0.9975	2.8206

	Coefficient	Std. Error	t	P
a	148.1574	2.9590	50.0702	<0.0001
b	0.3707	0.0201	18.4077	<0.0001
x0	7.5187	0.0197	381.5523	<0.0001
y0	73.0936	2.1715	33.6602	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	375152.5518	93788.1380
Residual	12	95.4700	7.9558
Total	16	375248.0218	23453.0014

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	47470.7226	15823.5742	1988.9272	<0.0001
Residual	12	95.4700	7.9558		
Total	15	47566.1926	3171.0795		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.3841)

W Statistic= 0.9428 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.2766)

Fit Equation Description:
[Variables]
x = col(6)
y = col(7)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 148.157}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.370658}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 7.51872}}
y0 = min(y) "Auto {{previous: 73.0936}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Sorbitol_344nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 1:07:54 PM**

Data Source: Data 2 in Sorbitol Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9966	0.9932	0.9918	31.2642

	Coefficient	Std. Error	t	P
a	739.8470	18.8404	39.2692	<0.0001
b	0.3590	0.0412	8.7154	<0.0001
x0	6.7244	0.0494	136.0571	<0.0001
y0	26.7927	12.3274	2.1734	0.0462

Analysis of Variance:

	DF	SS	MS
Regression	4	5353478.9203	1338369.7301
Residual	15	14661.7901	977.4527
Total	19	5368140.7104	282533.7216

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	2132030.0898	710676.6966	727.0702	<0.0001
Residual	15	14661.7901	977.4527		
Total	18	2146691.8799	119260.6600		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0313)

W Statistic= 0.8893 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0309)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 739.847}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.358981}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.72438}}
y0 = min(y) "Auto {{previous: 26.7927}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Sorbitol_344nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 12:51:35 PM**

Data Source: Data 1 in Sorbitol Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9993	0.9987	0.9984	15.1430

	Coefficient	Std. Error	t	P
a	856.1416	10.3947	82.3635	<0.0001
b	0.4450	0.0190	23.4376	<0.0001
x0	6.7965	0.0235	288.6634	<0.0001
y0	21.4203	6.4392	3.3265	0.0046

Analysis of Variance:

	DF	SS	MS
Regression	4	6421046.5269	1605261.6317
Residual	15	3439.6558	229.3104
Total	19	6424486.1827	338130.8517

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	2568757.0373	856252.3458	3734.0321	<0.0001
Residual	15	3439.6558	229.3104		
Total	18	2572196.6931	142899.8163		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0009)

W Statistic= 0.7929 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.1862)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 856.142}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.445004}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.79655}}
y0 = min(y) "Auto {{previous: 21.4203}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Sorbitol_378nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 1:08:04 PM**

Data Source: Data 2 in Sorbitol Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8006	0.6410	0.3717	7.2684

	Coefficient	Std. Error	t	P
a	16.7530	10.0720	1.6633	0.1716
b	-0.3581	0.6138	-0.5833	0.5910
x0	4.2745	0.7258	5.8891	0.0042
y0	116.6072	6.4332	18.1258	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	126312.2583	31578.0646
Residual	4	211.3165	52.8291
Total	8	126523.5748	15815.4468

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	377.2664	125.7555	2.3804	0.2105
Residual	4	211.3165	52.8291		
Total	7	588.5828	84.0833		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.2072)

W Statistic= 0.8844 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0096)

Fit Equation Description:

```
[Variables]
x = col(3)
y = col(4)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 16.753}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.358085}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 4.27446}}
y0 = min(y) "Auto {{previous: 116.607}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_Sorbitol_378nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 1:08:18 PM**

Data Source: Data 2 in Sorbitol Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9856	0.9713	0.9627	12.0657

	Coefficient	Std. Error	t	P
a	146.5266	9.6877	15.1250	<0.0001
b	0.2547	0.0695	3.6646	0.0044
x0	6.7336	0.0840	80.1377	<0.0001
y0	115.0223	8.0590	14.2725	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	722378.6590	180594.6647
Residual	10	1455.8166	145.5817
Total	14	723834.4756	51702.4625

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	49343.8637	16447.9546	112.9810	<0.0001
Residual	10	1455.8166	145.5817		
Total	13	50799.6803	3907.6677		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0093)

W Statistic= 0.8213 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.6047)

Fit Equation Description:

```
[Variables]
x = col(5)
y = col(6)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 146.527}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.254708}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.7336}}
y0 = min(y) "Auto {{previous: 115.022}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_Sorbitol_378nm_3

Nonlinear Regression **Wednesday, September 21, 2011, 12:51:52 PM**

Data Source: Data 1 in Sorbitol Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9686	0.9381	0.8762	2.5456

	Coefficient	Std. Error	t	P
a	14.0573	2.5839	5.4403	0.0122
b	-0.1488	0.6255	-0.2378	0.8274
x0	3.9698	0.2467	16.0937	0.0005
y0	121.1281	1.5539	77.9534	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	114940.5940	28735.1485
Residual	3	19.4409	6.4803
Total	7	114960.0349	16422.8621

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	294.5742	98.1914	15.1523	0.0257
Residual	3	19.4409	6.4803		
Total	6	314.0151	52.3358		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.4049)

W Statistic= 0.9113 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.3884)

Fit Equation Description:
[Variables]
x = col(4)
y = col(5)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 14.0573}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.148753}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 3.9698}}
y0 = min(y) "Auto {{previous: 121.128}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Sorbitol_378nm_4

Nonlinear Regression **Wednesday, September 21, 2011, 12:52:03 PM**

Data Source: Data 1 in Sorbitol Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9957	0.9915	0.9891	7.6972

	Coefficient	Std. Error	t	P
a	176.7081	6.7750	26.0825	<0.0001
b	0.3756	0.0459	8.1924	<0.0001
x0	6.8153	0.0584	116.7021	<0.0001
y0	117.8892	5.1770	22.7718	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	871478.9364	217869.7341
Residual	11	651.7172	59.2470
Total	15	872130.6536	58142.0436

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	75581.4482	25193.8161	425.2335	<0.0001
Residual	11	651.7172	59.2470		
Total	14	76233.1654	5445.2261		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.6029)

W Statistic= 0.9548 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.4731)

Fit Equation Description:
[Variables]
x = col(6)
y = col(7)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 176.708}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.375643}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.81528}}
y0 = min(y) "Auto {{previous: 117.889}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Tagatose_344nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 12:56:23 PM**

Data Source: Data 2 in Tagatose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9999	0.9997	0.9997	3.9214

	Coefficient	Std. Error	t	P
a	470.7155	2.8014	168.0278	<0.0001
b	0.4709	0.0106	44.5243	<0.0001
x0	6.8783	0.0123	557.5450	<0.0001
y0	17.4612	1.6218	10.7663	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	1577520.2610	394380.0653
Residual	13	199.9071	15.3775
Total	17	1577720.1681	92807.0687

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	710564.7623	236854.9208	15402.7265	<0.0001
Residual	13	199.9071	15.3775		
Total	16	710764.6694	44422.7918		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.0513)

W Statistic= 0.8927 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.4257)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 470.715}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.470886}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.87826}}
y0 = min(y) "Auto {{previous: 17.4612}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_Tagatose_344nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 1:00:28 PM**

Data Source: Data 1 in Tagatose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9998	0.9996	0.9995	5.0748

	Coefficient	Std. Error	t	P
a	514.9725	3.3805	152.3361	<0.0001
b	0.4335	0.0115	37.6488	<0.0001
x0	6.8608	0.0137	501.2176	<0.0001
y0	20.0916	1.9366	10.3747	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	1920572.9752	480143.2438
Residual	14	360.5554	25.7540
Total	18	1920933.5306	106718.5295

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	920696.8033	306898.9344	11916.5745	<0.0001
Residual	14	360.5554	25.7540		
Total	17	921057.3587	54179.8446		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.9857)

W Statistic= 0.9848 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.4869)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 514.972}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.433482}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.86083}}
y0 = min(y) "Auto {{previous: 20.0916}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

7-IQBA_Tagatose_378nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 12:56:30 PM**

Data Source: Data 2 in Tagatose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9974	0.9949	0.9937	6.0477

	Coefficient	Std. Error	t	P
a	184.7042	5.2863	34.9403	<0.0001
b	0.5932	0.0547	10.8462	<0.0001
x0	5.2163	0.0594	87.7818	<0.0001
y0	106.1662	4.2286	25.1068	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	952409.8870	238102.4718
Residual	13	475.4765	36.5751
Total	17	952885.3635	56052.0802

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	92201.0867	30733.6956	840.2898	<0.0001
Residual	13	475.4765	36.5751		
Total	16	92676.5632	5792.2852		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.1419)

W Statistic= 0.9190 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0091)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(3)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 184.704}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.593175}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 5.21632}}
y0 = min(y) "Auto {{previous: 106.166}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

7-IQBA_Tagatose_378nm_2

Nonlinear Regression **Wednesday, September 21, 2011, 1:00:35 PM**

Data Source: Data 1 in Tagatose Titration
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9969	0.9938	0.9925	7.4277

	Coefficient	Std. Error	t	P
a	206.1214	5.5507	37.1341	<0.0001
b	0.5019	0.0494	10.1617	<0.0001
x0	5.0792	0.0499	101.7657	<0.0001
y0	109.9059	4.4670	24.6039	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	1179972.8110	294993.2028
Residual	14	772.3826	55.1702
Total	18	1180745.1936	65596.9552

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	124399.7924	41466.5975	751.6124	<0.0001
Residual	14	772.3826	55.1702		
Total	17	125172.1751	7363.0691		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.2259)

W Statistic= 0.9337 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.1693)

Fit Equation Description:
[Variables]
x = col(1)
y = col(3)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 206.121}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: 0.501877}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 5.07922}}
y0 = min(y) "Auto {{previous: 109.906}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

4-POBA_430nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 2:43:00 PM**

Data Source: Data 1 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9995	0.9989	0.9987	3.6786

	Coefficient	Std. Error	t	P
a	241.1165	3.2042	75.2513	<0.0001
b	-0.4891	0.0219	-22.3752	<0.0001
x0	7.9988	0.0243	329.8446	<0.0001
y0	7.8607	2.5946	3.0297	0.0105

Analysis of Variance:

	DF	SS	MS
Regression	4	588552.6652	147138.1663
Residual	12	162.3838	13.5320
Total	16	588715.0491	36794.6906

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	150667.7267	50222.5756	3711.3977	<0.0001
Residual	12	162.3838	13.5320		
Total	15	150830.1105	10055.3407		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.6650)

W Statistic= 0.9602 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0120)

Fit Equation Description:
[Variables]
x = col(11)
y = col(12)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 241.116}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.489059}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 7.99879}}
y0 = min(y) "Auto {{previous: 7.86071}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

4-POBA_430nm_2

Nonlinear Regression

Data Source: Data 1 in All for POBA.JNB
Equation: Standard Curves, Four Parameter Logistic Curve
f1=min + (max-min)/(1 + abs(x/EC50)^Hillslope)
f2=min + (max-min)*(abs(x/EC50)^abs(Hillslope))/(1 + (abs(x/EC50)^abs(Hillslope)))
f = if(x<=0, if(Hillslope>0,max,min), if(Hillslope>0, f1, f2))

R	**Rsqr**	**Adj Rsqr**	**Standard Error of Estimate**
0.9994	0.9987	0.9985	4.2208

	Coefficient	**Std. Error**	**t**	**P**	**VIF**
min	7.5784	2.0164	3.7583	0.0019	1.6971
max	247.6670	1.5980	154.9899	<0.0001	1.3658
EC50	7.9922	0.0249	320.9172	<0.0001	1.4909
Hillslope	16.6127	0.7875	21.0942	<0.0001	1.5182

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	**SS**	**MS**
Regression	4	588732.2475	147183.0619
Residual	15	267.2276	17.8152
Total	19	588999.4752	30999.9724

Corrected for the mean of the observations:

	DF	**SS**	**MS**	**F**	**P**
Regression	3	211818.4923	70606.1641	3963.2596	<0.0001
Residual	15	267.2276	17.8152		
Total	18	212085.7200	11782.5400		

Statistical Tests:

PRESS 410.6307

Durbin-Watson Statistic 0.7963 Failed

Normality Test Passed (P = 0.8908)

K-S Statistic = 0.1285 Significance Level = 0.8908

Constant Variance Test Failed (P = <0.0001)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	**Std. Res.**	**Stud. Res.**	**Stud. Del. Res.**
1	1.1917	1.2876	1.3189
2	0.8219	0.8880	0.8813
3	1.8073	1.9526	2.1843<
4	0.6737	0.7273	0.7153
5	-1.1161	-1.1991	-1.2183

4-POBA_Fructose_430nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 2:45:05 PM**

Data Source: Data 1 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9999	0.9997	0.9996	2.0953

	Coefficient	Std. Error	t	P
a	236.5612	1.8135	130.4412	<0.0001
b	-0.4697	0.0131	-35.8096	<0.0001
x0	6.2555	0.0179	349.7777	<0.0001
y0	10.6522	1.0637	10.0144	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	221923.3437	55480.8359
Residual	7	30.7319	4.3903
Total	11	221954.0757	20177.6432

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	112010.3265	37336.7755	8504.4234	<0.0001
Residual	7	30.7319	4.3903		
Total	10	112041.0585	11204.1058		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.1772)

W Statistic= 0.8985 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.0653)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 236.561}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.469716}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.25551}}
y0 = min(y) "Auto {{previous: 10.6522}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

4-POBA_Fructose_430nm_2

Nonlinear Regression

Data Source: Data 1 in All for POBA.JNB
Equation: Standard Curves, Four Parameter Logistic Curve
f1=min + (max-min)/(1 + abs(x/EC50)^Hillslope)
f2=min + (max-min)*(abs(x/EC50)^abs(Hillslope))/(1 + (abs(x/EC50)^abs(Hillslope)))
f = if(x<=0, if(Hillslope>0,max,min), if(Hillslope>0, f1, f2))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9996	0.9993	0.9991	3.2307

	Coefficient	Std. Error	t	P	VIF
min	9.1445	1.2640	7.2345	<0.0001	1.4421
max	247.4246	1.3511	183.1299	<0.0001	1.3511
EC50	6.2539	0.0188	331.9677	<0.0001	1.4132
Hillslope	13.5616	0.4804	28.2314	<0.0001	1.3648

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	4	477917.2403	119479.3101
Residual	15	156.5604	10.4374
Total	19	478073.8007	25161.7790

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	214911.8416	71637.2805	6863.5442	<0.0001
Residual	15	156.5604	10.4374		
Total	18	215068.4020	11948.2446		

Statistical Tests:

PRESS 246.6774

Durbin-Watson Statistic 1.8329 Passed

Normality Test Passed (P = 0.6535)

K-S Statistic = 0.1631 Significance Level = 0.6535

Constant Variance Test Failed (P = 0.0050)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Std. Res.	Stud. Res.	Stud. Del. Res.
1	0.5626	0.6193	0.6061
2	-0.2084	-0.2294	-0.2220
3	2.0125	2.2148<	2.6083<
4	0.7286	0.7999	0.7898
5	-1.3320	-1.4505	-1.5113

4-POBA_Glucose_430nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 2:48:55 PM**

Data Source: Data 1 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9997	0.9995	0.9993	2.6249

	Coefficient	Std. Error	t	P
a	222.0919	1.7381	127.7751	<0.0001
b	-0.4572	0.0148	-30.9273	<0.0001
x0	6.3011	0.0167	377.1864	<0.0001
y0	9.6617	1.1751	8.2217	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	421181.3785	105295.3446
Residual	13	89.5721	6.8902
Total	17	421270.9506	24780.6442

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	164111.8557	54703.9519	7939.4267	<0.0001
Residual	13	89.5721	6.8902		
Total	16	164201.4278	10262.5892		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = <0.0001)

W Statistic= 0.6309 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0051)

Fit Equation Description:
[Variables]
x = col(3)
y = col(4)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 222.092}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.457246}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.30113}}
y0 = min(y) "Auto {{previous: 9.66166}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

4-POBA_Glucose_430nm_2

Nonlinear Regression

Data Source: Data 1 in All for POBA.JNB
Equation: Standard Curves, Four Parameter Logistic Curve
f1=min + (max-min)/(1 + abs(x/EC50)^Hillslope)
f2=min + (max-min)*(abs(x/EC50)^abs(Hillslope))/(1 + (abs(x/EC50)^abs(Hillslope)))
f = if(x<=0, if(Hillslope>0,max,min), if(Hillslope>0, f1, f2))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9996	0.9993	0.9992	2.9600

	Coefficient	Std. Error	t	P	VIF
min	8.6051	1.1750	7.3233	<0.0001	1.4674
max	230.5306	1.2364	186.4484	<0.0001	1.3690
EC50	6.2945	0.0183	343.3000	<0.0001	1.4097
Hillslope	13.8602	0.4935	28.0865	<0.0001	1.3965

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	4	421314.0008	105328.5002
Residual	15	131.4218	8.7615
Total	19	421445.4226	22181.3380

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	187179.0354	62393.0118	7121.3089	<0.0001
Residual	15	131.4218	8.7615		
Total	18	187310.4572	10406.1365		

Statistical Tests:

PRESS 195.8019

Durbin-Watson Statistic 1.6542 Passed

Normality Test Passed (P = 0.6837)

K-S Statistic = 0.1591 Significance Level = 0.6837

Constant Variance Test Failed (P = 0.0053)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Std. Res.	Stud. Res.	Stud. Del. Res.
1	0.7843	0.8632	0.8555
2	1.0015	1.1022	1.1107
3	1.0480	1.1529	1.1667
4	0.8603	0.9440	0.9403
5	-2.9266<	-3.1877<	-5.4220<

4-POBA_Mannose_430nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 2:50:15 PM**

Data Source: Data 1 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9995	0.9989	0.9987	3.1373

	Coefficient	Std. Error	t	P
a	193.3807	2.2912	84.4018	<0.0001
b	-0.4918	0.0218	-22.5888	<0.0001
x0	6.4266	0.0248	259.3023	<0.0001
y0	19.2942	1.6043	12.0265	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	353880.1428	88470.0357
Residual	12	118.1149	9.8429
Total	16	353998.2576	22124.8911

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	111715.7644	37238.5881	3783.2927	<0.0001
Residual	12	118.1149	9.8429		
Total	15	111833.8792	7455.5919		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.4057)

W Statistic= 0.9443 Significance Level = 0.0500

Constant Variance Test Failed (P = <0.0001)

Fit Equation Description:
```
[Variables]
x = col(5)
y = col(6)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 193.381}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.491755}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.42663}}
y0 = min(y) "Auto {{previous: 19.2942}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

4-POBA_Mannose_430nm_2

Nonlinear Regression

Data Source: Data 1 in All for POBA.JNB
Equation: Standard Curves, Four Parameter Logistic Curve
f1=min + (max-min)/(1 + abs(x/EC50)^Hillslope)
f2=min + (max-min)*(abs(x/EC50)^abs(Hillslope))/(1 + (abs(x/EC50)^abs(Hillslope)))
f = if(x<=0, if(Hillslope>0,max,min), if(Hillslope>0, f1, f2))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9993	0.9987	0.9984	3.5163

	Coefficient	Std. Error	t	P	VIF
min	16.3489	1.4381	11.3686	<0.0001	1.5486
max	211.5895	1.5130	139.8442	<0.0001	1.4267
EC50	6.4343	0.0267	241.2231	<0.0001	1.4760
Hillslope	12.8321	0.5943	21.5934	<0.0001	1.4604

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	4	354503.1801	88625.7950
Residual	15	185.4685	12.3646
Total	19	354688.6486	18667.8236

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	141062.0020	47020.6673	3802.8557	<0.0001
Residual	15	185.4685	12.3646		
Total	18	141247.4705	7847.0817		

Statistical Tests:

PRESS 306.2209

Durbin-Watson Statistic 1.2026 Failed

Normality Test Passed (P = 0.9899)

K-S Statistic = 0.0980 Significance Level = 0.9899

Constant Variance Test Failed (P = 0.0077)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Std. Res.	Stud. Res.	Stud. Del. Res.
1	1.4634	1.6211	1.7245
2	2.1066	2.3334<	2.8245<
3	-0.9170	-1.0148	-1.0159
4	-0.1987	-0.2187	-0.2116
5	-1.2372	-1.3434	-1.3837

4-POBA_Sorbitol_430nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 2:53:34 PM**

Data Source: Data 1 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	**Rsqr**	**Adj Rsqr**	**Standard Error of Estimate**
0.9997	0.9995	0.9994	2.7690

	Coefficient	**Std. Error**	**t**	**P**
a	242.5169	1.9306	125.6183	<0.0001
b	-0.4695	0.0150	-31.3599	<0.0001
x0	6.5998	0.0172	384.5378	<0.0001
y0	8.6625	1.3844	6.2572	<0.0001

Analysis of Variance:

	DF	**SS**	**MS**
Regression	4	498423.6230	124605.9057
Residual	12	92.0076	7.6673
Total	16	498515.6306	31157.2269

Corrected for the mean of the observations:

	DF	**SS**	**MS**	**F**	**P**
Regression	3	181431.8211	60477.2737	7887.6865	<0.0001
Residual	12	92.0076	7.6673		
Total	15	181523.8287	12101.5886		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.2562)

W Statistic= 0.9314 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0213)

Fit Equation Description:
[Variables]
x = col(7)
y = col(8)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 242.517}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.469452}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.59985}}
y0 = min(y) "Auto {{previous: 8.66252}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y

4-POBA_Sorbitol_430nm_2

Nonlinear Regression

Data Source: Data 1 in All for POBA.JNB
Equation: Standard Curves, Four Parameter Logistic Curve
f1=min + (max-min)/(1 + abs(x/EC50)^Hillslope)
f2=min + (max-min)*(abs(x/EC50)^abs(Hillslope))/(1 + (abs(x/EC50)^abs(Hillslope)))
f = if(x<=0, if(Hillslope>0,max,min), if(Hillslope>0, f1, f2))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9994	0.9988	0.9986	4.2373

	Coefficient	Std. Error	t	P	VIF
min	9.3925	1.6585	5.6632	<0.0001	1.4330
max	249.7408	1.7338	144.0402	<0.0001	1.3259
EC50	6.5847	0.0251	262.4509	<0.0001	1.3919
Hillslope	14.4402	0.6898	20.9351	<0.0001	1.3789

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	4	498788.4676	124697.1169
Residual	15	269.3226	17.9548
Total	19	499057.7902	26266.1995

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	222945.1680	74315.0560	4138.9981	<0.0001
Residual	15	269.3226	17.9548		
Total	18	223214.4906	12400.8050		

Statistical Tests:

PRESS 393.0862

Durbin-Watson Statistic 1.0801 Failed

Normality Test Passed (P = 0.7701)

K-S Statistic = 0.1474 Significance Level = 0.7701

Constant Variance Test Passed (P = 0.1712)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Std. Res.	Stud. Res.	Stud. Del. Res.
1	1.0469	1.1474	1.1606
2	0.4648	0.5094	0.4965
3	0.2514	0.2754	0.2668
4	1.2602	1.3792	1.4259
5	-0.6653	-0.7241	-0.7121

4-POBA_Tagatose_430nm_1

Nonlinear Regression **Wednesday, September 21, 2011, 2:55:12 PM**

Data Source: Data 1 in Titration Reports
Equation: Sigmoidal, Sigmoid, 4 Parameter
f = y0+a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9998	0.9996	0.9994	2.5450

	Coefficient	Std. Error	t	P
a	237.8174	1.7744	134.0243	<0.0001
b	-0.4706	0.0141	-33.4481	<0.0001
x0	6.5720	0.0158	415.6613	<0.0001
y0	8.8595	1.2716	6.9672	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	4	482109.9243	120527.4811
Residual	12	77.7221	6.4768
Total	16	482187.6463	30136.7279

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	172906.9226	57635.6409	8898.7299	<0.0001
Residual	12	77.7221	6.4768		
Total	15	172984.6447	11532.3096		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.2912)

W Statistic= 0.9349 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0238)

Fit Equation Description:
```
[Variables]
x = col(9)
y = col(10)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
reciprocal_pred = 1/abs(f)
reciprocal_predsqr = 1/f^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 237.817}}
b = if(xwtr(x,y-min(y),.5)<>0, xwtr(x,y-min(y),.5)/4, 1) "Auto {{previous: -0.470598}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 6.57201}}
y0 = min(y) "Auto {{previous: 8.85953}}
[Equation]
f = y0+a/(1+exp(-(x-x0)/b))
fit f to y
```

4-POBA_Tagatose_430nm_2

Nonlinear Regression

Data Source: Data 1 in All for POBA.JNB
Equation: Standard Curves, Four Parameter Logistic Curve
f1=min + (max-min)/(1 + abs(x/EC50)^Hillslope)
f2=min + (max-min)*(abs(x/EC50)^abs(Hillslope))/(1 + (abs(x/EC50)^abs(Hillslope)))
f = if(x<=0, if(Hillslope>0,max,min), if(Hillslope>0, f1, f2))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9997	0.9994	0.9993	2.9637

	Coefficient	Std. Error	t	P	VIF
min	7.5522	1.1740	6.4328	<0.0001	1.4501
max	245.5562	1.2201	201.2518	<0.0001	1.3446
EC50	6.5654	0.0176	371.9841	<0.0001	1.3989
Hillslope	13.9610	0.4634	30.1293	<0.0001	1.3682

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	4	482259.5182	120564.8796
Residual	15	131.7504	8.7834
Total	19	482391.2687	25389.0141

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	216070.7186	72023.5729	8199.9995	<0.0001
Residual	15	131.7504	8.7834		
Total	18	216202.4691	12011.2483		

Statistical Tests:

PRESS 251.6510

Durbin-Watson Statistic 2.2183 Passed

Normality Test Passed (P = 0.9933)

K-S Statistic = 0.0948 Significance Level = 0.9933

Constant Variance Test Failed (P = <0.0001)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Std. Res.	Stud. Res.	Stud. Del. Res.
1	1.0790	1.1840	1.2014
2	0.9096	0.9981	0.9979
3	-0.7531	-0.8262	-0.8170
4	1.6475	1.8040	1.9696
5	-1.6783	-1.8272	-2.0021<