Using Quenching Kinetics and Thermodynamics of Amino-Fluorophores As Empirical Tools for Predicting Boronic Acid Sensors Suitable for Use in Physiological Conditions

Nicholas McGregor,¹ Christophe Pardin,¹,² W.G. Skene*¹

¹Laboratoire de caractérisation photophysique des matériaux conjugués
Department of Chemistry, Pavillon JA Bombardier,
Université de Montréal, CP 6128, succ. Centre-ville,
Montreal, Quebec, Canada H3C 3J6

²Department of Chemistry, D’Iorio Hall,
University of Ottawa, 10 Marie Curie,
Ottawa, Ontario, Canada K1N 6N5
Tables of contents

Figure 1: Normalized absorbance (black) and fluorescence (red) spectra of 1. ......................... 5
Figure 2: Normalized absorbance (black) and fluorescence (red) spectra of 2. ....................... 6
Figure 3: Normalized absorbance (black) and fluorescence (red) spectra of 3. ....................... 7
Figure 4: Normalized absorbance (black) and fluorescence (red) spectra of 4. ....................... 8
Figure 5: Normalized absorbance (black) and fluorescence (red) spectra of 5. ....................... 9
Figure 6: Normalized absorbance (black) and fluorescence (red) spectra of 6. ....................... 10
Figure 7: Normalized absorbance (black) and fluorescence (red) spectra of 11. ..................... 11
Figure 8: Normalized absorbance (black) and fluorescence (red) spectra of 12. ..................... 12
Figure 9: Normalized absorbance (black) and fluorescence (red) spectra of 13. ..................... 13
Figure 10: Normalized absorbance (black) and fluorescence (red) spectra of 14..................... 14
Figure 11: Fluorescence spectra of 21 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing diffusion limited fluorescence quenching of 21 with 10. ................. 15
Figure 12: Fluorescence spectra of 11 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing the diffusion limited relationship between imine concentration and fluorescence quenching. ................................................................................................. 16
Figure 13: Fluorescence spectra of 11 with increasing concentrations of phenylboronic acid in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [phenylboronic acid] including linear fit and basic statistics showing the diffusion limited relationship between phenylboronic acid concentration and fluorescence quenching. ........................................................................................................ 17
Figure 14: Fluorescence spectra of 12 with increasing concentrations of phenylboronic acid in pH 7.4 PBS. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [phenylboronic acid] including linear fit and basic statistics showing the lack of fluorescence quenching of 12 with phenylboronic acid. ........................................................................................................ 18
Figure 15: Fluorescence spectra of 13 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing the lack of fluorescence quenching of 13 with 10. ......................... 19
Figure 16: Fluorescence spectra of 14 with increasing concentrations of phenylboronic acid in pH 7.4 PBS. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [phenylboronic acid] including linear fit and basic statistics showing the lack of fluorescence quenching of 14 with phenylboronic acid. ........................................................................................................ 20
Figure 17: Fluorescence spectra of 20 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing diffusion limited fluorescence quenching of 20 with 10. ......................... 21
Figure 18: Fluorescence spectra of 1 in pH 7.4 PBS with added fructose demonstrating the fluorescence revival of 1 upon saccharide addition. Inset: Plot of [1:fructose] vs. [fructose] with a one-site binding fit overlayed and basic statistics used to determine the binding constant of 1 with fructose. ........................................................................................................ 22
Figure 19: Fluorescence spectra of 5 in pH 7.4 PBS with added fructose demonstrating the fluorescence revival of 5 upon saccharide addition. Inset: Plot of [5:fructose] vs. [fructose] with a one-site binding fit overlayed and basic statistics used to determine the binding constant of 5 with fructose. ........................................................................................................ 23
Figure 20: Fluorescence spectra of 6 in pH 7.4 PBS with added fructose demonstrating the fluorescence revival of 6 upon saccharide addition. Inset: Plot of [6:fructose] vs.
[fructose] with a two-site binding fit overlayed and basic statistics used to determine the binding constant of 6 with fructose.......................................................... 24
Figure 21: Fluorescence pH titrations of 1 without saccharide (■), with 100 mM glucose (■) and with 50 mM fructose (■) (~saturation) demonstrating the reduction of pKa of the boronic acid by formation of the boronic ester.......................................................... 25
Figure 22: Fluorescence pH titrations of 1 without saccharide (■), with 10 mM fructose (■) and with 100 mM fructose (■) (~saturation) demonstrating the reduction of pKa of the boronic acid by formation of the boronic ester.......................................................... 26
Figure 23: Fluorescence pH titrations of 1 without saccharide (■), with 10 mM fructose (■) and with 100 mM fructose (■) (~saturation) demonstrating the reduction of pKa of the boronic acid by formation of the boronic ester.......................................................... 27
Figure 24: Cyclic voltammogram of the cathodic process of phenylboronic acid in anhydrous dimethylformamide........................................................................................................ 28
Figure 25: Cyclic voltammogram of the anodic process of 9 in anhydrous dimethylformamide. 29
Figure 26: Cyclic voltammogram of the cathodic process of 10 in anhydrous dimethylformamide........................................................................................................ 30
Figure 27: Cyclic voltammogram of the anodic process of 11 in anhydrous dimethylformamide. ........................................................................................................ 31
Figure 28: Cyclic voltammogram of the anodic process of 12 in anhydrous dimethylformamide. ........................................................................................................ 32
Figure 29: Cyclic voltammogram of the anodic process of 13 in anhydrous dimethylformamide. ........................................................................................................ 33
Figure 30: Cyclic voltammogram of the anodic process of 20 in anhydrous dimethylformamide. ........................................................................................................ 34
Figure 31: Cyclic voltammogram of the anodic process of 21 in anhydrous dimethylformamide. ........................................................................................................ 35
Figure 32: 1H NMR of N-Boc-5-aminonaphthalene-1-sulfonic acid in CDCl3.......................................................... 36
Figure 33: 13C NMR of N-Boc-5-aminonaphthalene-1-sulfonic acid in CDCl3.......................................................... 37
Figure 34: 1H NMR of N-Boc-5-aminonaphthalene-1-sulfonyl chloride in CDCl3.......................................................... 38
Figure 35: 13C NMR of N-Boc-5-aminonaphthalene-1-sulfonyl chloride in CDCl3.......................................................... 39
Figure 36: 1H NMR of N-Boc-5-aminonaphthalene-1-tert-butylsulfonamide in CDCl3.......................................................... 40
Figure 37: 1H NMR of 5-aminonaphthalene-1-sulfonamide in 5% D2O in CD3CN.......................................................... 42
Figure 38: 1H NMR of N-phenylborono-5-aminonaphthalene-1-sulfonamide in CD3OD.......................................................... 43
Figure 39: 1H NMR of N-phenylborono-5-aminonaphthalene-1-sulfonamide in CD3OD+Et3N. 46
Figure 40: 1H NMR of N-benzyl-5-aminonaphthalene-1-sulfonamide in D6-DMSO.......................................................... 44
Figure 41: 1H-NMR of N-benzylidene-5-aminonaphthalene-1-sulfonamide in D6-DMSO.......................................................... 45
Figure 42: 1H NMR of N-phenylborono-5-aminonaphthalene-1-sulfonic acid in CD3OD+Et3N. 46
Figure 43: 1H NMR of N-phenylborono-5-amin-1-naphthol-3-sulfonic acid in CD3OD+Et3N. 48
Figure 44: 1H NMR of FluoHc in CD3OD........................................................................................................ 51
Figure 45: 1H-NMR of tBuFluoHa in CDCl3.......................................................... 49
Figure 46: 13C NMR of tBuFluoHa in CDCl3.......................................................... 50
Figure 47: 1H NMR of FluoHc in CD3OD.......................................................... 51
Figure 48: 1H NMR of tBuFluoNH2a in CDCl3.......................................................... 52
Figure 49: 13C NMR of tBuFluoNH2a in CDCl3.......................................................... 53
Figure 50: 1H NMR of FluoNH2c in CD3OD.......................................................... 54
Figure 51: (-)ESI-HRMS analysis of N-Boc-5-aminonaphthalene-1-sulfonic acid.......................................................... 55
Figure 52: (+)ESI-HRMS of N-phenylborono-5-aminonaphthalene-1-sulfonamide.......................................................... 56
Figure 53: (+)ESI-HRMS of N-benzyl-5-aminonaphthalene-1-sulfonamide.......................................................... 57
Figure 54: (+)ESI-HRMS of N-benzylidene-5-aminonaphthalene-1-sulfonamide.......................................................... 58
Figure 55: (+)ESI-HRMS of N-phenylborono-5-aminonaphthalene-1-sulfonic acid. .......... 59
Figure 56: (+)ESI-HRMS of N-phenylborono-5-amino-1-naphthol. ......................................... 60
Figure 57: (+)ESI-HRMS of N-phenylborono-5-amino-1-naphthol-3-sulfonic acid. .............. 61
Figure 58: (+)ESI-HRMS of FluoHa. .................................................................................... 62
Figure 59: (+)ESI-HRMS of FluoHc. ....................................................................................... 63
Figure 60: (+)ESI-HRMS of FluoNH2a. .................................................................................. 64
Figure 61: (-)ESI-HRMS of FluoHc. ....................................................................................... 65
Figure 62: (-)ESI-MS N-Boc-5-aminonaphthalene-1-sulfonyl chloride. ................................ 66
Figure 64: (-)ESI-MS of 5-aminonaphthalene-1-sulfonamide. ............................................. 68
Figure 65: (+)ESI-MS of monoaminofluorene. ........................................................................ 69
Figure 66: (+)ESI-MS of diaminofluorene. ............................................................................. 70
Figure 67: (-)ESI-MS of dinitrofluorene. .................................................................................. 71
Figure 1: Normalized absorbance (black) and fluorescence (red) spectra of 1.
Figure 2: Normalized absorbance (black) and fluorescence (red) spectra of 2.
Figure 3: Normalized absorbance (black) and fluorescence (red) spectra of 3.
Figure 4: Normalized absorbance (black) and fluorescence (red) spectra of 4.
Figure 5: Normalized absorbance (black) and fluorescence (red) spectra of 5.
Figure 6: Normalized absorbance (black) and fluorescence (red) spectra of 6.
Figure 7: Normalized absorbance (black) and fluorescence (red) spectra of 11.
Figure 8: Normalized absorbance (black) and fluorescence (red) spectra of 12.
Figure 9: Normalized absorbance (black) and fluorescence (red) spectra of 13.
Figure 10: Normalized absorbance (black) and fluorescence (red) spectra of 14.
Figure 11: Fluorescence spectra of 21 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing diffusion limited fluorescence quenching of 21 with 10.
Figure 12: Fluorescence spectra of 11 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing the diffusion limited relationship between imine concentration and fluorescence quenching.
Figure 13: Fluorescence spectra of 11 with increasing concentrations of phenylboronic acid in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [phenylboronic acid] including linear fit and basic statistics showing the diffusion limited relationship between phenylboronic acid concentration and fluorescence quenching.
Figure 14: Fluorescence spectra of 12 with increasing concentrations of phenylboronic acid in pH 7.4 PBS. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [phenylboronic acid] including linear fit and basic statistics showing the lack of fluorescence quenching of 12 with phenylboronic acid.
Figure 15: Fluorescence spectra of 13 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing the lack of fluorescence quenching of 13 with 10.
Figure 16: Fluorescence spectra of 14 with increasing concentrations of phenylboronic acid in pH 7.4 PBS. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [phenylboronic acid] including linear fit and basic statistics showing the lack of fluorescence quenching of 14 with phenylboronic acid.
Figure 17: Fluorescence spectra of 20 with increasing concentrations of 10 in anhydrous acetonitrile. Inset: Stern-Volmer plot of $\Phi_0/\Phi$ vs. [10] including linear fit and basic statistics showing diffusion limited fluorescence quenching of 20 with 10.
Figure 18: Fluorescence spectra of 1 in pH 7.4 PBS with added fructose demonstrating the fluorescence revival of 1 upon saccharide addition. Inset: Plot of [1:fructose] vs. [fructose] with a one-site binding fit overlayed and basic statistics used to determine the binding constant of 1 with fructose.
Figure 19: Fluorescence spectra of 5 in pH 7.4 PBS with added fructose demonstrating the fluorescence revival of 5 upon saccharide addition. Inset: Plot of [5:fructose] vs. [fructose] with a one-site binding fit overlayed and basic statistics used to determine the binding constant of 5 with fructose.
Figure 20: Fluorescence spectra of 6 in pH 7.4 PBS with added fructose demonstrating the fluorescence revival of 6 upon saccharide addition. Inset: Plot of [6:fructose] vs. [fructose] with a two-site binding fit overlayed and basic statistics used to determine the binding constant of 6 with fructose.
Figure 21: Fluorescence pH titrations of 1 without saccharide (■), with 100 mM glucose (■) and with 50 mM fructose (■) (~saturation) demonstrating the reduction of pKa of the boronic acid by formation of the boronic ester.
Figure 22: Fluorescence pH titrations of 1 without saccharide (■), with 10 mM fructose (■) and with 100 mM fructose (■) (~saturation) demonstrating the reduction of pKa of the boronic acid by formation of the boronic ester.
Figure 23: Fluorescence pH titrations of I without saccharide (■), with 10 mM fructose (■) and with 100 mM fructose (■) (~saturation) demonstrating the reduction of pKa of the boronic acid by formation of the boronic ester.
Figure 24: Cyclic voltammogram of the cathodic process of phenylboronic acid in anhydrous dimethylformamide.
Figure 25: Cyclic voltammogram of the anodic process of 9 in anhydrous dimethylformamide.
Figure 26: Cyclic voltammogram of the cathodic process of 10 in anhydrous dimethylformamide.
Figure 27: Cyclic voltammogram of the anodic process of 11 in anhydrous dimethylformamide.
Figure 28: Cyclic voltammogram of the anodic process of 12 in anhydrous dimethylformamide.
Figure 29: Cyclic voltammogram of the anodic process of 13 in anhydrous dimethylformamide.
Figure 30: Cyclic voltammogram of the anodic process of 20 in anhydrous dimethylformamide.
Figure 31: Cyclic voltammogram of the anodic process of $21$ in anhydrous dimethylformamide.
Figure 32: $^1$H NMR of N-Boc-5-aminonaphthalene-1-sulfonic acid in CDCl$_3$. 
Figure 33: $^{13}$C NMR of N-Boc-5-aminonaphthalene-1-sulfonic acid in CDCl$_3$. 
Figure 34: $^1$H NMR of N-Boc-5-aminonaphthalene-1-sulfonyl chloride in CDCl$_3$. 
Figure 35: $^{13}$C NMR of N-Boc-5-aminonaphthalene-1-sulfonyl chloride in CDCl$_3$. 
Figure 36: $^1$H NMR of N-Boc-5-aminonaphthalene-1-tert-butylsulfonamide in CDCl$_3$. 
Figure 37: $^{13}$C NMR of N-Boc-5-aminonaphthalene-1-tert-butylsulfonamide in CDCl$_3$. 
Figure 38: $^1$H NMR of 5-aminonaphthalene-1-sulfonamide in 5% $D_2$O in CD$_3$CN.
Figure 39: $^1$H NMR of N-phenylborono-5-aminonaphthalene-1-sulfonamide in CD$_3$OD.
Figure 40: $^1$H NMR of N-benzyl-5-aminonaphthalene-1-sulfonamide in D$_6$-DMSO.
Figure 41: $^1$H-NMR of N-benzylidine-5-aminonaphthalene-1-sulfonamide in D$_6$-DMSO.
Figure 42: $^1$H NMR of N-phenylborono-5-aminonaphthalene-1-sulfonic acid in CD$_3$OD+Et$_3$N.
Figure 43: $^1$H NMR of N-phenylborono-5-amino-1-naphthol in CD$_3$OD.
Figure 44: $^1$H NMR of N-phenylborono-5-amino-1-naphthol-3-sulfonic acid in CD$_3$OD+Et$_3$N.
Figure 45: $^1$H NMR of 'BuFluoHa in CDCl$_3$. 
Figure 46: $^{13}$C NMR of $^{t}$BuFluoHa in CDCl$_3$. 
Figure 47: $^1$H NMR of FluoHe in CD$_3$OD.
Figure 48: $^1$H NMR of $t$BuFluoNH$_2$a in CDCl$_3$. 
Figure 49: $^{13}$C NMR of $t^3$BuFluNH$_2$a in CDCl$_3$. 
Figure 50: $^1$H NMR of FluoNH$_2$c in CD$_3$OD.
Figure 51: (-)ESI-HRMS analysis of N-Boc-5-aminonaphthalene-1-sulfonic acid.
Figure 52: (+)ESI-HRMS of N-phenylborono-5-aminonaphthalene-1-sulfonamide.
Figure 53: (+)ESI-HRMS of N-benzyl-5-aminonaphthalene-1-sulfonamide.
Figure 54: (+)ESI-HRMS of N-benzylidine-5-aminonaphthalene-1-sulfonamide.
Figure 55: (+)ESI-HRMS of N-phenylborono-5-aminonaphthalene-1-sulfonic acid.
Figure 56: (+)ESI-HRMS of N-phenylborono-5-amino-1-naphthol.
Figure 57: (+)ESI-HRMS of N-phenylborono-5-amino-1-naphthol-3-sulfonic acid.
Figure 58: (+)ESI-HRMS of FluoHa.
Figure 59: (+)ESI-HRMS of FluoHc.
Figure 60: (+)ESI-HRMS of FluoNH2a.
Figure 61: (-)ESI-HRMS of FluoHc.
Figure 62: (-)ESI-MS N-Boc-5-aminonaphthalene-1-sulfonyl chloride.
Figure 63: (-)ESI-MS of N-Boc-5-aminonaphthalene-1-tert-butylsulfonamide.
Figure 64: (-)ESI-MS of 5-aminonaphthalene-1-sulfonamide.
Figure 65: (+)ESI-MS of monoaminofluorene.
Figure 66: (+)ESI-MS of diaminofluorene.
Figure 67: (-)ESI-MS of dinitrofluorene.