Proton-in-Flight Mechanism for the Spontaneous Hydrolysis of *N*-Methyl *O*-Phenyl Sulfamate: Implications for the Design of Steroid Sulfatase Inhibitors

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Contents of Supporting Information

UV-visible pH Titration of 1:

A series of UV-visible spectra were obtained on an aqueous solution of **1** (7.4×10^{-5} M) from pH 9.2 – 11.4 at 25 ^{o}C and the absorbance values at 220 nm recorded. Corrections were applied for absorbances due to the pH buffer or KOH. A fit of the data to a standard equation yields a pK_a of 9.65 ± 0.11.

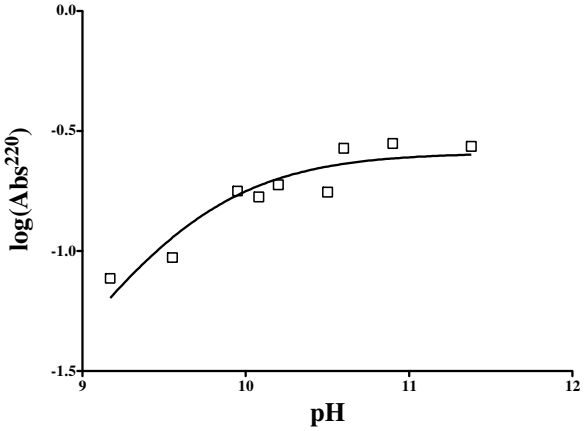

Figure S1. A plot of log(Abs^{220}) versus pH determined on an aqueous solution of **1** at 25 ^{o}C. The data are fit to a standard equation to yield a pK_a of 9.65 ± 0.11.

Eyring Plots for the hydrolysis of 1 and 2:

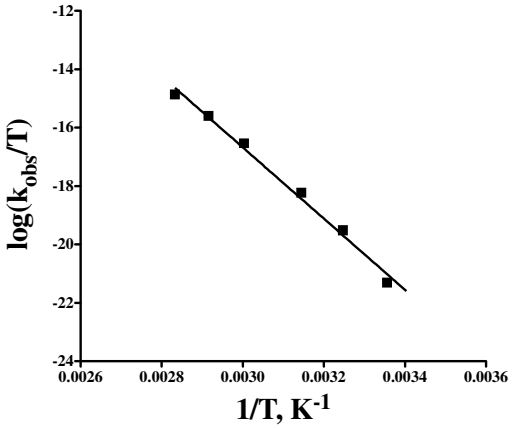

Figure S2. An Eyring plot for the hydrolysis of **1** determined at pH 5.9 from 25 < *T* < 80 °C. The activation parameters are $\Delta H^{\ddagger}$ = 18.7 ± 0.5 kcal/mol and $\Delta S^{\ddagger}$ = -24 ± 1 cal/mol•K. The units for k_{obs} is s^{-1}.

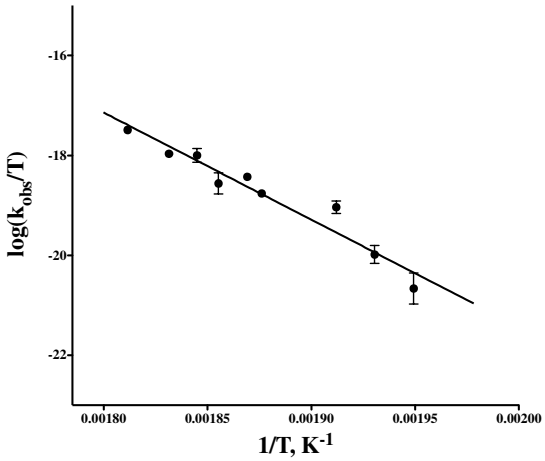

Figure S3. An Eyring plot for the hydrolysis of **2** determined at pH 5.9 from 240 < *T* < 279 °C. The activation parameters are $\Delta H^{\ddagger}$ = 38 ± 3 kcal/mol and $\Delta S^{\ddagger}$ = -14 ± 6 cal/mol•K. The units for k_{obs} is s^{-1}.

<u>Solvent Deuterium Kinetic Isotope Effect for the Hydrolysis of 1</u>:

A solvent deuterium kinetic isotope effect was determined on $k^{\mathbf{1}}$ for the hydrolysis of **1** at 60 °C. Observed first order rate constants were determined in duplicate for reactions run in 0, 20, 40, 60, 80 and 97.5% D_2O by monitoring phenol production for over 4 half-lives by UV-visible spectrophotometry. In this series of experiments, we assume that an equilibrium mixture of **1** and *d*-**1** is obtained quickly relative to product formation based on the fast H/D exchange observed below pH 6 for *N*-sulfo-D-glucoseamine by ^{1}H NMR.[1] A plot of k_{obs} versus deuterium content is shown in Figure S4 and from this k^H/k^D is calculated to be 1.8 ± 0.1.

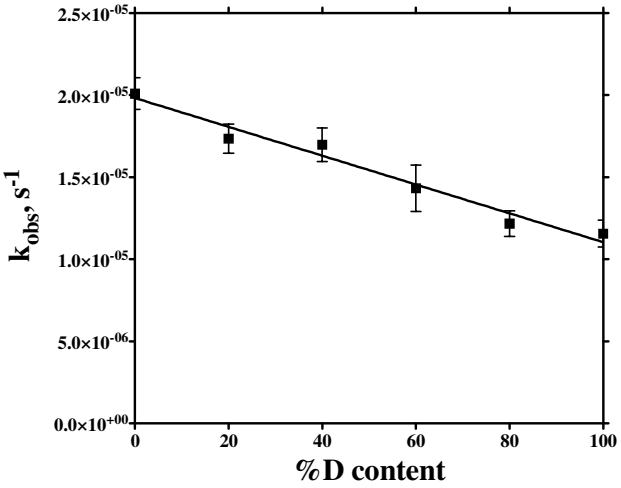

Figure S4. A plot of k_{obs} versus %D content calculated as [D_2O/(D_2O + H_2O) x100] for the hydrolysis of **1** at 60 ^{o}C, 0.02 M phosphate buffer at pH 5.9 from which $k^H/k^D = 1.8 \pm 0.1$ was calculated.

Table S1. Kinetic Data and References for the Construction of Figure 4 of the main text:

Substrate	pK_a^{ArO-H}	$pK_a^{ArOSO2X-H}$	k, s^{-1}
4-NO_2-$C_6H_4OSO_3^-$	7.14	-4.86[a]	(1.9×10^{-10})[b]
4-NO_2-$C_6H_4OSO_2N^-(CH_3)$	7.14	8.88[c]	(8.4×10^{-3})[c]
4-NO_2-$C_6H_4OSO_2N^-CH_2(C_6H_3Cl_2)$	7.14	7.83[d]	(2.7×10^{-3})[d]
4-NO_2-$C_6H_4OSO_2N^-CH_2(C_6H_4Cl)$	7.14	8.11[d]	(3.5×10^{-3})[d]
4-NO_2-$C_6H_4OSO_2N^-CH_2(C_6H_4Cl)$	7.14	7.89[d]	(2.8×10^{-3})[d]
4-NO_2-$C_6H_4OSO_2N^-CH_2(C_6H_4F)$	7.14	8.04[d]	(3.7×10^{-3})[d]
4-NO_2-$C_6H_4OSO_2N^-CH_2(C_6H_6)$	7.14	8.26[d]	(4.0×10^{-3})[d]
4-NO_2-$C_6H_4OSO_2N^-CH_2(4\text{-}CH_3C_6H_4)$	7.14	8.22[d]	(4.7×10^{-3})[d]
4-NO_2-$C_6H_4OSO_2N^-CH_2(4\text{-}CH_3OC_6H_4)$	7.14	8.26[d]	(4.7×10^{-3})[d]
4-NO_2-$C_6H_4OSO_2C^-HSO_2CH_3$	7.14	9.0[e]	(3.1×10^{-4})[e]
4-NO_2-$C_6H_4OSO_2C^-CH_3SO_2CH_3$	7.14	11.24[e]	(1.5×10^{-1})[e]
4-NO_2-$C_6H_4OSO_2C^-HPh$	7.14	21.7[f]	(5×10^{9})[f]
4-NO_2-$C_6H_4OSO_2N^-H$	7.14	7.29[g]	(7.7×10^{-4})[g]
3-NO_2-$C_6H_4OSO_3^-$	8.38	-4.6[h]	(6.7×10^{-12})[b]
3-NO_2-$C_6H_4OSO_2C^-HSO_2CH_3$	8.38	9.07[e]	(1.7×10^{-6})[e]
3-NO_2-$C_6H_4OSO_2C^-HPh$	8.38	22.2[f]	(3.3×10^{7})[f]
3-Cl-$C_6H_4OSO_3^-$	9.38	-4.4[g]	(1.3×10^{-14})[b]
3-Cl-$C_6H_4OSO_2C^-HSO_2CH_3$	9.38	9.7[i]	(6.2×10^{-8})[j]
3-Cl-$C_6H_4OSO_2C^-HPh$	9.38	22.6[f]	(2.7×10^{5})[f]
$C_6H_5OSO_3^-$	9.99	-3.88[h]	(2.3×10^{-15})[b]
$C_6H_5OSO_2C^-HSO_2CH_3$	9.99	9.99[e]	(5.6×10^{-9})[j]
$C_6H_5OSO_2C^-HPh$	9.99	22.8[f]	(2.9×10^{4})[f]

a) Guthrie, J. P. *J. Am. Chem. Soc.* **1980**, *102*, 5177. b) Edwards, D. R.; Lohman, D. C.; Wolfenden, R. J. Am. Chem. Soc. **2012**, *125*, 525. c) Willams, A.; Douglas, K. T. *J. Chem. Soc. Perkin Trans. 2* **1974**, 1727. d) Spilane, W. J.; Thea, S.; Cevasco, G.; Hynes, M. J.; McCaw, C. J. A.; Maguire, N. P. Org. Biomol. Chem. **2011**, 9, 523. e) Thea, S.; Guanti, G. Hopkins, A. R.; Williams, A. *J. Org. Chem.* **1985**, *50*, 5592. f) Davy, M. B.; Douglas, K. T.; Loran, J. S.; Steltner, A.; Williams, A. *J. Am. Chem. Soc.* **1977**, *99*, 1196. g) Thea. S.; Cevasco, G.; Guanti, G.; Williams, A. *J. Chem. Soc. Chem. Commun.* 1986, 1582. h) $pK_a^{ArOSO2X-H}$ estimated from the correlation shown in Figure S5 where $pK_a^{ArOSO3H} = (0.2 \pm 0.1)pK_a^{ROH} - (6.3 \pm 0.4)$. i) Calculated from equation 3 in Thea, S.; Guanti, G. Hopkins, A. R.; Williams, A. *J. Org. Chem.* **1985**, *50*, 5592. j) Calculated from equation 5 in Thea, S.; Guanti, G. Hopkins, A. R.; Williams, A. *J. Org. Chem.* **1985**, *50*, 5592.

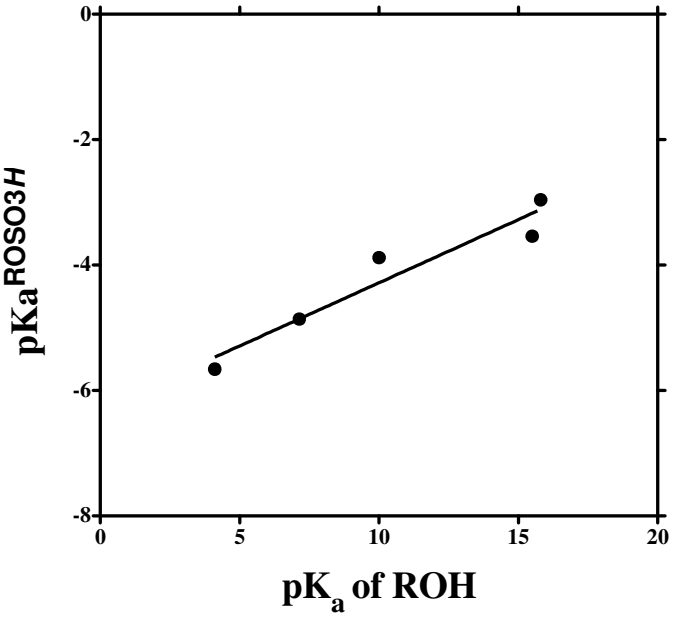

Figure S5. A plot of $pK_a^{ArOSO3H}$ versus pK_a^{ROH} corresponding to the acid base reactions: $ROSO_3$-H ←→ $ROSO_3^-$ + H^+ and RO-H ←→ RO^- + H^+, respectively. The best fit line through the data adheres to $pK_a^{ArOSO3H}$ = (0.2 ± 0.1)pK_a^{ROH} – (6.3 ± 0.4) with an r^2 = 0.92 (5 data). The data correspond to 2,4-dinitrophenyl sulfate, 4-nitrophenyl sulfate, phenyl sulfate, methyl sulfate and sulfuric acid. The data are from Guthrie, J. P. *Can. J. Chem.* **1978**, *56,* 2342 and Guthrie, J. P. *J. Am. Chem. Soc.* **1980**, *102*, 5177.

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1) Langeslay, D. J.; Beni, S.; Larive, C. K. *Anal. Chem.* **2011**, *83*, 8006.