Supplementary Information

A bodipy based hydroxylamine sensor

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Content

1. Fluorescence analysis

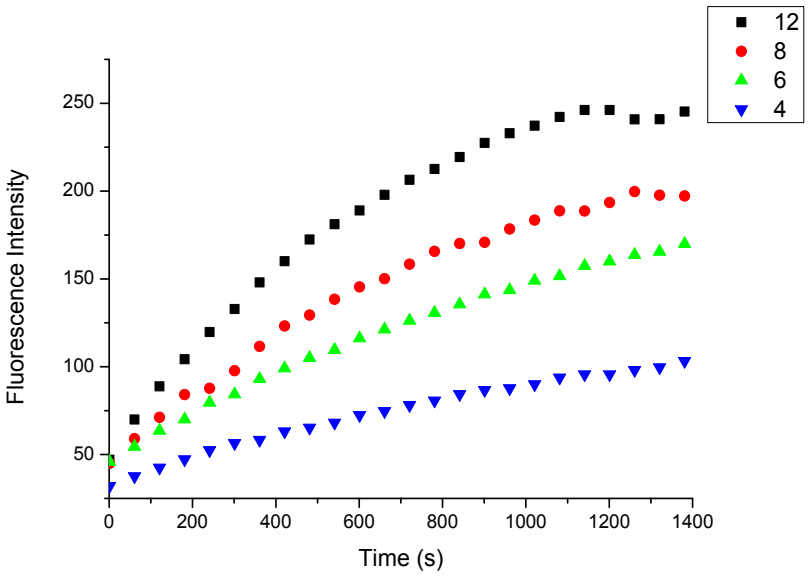


Figure S1 - Time curve of the fluorescence intensity with Probe **1** (0.5 μM), and various concentrations of hydroxylamine (4, 6, 8 and 12 μM). PBS Buffer, 1% DMSO, pH = 7.4. slit width ex = 5 nm, em = 2.5 nm. λ_{ex} = 465 nm. λ_{em} = 510 nm

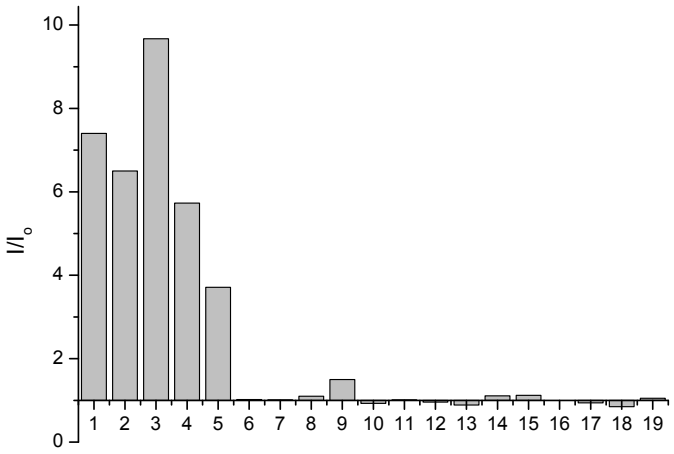


Figure S2 - Overall selectivity of probe **1** (0.5 µM) with various hydroxylamines, amino acids, amines and sulphur containing compounds (1 – H_2S (50 µM, 15 min), 2 – Hydroxylamine (50 µM, 15 min), 3 - N-(Methyl)Hydroxylamine (50 µM, 15 min), 4 – *N*-(Benzyl)Hydroxylamine (50 µM, 15 min), 5 - *N*-(Propargyl)Hydroxylamine (50 µM, 15 min), 6 - *N*-(tert-Butyl)Hydroxylamine (50 µM, 15 min), 7 - O-(Benzyl)Hydroxylamine (50 µM, 15 min), 8 – GSH (5 mM, 30 min), 9 – Cysteine (5 mM, 30 min), 10 - Methionine (5 mM, 30 min), 11 – Lysine (5 mM, 30 min), 12 – Serine (5 mM, 30 min), 13 – Histidine (5 mM, 30 min), 14 – Tyrosine (5 mM, 30 min), 15 – Arginine (5 mM, 30 min), 16 – Proline (5 mM, 30 min), 17 – Glycine (5 mM, 30 min), 18 – Glutamic acid (5 mM, 30 min), 19 – Blank in PBS Buffer, 1% DMSO pH = 7.4. slit width ex = 5 nm, em = 2.5 nm. λ_{ex} = 465 nm, λ_{em} = 510 nm.

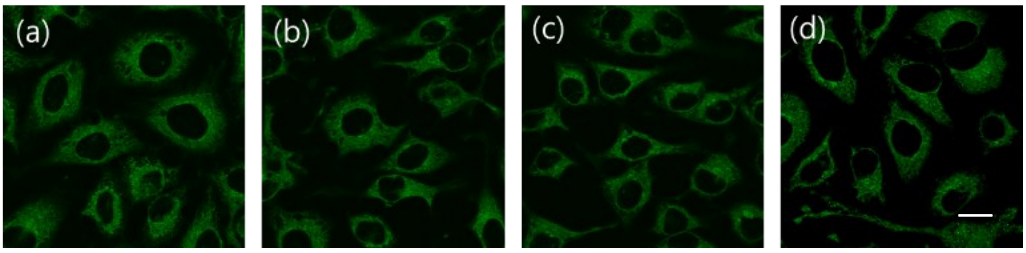
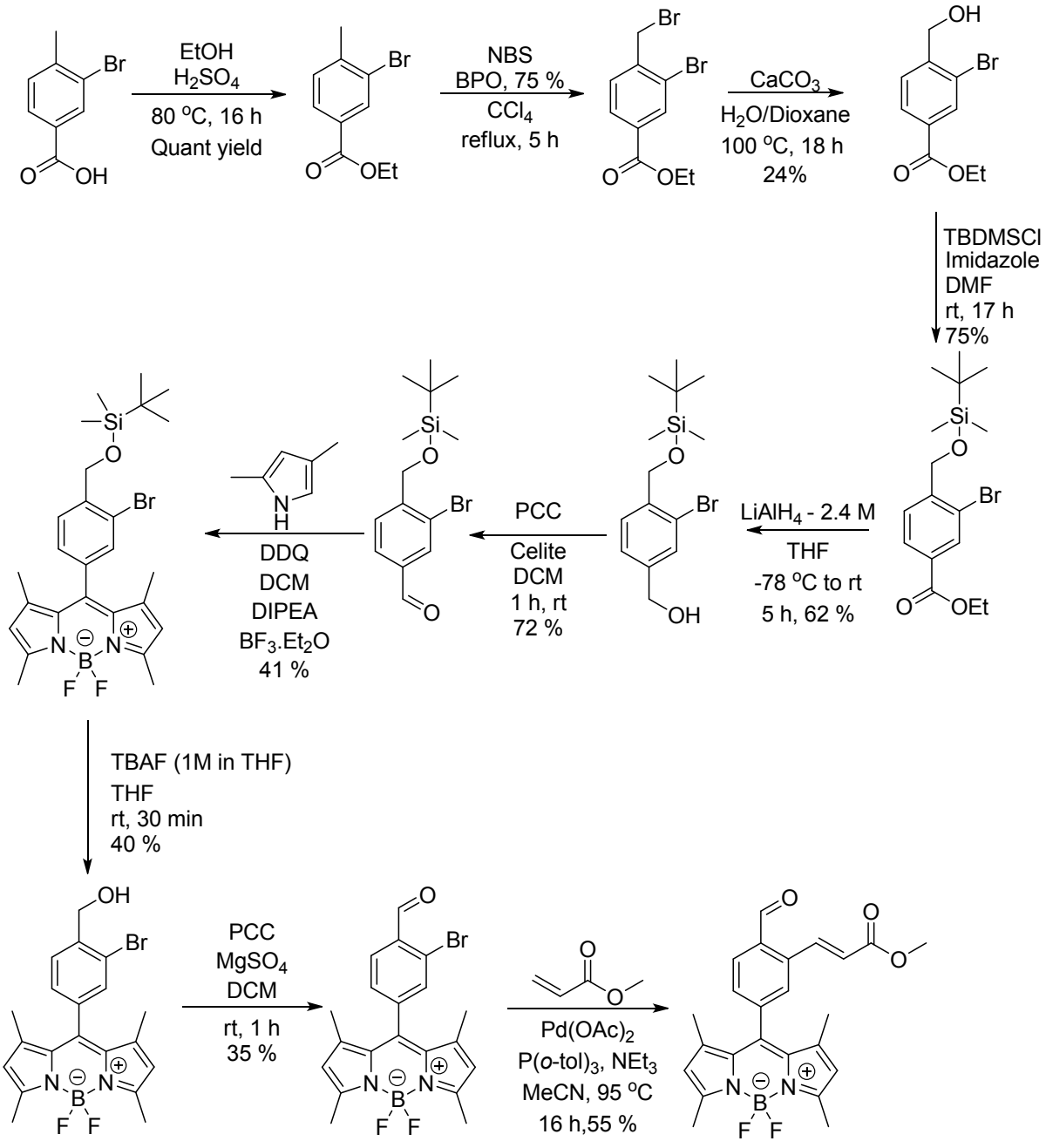


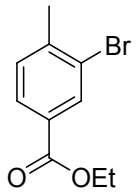
Figure S3 - Fluorescence images of HeLa cells with 0, 10, 50, 150 µM NH_2OH (30 min) and washed with DPBS then incubated with 1 µM of probe **1** (30 min) obtained by confocal microscopy. λex. 473 nm/ λex em. 490-590 nm. Scale bar 20 µM.

2. Experimental



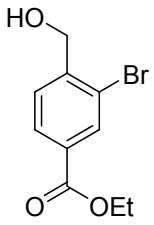
Experimental

Ethyl 3-bromo-4-methylbenzoate



3-Bromo-4-methylbenzoic acid (25 g, 116.3 mmol) was dissolved in EtOH (400 mL) and $^{Conc}H_2SO_4$ (10 mL), the solution was then heated at reflux for 18 h. The reaction mixture was cooled to rt and the solvent was removed under reduced pressure. The residue was slowly quenched with saturated $NaHCO_3$ solution and the aqueous layer was extracted three times with EtOAc. The combined organics were dried ($MgSO_4$) and concentrated *in vacuo* to afford an orange oil, no further purification was required (quantitative yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (s, Ar*H,* 1 H), 7.86 (d, Ar*H*, *J* = 7.8 Hz, 1 H), 7.28 (d, Ar*H, J* = 7.8 Hz, 1 H), 4.36 (q, COC*H*$_2$CH$_3$, *J* = 7.3 Hz, 2 H), 2.44 (s, ArCH$_3$, 3H), 1.39 (t, COCH$_2$C*H*$_3$, *J* = 7.1 Hz, 3 H); ^{13}C NMR (125.75 MHz $CDCl_3$) δ 165.32, 143.11, 133.36, 130.60, 129.82, 128.61, 124.71, 61.15, 23.15, 14.28; I.R (thinfilm) ν max (cm^{-1}): 1716.86 (C=O); HRMS (ESI): m/z calculated for $C_{10}H_{11}BrO_2$: requires: 264.9840 for $[M+Na]^+$; found: 264.9826

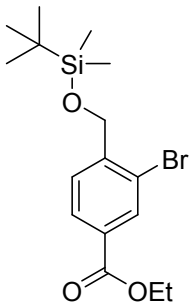
Ethyl 3-bromo-4-(hydroxymethyl)benzoate



A mixture of ethyl 3-bromo-4-methylbenzoate (26.00 g, 106.95 mmol), NBS (22.78 g, 128.34 mmol) and benzoyl peroxide (2.6 g, 10.695 mmol) were suspended in CCl_4 (300 mL), the reaction mixture was then heated to reflux for 5 h. After cooling to rt, the solid by-products were removed by filtration and the filtrate was concentrated *in vacuo*. The residue was dissolved in EtOAc (200 mL) and the organic was washed with H_2O (3 x 100 mL), brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude mixture which was predominantly the desired bromomethyl product and a small amount of undesired dibromoproduct. This was used directly in the next reaction without any further purification.

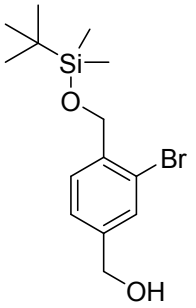
$CaCO_3$ (32 g, 328 mmol) was added to a solution of bromomethyl product in (150 mL) H_2O and (150 mL) 1,4-dioxane, The mixture was then stirred at 100 ^oC for 24 h. The reaction mixture was then cooled to rt and the solid was filtered. The solvent was concentrated *in vacuo* to remove the 1,4-dioxane. The residue was diluted with (400 mL) EtOAc and the organic layer was washed with H_2O (2 x 100 mL), Brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude material. The crude material was purified via column chromatography (5 to 20% EtOAc/Pentane) to afford the title compound as a white solid (7.5 g, 28.94 mmol, 27 %). Mp. 71-73 ^oC. ^1H NMR (500 MHz ,$CDCl_3$) δ 8.20 (s, Ar*H, 1* H), 8.00 (d, Ar*H*, J = 7.8 Hz, 1 H), 7.60 (d, Ar*H*, J = 7.8 Hz, 1 H), 4.80 (s, C*H*$_2$OH, 2 H), 4.39 (q, C*H*$_2$CH$_3$ *J* = 6.8 Hz, 2 H), 2.14 (br. s., CH$_2$O*H*, 1 H), 1.41 (t, CH$_2$C*H*$_3$, *J* = 7.1 Hz, 3 H); ^{13}C NMR (125.75 MHz $CDCl_3$) δ 165.21, 144.57, 133.46, 131.14, 128.67, 128.03, 121.78, 64.64, 61.37, 14.28; I.R (thinfilm) ν max (cm^{-1}): 3487.19 (O-H), 1694.97 (C=O); HRMS (ESI): m/z calculated for $C_{10}H_{11}BrO_3$: requires: 256.9813 for $[M-H]^-$; found: 256.9808.

Ethyl 3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)benzoate



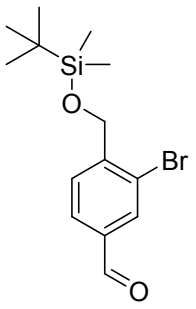
tert-Butyldimethylsilyl chloride (4.6 g, 30.39 mmol) was added to a mixture of Ethyl 3-bromo-4-(hydroxymethyl)benzoate (7.50 g, 28.94 mmol) and imidazole (3 g, 43.41 mmol) in CH_2Cl_2 (200 mL), the reaction was then stirred at rt for 16 h. The reaction mixture was partitioned with H_2O (200 mL) and the organic layer was washed with H_2O (2 x 100 mL), brine (100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to afford the title compound as a clear oil (9.47 g, 25.36 mmol, 88 %). No further purification was required. ^{1}H NMR (500MHz ,$CDCl_3$) ^{1}H NMR (500 MHz ,$CDCl_3$) δ 8.17 (s, Ar*H*, 1 H), 8.02 (d, Ar*H*, *J* = 7.8 Hz, 1 H), 7.65 (d, Ar*H*, *J* = 8.3 Hz, 1 H), 4.77 (s, CH_2OSi, 2 H), 4.38 (q, COCH_2CH$_3$, *J* = 7.3 Hz, 2 H), 1.40 (t, COCH$_2$CH_3, *J* = 7.1 Hz, 3 H), 0.98 (s, OSi(CH$_3$)$_2$C(CH_3)$_3$ 9 H), 0.15 (s, OSi(CH_3)$_2$C(CH$_3$)$_3$, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.40, 145.44, 133.06, 130.52, 128.48, 127.24, 120.58, 64.62, 61.23, 25.91, 18.38, 14.31, -5.37; I.R (thinfilm) ν max (cm^{-1}): 1722.22 (C=O); HRMS (FTMS+pNSI): m/z calculated for $C_{16}H_{25}BrO_3Si$: requires 373.0829 for $[M+H]^+$; found 373.0828.

(3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)methanol



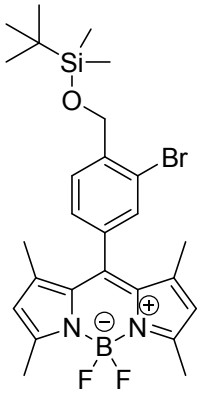
A solution of ethyl 3-bromo-4-(hydroxymethyl)benzoate (9.40 g, 25.18 mmol) in dry THF (250 mL) was cooled to -78 ^{o}C under N_2 followed by the dropwise addition of $LiAlH_4$ - 1 M in THF (25 mL, 60.44 mmol). The reaction was allowed to warm to rt and stirred for a further 5 h before being quenched at -78 ^{o}C with phosphate buffer. The quenched reaction mixture was immediately filtered through Celite® and the filtrate was concentrated *in vacuo* to afford the title compound as a clear oil. No further purification was required. (5.15 g, 25.18 mmol, 62 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 - 7.52 (m, Ar*H*, 2 H), 7.33 - 7.30 (m, Ar*H,* 1 H), 4.73 (s, C*H*$_2$OSi, 2 H), 4.66 (s, C*H*$_2$OH, 2 H), 0.96 (s, OSi(CH$_3$)$_2$C(C*H*$_3$)$_3$, 9 H), 0.13 (s, OSi(C*H*$_3$)$_2$C(CH$_3$)$_3$, 6 H); ^{13}C NMR (125.75 MHz $CDCl_3$) δ_C: 141.12, 139.91, 130.5, 127.67, 125.80, 121.08, 64.49, 64.38, 25.93, 18.39, -5.34; I.R (thinfilm) ν max (cm^{-1}): 3340.73 (br O-H); HRMS (ESI): m/z calculated for $C_{14}H_{22}BrO_2Si$: requires 329.0572 for [M-H]; found 329.0568.

3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)benzaldehyde



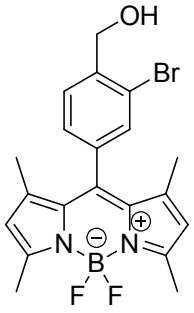
A solution of (3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)methanol (5.00 g, 15.09 mmol) in CH_2Cl_2 (50 mL) was cautiously added to a mixture of PCC (4. 87 g, 22.64 mmol) and Celite® (3.63 g) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at rt for 1 h before being filtered through Celite® and a silica pad (eluted with CH_2Cl_2) and then concentrated *in vacuo* to obtain the title compound as a clear oil. No further purification was necessary. (3.60 g, 10.93 mmol, 72 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.95 (s, C*H*O, 1 H), 8.01 (s, Ar*H*, 1 H), 7.85 (dd, *J* = 1.5, 7.9 Hz, Ar*H*, 1 H), 7.76 (d, *J* = 7.9 Hz, Ar*H*, 1 H), 4.77 (s, ArCH_2, 2 H,), 0.98 (s, OSi$(CH_3)_2$C(CH_3)$_3$, 9 H), , 0.16 (s, OSi(CH_3)$_2$C$(CH_3)_3$, 6 H,); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 190.7, 147.4, 136.4, 132.8, 128.9, 127.9, 121.5, 64.7, 25.9, 18.4, -5.4; I.R (thinfilm) ν max (cm^{-1}): 1702.11 (C=O); Mass spec was not observed.

10-(3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinine



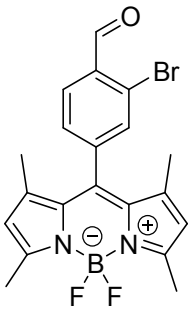
2,4-Dimethylpyrrole (0.693 g, 7.29 mmol) was added to a solution of 3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)benzaldehyde (1.20 g, 3.65 mmol) in anhydrous CH_2Cl_2 (150 mL) and stirred at rt for 16 h under a N_2 environment. DDQ (1.24 g, 5.48 mmol) was added to the reaction mixture and stirred for a further 2 h. The reaction mixture was then cooled to 0 ^oC before the addition of DIPEA (6.3 mL) and $BF_3.Et_2O$ (11.33 mL), the reaction was then stirred for a further 16 hrs. The solid impurities were filtered through Celite®, the filtrate was washed with H_2O (100 mL), brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude material. The crude material was purified via column chromatography 10 % (EtOAc/Pentane) to afford the title compound as a red gum (0.81 g, 41 %). ^1H NMR (500 MHz , $CDCl_3$) δ 7.69 (d, Ar*H*, *J* = 7.8 Hz, 1 H), 7.47 (d, Ar*H*, *J* = 2.0 Hz, 1 H), 7.28 (d, Ar*H*, *J* = 2.0 Hz, 1 H), 6.00 (s, (Pyr*H*)$_2$, 2 H), 4.82 (s, C*H*$_2$OSi, 2 H), 2.56 (s, ArC*H*$_3$ 6 H), 1.44 (s, ArC*H*$_3$, 6 H), 0.99 (s, Si(CH$_3$)$_2$C(C*H*$_3$)$_3$, 9 H), 0.17 (s, Si(C*H*$_3$)$_2$C(CH$_3$)$_3$, 6 H); ^{13}C NMR (125.75 MHz $CDCl_3$) δ 155.83, 142.97, 141.46, 139.67, 134.93, 131.42, 128.07, 127.14, 121.38, 121.24, 64.44, 25.92, 14.74, -5.31; I.R (thinfilm) ν max (cm^{-1}): No presence of carbonyl stretch; HRMS (ESI): m/z calculated for $C_{26}H_{34}BBrF_2N_2OSi$: requires: 547.1763 for $[M+H]^+$, found: 547.1768. requires: 569.1582 for $[M+Na]^+$, found 569.1607.

(2-bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)phenyl)methanol



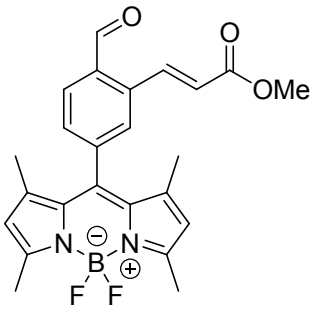
TBAF – 1M in THF (2.1 mL, 2.08 mmol) was added dropwise to a solution of 10-(3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (1.14 g, 2.08 mmol) and in THF (20 mL) at rt the reaction was then stirred for 30 min. The reaction mixture was quenched with saturated $NaHCO_3$ solution and extracted EtOAc (3 x 100 mL). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo*, the residue was then purified *via* column chromatography (40 to 60 % EtOAc/Pentane) to afford the title compound as an orange solid (0.36 g, 40%). Mp. 236-237 ^{o}C. ^{1}H NMR (500 MHz ,$CDCl_3$) δ 7.66 (d, Ar*H*, *J* = 7.8 Hz, 1 H), 7.53 (d, Ar*H*, *J* = 2.0 Hz, 1 H), 7.30 (dd, Ar*H*, *J* = 2.0, 7.8 Hz, 1 H), 6.00 (s, (Pyr*H)*$_2$, 2 H), 4.86 (d, CH_2OH, *J* = 5.9 Hz, 2 H), 2.56 (s, ArCH_3, 6 H), 1.44 (s, ArCH_3, 6 H); ^{13}C NMR (125.75 MHz $CDCl_3$) δ 156, 142.88, 140.77, 139.23, 135.80, 131.99, 131.22, 128.99, 127.46, 122.54, 121.47, 64.63, 14.75; I.R (thinfilm) ν max (cm^{-1}): 3529.29 (O-H); HRMS (ESI): m/z calculated for $C_{20}H_{21}BBrF_2N_2O$: requires 433.0894 for $[M+H]^+$, found 433.0872.

2-bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)benzaldehyde



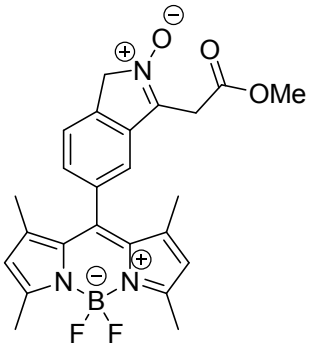
(2-bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenyl)methanol (0.57 g, 1.32 mmol) in CH_2Cl_2 (100 mL) was poured cautiously into a solution of PCC (0.431 g, 2.00 mmol) and $MgSO_4$ (0.550 g) in CH_2Cl_2 (100 mL). The reaction mixture was stirred for 1.5 h before being filtered through Celite® and a silica pad and then concentrated *in vacuo* to afford the title compound as a red solid in 43 % yield (0.245 g, 0.57 mmol). No purification was required. m.p. 217 – 219 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.44 (s, C*H*O, 1 H), 8.06 (d, Ar*H*, *J* = 7.8 Hz, 1 H), 7.68 (s, Ar*H*, 1 H), 7.43 (d, Ar*H*, *J* = 7.8 Hz, 1 H), 6.03 (s, (Pyr*H)*$_2$, 2 H), 2.57 (s, ArCH_3, 6 H), 1.45 (s, ArCH_3, 6 H); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 185.7, 151.5, 137.4, 137.2, 132.5, 128.5, 125.3, 123.0, 122.1, 116.7, 9.5; I.R (thinfilm) ν max (cm^{-1}): 1695.49 (C=O); HRMS (EI): m/z calculated for $C_{20}H_{18}BBrF_2N_2O$: requires 429.0694 for $[M+]^+$, found 429.0697

Methyl(E)-3-(5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)-2-formylphenyl)acrylate



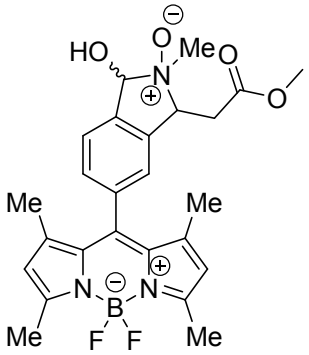
2-Bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'*f*][1,3,2]diazaborinin-10-yl)benzaldehyde (0.24 g, 0.57 mmol) in anhydrous MeCN (5 mL) was bubbled with argon for 30 min in a sealed tube before the addition of methyl acrylate (0.956 mL, 2.85 mmol), P(*O*-Tol)$_3$ (0.052 g, 0.171 mmol), NEt_3 (0.07 mL, 0.86 mmol) and $Pd(OAc)_2$ (0.019 g, 0.086 mmol). The tube was sealed and the reaction mixture was heated at 95 ˚C for 16 h in a sealed vessel. The reaction was cooled to rt and diluted with diethyl ether (50 mL), filtered through Celite® and washed with H_2O (2 x 50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude material that was purified via column chromatography (20 to 40 % EtOAc/Pentane) to afford the title compound as a red gum (0.15 g, 0.34 mmol, 60 %). ^{1}H NMR (500 MHz ,$CDCl_3$) δ 10.39 (s, CHO, 1 H), 8.56 (d, C*H*CHCOOMe, *J* = 16.1 Hz, 1 H), 8.04 (d, Ar*H*, *J* = 7.8 Hz, 1 H), 7.67 - 7.61 (m, Ar*H*, 1 H), 7.55 (dd, Ar*H*, *J* = 1.5, 7.8 Hz, 1 H), 6.39 (d, CHC*H*COOMe, *J* = 15.7 Hz, 1 H), 6.02 (s, (PyrC*H*)$_2$ 2 H), 3.83 (s, COOMe, 3 H), 2.57 (s, ArC*H*$_3$, 6 H), 1.39 (s, $ArCH_3$, 6 H); ^{13}C NMR (125.75 MHz $CDCl_3$) δ: 190.9, 166.22, 156.65, 142.48, 139.98, 137.45, 133.92, 132.85, 129.82, 127.93, 123.76, 121.79, 52.04, 14.75; I.R (thinfilm) ν max (cm^{-1}): 1687.6 (C=O); HRMS (ESI): m/z calculated for $C_{24}H_{23}BF_2N_2O_3$: requires 437.1848 for $[M+H]^+$, found 437.1885.

5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)-3-(2-methoxy-2-oxoethyl)-1*H*-isoindole 2-oxide



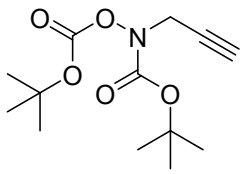
A solution of methyl(E)-3-(5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)-2-formylphenyl)acrylate (0.07 g, 0.16 mmol) in THF (2 mL) was cooled to -20 ^{o}C followed by the addition of NH_2OH- 50 % in H_2O (0.012 ml, 0.24 mmol). The reaction was stirred at -20 ^{o}C for 30 min then it was allowed to warm to rt for 30 min. The reaction mixture was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The aqueous layer was extracted twice with CH_2Cl_2 (2 x 50 ml) and the combined organics were dried ($MgSO_4$) and concentrated *in vacuo* to afford the title compound as a shiny red/green solid (quantitative yield). M.p 143-144 ^{o}C ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (d, Ar*H*, *J* = 6.8 Hz, 1 H), 7.31 (dd, Ar*H*, *J* = 1.5, 7.3 Hz, 1 H), 7.26 (s, Ar*H*, 1 H), 6.00 (s, $(PyrH)_2$, 2 H), 5.11 (s, CH_2COOMe, 2 H), 3.91 (s, CH_2NO, 2 H), 3.70 (s, COOMe, 3 H), 2.55 (s, ArCH_3, 6 H), 1.41 (s, ArCH_3, 6 H); ^{13}C NMR (125.75 MHz $CDCl_3$) δ 167.49, 156.04, 142.81, 140.85, 140.03, 137.11, 135.66, 133.45, 131.26, 127.56, 122.25, 121.52, 119.15, 66.09, 52.63, 29.34, 14.66, 14.60; I.R (thinfilm) ν max (cm^{-1}): 1738.03 (C=O); HRMS (ESI): m/z calculated for $C_{24}H_{24}BF_2N_3O_3$: requires 452.20 for $[M+H]^+$, found 452.1969.

5,5-difluoro-10-(1-hydroxy-3-(2-methoxy-2-oxoethyl)-2-methyl-2-oxidoisoindolin-5-yl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide



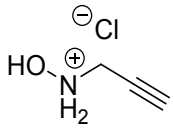
To a solution of Methyl(E)-3-(5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-2-formylphenyl)acrylate (20 mg, 0.046 mmol) in 5:1 THF:H2O (3 mL) was added *N*-methylhydroxylamine hydrochloride (3.8 mg, 0.046 mmol) and Et_3N (012 μL, 0.092 mmol). The reaction was left to stir at room temperature for 2 h. The crude mixture was then taken up in DCM and water and separated. The aqueous layer was washed with DCM (3 x 20 mL), the combined organics were dried (MgSO4), and the solvent was removed under vacuum to yield the title compound as a shiny red oil in 92% yield (20.5 mg, 0.042 mmol) as a mixture of diastereomers. **Major Diastereomer:** ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.47 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar*H*), 7.22 (ddd, *J* = 11.0, 7.8, 1.7 Hz, 1H, Ar*H*), 7.10 (dd, *J* = 20.7, 1.5 Hz, 1H, Ar*H*), 5.98 (d, *J* = 6.0 Hz, 2H, Pyr*H*), 5.82 (s, 1H, C*H*OH), 4.37 (t, *J* = 5.9 Hz, 1H, C*H*CH$_2$), 3.63 (s, 3H, C(O)OCH_3), 3.11 (dd, *J* = 15.7, 5.7 Hz, 1H, CHCH_aH$_b$), 2.80 (s, 3H, NCH_3), 2.55 (s, 6H, ArCH_3), 2.51 (dd, *J* = 15.8, 6.2 Hz, 1H, CHCH$_a$$H_b$), 1.43 – 1.35 (m, 6H, ArCH_3). ^{13}C NMR (126 MHz, Chloroform-d) δ 172.47, 156.11, 142.80, 138.90, 135.37, 135.25, 131.43, 128.44, 127.39, 125.87, 124.79, 121.54, 93.69, 60.11, 52.23, 42.06, 33.50, 30.48, 14.85, 14.73, 14.54. **Minor Diastereomer:** ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.47 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar*H*), 7.22 (ddd, *J* = 11.0, 7.8, 1.7 Hz, 1H, Ar*H*), 7.10 (dd, *J* = 20.7, 1.5 Hz, 1H, Ar*H*), 5.98 (d, *J* = 6.0 Hz, 2H, Pyr*H*), 5.90 (s, 1H, C*H*OH), 4.22 (t, *J* = 4.6 Hz, 1H, C*H*CH$_2$), 3.62 (s, 3H, C(O)OCH_3), 2.95 (dd, *J* = 16.9, 4.4 Hz, 1H, CHCH_aH$_b$), 2.84 (s, 3H, NCH_3), 2.55 (s, 6H, ArCH_3), 2.51 (dd, *J* = 15.8, 6.2 Hz, 1H, CHCH$_a$$H_b$), 1.42 (d, *J* = 12.2 Hz, 6H, ArCH_3). ^{13}C NMR (126 MHz, Chloroform-d) δ 172.47, 155.79, 143.21, 140.70, 136.69, 129.14, 127.57, 126.91, 125.66, 124.79, 121.39, 93.97, 62.03, 52.11, 43.31, 34.38, 29.85, 14.92, 14.76, 14.75. I.R (thinfilm) ν max (cm^{-1}): 3456 (OH), 1732 (C=O); HRMS (ESI): m/z calculated for $C_{25}H_{28}BF_2N_3O_4$: requires 484.2218 for $[M+H]^+$, found 484.2252, $C_{25}H_{28}BF_2N_3O_4$: requires 482.2073 for $[M-H]^-$, found 482.2076.

tert-Butyl ((tert-butoxycarbonyl)oxy)(prop-2-yn-1-yl)carbamate



Propargyl bromide (80 % solution in PhMe, 0.23 mL, 2.07 mmol) was added to a mixture of N,O-dibochydroxlamine (0.435 g, 1.86 mmol) and K_2CO_3 (0.343 g, 2.48 mmol) in DMF (15 mL). The reaction mixture was stirred for 16 h before the addition of H_2O (100 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organics were washed with H_2O (2 x 100 mL), brine (100 mL) and dried ($MgSO_4$). The solvent was removed *in-vacuo* to afford the title compound as a colourless oil (0.304 g, 1.12 mmol, 60 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.33 (br. s., NC*H*$_2$CCH, 2 H), 2.28 (t, *J* = 2.4 Hz, NCH$_2$CC*H*, 1 H), 1.54 (s, BOC, 9 H), 1.50 (s, BOC, 9 H). The ^{1}H NMR data matches the data reported in the literature.[1]

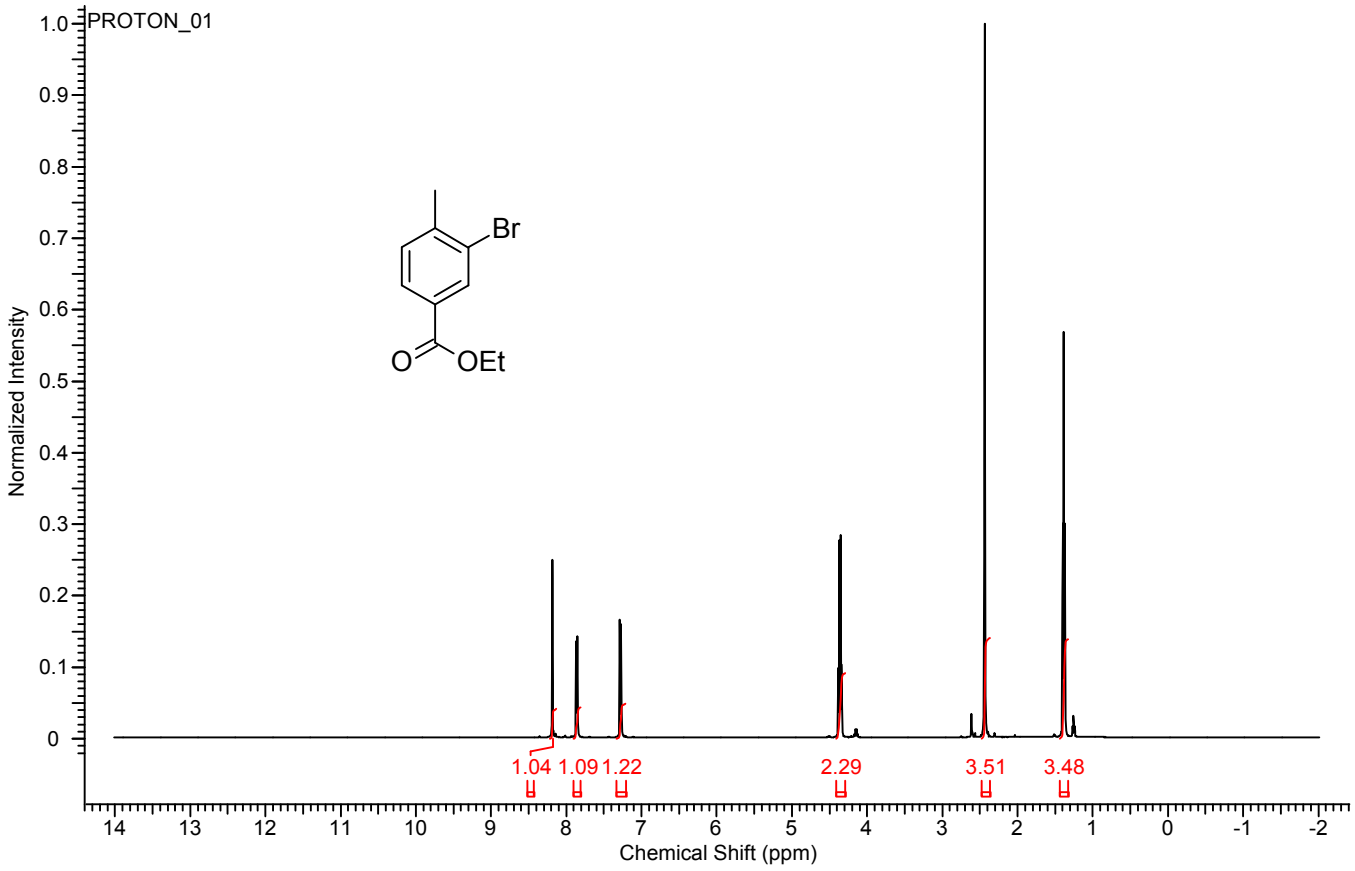
N-(Prop-2-yn-1-yl)hydroxylammonium chloride



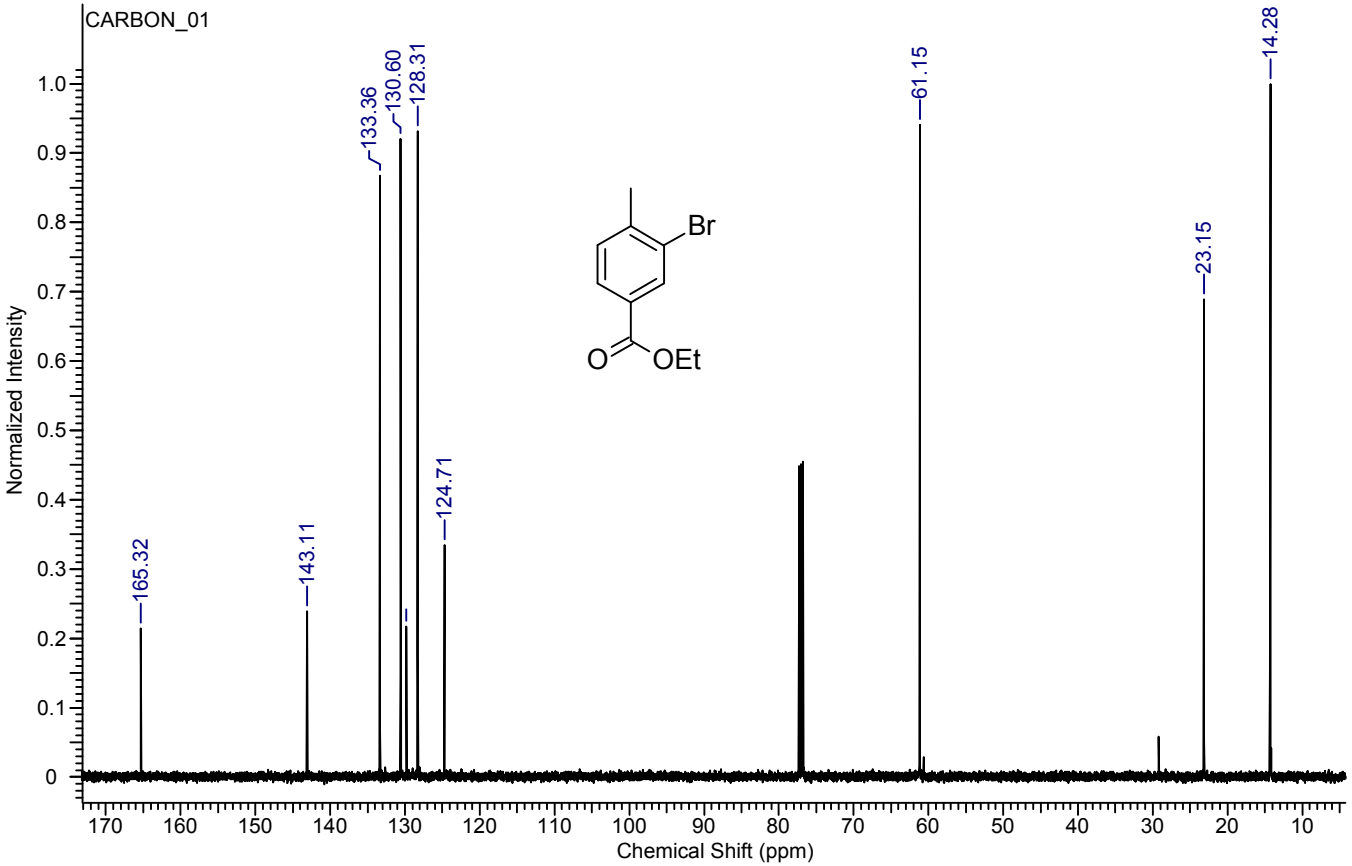
tert-Butyl ((tert-butoxycarbonyl)oxy)(prop-2-yn-1-yl)carbamate (0.304 g, 1.12 mmol) was dissolved in EtOAc (10 mL) and H_2O (5 mL). $^{Conc.}$HCl (5 mL) was added dropwise and the reaction mixture was stirred for 2 h. The solvent was then removed *in-vacuo* to afford the title compound as a brown solid (quant. Yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.90 (br. s., *H*$_2$N(OH)CH$_2$, 2 H), 11.11 (br. s., H$_2$N(O*H*)CH$_2$, 1 H), 4.04 (d, *J* = 2.6 Hz, NC*H*$_2$CCH, 2 H), 3.67 (t, *J* = 2.5 Hz, NCH$_2$CC*H,* 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 79.7, 74.2; I.R (thinfilm) ν max (cm^{-1}): 3094.6 (Br, O-H); HRMS (FTMS): m/z calculated for C_3H_6ON: requires 72.0444 for [M-Cl]$^+$, found 72.0443.

3. NMR

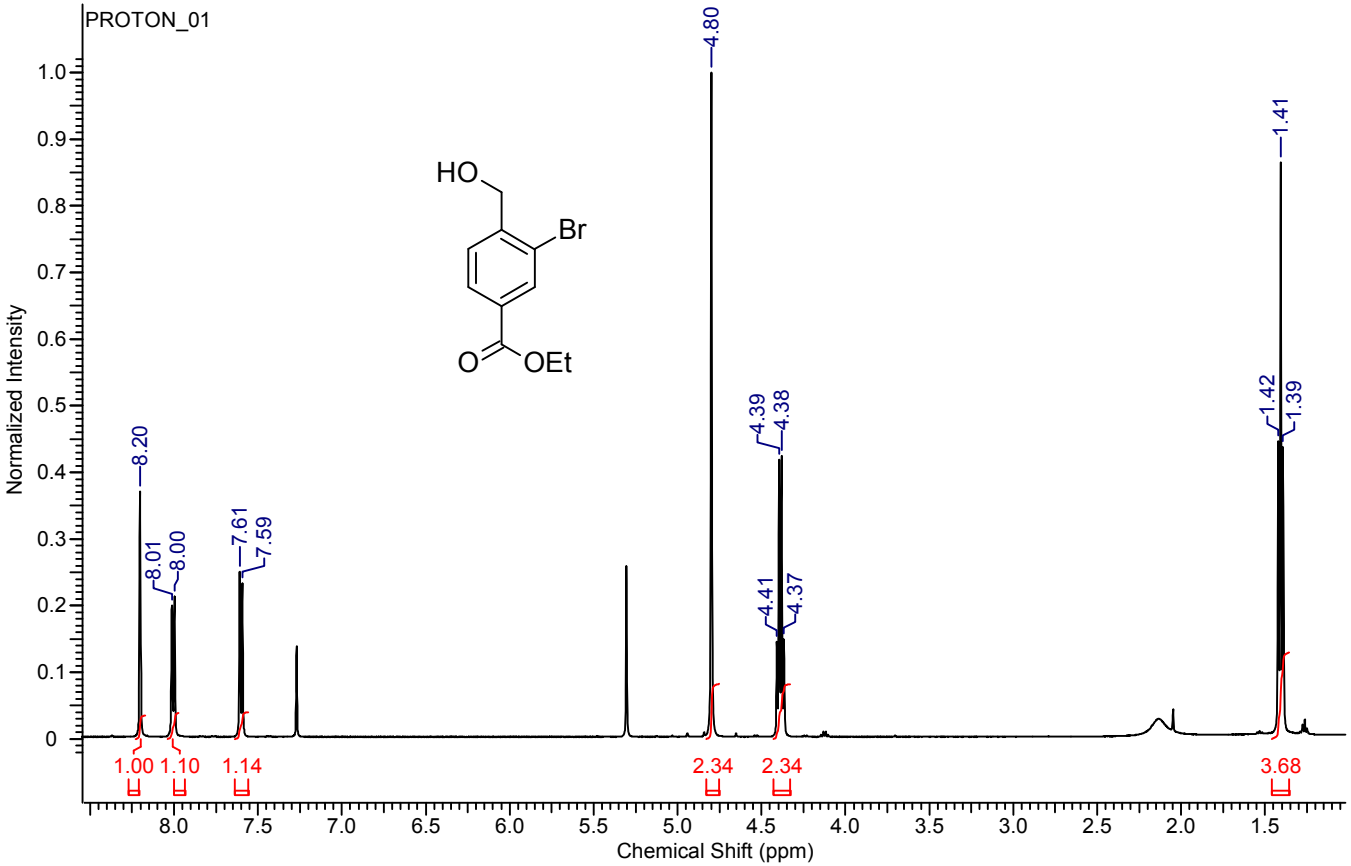
Ethyl 3-bromo-4-methylbenzoate (500 MHz, $CDCl_3$)



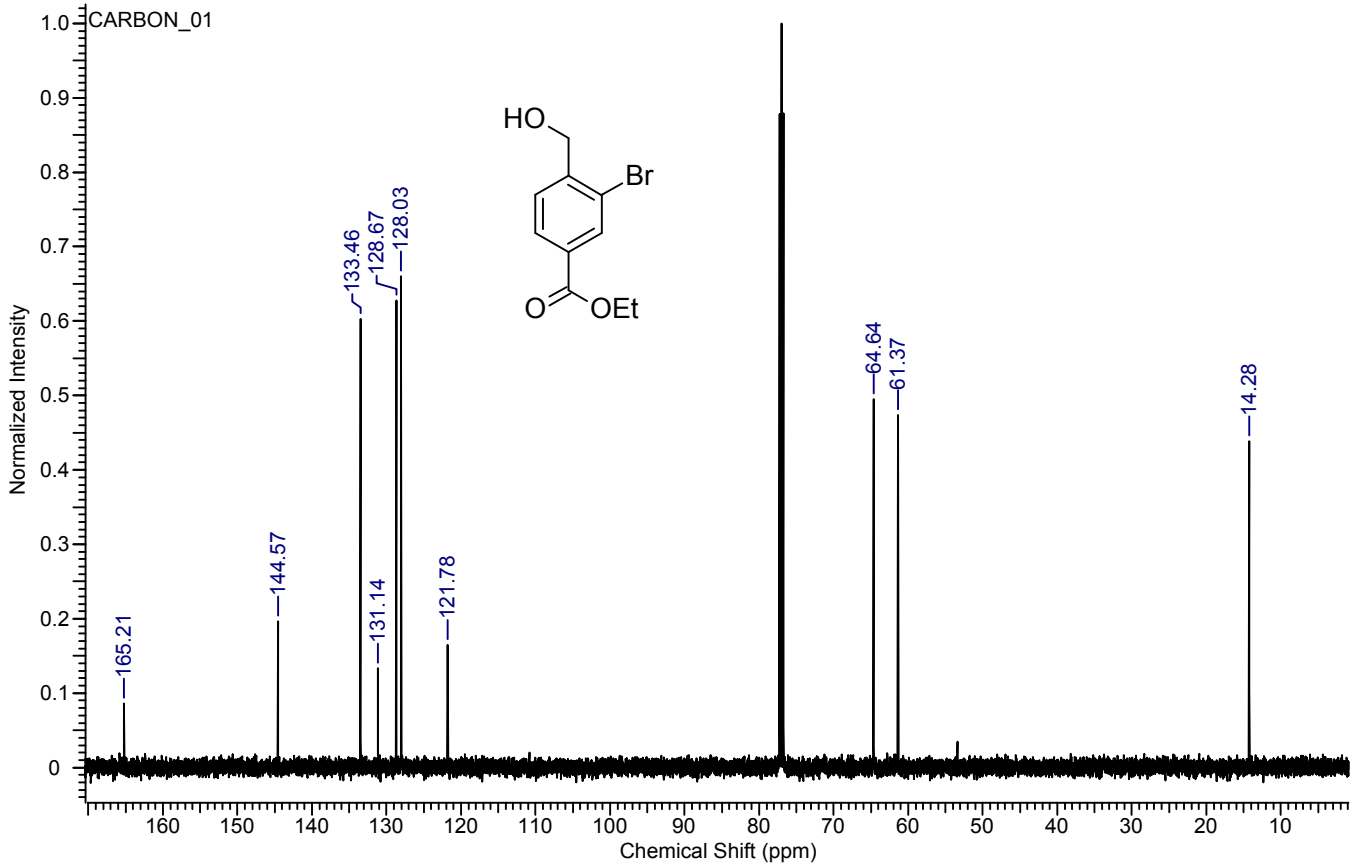
Ethyl 3-bromo-4-methylbenzoate (125.5 MHz, $CDCl_3$)



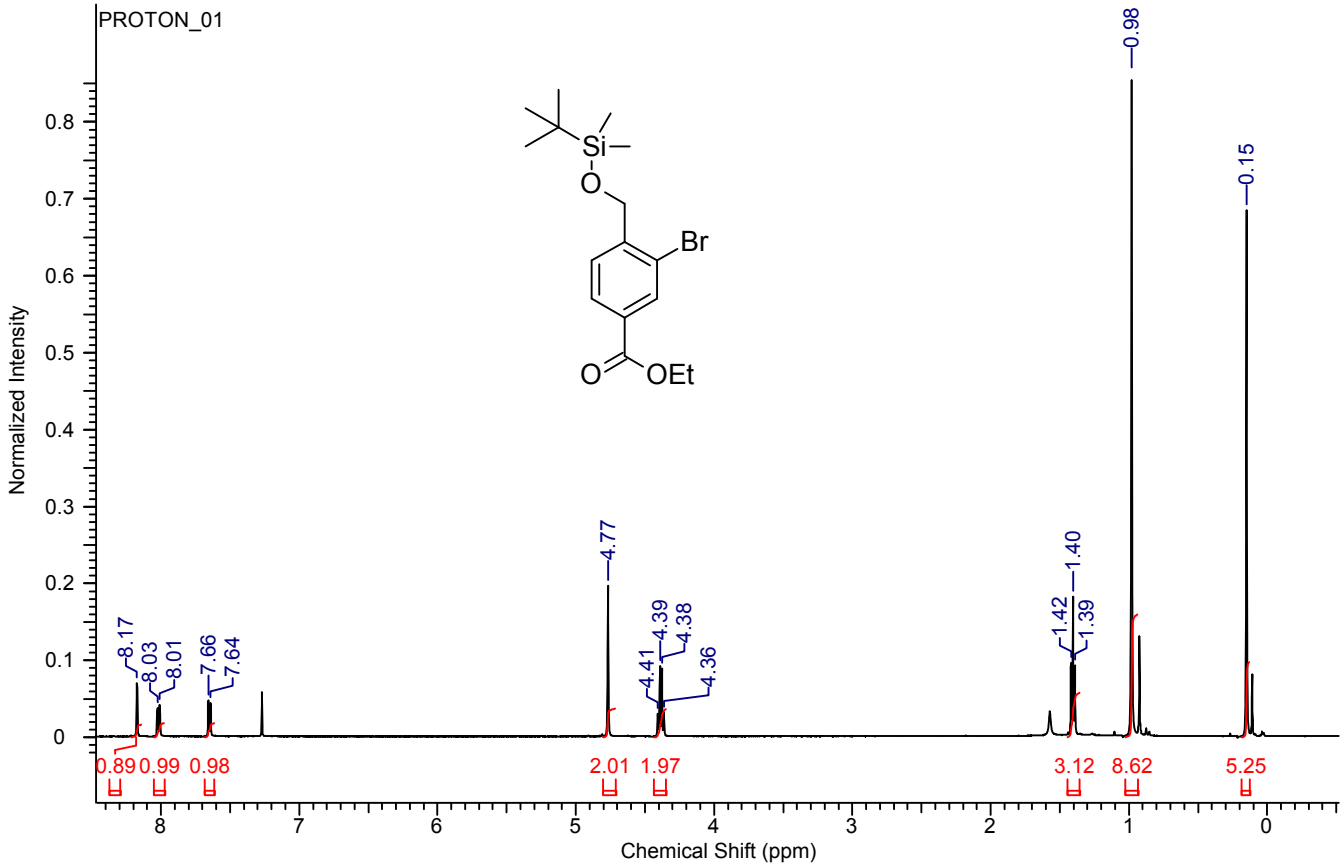
Ethyl 3-bromo-4-(hydroxymethyl)benzoate (500 MHz, $CDCl_3$)



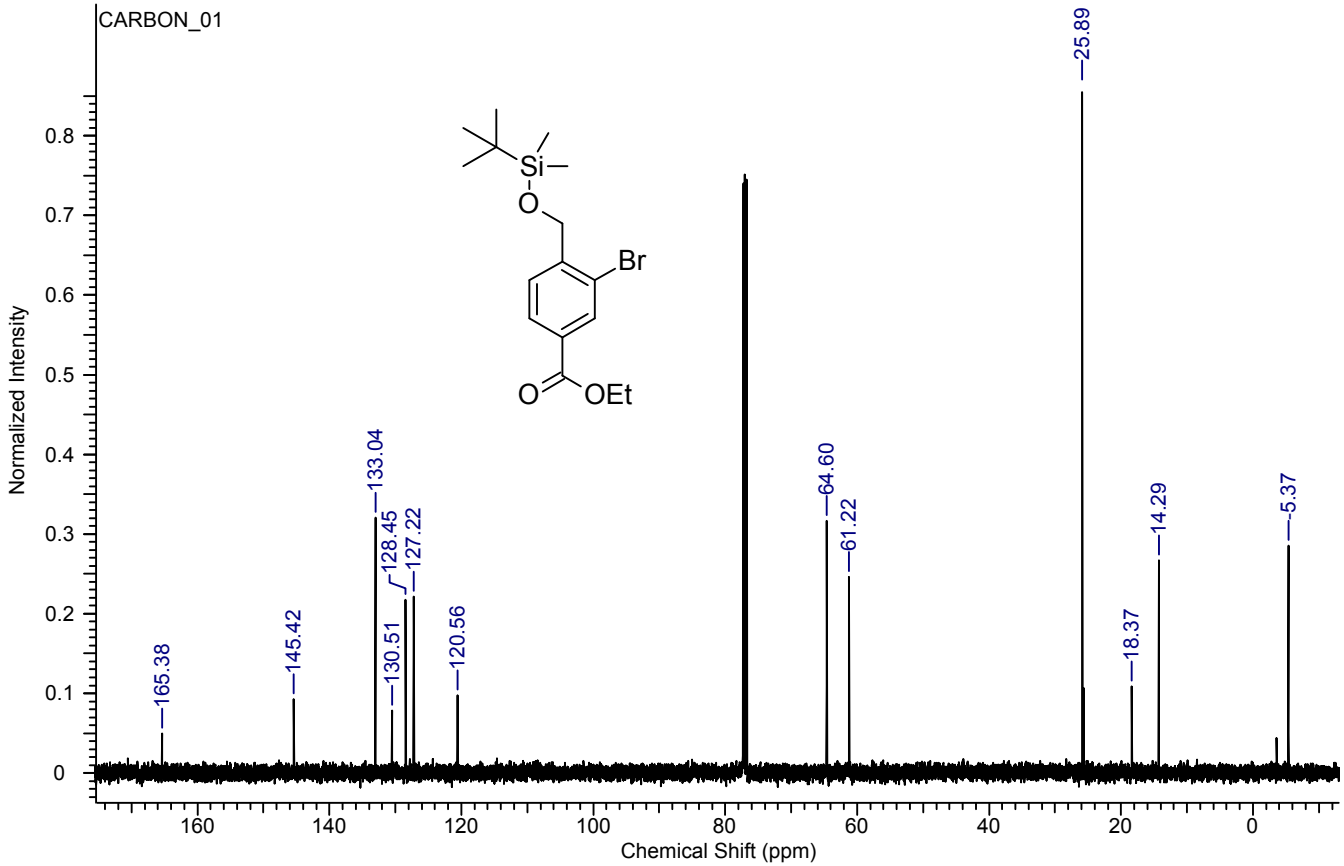
Ethyl 3-bromo-4-(hydroxymethyl)benzoate (125.5 MHz, $CDCl_3$)



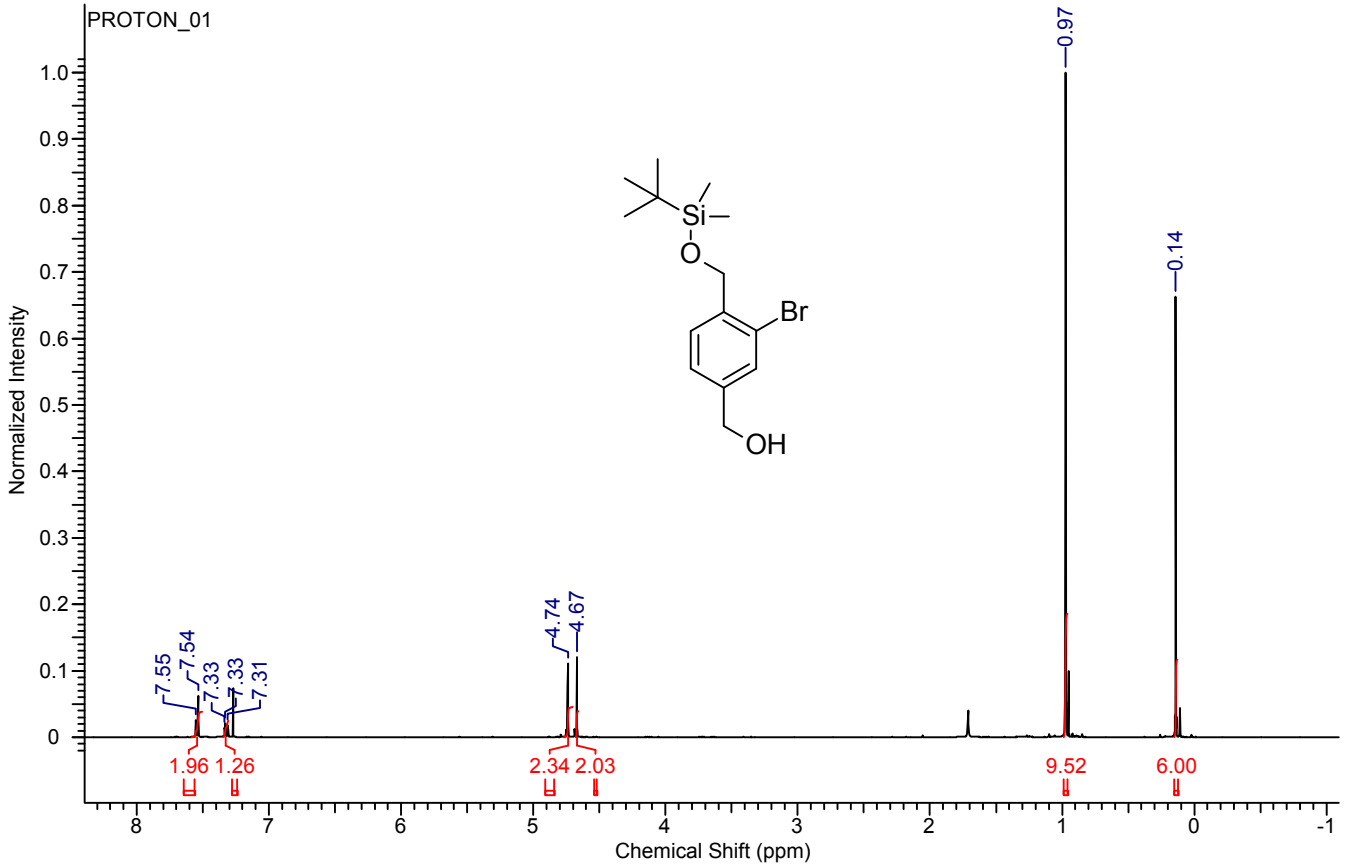
Ethyl 3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)benzoate (500 MHz, $CDCl_3$)



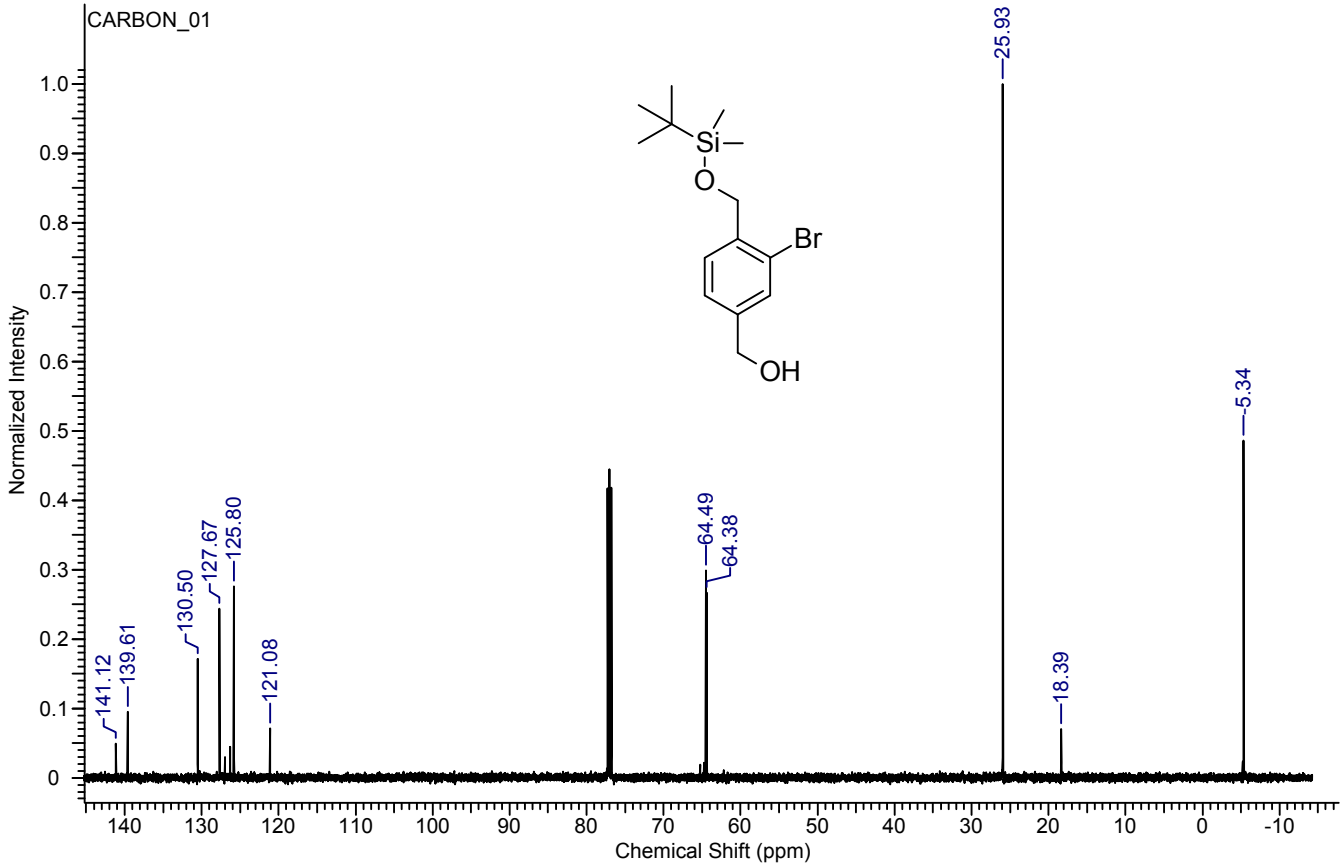
Ethyl 3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)benzoate (125.5 MHz, $CDCl_3$)



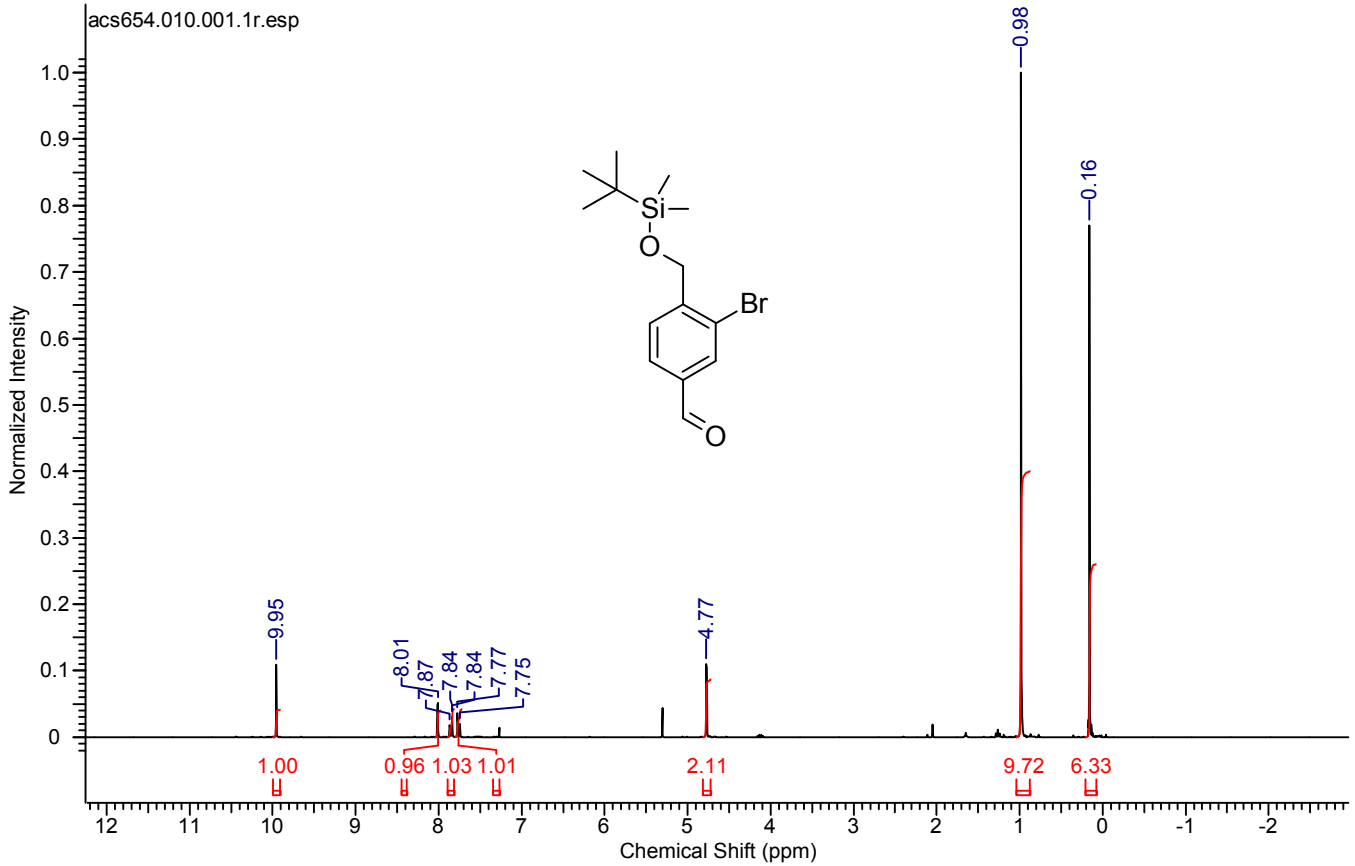
(3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)methanol (500 MHz, $CDCl_3$)



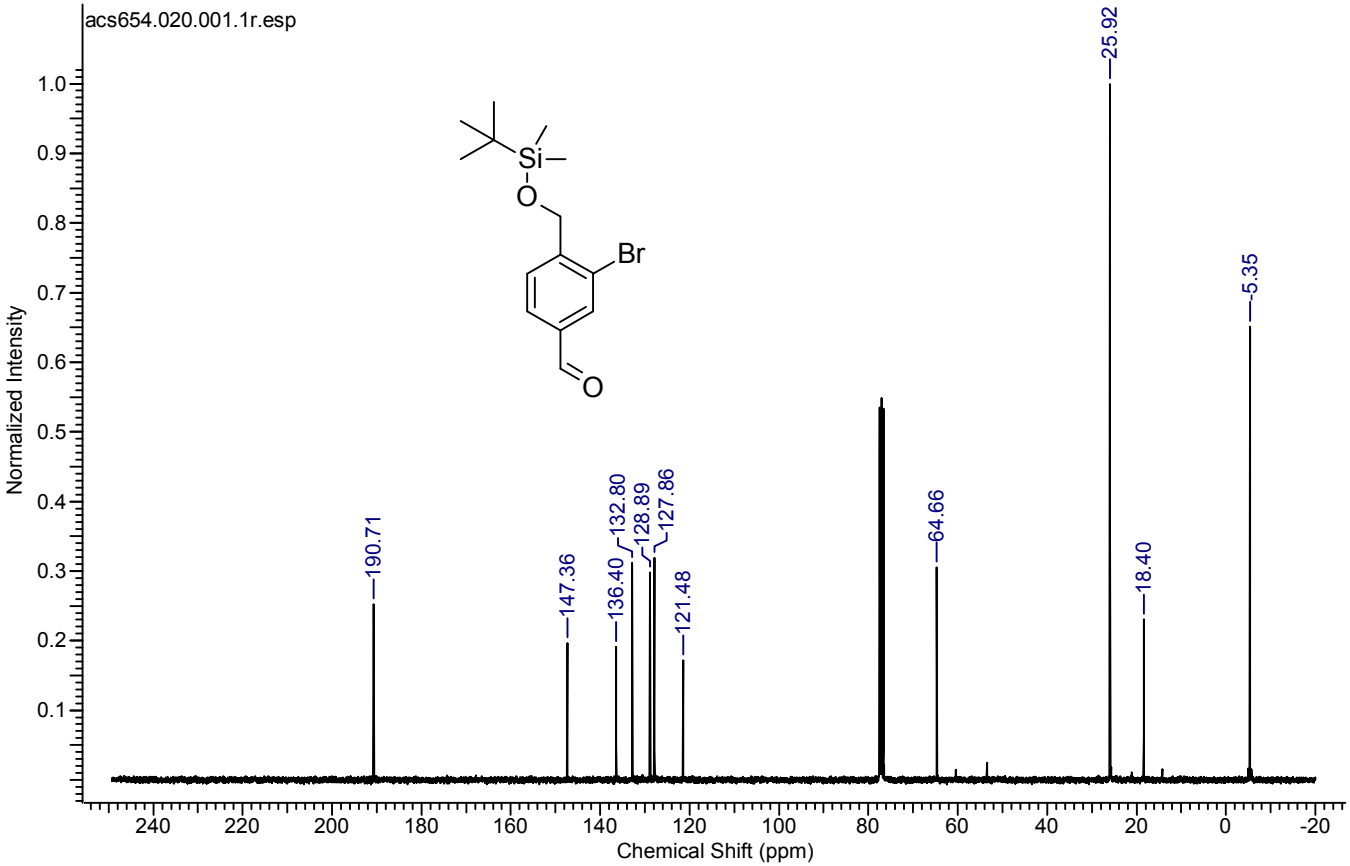
(3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)methanol (125.5 MHz, $CDCl_3$)



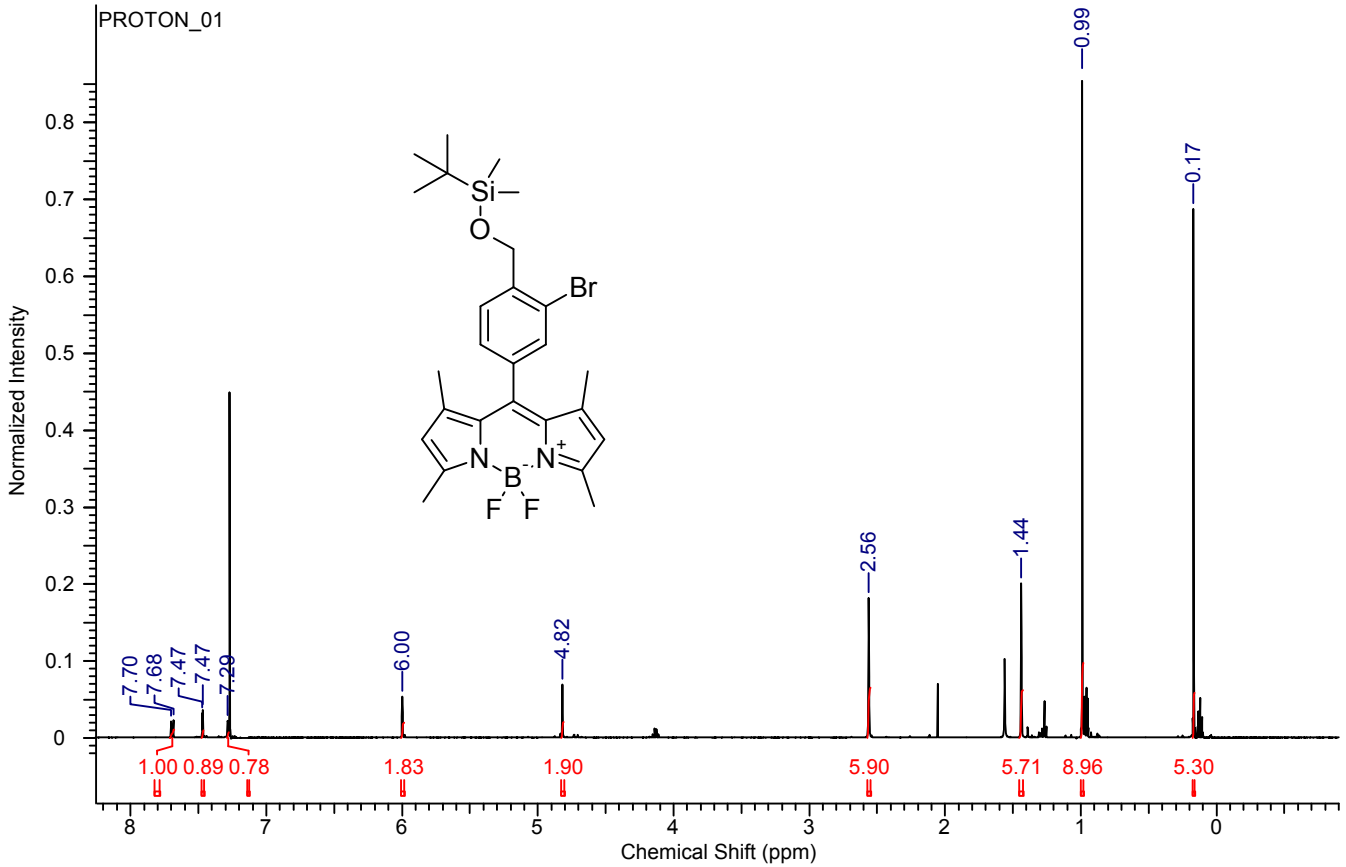
3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)benzaldehyde (300 MHz, $CDCl_3$)



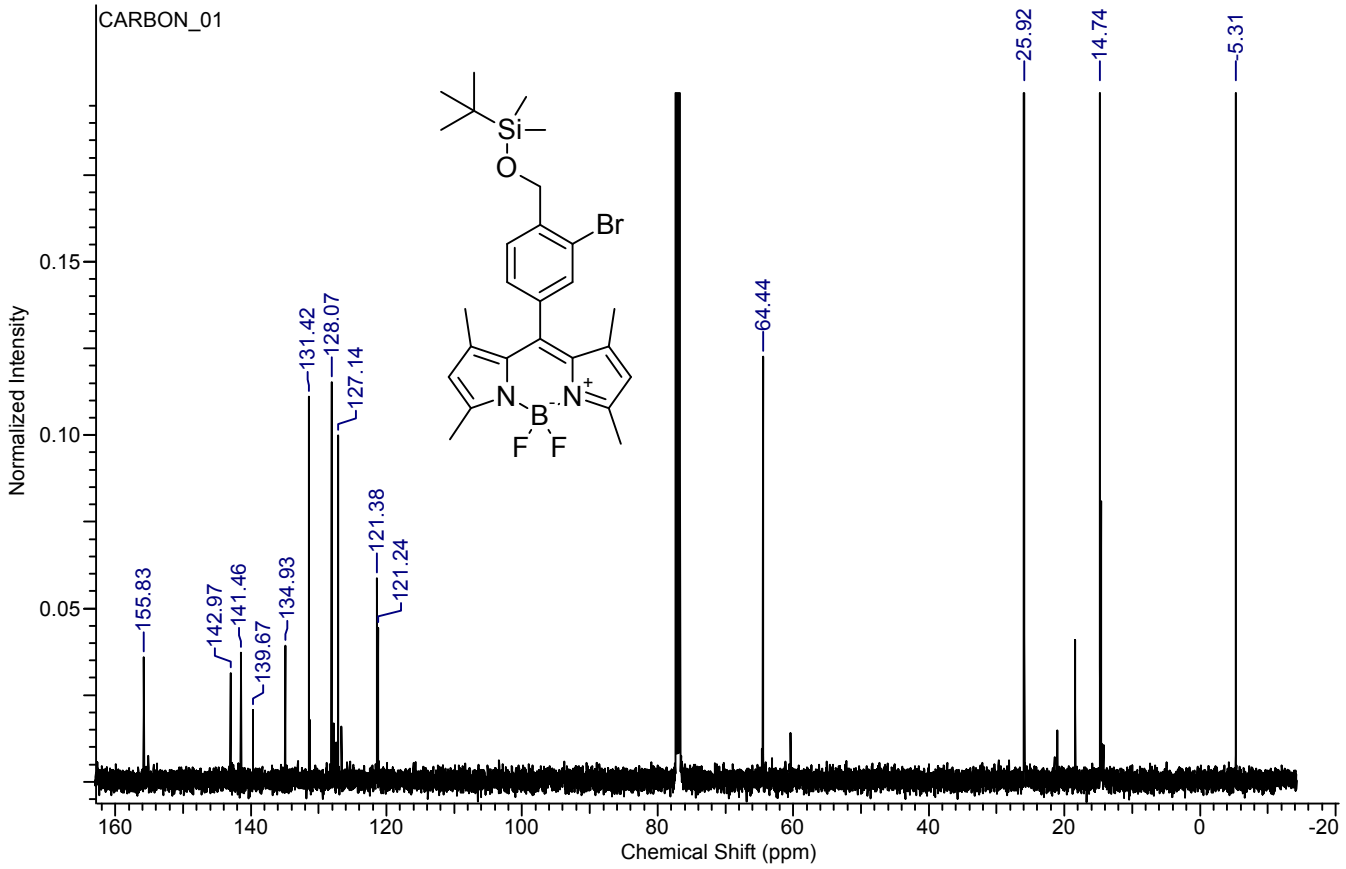
3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)benzaldehyde (75.5 MHz, $CDCl_3$)



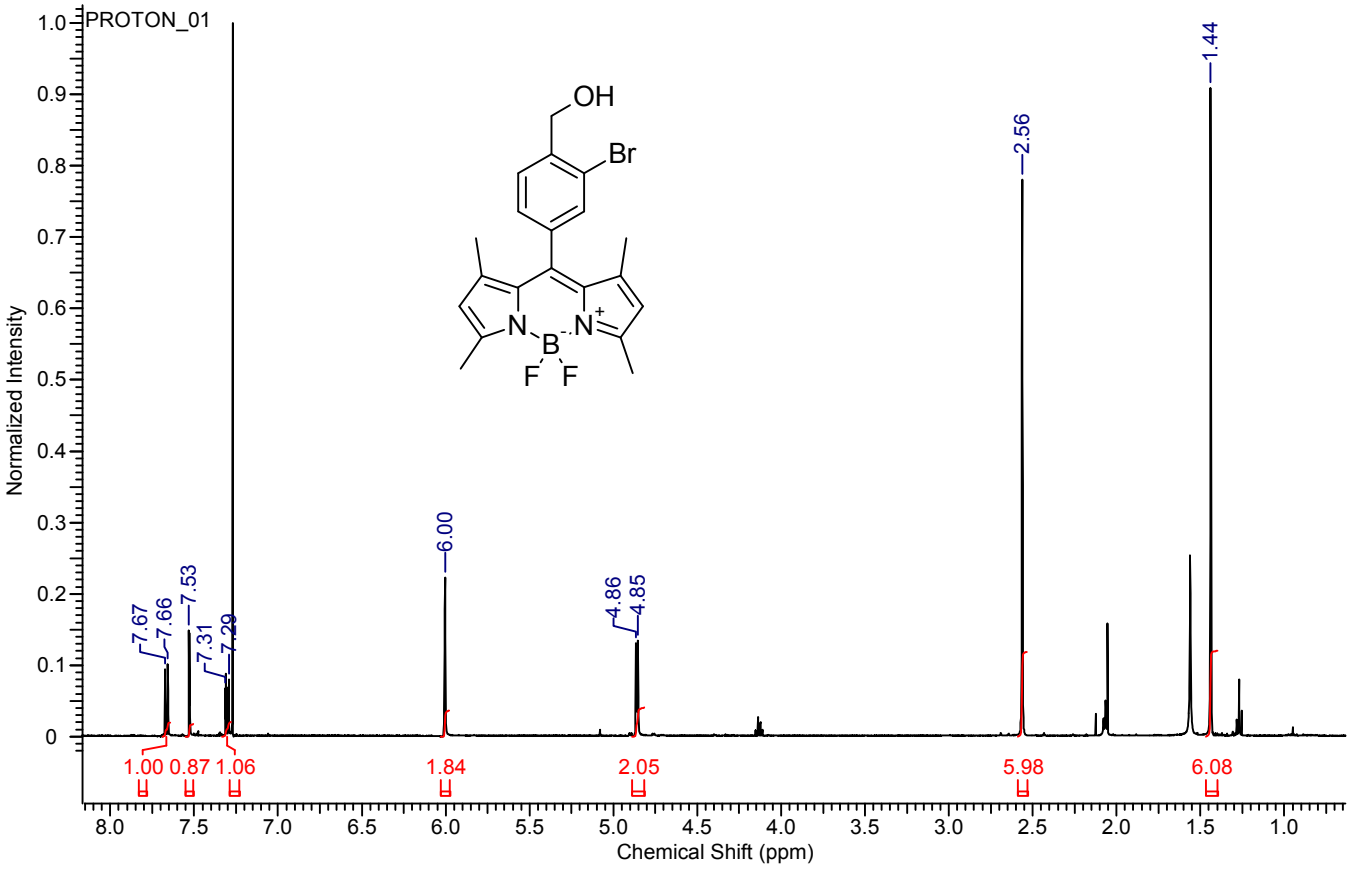
10-(3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinine (500 MHz, $CDCl_3$)



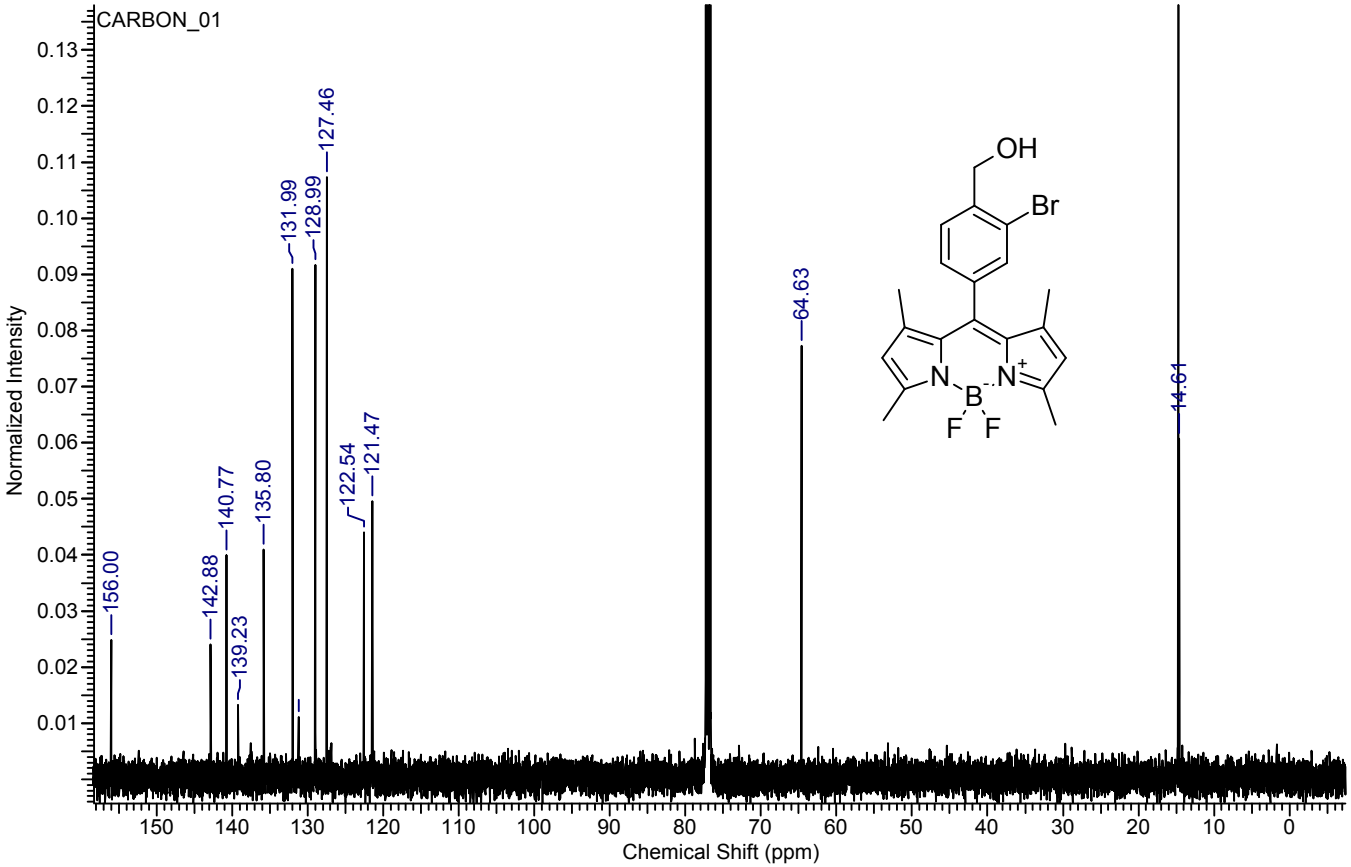
10-(3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinine (125.5 MHz, $CDCl_3$)



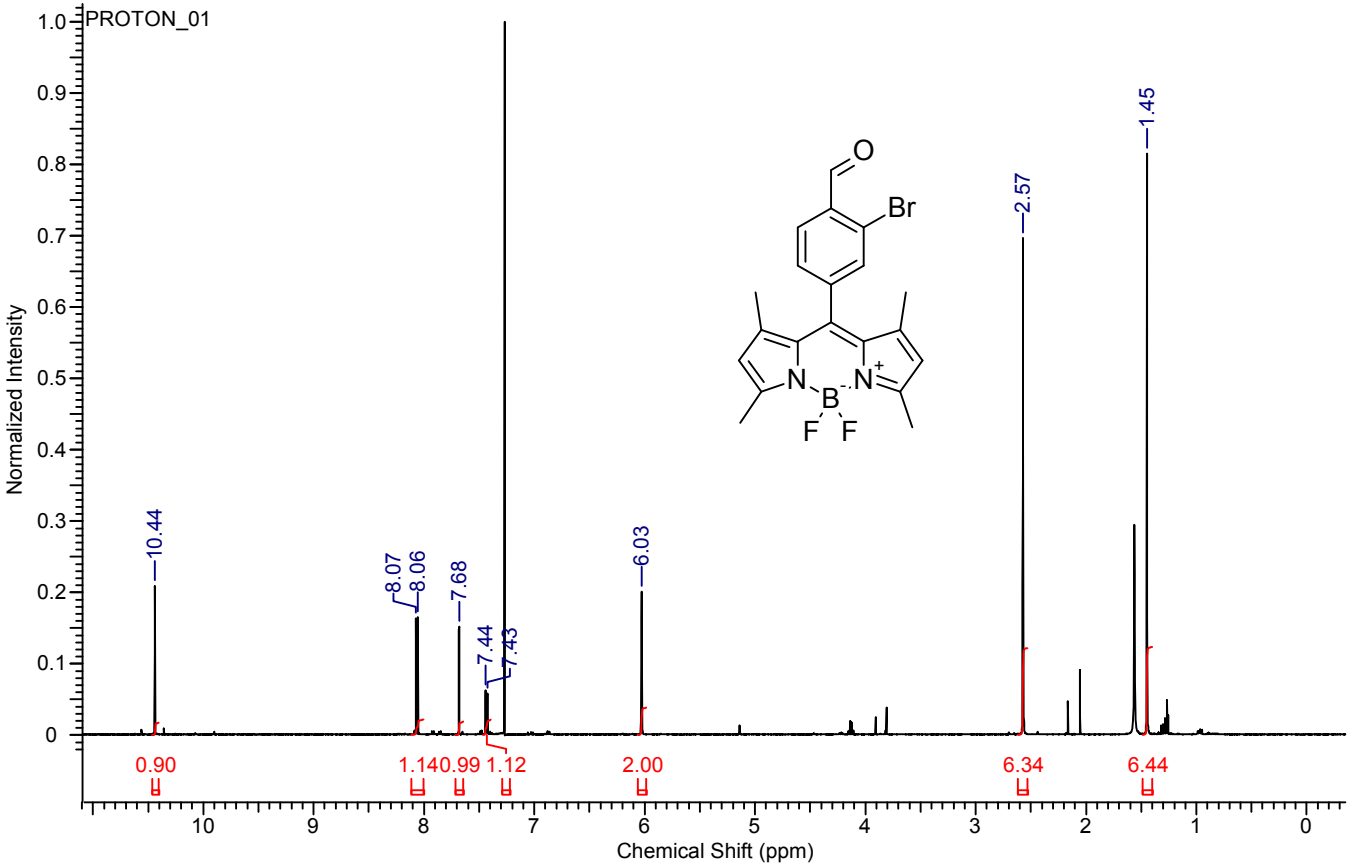
(2-bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)phenyl)methanol (500 MHz, $CDCl_3$)



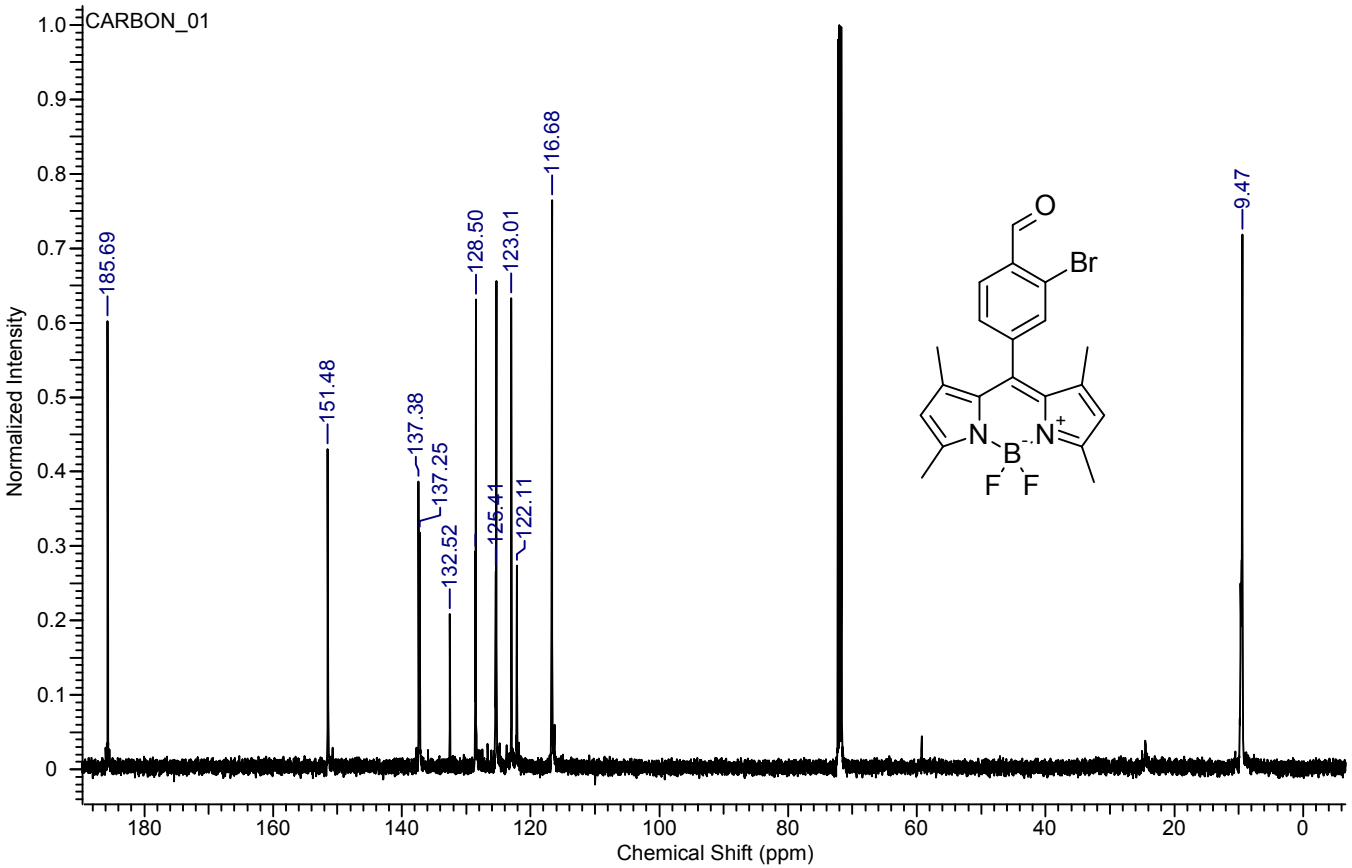
(2-bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)phenyl)methanol (125.5 MHz, $CDCl_3$)



2-bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)benzaldehyde (300 MHz, $CDCl_3$)

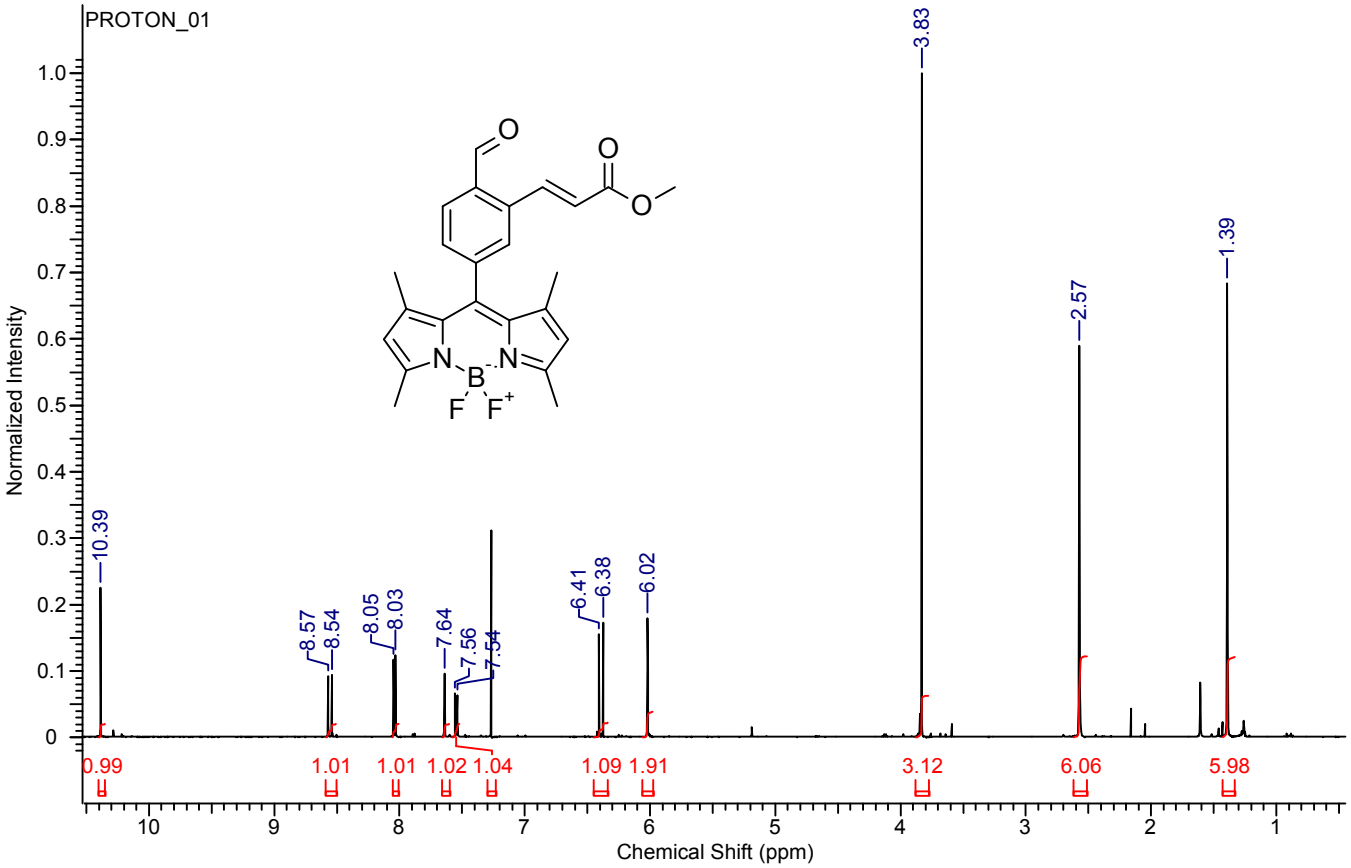


2-bromo-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)benzaldehyde (75.5 MHz, $CDCl_3$)



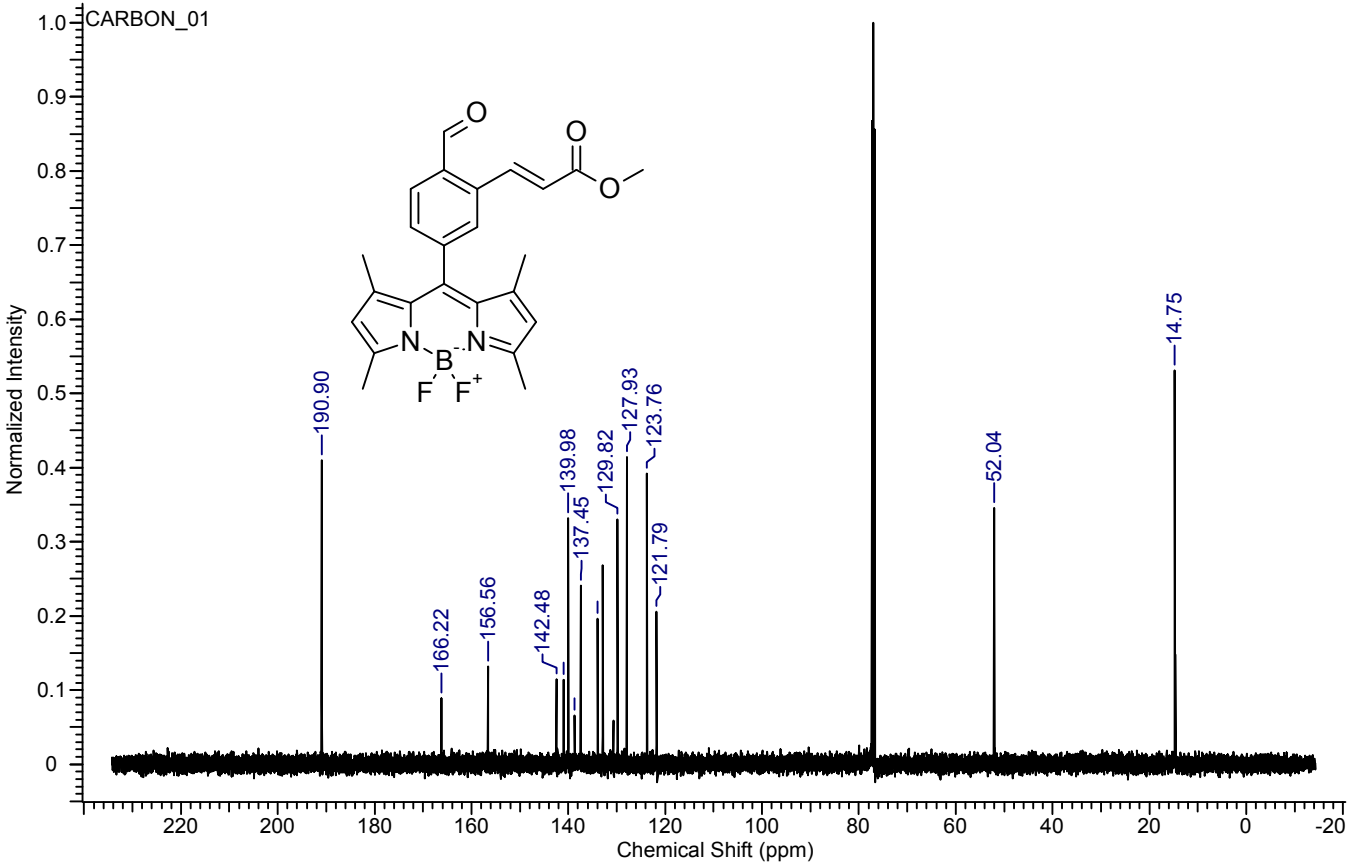
Methyl(E)-3-(5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)-2-formylphenyl)acrylate

(500 MHz, $CDCl_3$)

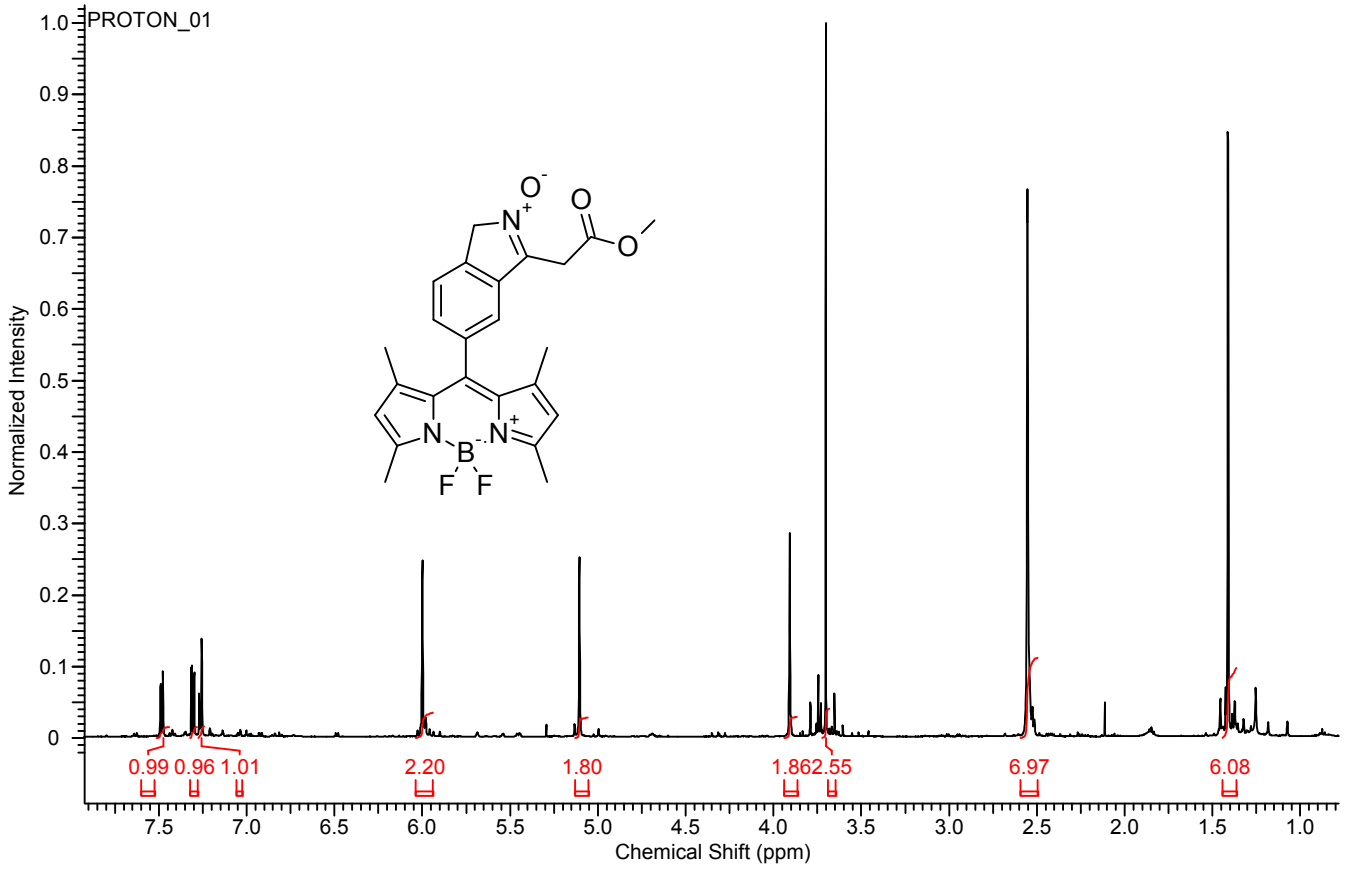


Methyl(E)-3-(5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)-2-formylphenyl)acrylate

(125.5 MHz, $CDCl_3$)

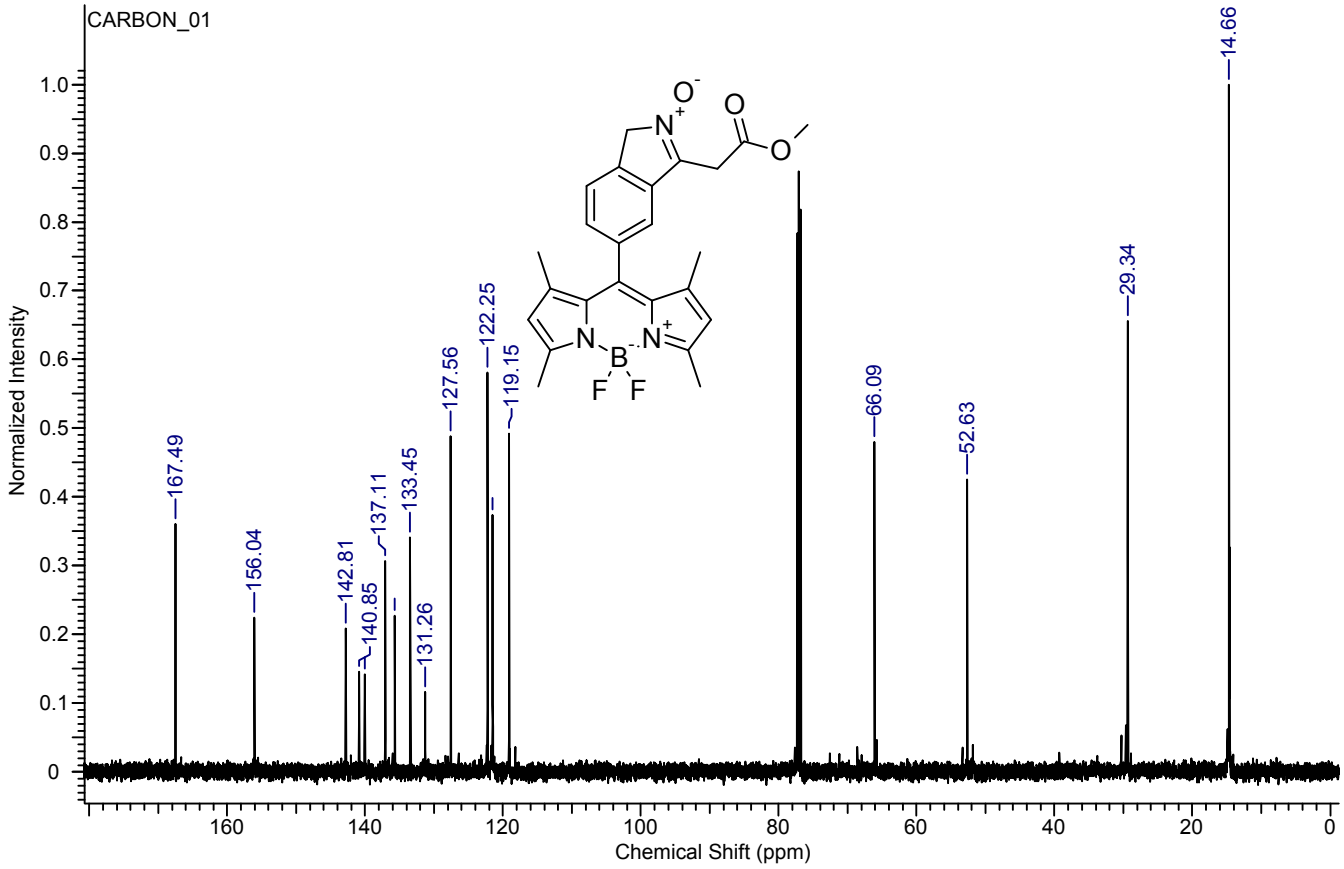


5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)-3-(2-methoxy-2-oxoethyl)-1*H*-isoindole 2-oxide (500 MHz, $CDCl_3$)

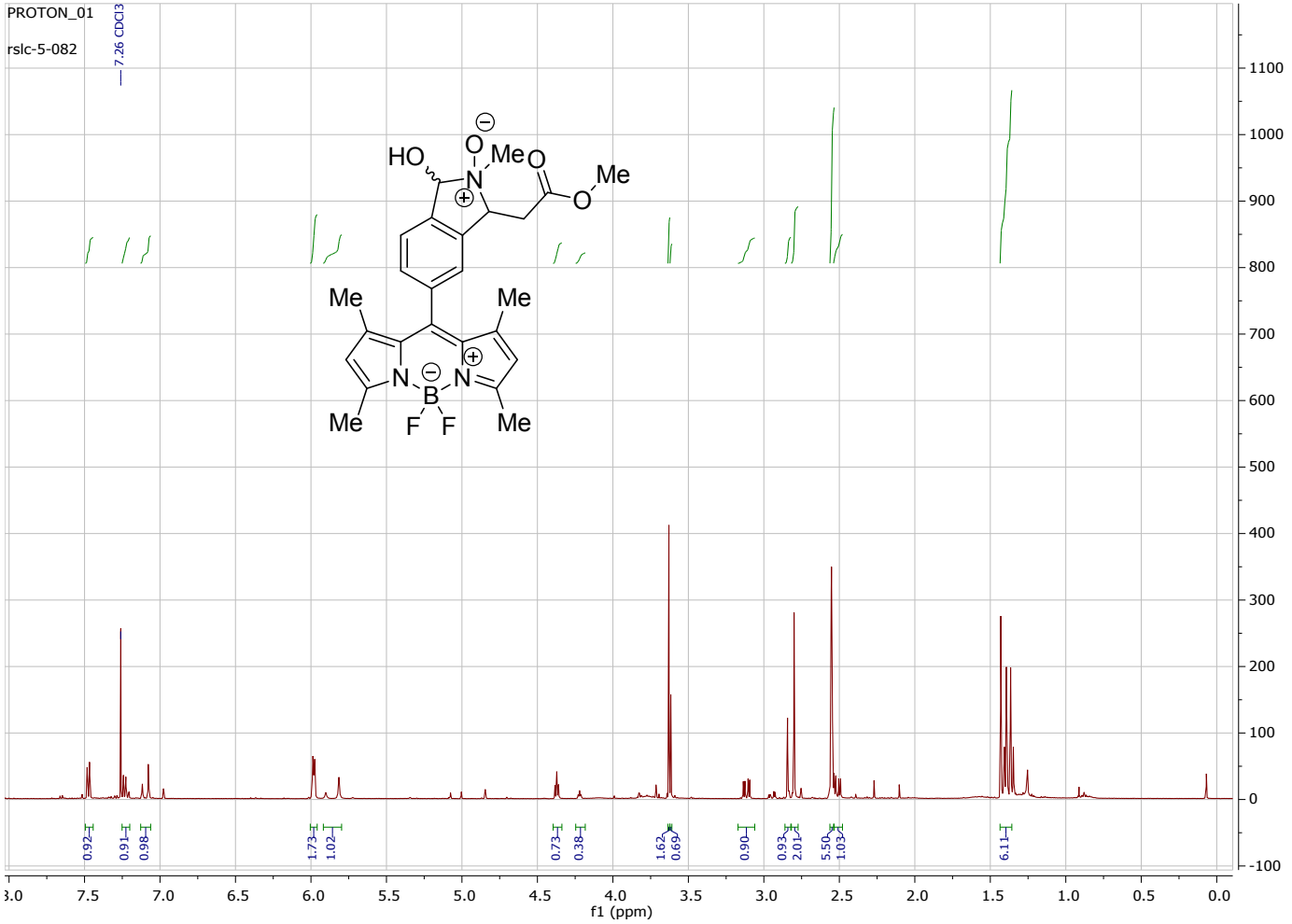


5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-10-yl)-3-(2-methoxy-2-oxoethyl)-1*H*-isoindole 2-oxide

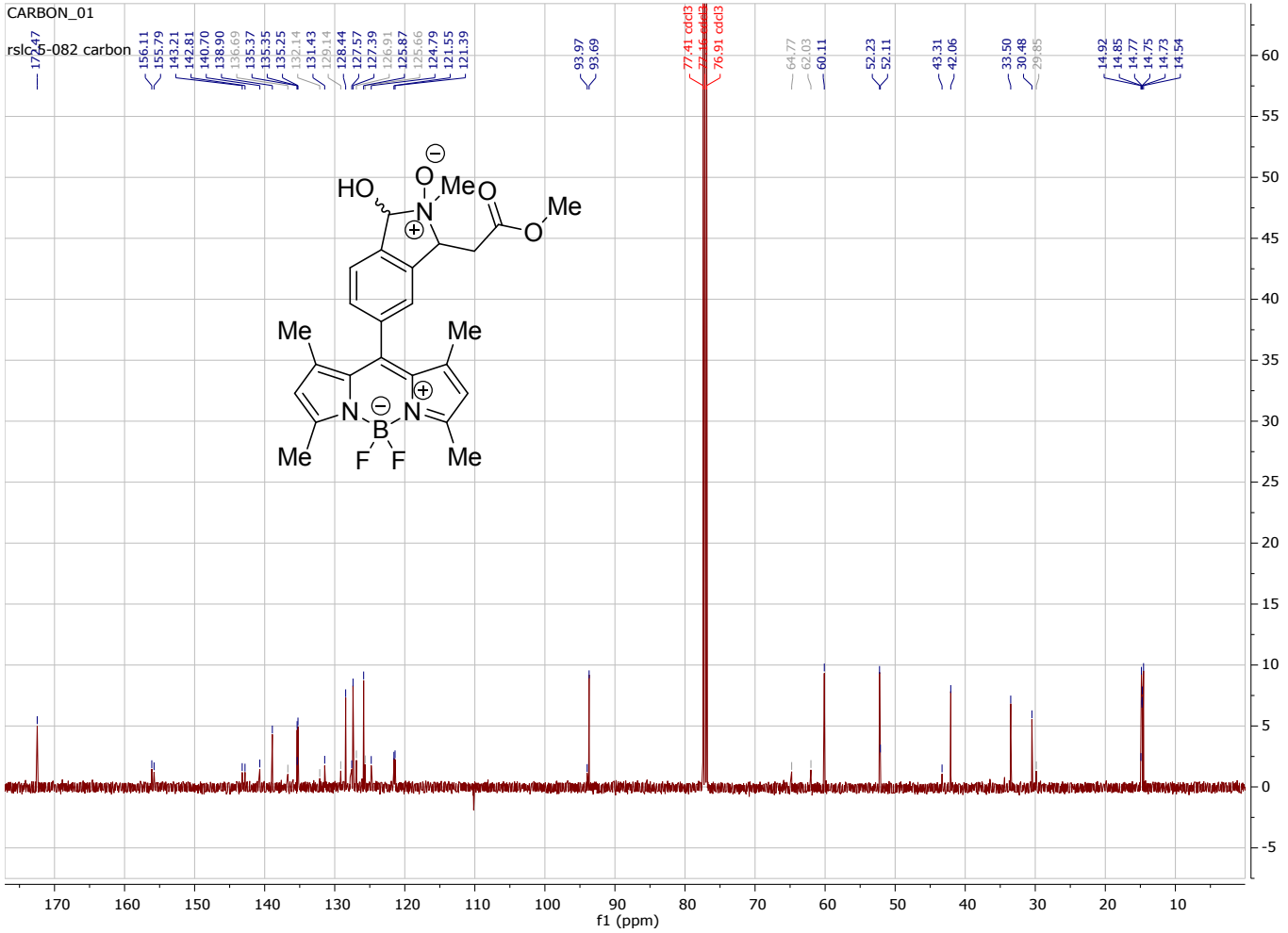
(125.5 MHz, $CDCl_3$)



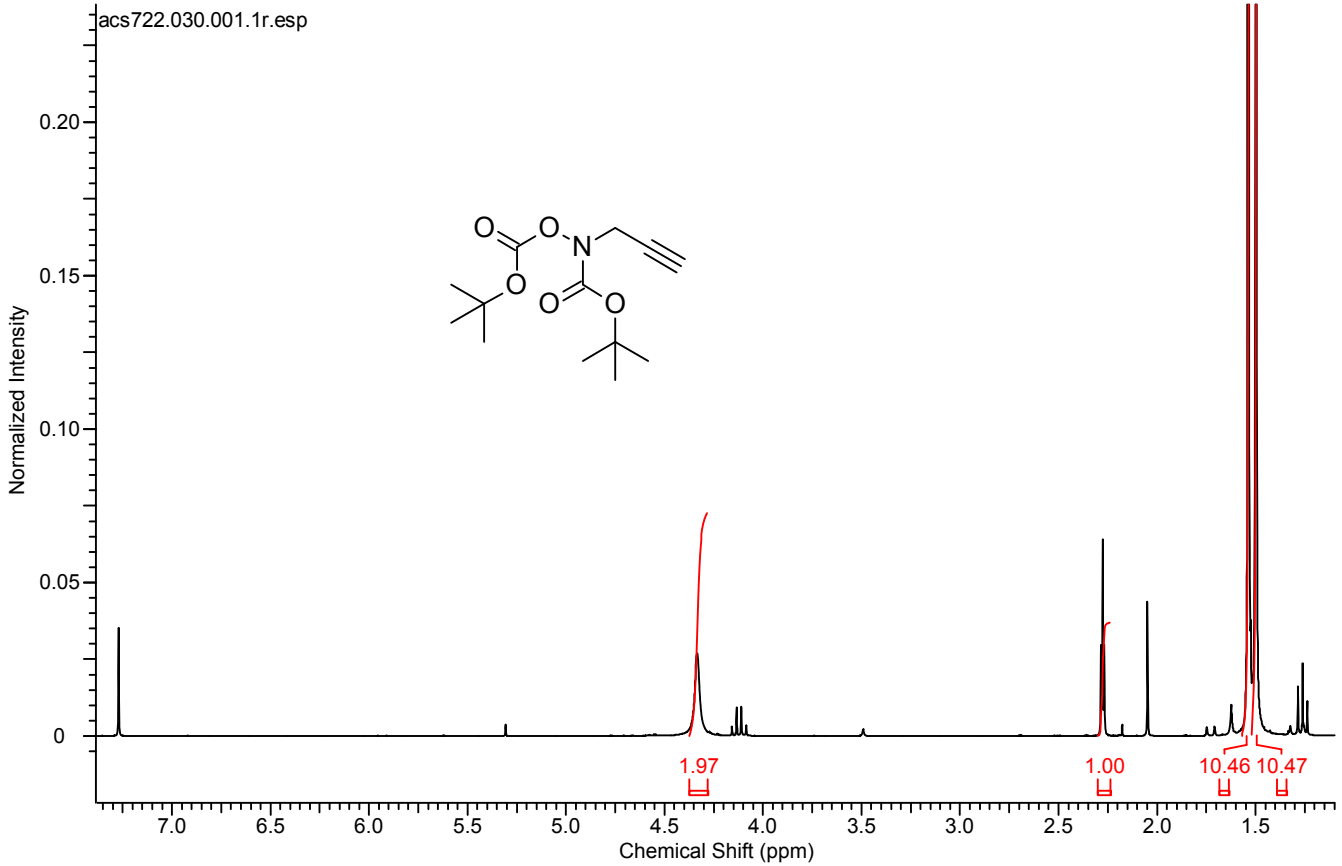
5,5-difluoro-10-(1-hydroxy-3-(2-methoxy-2-oxoethyl)-2-methyl-2-oxidoisoindolin-5-yl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (500 MHz, $CDCl_3$)



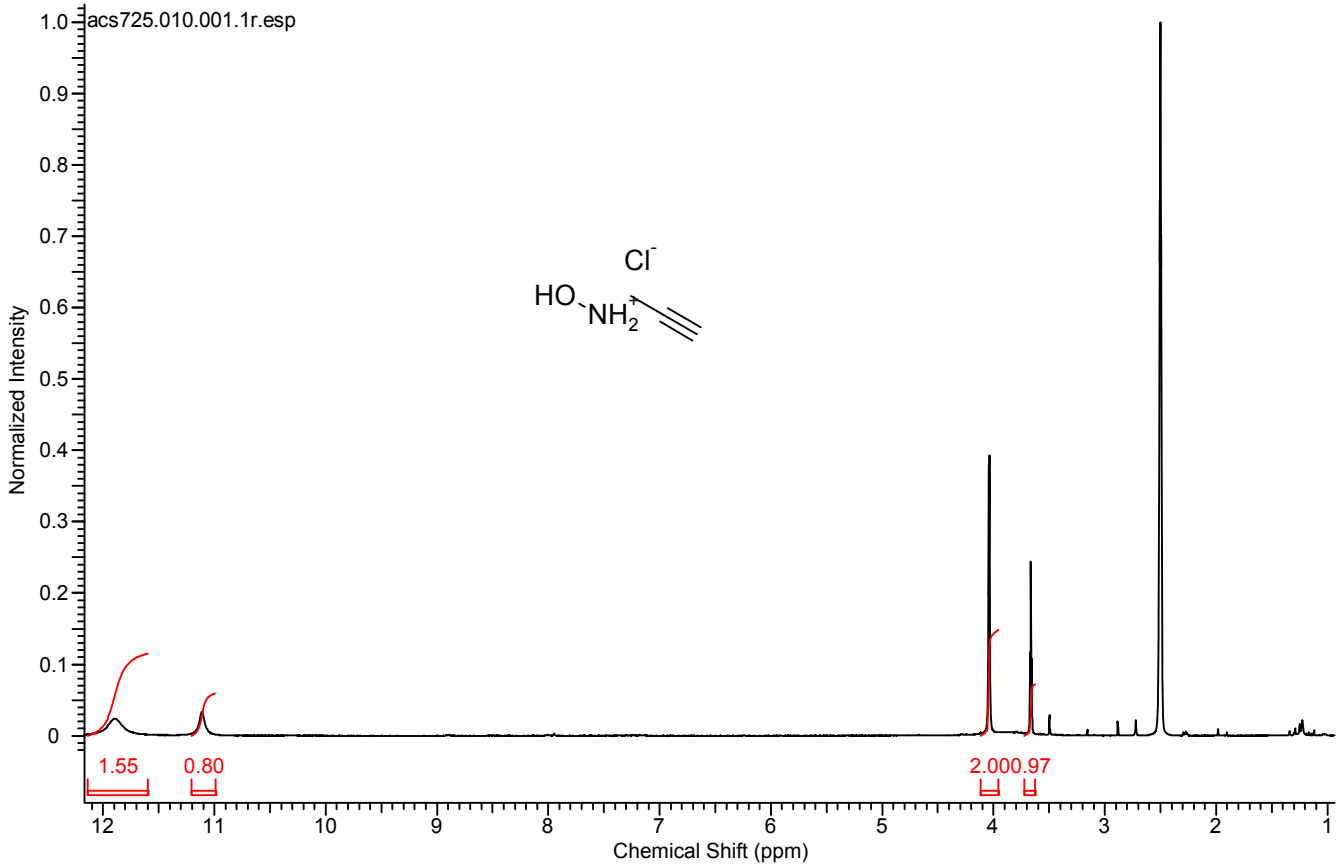
5,5-difluoro-10-(1-hydroxy-3-(2-methoxy-2-oxoethyl)-2-methyl-2-oxidoisoindolin-5-yl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (126 MHz, $CDCl_3$)



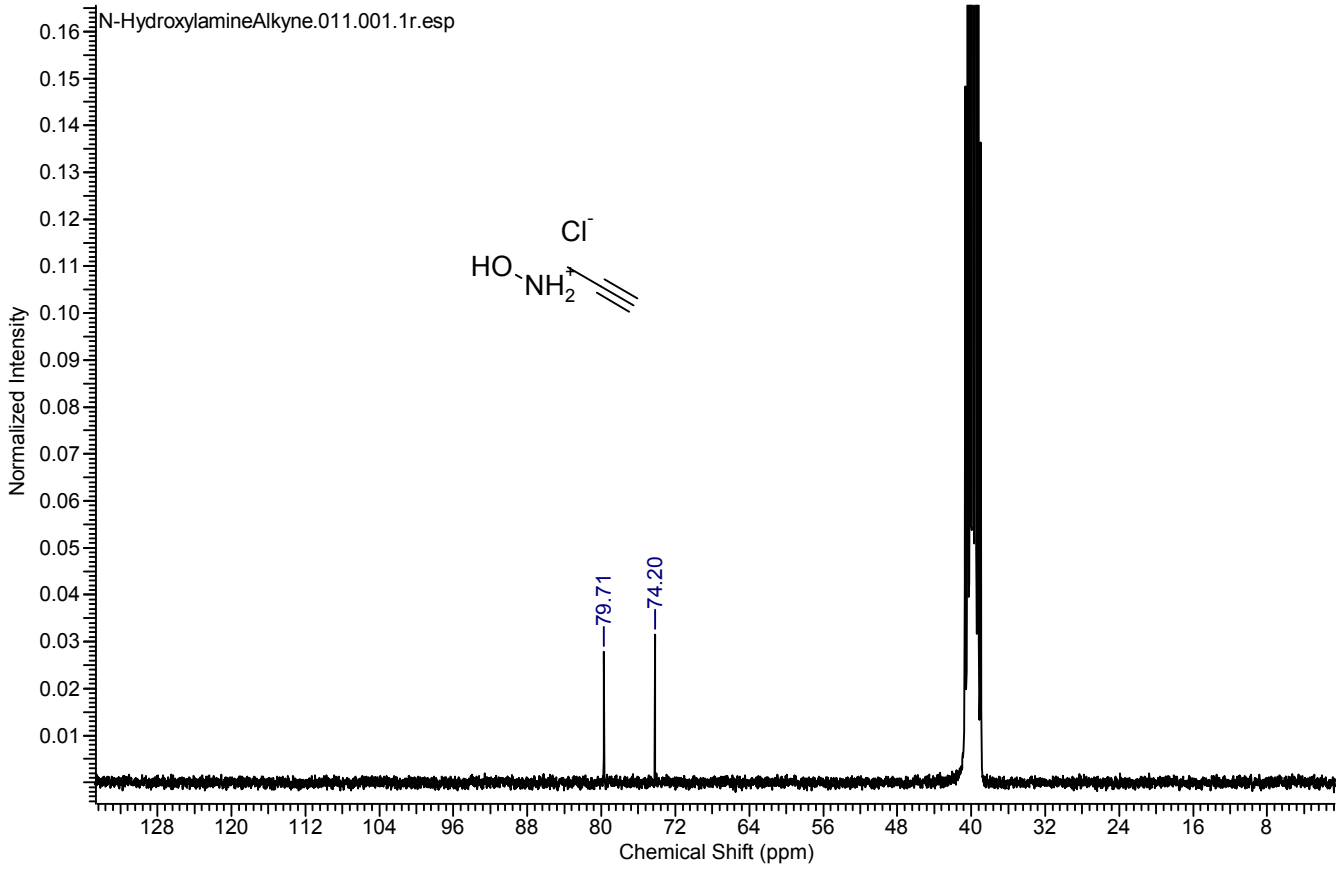
tert-Butyl ((tert-butoxycarbonyl)oxy)(prop-2-yn-1-yl)carbamate (300 MHz, $CDCl_3$)



N-(Prop-2-yn-1-yl)hydroxylammonium chloride (300 MHz, DMSO-d6)



N-(Prop-2-yn-1-yl)hydroxylammonium chloride (75.5 MHz, $CDCl_3$)



1. R. P. Temming, L. Eggermont, M. B. van Eldijk, J. C. M. van Hest and F. L. van Delft, *Org. Biomol. Chem.* 2013, **11**, 2772-2779.