Supplementary Information

Boronate based fluorescence (ESIPT) probe for peroxynitrite

Adam C. Sedgwick,[a] Xiaolong Sun,[a] Gyoungmi Kim,[b] Juyoung Yoon,*[b] Steven D. Bull*[a] and Tony D. James*[a]

[a] Department of Chemistry, University of Bath, BA2 7AY, UK. Email: S.D.Bull@bath.ac.uk, T.D.James@bath.ac.uk
[b] Department of Chemistry and Nano Science, Ewha Womans University, Seoul 120-750, Korea. E-mail: jyoon@ewha.ac.kr.

Content

1. Preparation of ROS/RNS

$NO^{\cdot}$

Nitric oxide (NO) was prepared by treating sulfuric acid (3.6 M) solution with sodium nitrite solution (7.3M) and its stock solution (2.0 mM) was prepared by bubbling NO into deoxygenated deionized water for 30 min

ROO•

$ROO^{\cdot}$ was generated from 2,2'-azobis (2-amidinopropane) dihydrochloride. AAPH (2, 2' azobis (2-amidinopropane) dihydrochloride,1 M) was added into deionizer water, and then stirred at 37 °C for 30 min;

$^{-}O_2$

Superoxide was generated from KO_2. KO_2 and 18-crown-6 ether (2.5 eq) was dissolved in DMSO to afford a 0.25 M solution.

•HO

Hydroxyl radical was generated by the Fenton reaction. To prepare •OH solution, hydrogen peroxide (H_2O_2, 10 eq) was added to $Fe(ClO_4)_2$ in deionised water.

$ONOO^{-}$

Simultaneously, 0.6 M KNO_2, 0.6 M in HC1, 0.7 M in H_2O_2 was added at to a 3 M NaOH solution at 0 ºC. The concentration of peroxynitrite was estimated by using extinction co-efficient of 1670 $cm^{-1}M^{-1}$ at 302 nm in 0.1 M sodium hydroxide aqueous solutions

^{-}OCl

The concentration of ^{-}OCl was determined from the absorption at 292 nm (ε = 350 $M^{-1}cm^{-1}$).

H_2O_2

The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 M-1cm-1).

2. Fluorescence analysis

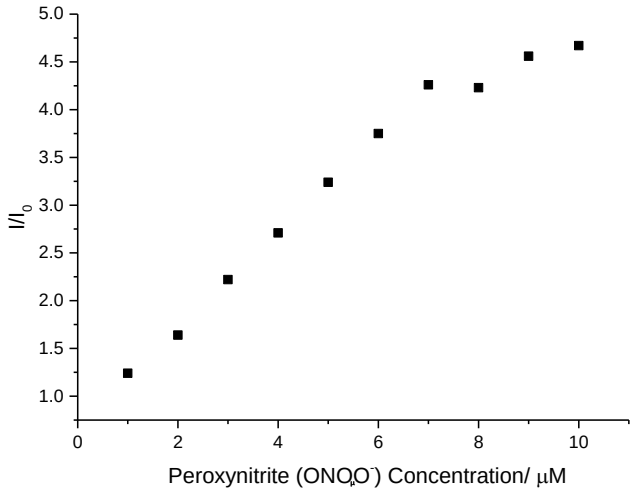


Fig S1: Fluorescence intensity changes (I/I_0) for probe **3** (0.25 μM) with increasing concentrations of peroxynitrite ($ONOO^-$) λ_{ex} 400 nm/ λ_{em} 461 nm in pH 8.2 buffer solution [52 wt% methanol]. each measurement was made after 1 minute.

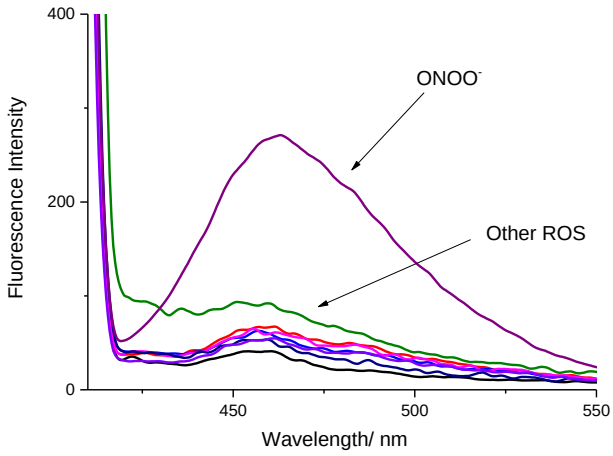


Fig S2: Fluorescence spectra of probe **3** (0.25 μM) in the presence of various ROS/RNS: $ONOO^-$ (10 μM, 1 min), ^-OCl (100 μM, 30 min), H_2O_2 (100 μM, 30 min), $ROO^\cdot$ (100 μM, 30 min), NO (100 μM, 1 min) $^-O_2$ (100 μM, 1 min), $^\cdot OH$ (100 μM, 1 min) λ_{ex} 400 nm in pH 8.2 buffer solution [52 wt% methanol].

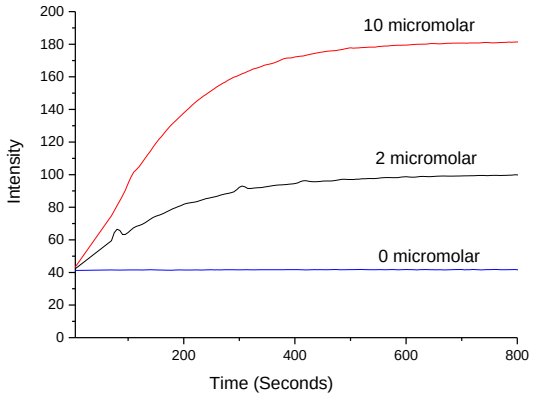


Fig S3: Fluorescence intensity changes for probe **3** (0.25 μM) as a function of time (Seconds) with increasing concentrations of $ONOO^-$ (0, 2, 10 μM), λ_{ex} 400nm, λ_{em} 460 nm in pH 8.2 buffer solution [52 wt% methanol].

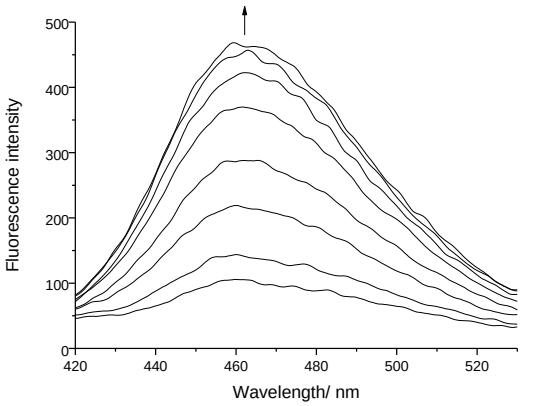


Fig S4: Fluorescence spectra of probe **3** (0.5 μM) with the addition of hydrogen peroxide (0-14 mM), λ_{ex} 400 nm in pH 8.2 buffer solution [52 wt% methanol] each measurement was made after 30 minutes.

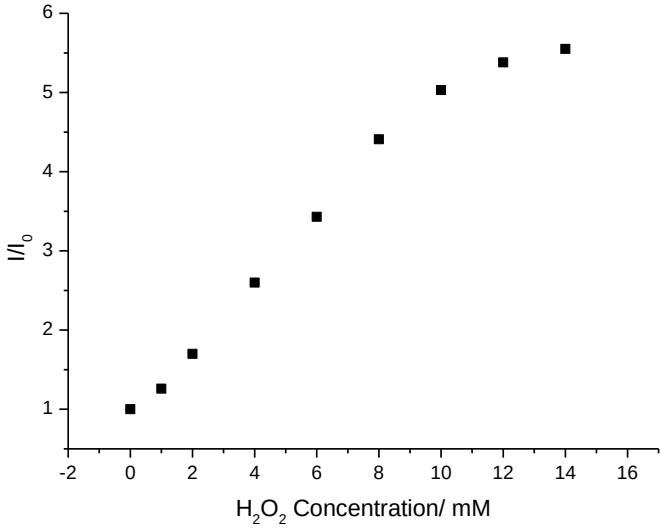


Fig S5: Fluorescence intensity changes (I/I_0) for probe **3** (0.5 µM) with increasing concentrations of hydrogen peroxide (H_2O_2) λ_{ex} 400 nm/ λ_{em} 461 nm in pH 8.2 buffer solution [52 wt% methanol] each measurement was made after 30 minutes.

3. UV analysis

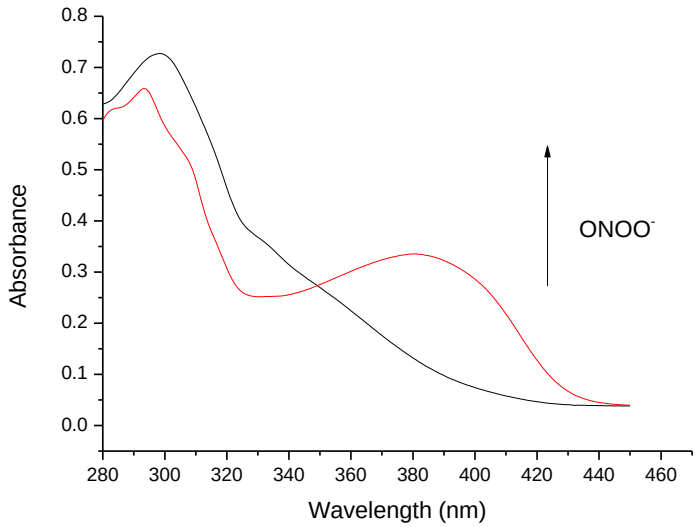


Fig S6: UV spectra of probe **3** (10 µM), with and without $ONOO^-$ in pH 8.2 buffer solution [52 wt% methanol].

4. Cell images and viability

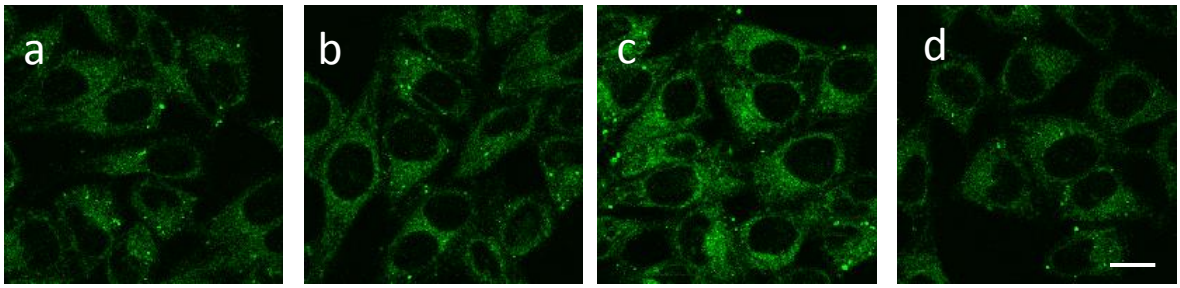


Fig S7: Fluorescence property in the live cell. HeLa cells were incubated with 20 μM probe **3** for 30 min and washed with DPBS and added (a) 0, (b) 30, (c) 100 μM ONOO- and (d) after pretreatment with 100 μM ebselen, add 100 μM ONOO- for 30 min. Fluorescence images were acquired by confocal microscopy. λ_{ex}. 473 nm/ λ_{em} 490 – 590 nm. Scale bar: 10 μm

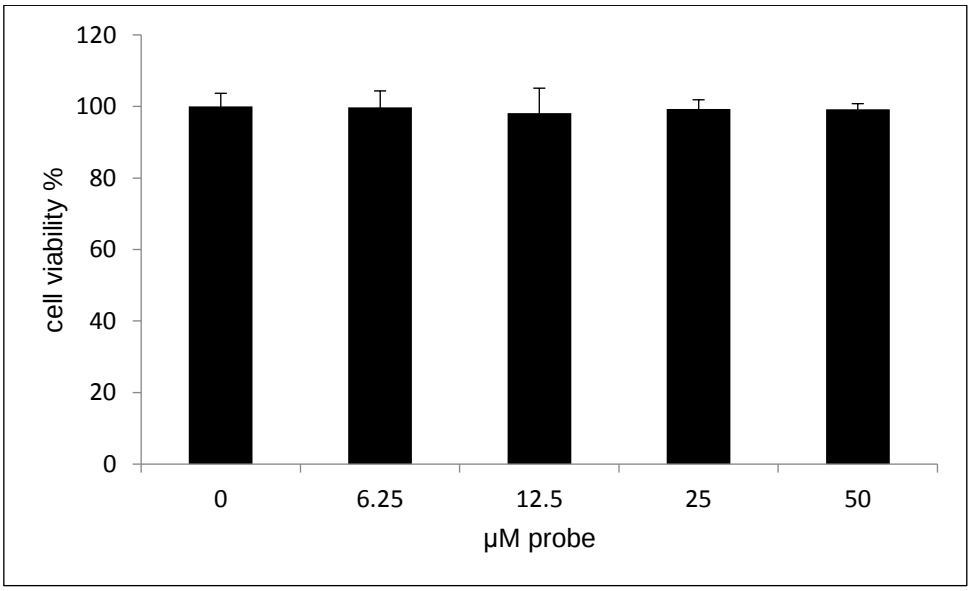
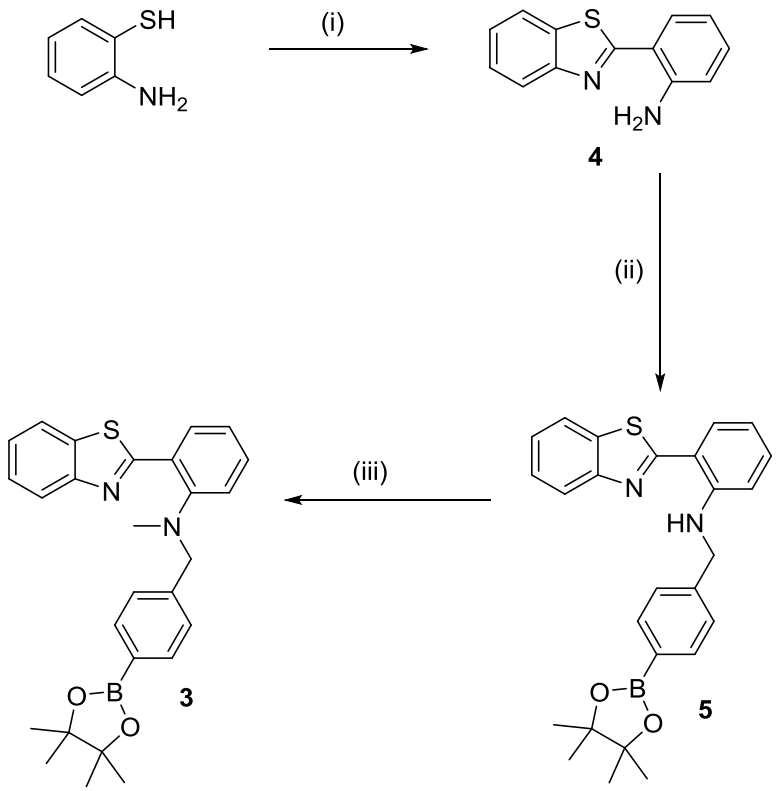


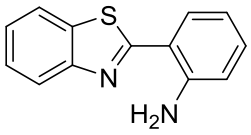
Fig S8: HeLa cells were incubated with each concentration of probe for 24hr. Cell viability was assayed by MTT test. Results are expressed as mean ± standard deviation of three independent experiments.

5. Experimental



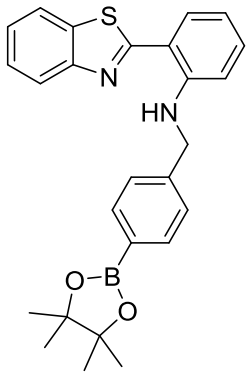
Scheme S1: (i) Isatoic anyhydride, NaOAc, AcOH, 1.5 h, 72 % yield (ii) **4**, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde, $NaBH(OAc)_3$, AcOH, DCE, 16 h, 78 % yield (iii) **5**, $NaCNBH_3$, Formaldehyde – 35 % in H_2O, AcOH, MeCN, 17 h, 14 % yield.

2-(benzo[d]thiazol-2-yl)aniline (4)



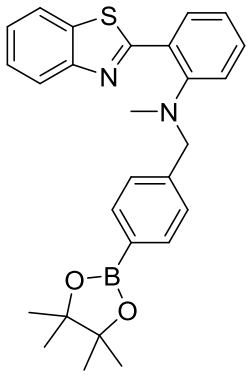
Isatoic anhydride (2.93 g, 15.27 mmol), 2-Aminothiophenol (2.55 mL, 20.37 mmol), Sodium acetate (1.2 g, 12.43 mmol) in AcOH (120 mL) was refluxed for 1.5 h under Argon. The reaction was cooled to rt and EtOAc (100 mL) was added and the solution was quenched with solid $NaHCO_3$. The organic layer was washed with H_2O (2 x 50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude material. The crude product was purified via column chromatography 5:95 (EtOAc:Pet ether) to afford a cream solid (3.33 g, 14.7 mmol, 72 %). M.p. 123-127 ^oC. ^1H NMR (500MHz, $CDCl_3$) δ_H 7.97 (d, *J* = 8.8 Hz, 1 H, Ar*H*), 7.88 (d, *J* = 9.3 Hz, 1 H, Ar*H*), 7.71 (dd, *J* = 1.5, 7.8 Hz, 1 H, Ar*H*), 7.50 - 7.42 (m, 1 H, Ar*H*), 7.40 - 7.33 (m, 1 H, Ar*H*), 7.23 (ddd, *J* = 1.5, 7.2, 8.4 Hz, 1 H, Ar*H*), 6.79 (dd, *J* = 1.0, 8.3 Hz, 1 H, Ar*H*), 6.76 - 6.73 (m, 1 H, Ar*H*), 6.40 (br. s., 2 H, Ar-N*H*$_2$); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ_C 169.2, 153.7, 146.7, 133.3, 131.5, 130.3, 126.0, 124.8, 122.4, 121.2, 116.9, 116.8, 115.3; I.R (thin film) ν max (cm^{-1}): 3461.97 (N-H); HRMS (ESI): m/z calculated for $C_{13}H_{10}N_2S$: requires 227.0643 for $[M+H]^+$, found 227.0637; requires 249.0462 for $[M+Na]^+$, found 249.0443

2-(benzo[d]thiazol-2-yl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)aniline (5)



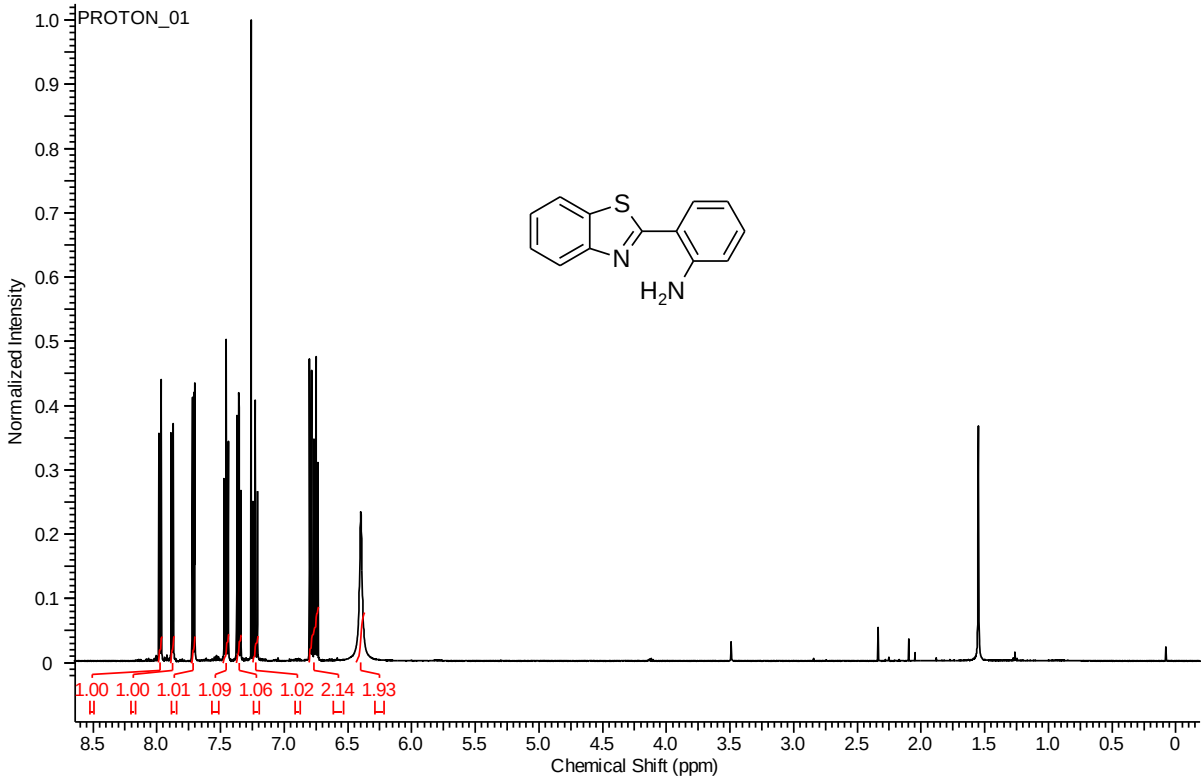
4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (1.18 g, 5.08 mmol) was added to a solution of **4** (0.72 g, 3.18 mmol) in DCE (10 mL) and AcOH (1.11 mL). The reaction mixture was stirred for 1 h before the addition of $NaBH(OAc)_3$ (1.75 g, 8.26 mmol). The reaction mixture was stirred for 24 h before being quenched with saturated $NaHCO_3$ solution. The organic layer was washed with H_2O (3 x 50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude product. The product was purified via column chromatography 10:90 (EtOAc:Pet ether) to afford a yellow oil (1.10 g, 2.49 mmol, 78 %). ^{1}H NMR (500MHz, $CDCl_3$) δ_H 9.52 (t, *J* = 5.4 Hz, 1 H, N-*H*), 7.93 (d, *J* = 7.8 Hz, 1 H, Ar*H*), 7.89 (d, *J* = 7.3 Hz, 1 H, Ar*H*), 7.83 (d, *J* = 7.8 Hz, 2 H, Ar*H*), 7.78 (dd, *J* = 1.5, 8.3 Hz, 1 H, Ar*H*), 7.27 - 7.20 (m, 1 H, Ar*H*), 6.74 - 6.68 (m, 2 H, Ar*H*), 4.65 (d, *J* = 5.4 Hz, 2 H, NHCH_2Ar), 1.36 (s, 12 H, BPin); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ_C 169.5, 153.5, 147.3, 142.5, 135.1, 133.1, 132.0, 130.6, 126.2, 126.0, 124.8, 122.3, 121.1, 115.6, 115.0, 111.9, 83.7, 47.3, 24.9; I.R (thinfilm) ν max (cm^{-1}): 3282.94 (N-H); HRMS (ESI): m/z calculated for $C_{26}H_{27}BN_2O_2S$: requires 443.1965 for $[M+H]^+$, found 443.2007

2-(benzo[d]thiazol-2-yl)-N-methyl-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)aniline (3)

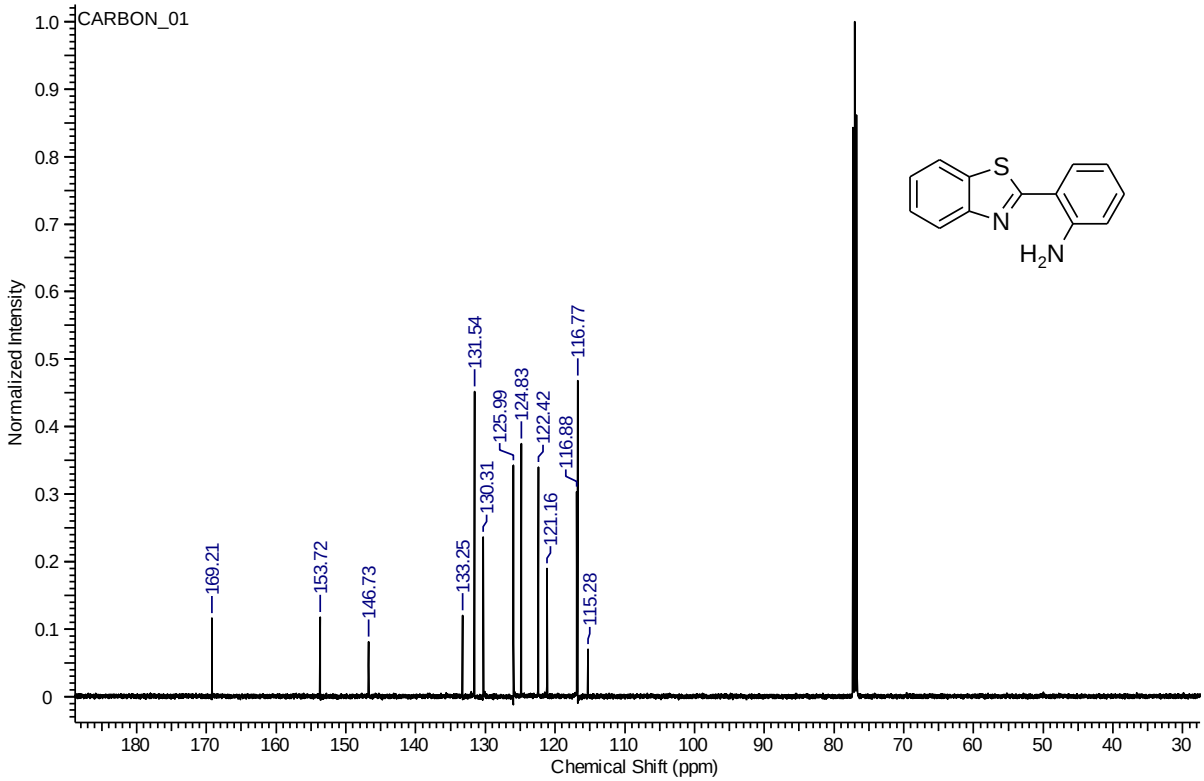


A solution of **5** (0.82 g, 1.85 mmol), formaldehyde – 37 % in H_2O (1.3 mL, 18.54 mmol), AcOH (1.6 mL, 27.75 mmol) in MeCN (20 mL) was cooled to 0 ^oC before the portion wise addition of $NaBH_3CN$ (0.3 g, 4.81 mmol). The reaction was stirred for 24 h before being quenched with saturated $NaHCO_3$ solution. Ether (100 mL) was then added and the organic layer was washed with H_2O (3 x 50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude material. The product was purified *via* column chromatography 5:95 (EtOAc:Pet ether) to afford a pale green foam (0.12 g, 0.263 mmol, 14 %). ^1H NMR (500MHz , $CDCl_3$) δ = 8.39 (dd, *J* = 2.0, 7.8 Hz, 1 H, Ar*H*), 8.10 (d, *J* = 8.3 Hz, 1 H, Ar*H*), 7.93 (d, *J* = 7.3 Hz, 1 H, Ar*H*), 7.79 (d, *J* = 7.8 Hz, 2 H, Ar*H*), 7.53 - 7.46 (m, 1 H, Ar*H*), 7.42 - 7.33 (m, 4 H, Ar*H*), 7.26 - 7.20 (m, 1 H, Ar*H*), 7.16 (d, *J* = 7.8 Hz, 1 H, Ar*H*), 4.20 (s, 2 H, CH_3NCH_2Ar), 2.66 (s, 3 H, CH_3NCH_2Ar), 1.36 (s, 12 H, BPin); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ = 165.0, 152.2, 151.4, 140.0, 136.7, 134.6, 130.9, 130.4, 129.0, 125.8, 124.9, 124.3, 122.9, 122.1, 121.4, 83.8, 61.1, 43.0, 24.9; HRMS (ESI): m/z calculated for $C_{27}H_{29}BN_2O_2S$: requires 479.1940 for $[M+Na]^+$, found 479.1963

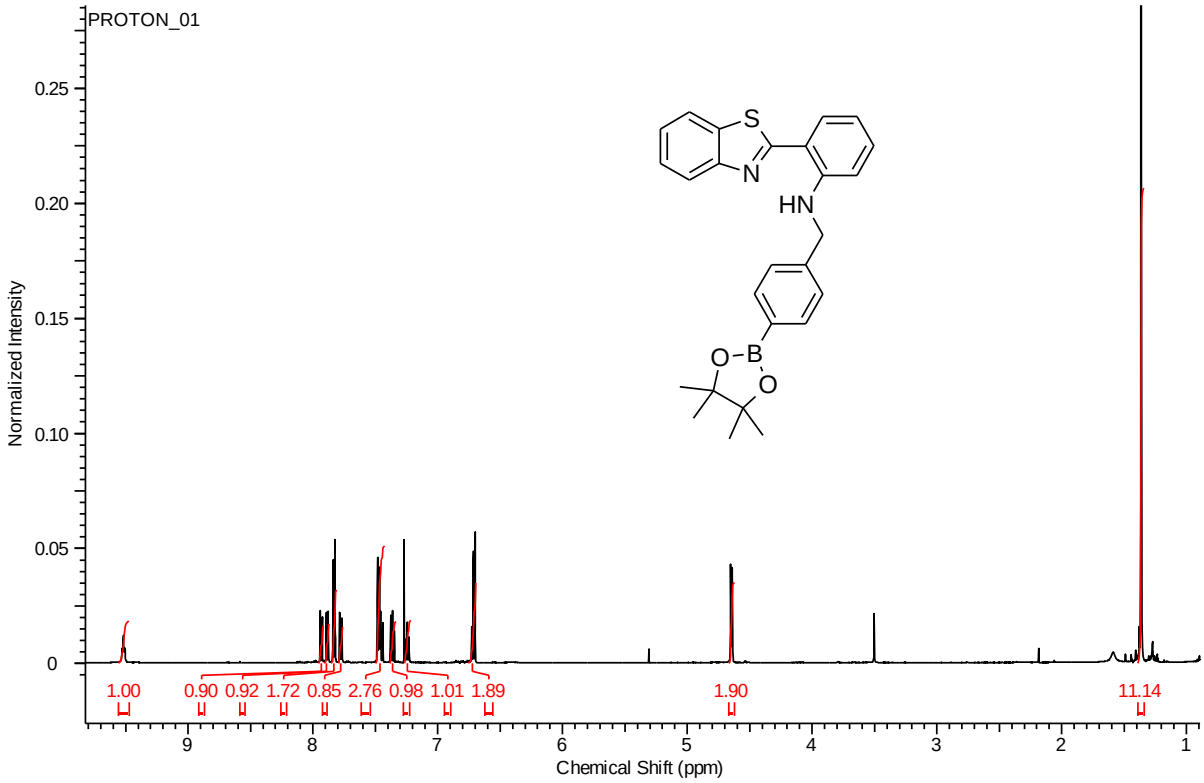
2-(benzo[d]thiazol-2-yl)aniline (4) (500 MHz, $CDCl_3$)



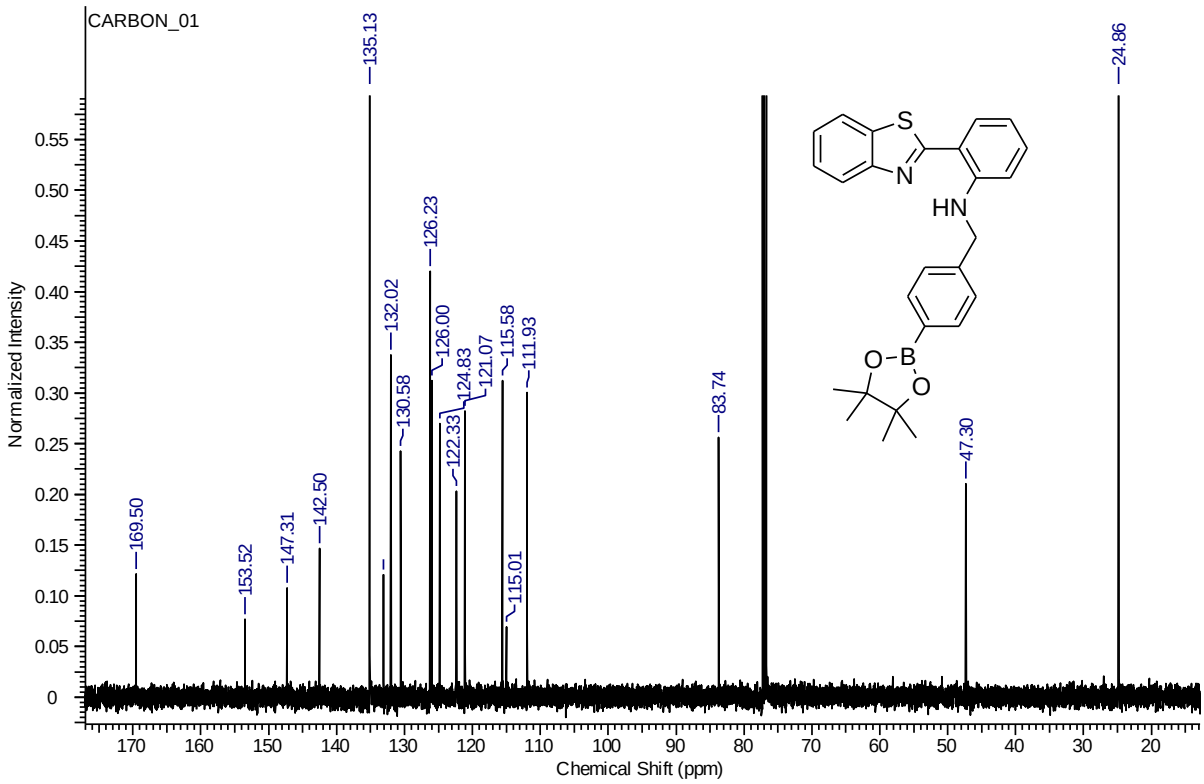
2-(benzo[d]thiazol-2-yl)aniline (4) (125.75 MHz, $CDCl_3$)



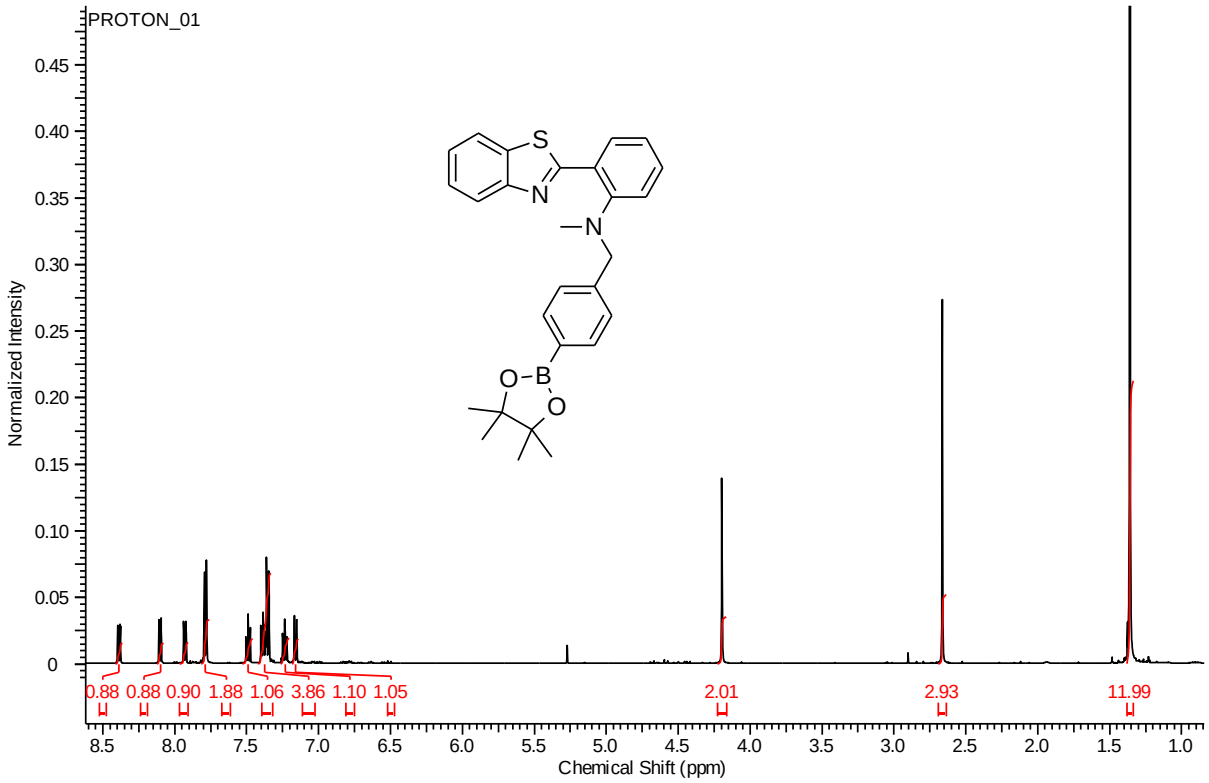
2-(benzo[d]thiazol-2-yl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)aniline (5) (500 MHz, $CDCl_3$)



2-(benzo[d]thiazol-2-yl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)aniline (5) (125.75 MHz, $CDCl_3$)



2-(benzo[d]thiazol-2-yl)-N-methyl-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)aniline (3) (500 MHz, $CDCl_3$)



2-(benzo[d]thiazol-2-yl)-N-methyl-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)aniline (3) (125.75 MHz, $CDCl_3$)