Supporting Information for

Novel Dual-Enzyme System for Synthesis of 2-Alkyl and 2-Arylbenzoxazoles via Aerobic Oxidation

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Experimental procedures

Materials. All the chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, Bide Pharmatech, Aladdin, Energy Chemical, TCI) and used without any further purification, unless otherwise stated. Jack Bean Urease purchased from Macklin (300 U/mg, One unit of urease activity is defined as that amount of enzyme which will produce 1 mg of ammonia nitrogen from urea in 5 min at pH 7.0 at 30°C). *E. coli* strain RP523 was obtained from the *E. coli* Genetic Stock Center (CGSC) at Yale University. Ni-NTA Superflow resin obtained from Solarbio. *E. coli* BL21(DE3) Competent Cell, Spin Miniprep, and Gel Extraction Kits were all obtained from Tiangen. Co(ppIX) was synthesized according to a previously reported procedure [1]. All reactions were carried out in oven-dried glassware with magnetic stirring. Silica gel chromatography purifications were carried out using AMD Silica Gel 60 230- 400 mesh. Thin Layer Chromatography (TLC) and preparative TLC were carried out using Merck Millipore TLC silica gel 60 F254 glass plates. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a 400 MHz spectrometer in $CDCl_3$. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane (TMS) and are referenced to residual protium in the NMR solvent ($CHCl_3$ = δ 7.26 ppm). NMR data are presented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant in Hertz (Hz), integration. The experiments were performed triplicate, and all data were obtained based on the average values. Mass spectra were recorded on the Bruker MicrOTOF Q II and an Orbitrap Fusion™ Tribrid™ mass spectrometer (Thermo Scientific, San Jose, CA, U.S.A.) coupled with HESI ion source.

Growth Media. Terrific Broth media was prepared as follows. For 1 L Terrific Broth media, deionized H_2O was added with 11.8 g of peptone 140 (pancreatic digest of casein), 23.6 g of yeast extract autolyzed low sodium, 9.4 g of dipotassium hydrogen phosphate, 2.2 g of potassium dihydrogen phosphate, 4 ml of glycerol and supplemented glucose (0.2 % w/v). Terrific Broth agar plates were prepared by adding 15 g agar to 1 L Terrific Broth media with ampicillin antibiotic selection and hemin (35 μg/mL). To media and plates was added ampicillin to a final concentration of 100 mg/L.

General Methods for Expression of *Vitreoscilla* Hemoglobin. The VHb gene and promoter region (promoter-VHb) were amplified by polymerase chain reaction (PCR) with Pfu polymerase and a pair of primers (5′-CCCAAGCTTACAGGACGCTGGGGAAAGT-3′; 5′-CCGGAATTCTTAAT GATGATGATGATGATGTTCAACCGCTTGAGCGTACAAATCT-3′). The promoter-VHb fragment was retrieved from plasmid DNA by using restriction enzymes EcoRI and HindIII. Finally, the promoter-VHb fragment was cloned into the pUC-19 plasmid and transformed into E. coli BL21 (DE3) cells for protein expression. All VHb proteins contained a C-terminal polyhistidine tag. BL21(DE3) competent cells was used for expression of normal VHb and variants with natural hemin cofactor. Competent cells were transformed with the PUC-19 vector encoding for the appropriate VHb variant and selected on Terrific Broth agar plates containing ampicillin (100 mg L^{-1}). Single colonies were used to inoculate 5 mL of Terrific Broth media supplemented with ampicillin (100 mg L^{-1}), followed by incubation at 37°C with shaking (180 rpm) for 10 to 15 hours. For expression of VHb, the overnight cultures were transferred to 1 L Terrific Broth media containing ampicillin, followed by incubation at 37°C with shaking (180 rpm). At an OD_{600} of 1.5, cells were induced by its anaerobic promoter in an anaerobic environment and incubated at 25°C with shaking (110 rpm) for 30 hours. Cell cultures were harvested by centrifugation at 5000 rpm. The overall pelleted bacteria were dissolved in 50 mL of 20 mM phosphate buffer (pH 7.4). After sonication for 30 min on ice, the cell lysates were centrifuged at 12,000 rpm for 30 min.

Expression-based Porphyrin Substitution of *Vitreoscilla* Hemoglobin Containing Artificial Metalloporphyrins. *E. coli* strain RP523 was used for all expression-based porphyrin substitution studies. *E. coli* strain RP523 contains a hemB (porphobilinogen synthase) gene disruption and an uncharacterized permeability mutation that renders the bacteria heme-permeable. The inability to biosynthesize heme is an auxotrophic mutation that requires supplementation of growth media with heme under aerobic conditions [2]. As a result of it, we can take advantage of this defect that supply different porphyrins to produce foreign porphyrin containing protein. Co(ppIX) was dissolved in DMSO (10 mM). These concentrated stock solutions were diluted to 2 mM using a solution of 100 mM NaOH and 200 mM Na_2PO_4 (pH 12). The final stock was directly added to expression cultures at a final concentration of 9 μM. Co(ppIX) solution was made directly before use. RP523 competent

cell were transformed with the appropriate expression plasmids. Positive transformants were selected using Terrific Broth agar plates with ampicillin (100 μg/mL) antibiotic selection and hemin (35 μg/mL) in anaerobic condition. Isolated colonies from agar plates were used to grow starter cultures in Hungate tubes containing Terrific Broth (14 mL) supplemented with ampicillin. Cultures were degassed with nitrogen for 15 minutes to remove oxygen and were subsequently grown at 37 °C for 12 h. Following anaerobic growth, starter cultures were used to inoculate 1 L Terrific Broth culture medium containing ampicillin. Culture was degassed for 15 minutes with nitrogen followed by incubation at 37°C with shaking (180 rpm). After that, we add Co(ppIX) solution to the culture, cells were induced by its anaerobic promoter in an anaerobic environment and incubated at 25°C with shaking (110 rpm) for 30 hours. Cell cultures were harvested by centrifugation at 5000 rpm. The overall pelleted bacteria were dissolved in 50 mL of 20 mM phosphate buffer (pH 7.4). After sonication for 30 min on ice, the cell lysates were centrifuged at 12,000 rpm for 30 min.

Protein Purification. The clarified lysate was transferred to a Ni-NTA column equilibrated with Ni-NTA Lysis Buffer. The resin was washed with 50 mL of Ni-NTA Lysis Buffer and then 50 mL of Ni-NTA Wash Buffer (20 mM phosphate buffer, 20 mM imidazole, pH = 7.4). Proteins were eluted with Ni-NTA Elution Buffer (20 mM phosphate buffer, 250 mM imidazole, pH = 7.4). After elution from the Ni-NTA column, the protein was loaded into a 5ml PD10 desalting column to remove imidazole. The concentration of the protein was tested by NanoDrop.

General procedure for dual-enzyme system synthesis of 2-alkyl and 2-arylbenzoxazoles: To a mixture of catechols (0.5 mmol), aldehydes (1 equiv.), urea (0.3 mmol), in water (2 ml), urease (300 U), ArVHbM (heme concentration: 0.02 mol%), DMSO (10% v/v) was added and stirred in a pre-heated constant temperature shaker at the 25 ℃ until completion of the reaction as indicated by TLC. The reaction mixture was extracted with ethyl acetate (2 x 5 mL). The combined organic layer was washed with an aqueous solution of NH_4Cl and dried over $MgSO_4$. Volatiles were removed under reduced pressure, and residue was purified on silica column with EA/PE (1:99~1:20) to afford the desired products. All the isolated products were well characterized by their NMR analysis.

Supporting Experimental Tables

Table S1. optimization of the reaction conditions [a]

Entry	Dosage of urea	Urease (U)	Heme concentration (mol%)	Yield (%)
1	0.3 mmol	300	0.02	91
2	0.1 mmol	300	0.02	37
3	0.2 mmol	300	0.02	74
4	0.4 mmol	300	0.02	93
5	0.3 mmol	100	0.02	55
6	0.3 mmol	200	0.02	82
7	0.3 mmol	400	0.02	84
8	0.3 mmol	500	0.02	75
9	0.3 mmol	300	0.04	94
10	0.3 mmol	300	0.06	96
11	0.3 mmol	300	0.01	61
12 [b]	0.3 mmol	300	0.02	93

a. Reaction condition: **1a** (0.5 mmol), **2a** (0.5 mmol), **3** (urea), Urease, ArVHbM, air, water (10% DMSO v/v), stirred at r.t. for 12 h; b. instead of O_2, reaction time: 10 h.

Table S2. Screening of hemoprotein [a]

Entry	Hemoprotein (mol%)	Yield (%)
1	Mn (ppIX) (1)	14
2	Co (TPP) (1)	15
3	Fe (TPP)Cl (1)	9
4	VB_{12} (0.02)	21
5	Mb (0.05)	42
6	HbBv (0.05)	46
7	HRP (0.05)	31
8	Cyt.c (0.05)	34
9	VHb (0.02)	26
10	VHb (0.05)	57

a. Reaction condition: **1a** (0.5 mmol), **2a** (0.5 mmol), **3** (urea, 0.3 mmol), Urease (300 U), metalloporphyrin or hemoprotein, air, water (10% DMSO v/v), stirred at r.t. for 12 h。

UV−vis absorption spectra of artificial VHb protein

After incorporating Co(ppIX) into the VHb scaffold, the UV−vis absorption spectra of Co(ppIX) containing VHb showed a strong absorption band at 425 nm and two weak absorption bands at 535

and 570 nm, respectively corresponding to protein's Soret and Q-bands (Figure S1, red line).

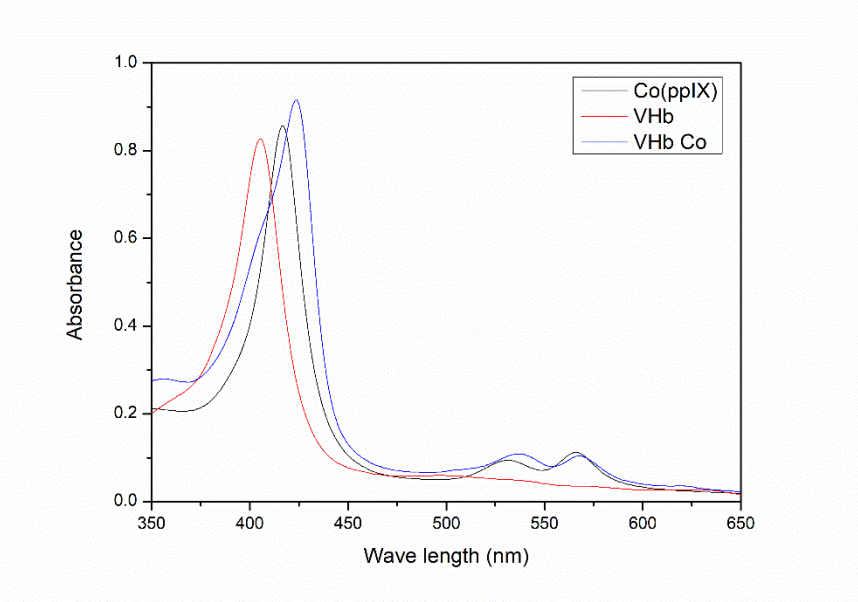


Figure S1: Overlay of the electronic absorption spectra of Co(ppIX) (black), VHb (red), VHb_{Co} (Blue).

Detection of H_2O_2 in the reaction mixture

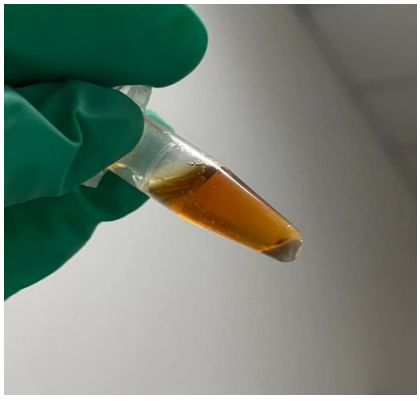
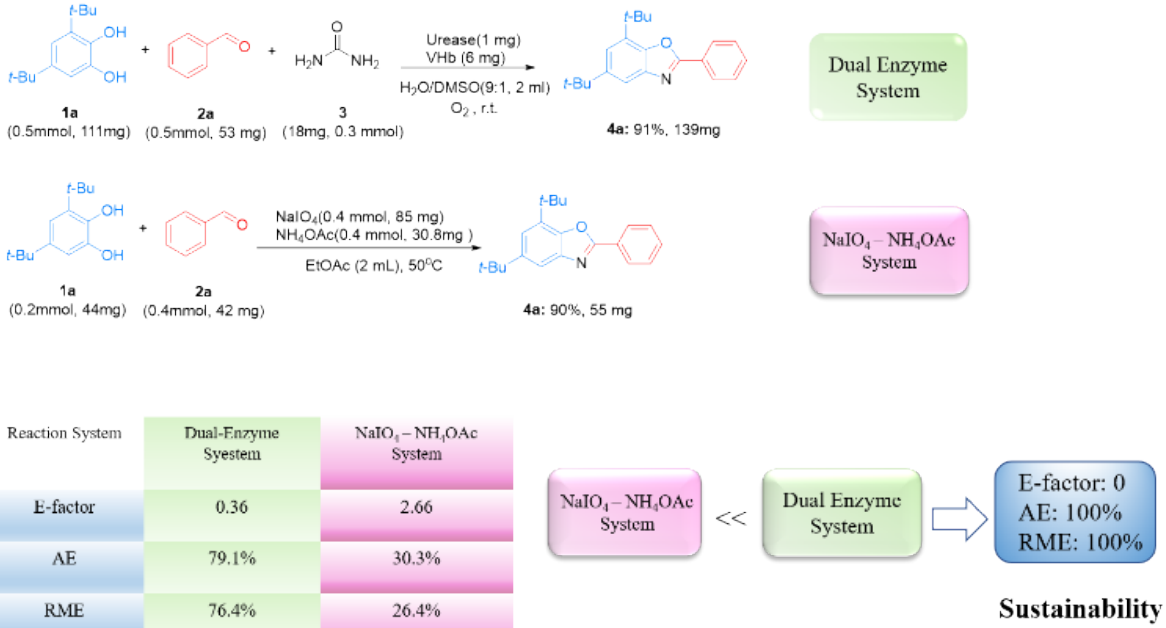
a).  b). 

Figure S2. a). Rection condition; b). Mohr's salt added.

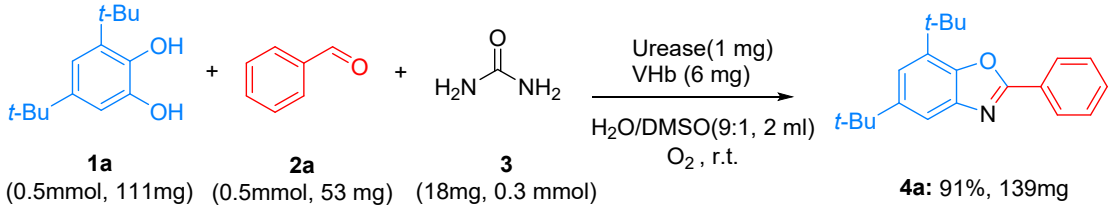
During the detection of H_2O_2 in this reaction, we chose the reaction condition as **Entry 1** in **Table 1** (solvent: H_2O/DMSO (9:1)). After 12 h of reaction, the reaction system was centrifuged at 12,000 rpm for 1 min, a 100 µL solution of Mohr's salt (10 mg in 100 µL H_2O) was added to the reaction mixture. Then, a rapid formation of $Fe(OH)_3$ floc was observed. The floc was observed due to the rapid oxidation of Fe(II) to Fe(III) by H_2O_2 in the reaction mixture.

Green chemistry metrics analysis



Scheme S1. Green chemistry metrics for dual enzyme system and NH_4OAc-$NaIO_4$

E-Factor for 4a using ArVHbM/Urease (This Work):



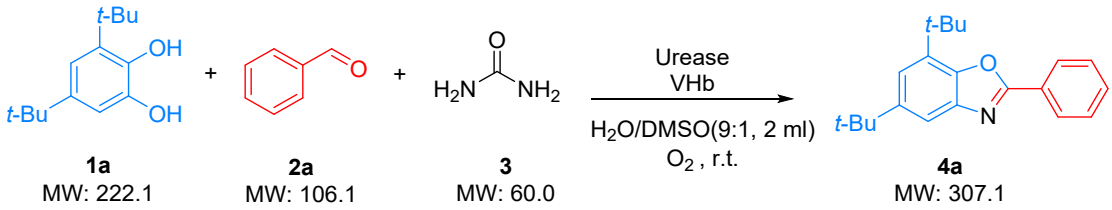
Total amount of reactants: 111 mg + 53 mg + 18 mg + 1 mg + 6 mg = 189 mg

Amount of final product: 139 mg

Amount of waste: 189 mg – 139 mg = 50 mg

E-Factor = Amount of waste/Amount of product = 50/139 = 0.36

AE for 4a using ArVHbM/Urease (This Work):

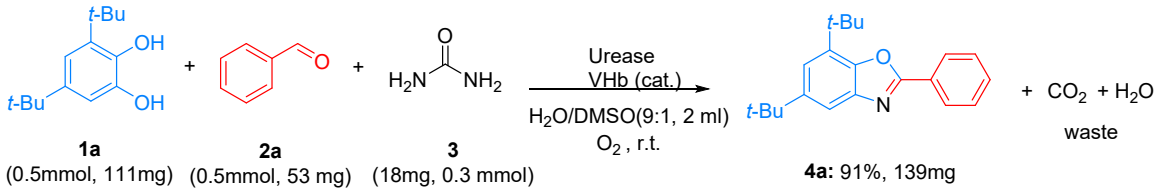


Molecular weight of product: 307.1

Sum of molecular weight of reagent: 222.1 + 106.1 + 60.0 = 388.2

Atom economy = Molecular weight of product/Sum of molecular weight of reagent = 307.1/388.2 = 79.1%

RME for 4a using ArVHbM/Urease (This Work):



1a (0.5mmol, 111mg) **2a** (0.5mmol, 53 mg) **3** (18mg, 0.3 mmol)

4a: 91%, 139mg

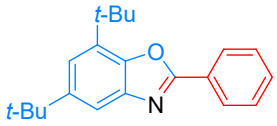
Mass of product: 139 mg

Total mass of reagent: 111 mg + 53 mg + 18 mg = 182 mg

RME = Mass of product/Total mass of reagent = 52.4/81.8 = 76.4 %

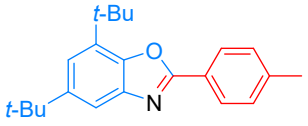
^{1}H Data of Product

The NMR spectra of all products were identical to those reported [3-6].



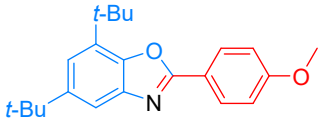
4a

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.35 – 8.27 (m, 2H), 7.71 (d, *J* = 2.0 Hz, 1H), 7.60 – 7.52 (m, 3H), 7.36 (d, *J* = 2.0 Hz, 1H), 1.60 (s, 9H), 1.45 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{26}NO$: 308.2071, found: 308.1946;



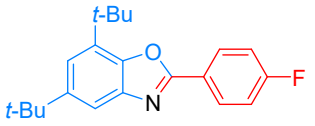
4b

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 – 8.14 (m, 2H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.44 – 7.33 (m, 3H), 2.48 (s, 3H), 1.60 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{22}H_{28}NO$: 322.4521, found: 322.4522.



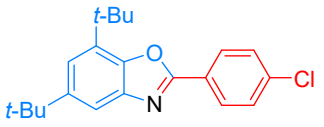
4c

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.28 – 8.21 (m, 2H), 7.66 (d, *J* = 2.0 Hz, 1H), 7.32 (d, *J* = 2.0 Hz, 1H), 7.11 – 7.05 (m, 2H), 3.93 (d, *J* = 4.4 Hz, 3H), 1.59 (s, 9H), 1.43 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{22}H_{28}NO_2$: 338.2142; found: 338.2131.



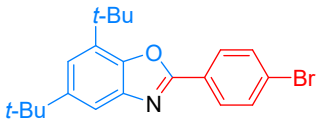
4d

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 – 8.22 (m, 2H), 7.68 (d, *J* = 2.0 Hz, 1H), 7.34 (d, *J* = 2.0 Hz, 1H), 7.25 (t, *J* = 8.4 Hz, 2H), 1.58 (s, 9H), 1.43 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{25}FNO$: 326.1915, found: 326.1914.



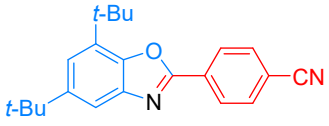
4e

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.25 – 8.20 (m, 2H), 7.69 (dd, *J* = 2.0, 0.8Hz, 1H), 7.56 – 7.52 (m, 2H), 7.36 (d, *J* =2.0 Hz, 1H), 1.59 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{25}ClNO$: 342.1549; found: 342.1547.



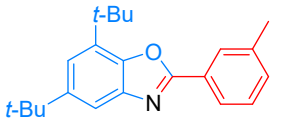
4f

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 – 8.13 (m, 2H), 7.72 – 7.68 (m, 3H), 7.37 (d, *J* = 2.0Hz, 1H), 1.59 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{25}BrNO$: 386.1114 m/z, found: 386.1108.



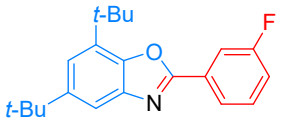
4g

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 – 8.36 (m, 2H), 7.88 – 7.83 (m, 2H), 7.71 (d, *J* = 2.0Hz, 1H), 7.42 (d, *J* = 2.0Hz, 1H), 1.59 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{22}H_{25}N_2O$: 333.1986; found: 333.1991.



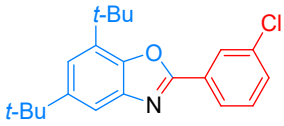
4h

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.14 – 8.07 (m, 2H), 7.70 (d, *J* = 2.0Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.40 – 7.34 (m, 2H), 2.51 (s, 3H), 1.60 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{22}H_{28}NO$: 322.4521, found: 322.4522.



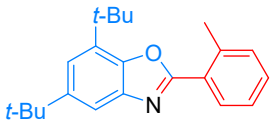
4i

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 (dt, *J* = 7.6, 1.2 Hz, 1H), 7.98 (ddd, *J* = 9.6, 2.8, 1.6 Hz, 1H), 7.71 (d, *J* = 2.0 Hz, 1H), 7.54 (td, *J* = 8.0, 5.6 Hz, 1H), 7.38 (d, *J* = 1.6 Hz, 1H), 7.26 (tdd, *J* = 8.4, 2.8, 1.2 Hz, 1H), 1.59 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{25}FNO$: 326.1915, found: 326.1915.



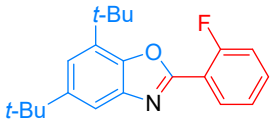
4j

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.26 (t, *J* = 1.6 Hz, 1H), 8.18 (dt, *J* = 7.2, 1.6 Hz, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.57 – 7.48 (m, 2H), 7.37 (d, *J* = 2.0 Hz, 1H), 1.59 (s, 9H), 1.43 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{25}ClNO$: 342.1549; found: 342.1543.



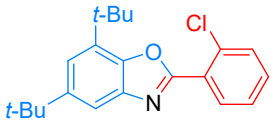
4k

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.28 – 8.19 (m, 1H), 7.75 (d, *J* = 2.0Hz, 1H), 7.50 – 7.33 (m, 4H), 2.88 (s, 3H), 1.60 (s, 9H), 1.46 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{22}H_{28}NO$: 322.4521, found: 322.4522.



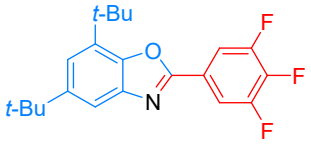
4l

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 (td, *J* = 7.6, 2.0 Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.60 – 7.49 (m, 1H), 7.40 – 7.25 (m, 3H), 1.59 (s, 9H), 1.43 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{25}FNO$: 326.1916, found: 326.1915.



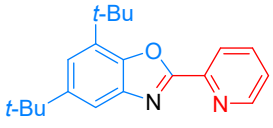
4m

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.22 (d, *J* = 7.2 Hz, 1H), 7.76 (s, 1H), 7.61 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.39 (s, 1H), 1.58 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{25}ClNO$: 342.1548; found: 342.1542.



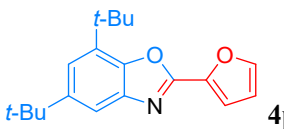
4n

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.93 – 7.89 (m, 2H), 7.68 (d, *J* = 2.0 Hz, 1H), 7.39 (d, *J* =2.0Hz, 1H), 1.58 (s, 9H), 1.43 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{21}H_{23}F_3NO$: 361.1710; found: 375.1708.



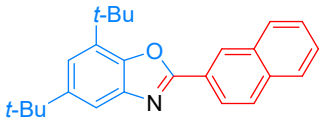
4o

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.87 (ddd, *J* = 4.8, 2.0, 1.2 Hz, 1H), 8.32 (dt, *J* = 7.6, 1.2 Hz, 1H), 7.91 (td, *J* = 7.6, 2.0Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.46 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 1H), 7.40 (d, *J* = 2.0 Hz, 1H), 1.60 (s, 9H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{20}H_{25}N_2O$: 309.1892; found: 309.1882.



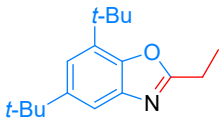
4p

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.70 (dd, *J* = 2.0, 1.2 Hz, 1H), 7.65 (d, *J* = 2.0Hz, 1H), 7.34 (d, *J* =2.0 Hz, 1H), 7.31 – 7.25 (m, 2H), 1.56 (s, 9H), 1.42 (s, 9H). HRMS (ESI): m/z = (M + Na^+) calcd for $C_{19}H_{23}NO_2Na$: 320.1621, found: 320.1627.



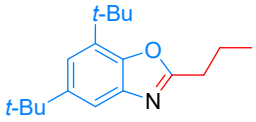
4q

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.45 (dd, *J* = 7.2, 1.2Hz, 1H), 8.11 – 8.05 (m, 1H), 8.02 – 7.96 (m, 1H), 7.83 (d, *J* = 2.0 Hz, 1H), 7.78 – 7.61 (m, 4H), 7.42 (d, *J* = 2.0Hz, 1H), 1.64 (s, 9H), 1.48 (s, 9H). HRMS (ESI): *m/z* = (M + H^+) calcd for $C_{25}H_{28}NO$: 358.2096; found: 358.2091.



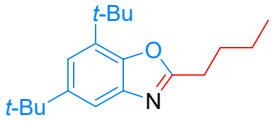
4r

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 2.0 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 3.00 (q, *J* = 7.6 Hz, 2H), 1.52 (s, 9H), 1.48 (d, *J* = 7.6 Hz, 3H), 1.41 (s, 9H). HRMS (ESI): *m/z* = (M + H^+) calcd for $C_{17}H_{26}NO$: 260.1938; found: 260.1934.



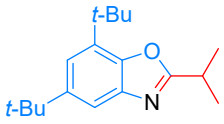
4s

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 2.0 Hz, 1H), 7.32 – 7.24 (m, 1H), 2.96 (t, *J* = 7.2 Hz, 2H), 1.96 (h, *J* = 7.2 Hz, 2H), 1.52 (s, 9H), 1.41 (s, 9H), 1.10 (t, *J* = 7.2 Hz, 3H). HRMS (ESI): *m/z* = (M + H^+) calcd for $C_{18}H_{28}NO$: 274.2093; found: 274.2084.



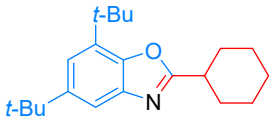
4t

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 (d, *J* =2.0 Hz, 1H), 7.29 (d, *J* = 2.0 Hz, 1H), 2.97 (t, *J* = 7.6 Hz, 2H), 1.93 (p, *J* = 7.6 Hz, 2H), 1.52 (s, 9H), 1.49(m,2H), 1.41 (s, 9H), 0.99 – 0.86 (m, 3H). HRMS (ESI): *m/z* = (M + H^+) calcd for $C_{19}H_{30}NO$: 288.2409; found: 288.2402.



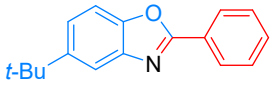
4u

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.60 (d, *J* = 2.0 Hz, 1H), 7.29 (d, *J* = 2.0 Hz, 1H), 3.30 (p, *J* = 7.2 Hz, 1H), 1.52 (s, 9H), 1.48 (d, *J* = 8.0 Hz, 6H), 1.40 (s, 9H). HRMS (ESI): *m/z* = (M + H^+) calcd for $C_{18}H_{28}NO$: 274.2094; found: 274.2085.



4v

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 2.0 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 3.00 (ddd, *J* = 11.2, 7.6, 3.6 Hz, 1H), 2.25 - 2.17 (m, 2H), 1.94 - 1.86 (m, 2H), 1.80 - 1.71 (m, 2H), 1.52(s, 9H), 1.49 - 1.42 (m, 4H), 1.40(s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{22}H_{32}NO$: 314.2484, found: 314.1581.

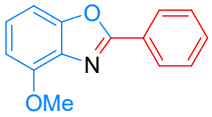


4w

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.29 (dd, *J* = 6.8, 2.8 Hz, 2H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 1.6 Hz, 1H), 7.56 (q, *J* = 2.8 Hz, 3H), 7.46 (dd, *J* = 8.4, 1.6 Hz, 1H), 1.44 (s, 9H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{17}H_{18}NO$: 252.1383; Found 252.1386.

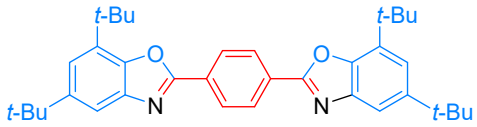
4x

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 (dd, *J* = 6.8, 2.8 Hz, 2H), 7.56 (d, *J* = 1.6 Hz, 1H), 7.51 (dd, *J* = 5.2, 2.0 Hz, 3H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.15 (dd, *J* = 8.4, 1.6 Hz, 1H), 2.48 (s, 3H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{14}H_{12}NO$: 210.0852; Found: 209.0848.



4y

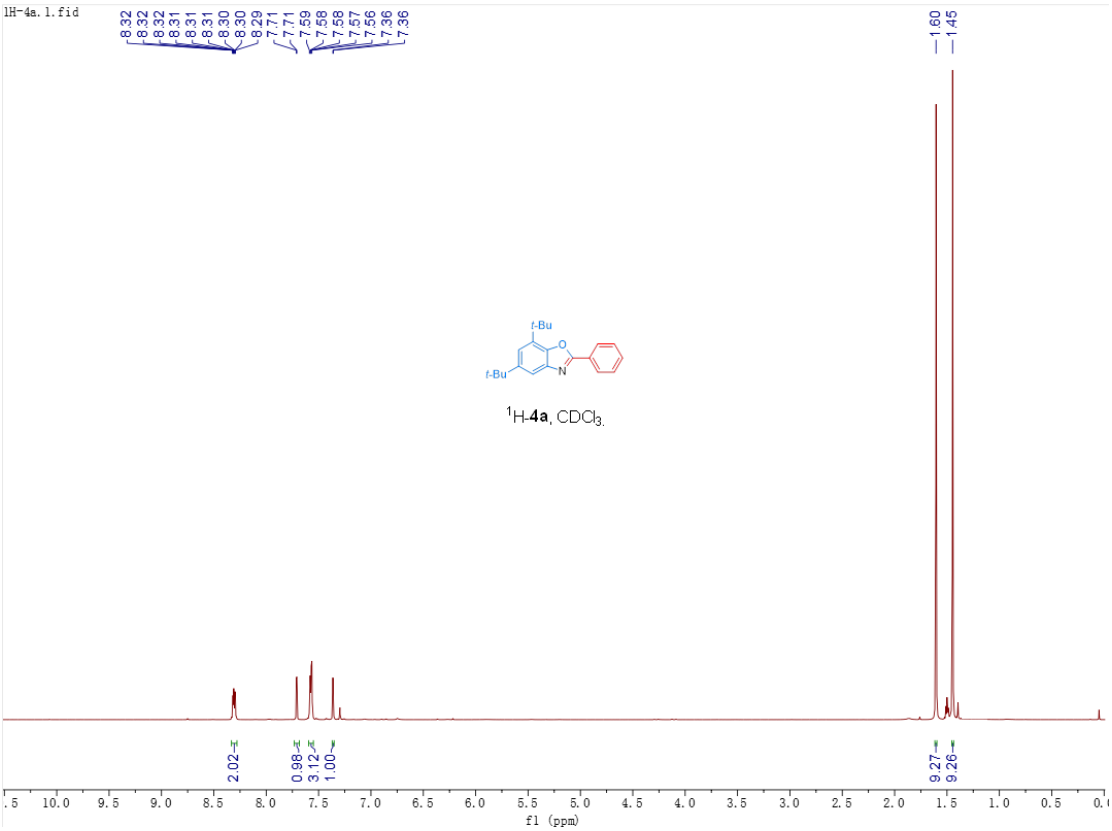
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 - 8.29 (m, 2H), 7.53 (s, 3H), 7.26 (t, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 4.07 (s, 3H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{14}H_{12}NO_2$: 226.1027; Found: 226.1023.



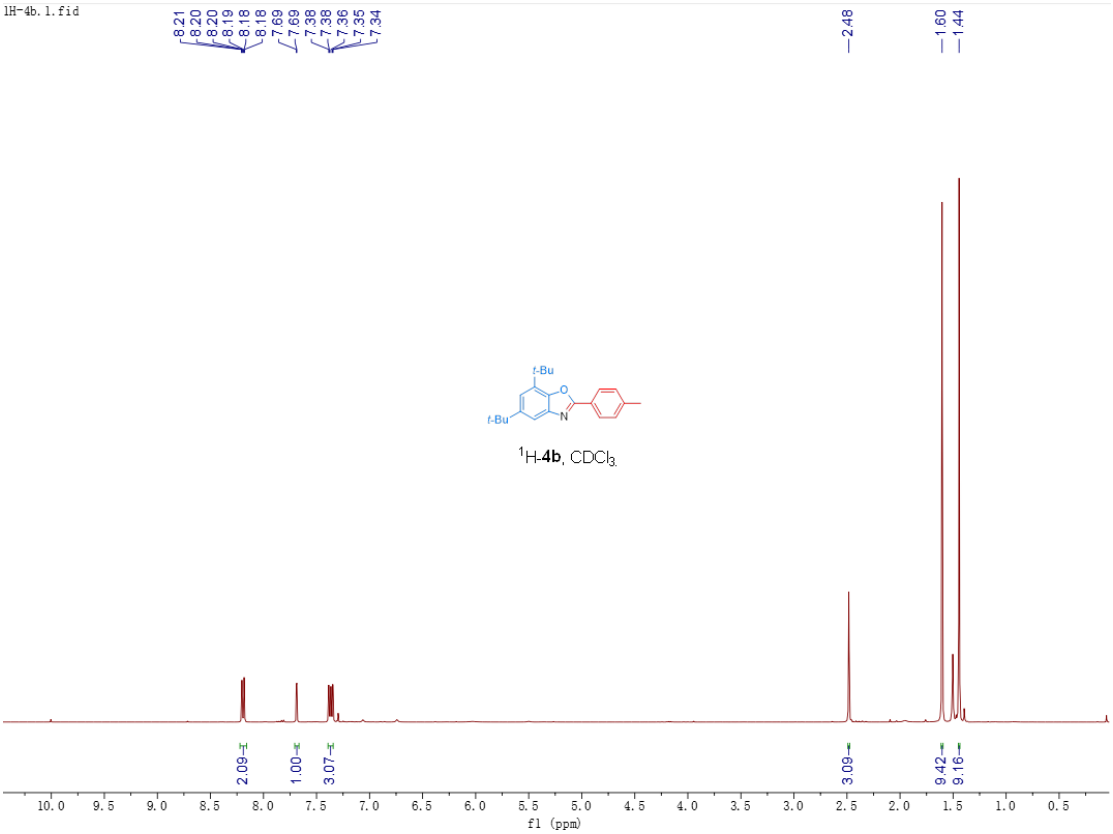
4ae

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.47 (s, 4H), 7.74 (d, *J* = 2.0 Hz, 2H), 7.39 (d, *J* = 2.0 Hz, 2H), 1.61 (s, 18H), 1.44 (s, 18H). HRMS (ESI): *m/z* = (M + H$^+$) calcd for $C_{36}H_{45}N_2O_2$: 537.3475; found: 537.3481.

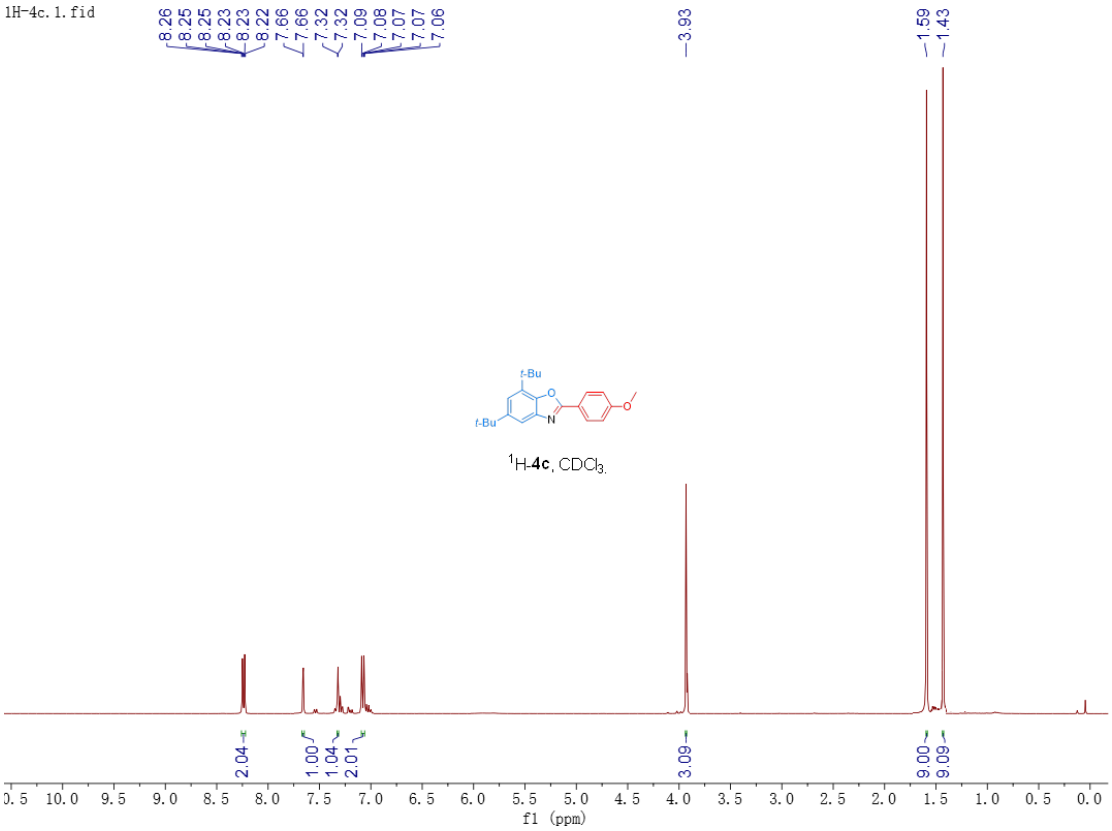
Copies NMR spectra of products

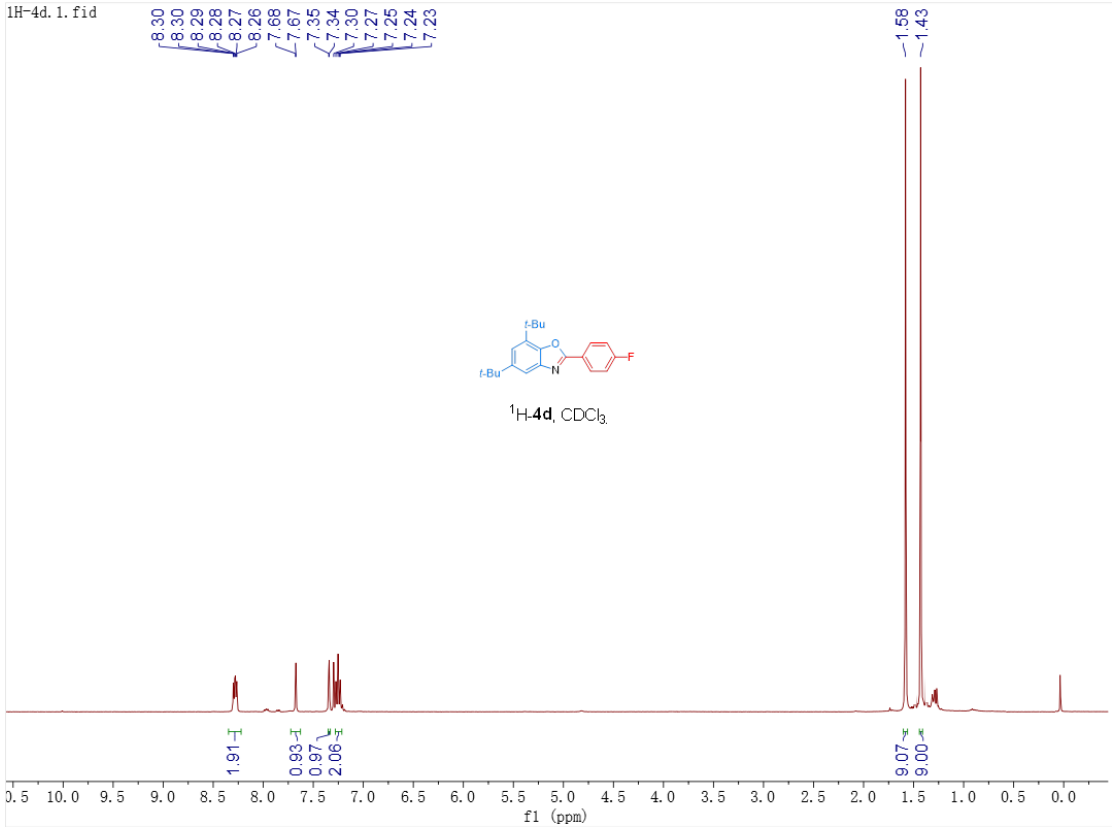


^{1}H-**4a**, $CDCl_3$



^{1}H-**4b**, $CDCl_3$


1H-4c.1.fid
8.26
8.25
8.25
8.23
8.23
8.22
7.66
7.66
7.32
7.32
7.09
7.08
7.07
7.07
7.06
3.93
1.59
1.43
t-Bu
t-Bu
O
N
O
1H-4c, CDCl3
2.04
1.00
1.04
2.01
3.09
9.00
9.09
f1 (ppm)


1H-4d.1.fid
8.30
8.30
8.29
8.28
8.27
8.26
7.68
7.67
7.35
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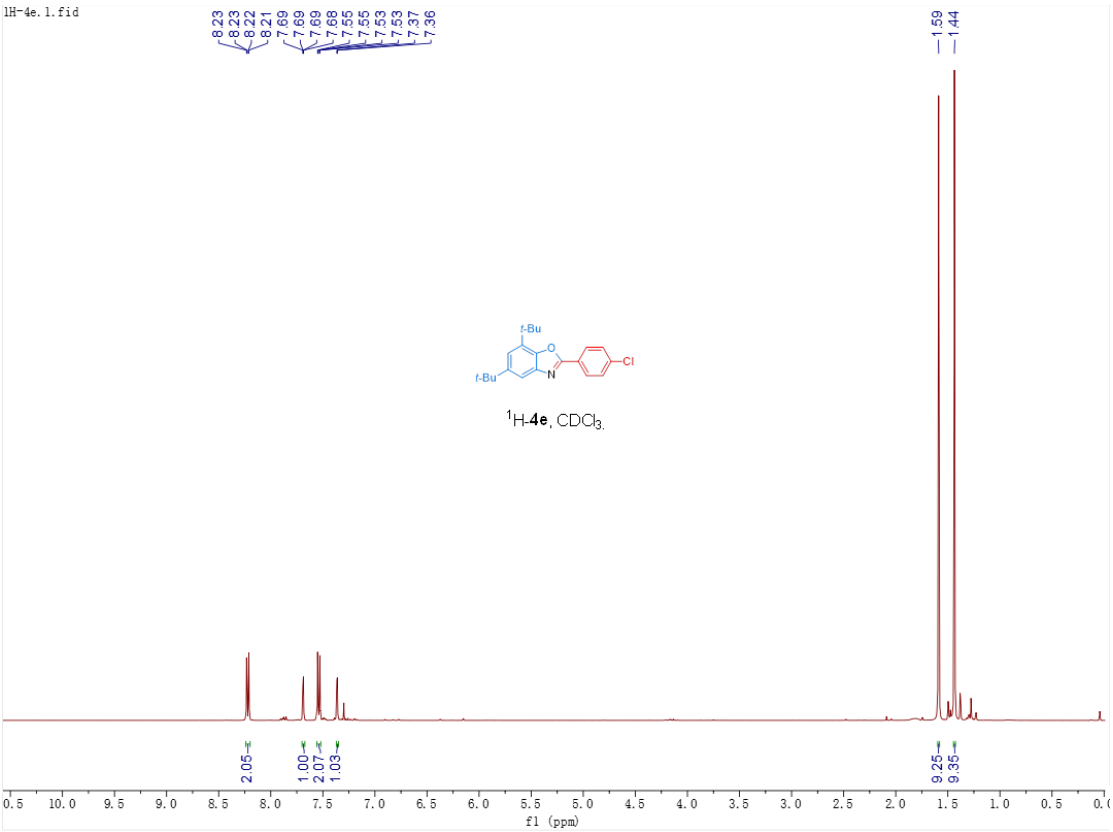
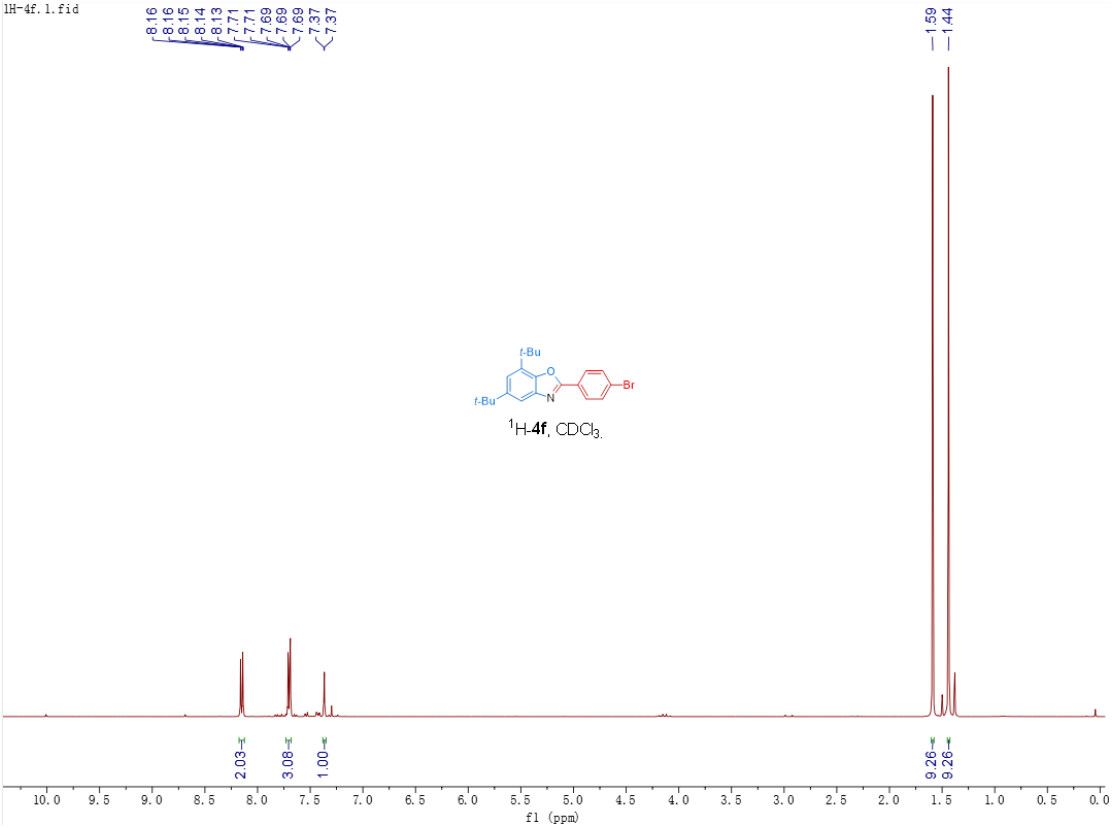
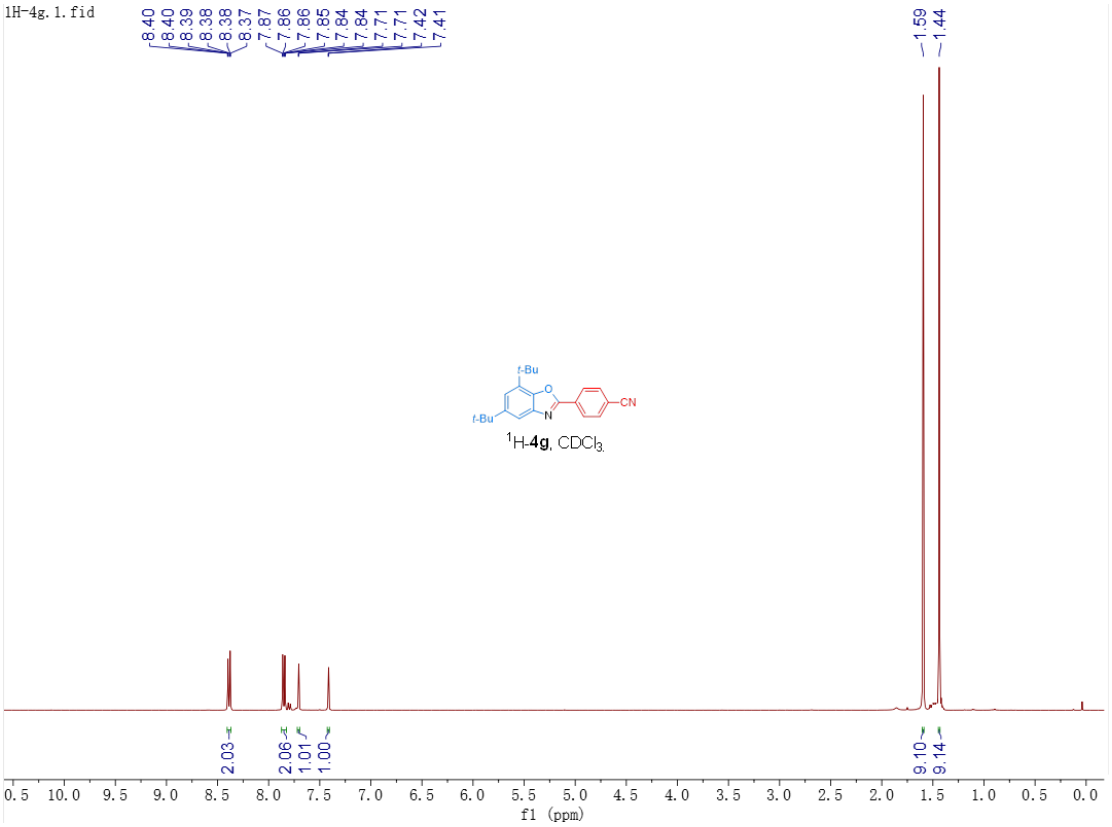
7.30
7.27
7.25
7.24
7.23
1.58
1.43
t-Bu
t-Bu
O
N
F
1H-4d, CDCl3
1.91
0.93
0.97
2.06
9.07
9.00
f1 (ppm)



^{1}H-**4e**, $CDCl_3$.



^{1}H-**4f**, $CDCl_3$.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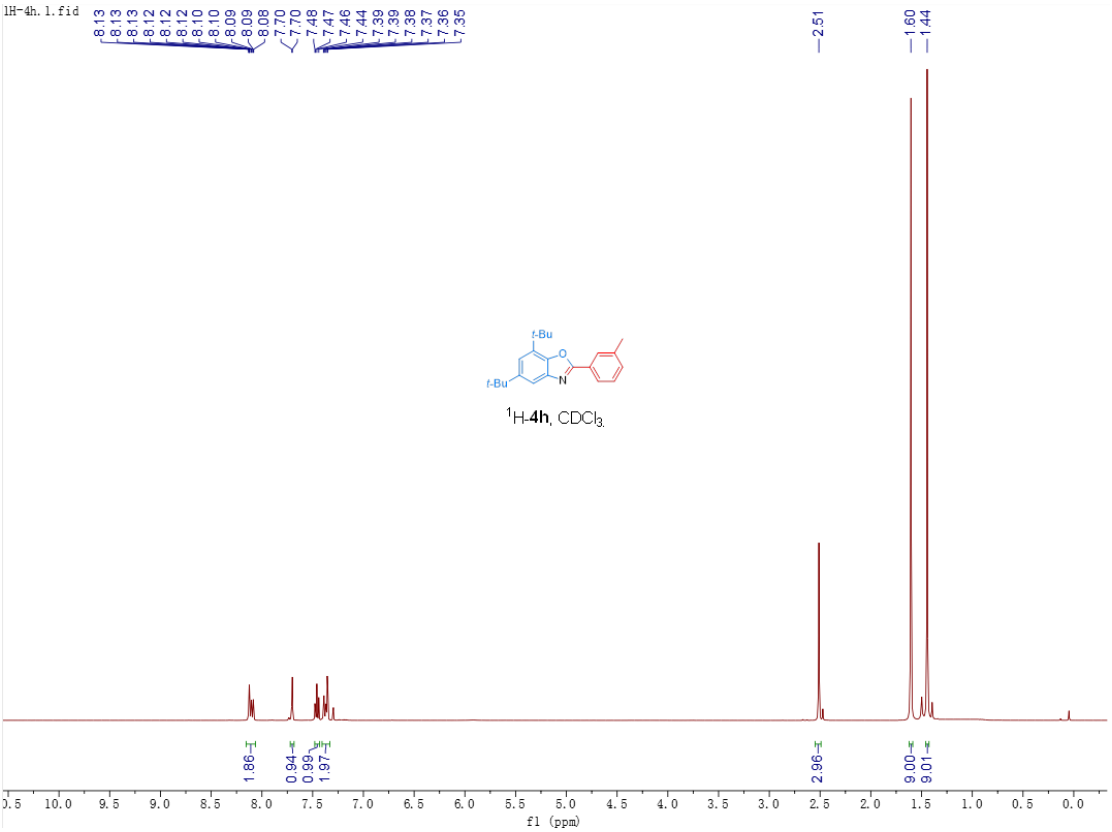
8.40
8.39
8.38
8.38
8.37
7.87
7.86
7.86
7.85
7.84
7.84
7.71
7.71
7.42
7.41
1.59
1.44
t-Bu
t-Bu
CN
1H-4g, CDCl3
2.03
2.06
1.01
1.00
9.10
9.14
f1 (ppm)


1H-4h.1.fid
8.13
8.13
8.13
8.12
8.12
8.12
8.10
8.10
8.09
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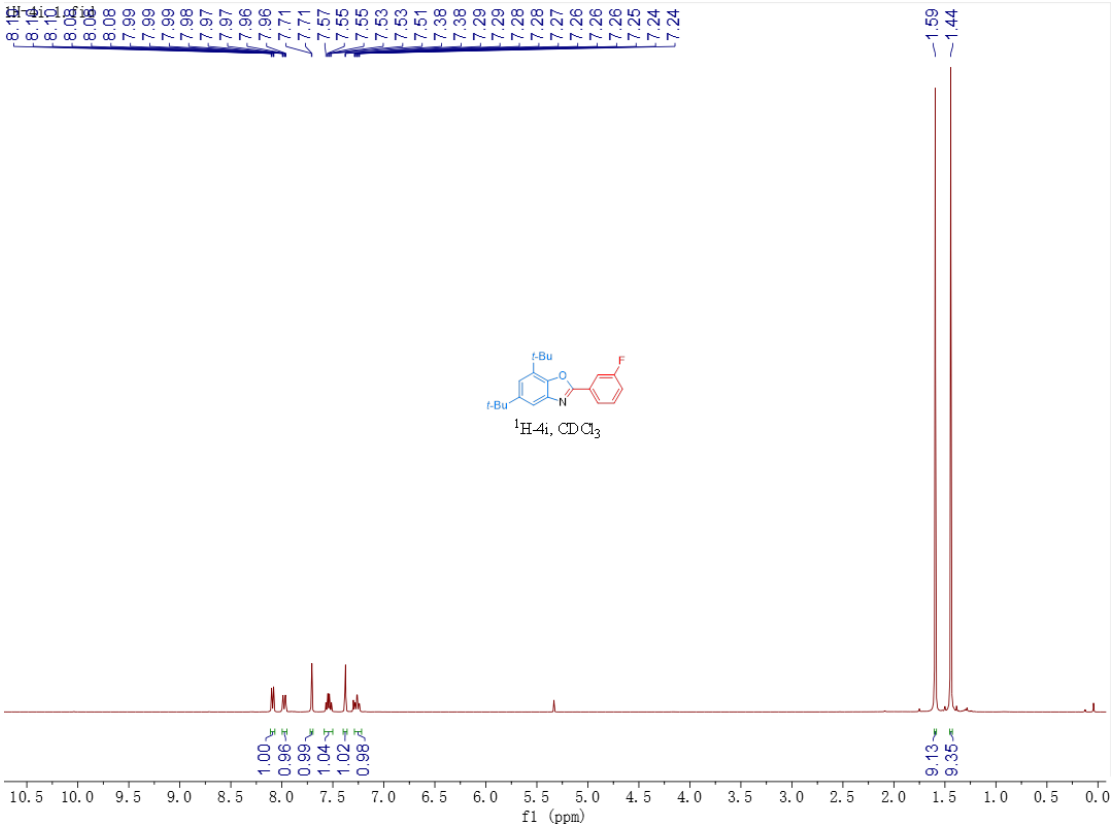
7.70
7.70
7.48
7.47
7.46
7.44
7.39
7.39
7.38
7.37
7.36
7.35
2.51
1.60
1.44
t-Bu
t-Bu
1H-4h, CDCl3
1.86
0.94
0.99
1.97
2.96
9.00
9.01
f1 (ppm)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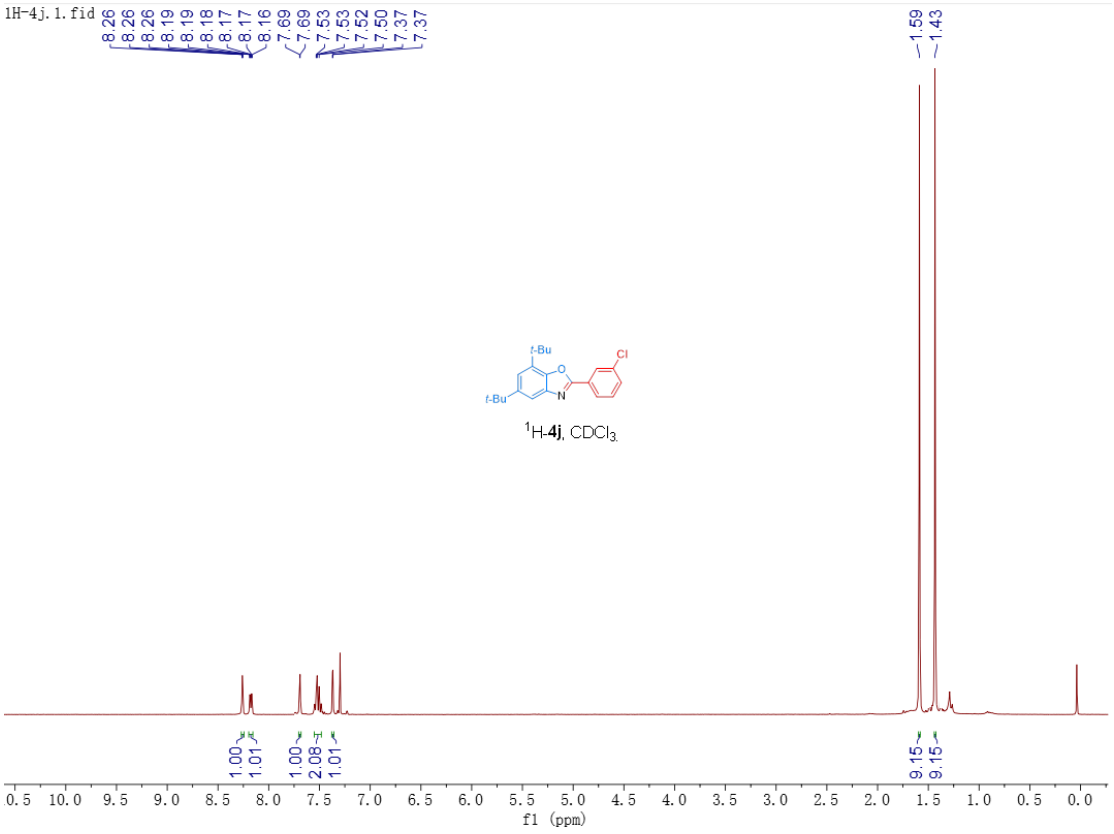
7.24
7.24
1.59
1.44
t-Bu
t-Bu
O
N
F
1H-4i, CDCl3
1.00
0.96
0.99
1.04
1.02
0.98
9.13
9.35
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


1H-4j.1.fid
8.26
8.26
8.26
8.19
8.19
8.18
8.17
8.17
8.16
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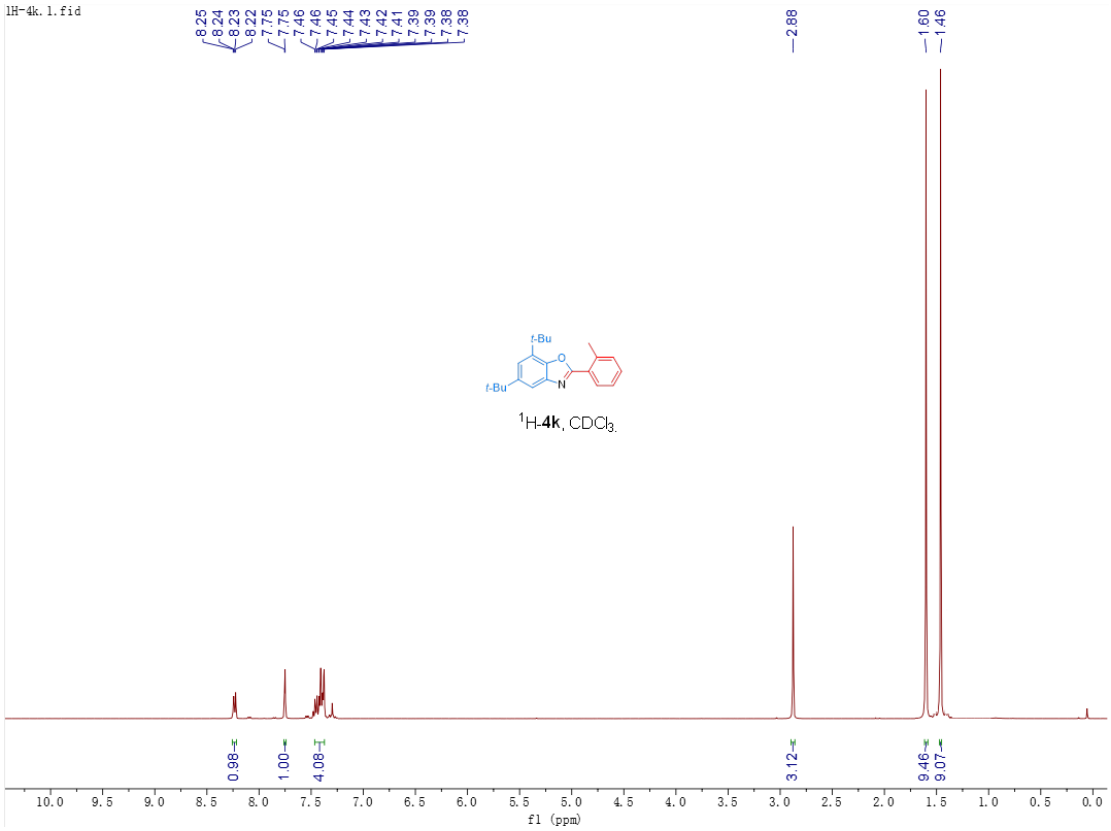
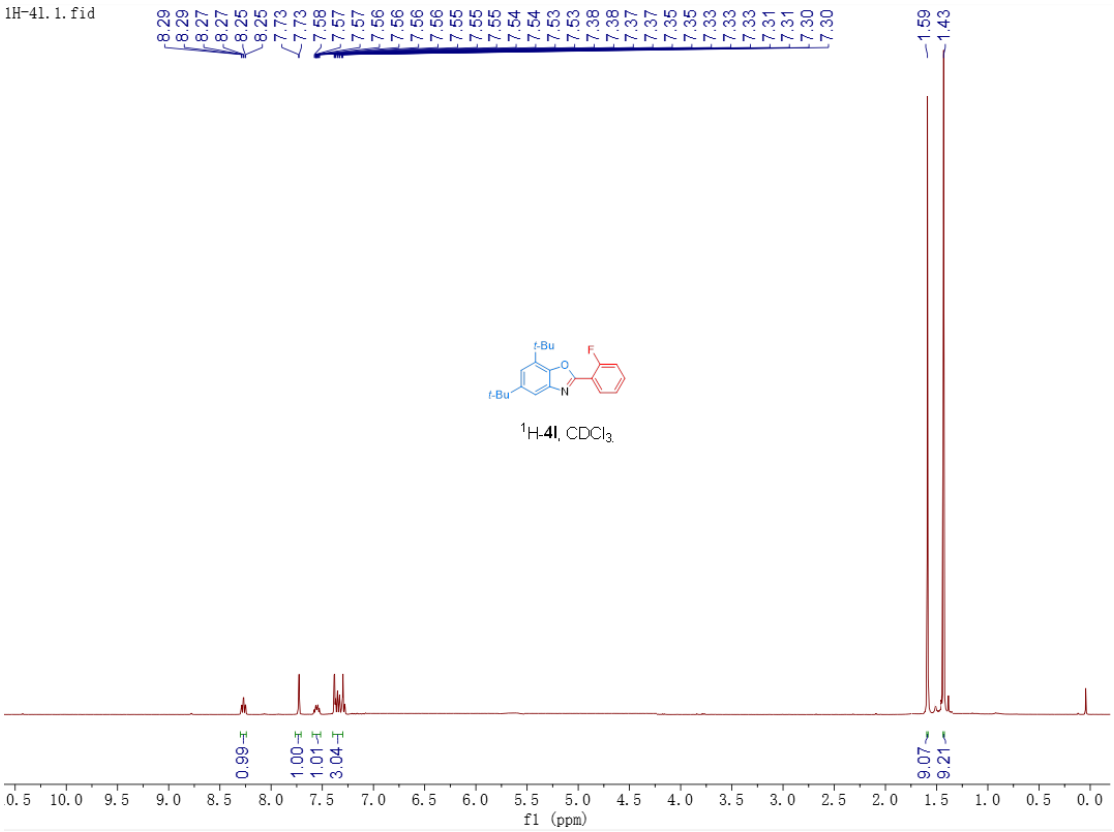
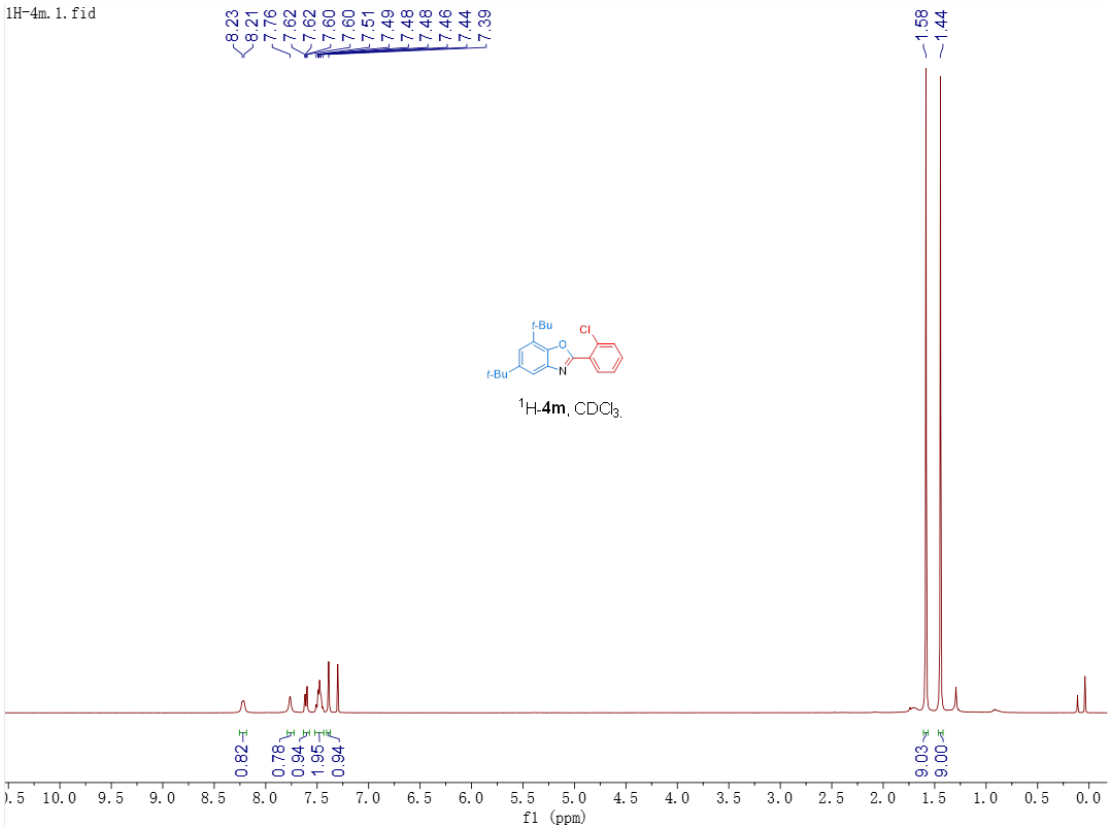
7.53
7.53
7.52
7.50
7.37
7.37
1.59
1.43
t-Bu
t-Bu
O
N
Cl
1H-4j, CDCl3
1.00
1.01
1.00
2.08
1.01
9.15
9.15
0.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)



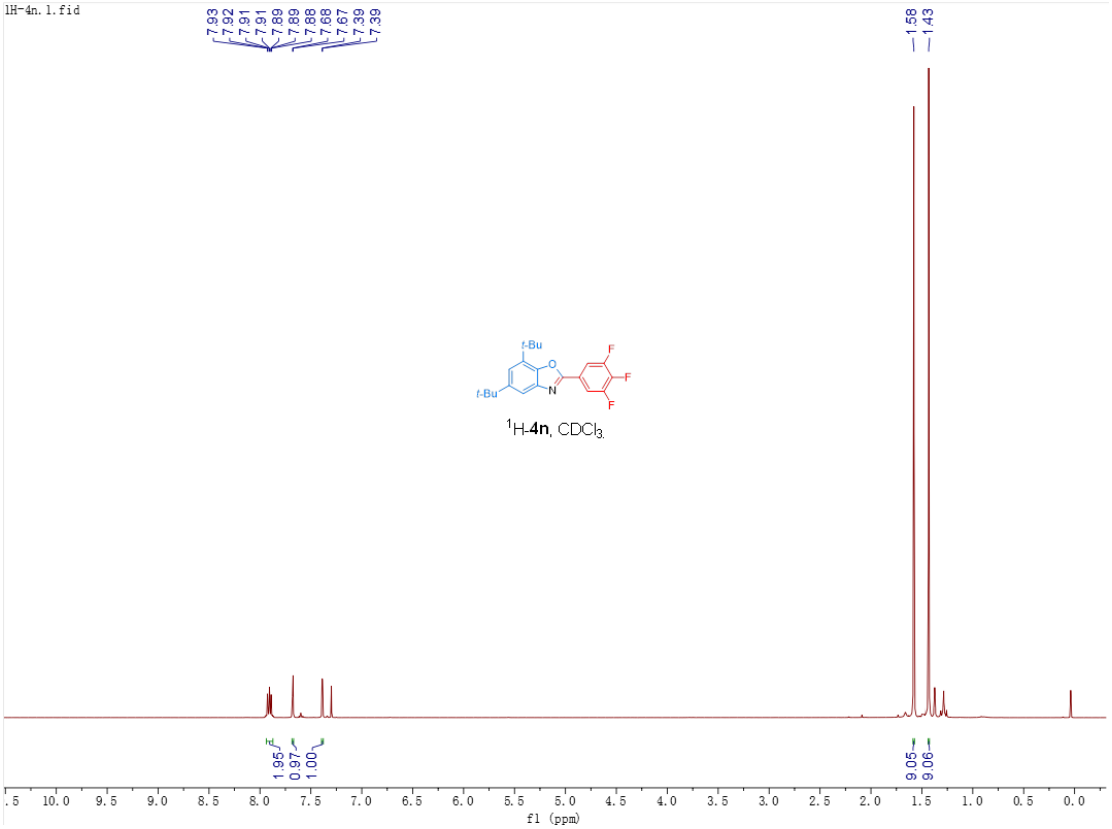
^{1}H-**4k**, $CDCl_3$.



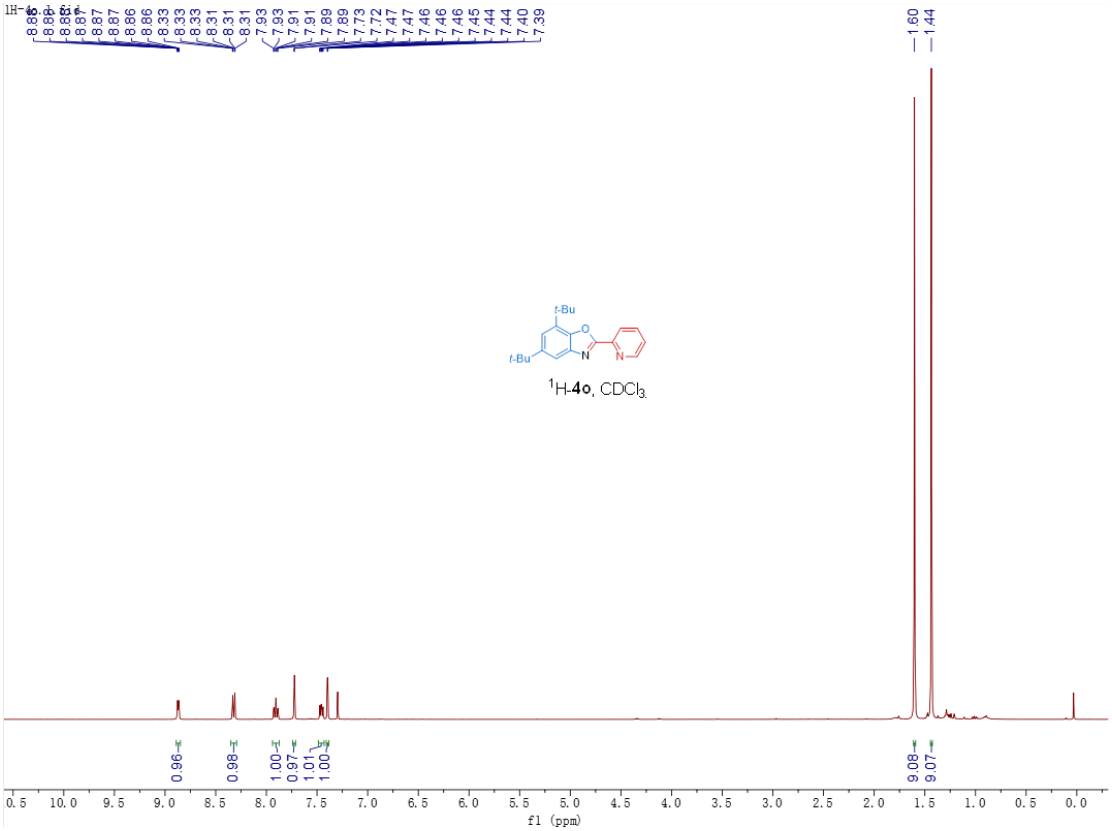
^{1}H-**4l**, $CDCl_3$.



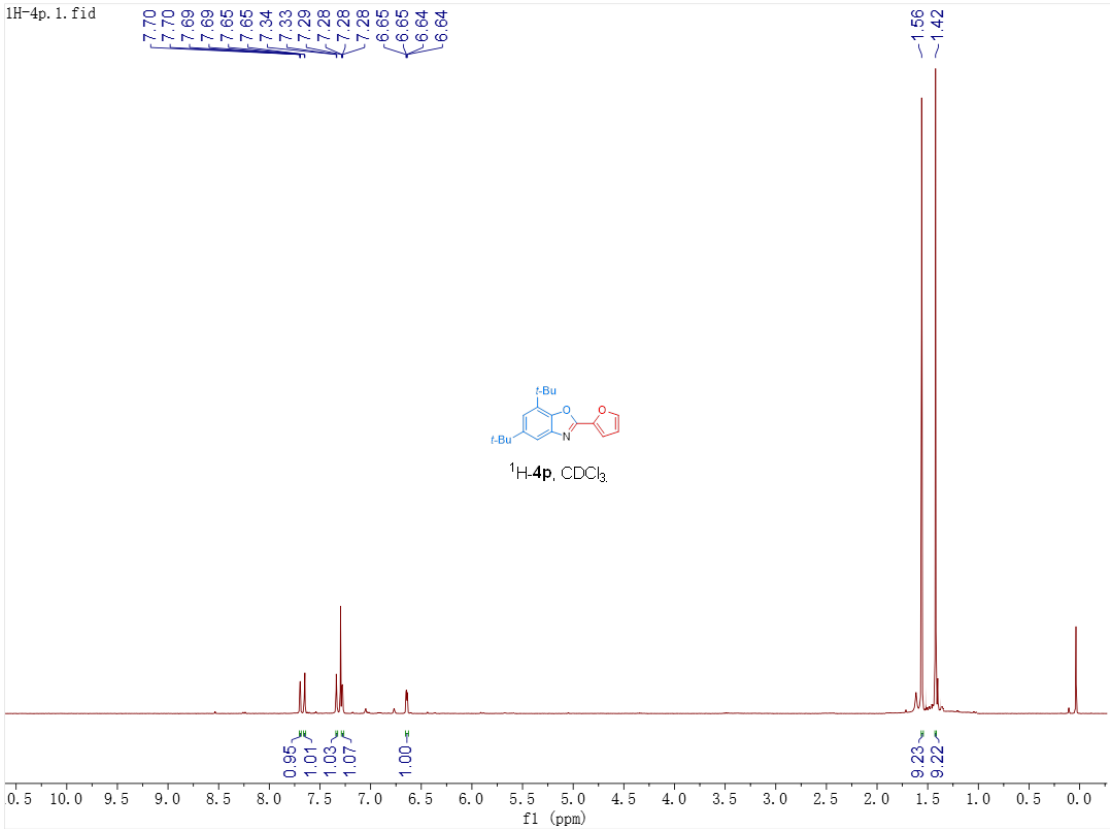
^{1}H-**4m**, $CDCl_3$



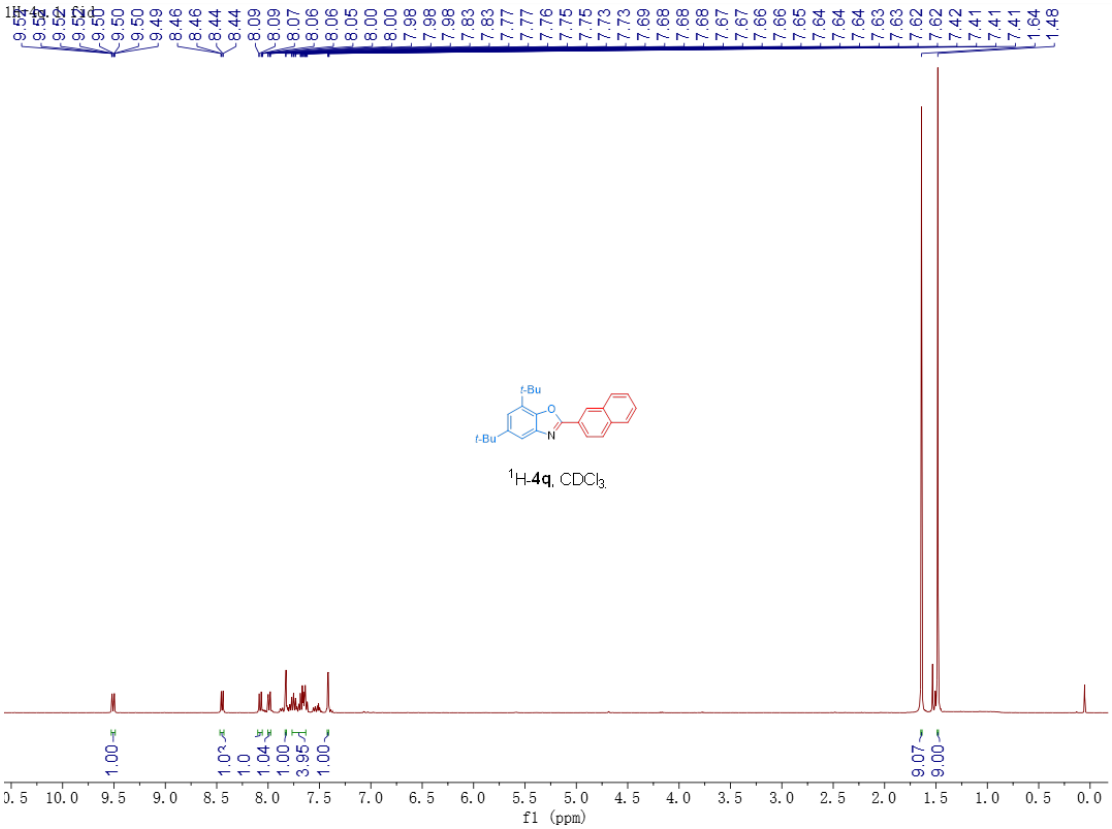
^{1}H-**4n**, $CDCl_3$



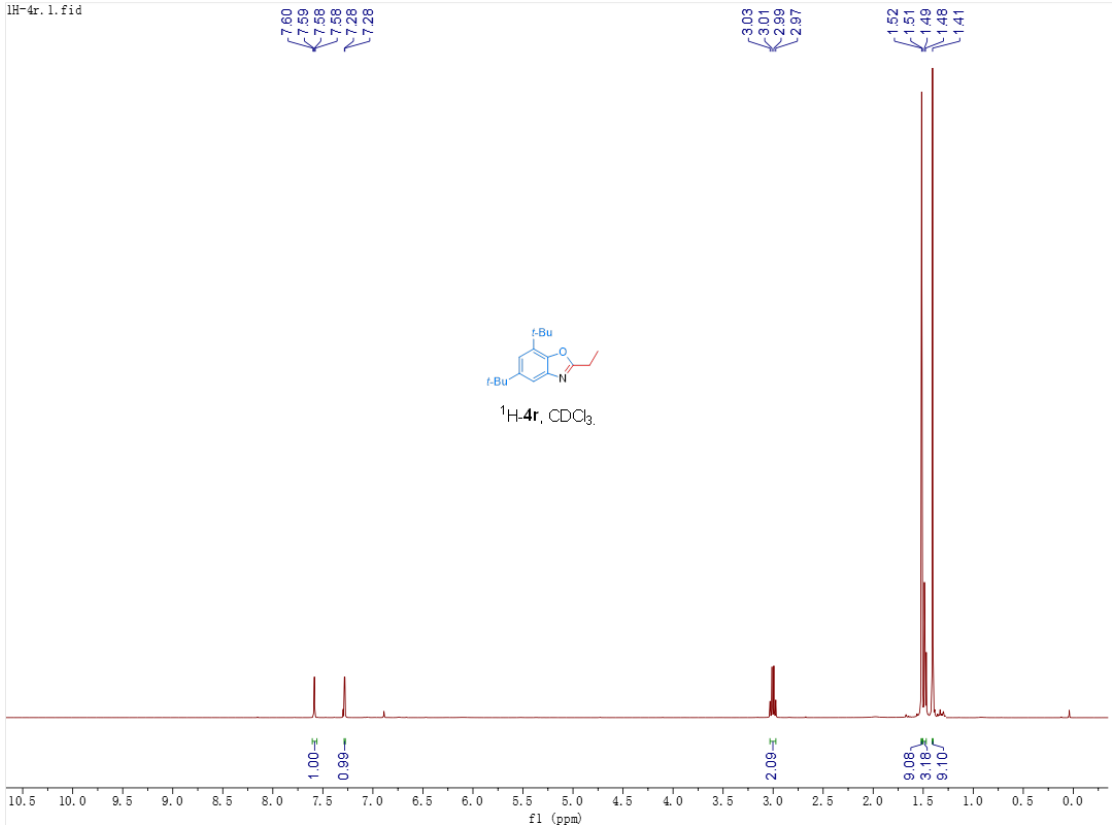
^{1}H-**4o**, $CDCl_3$



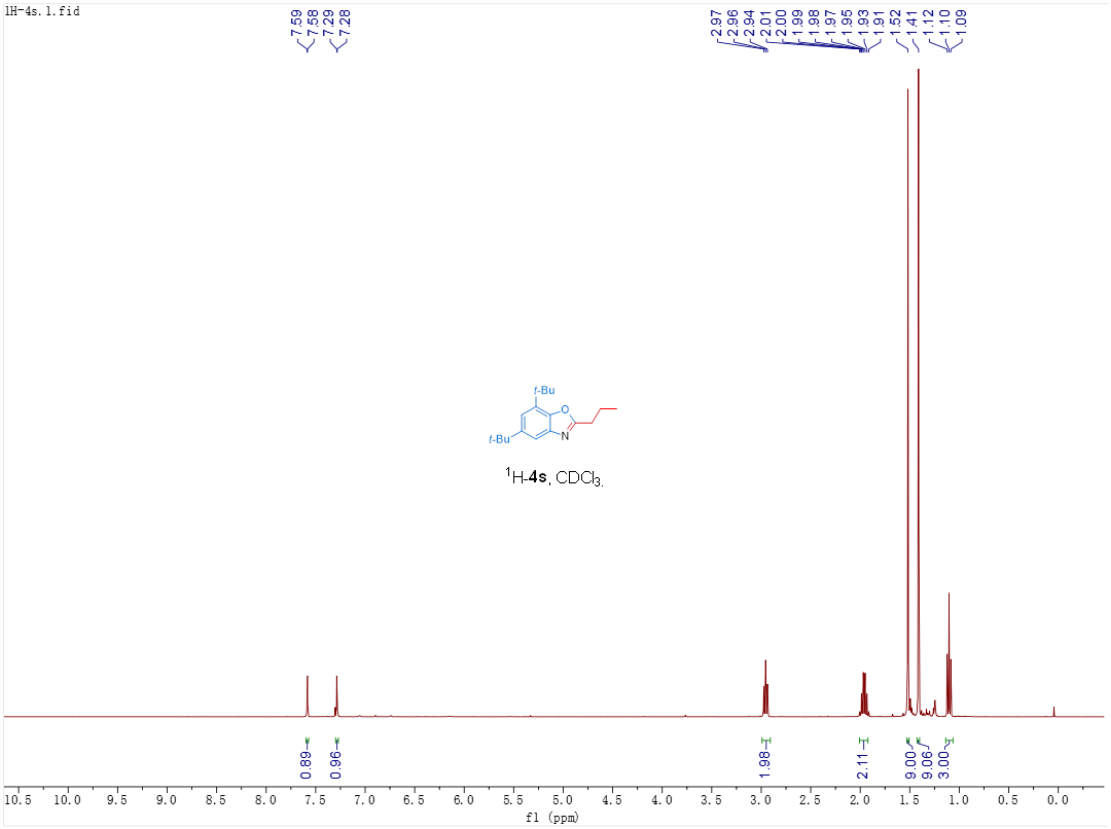
^{1}H-**4p**, $CDCl_3$



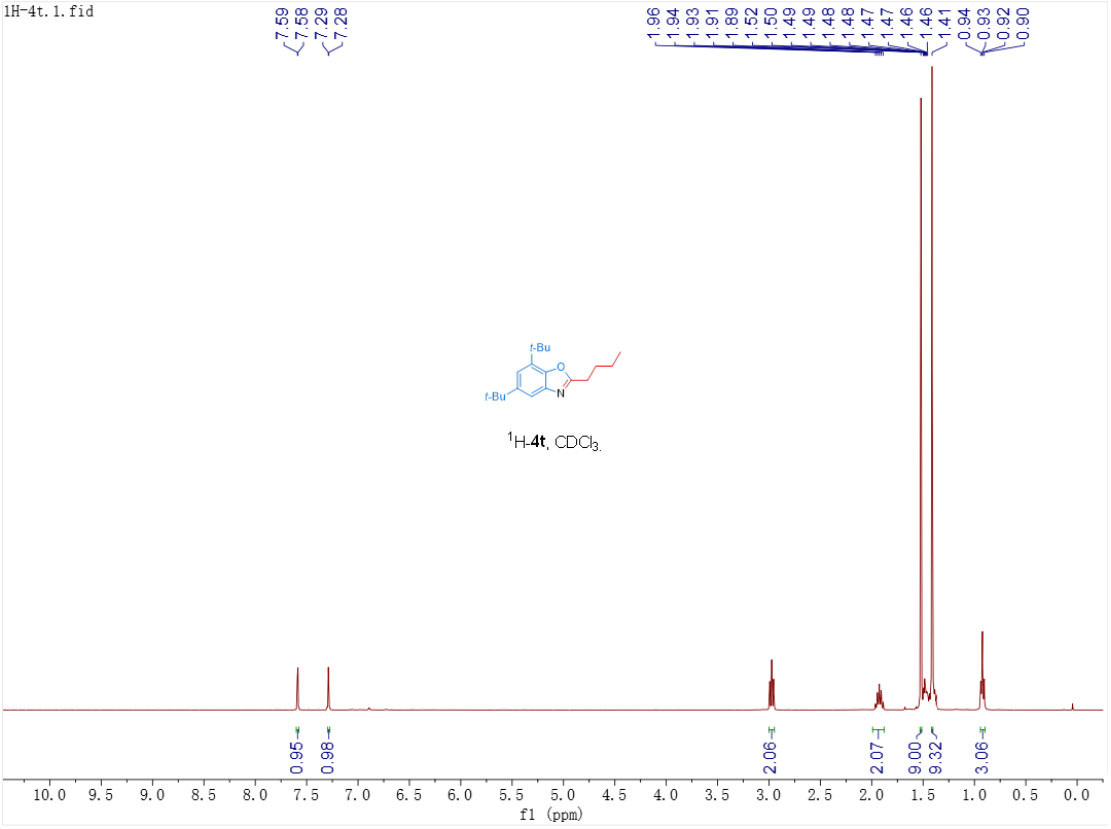
^{1}H-**4q**, $CDCl_3$



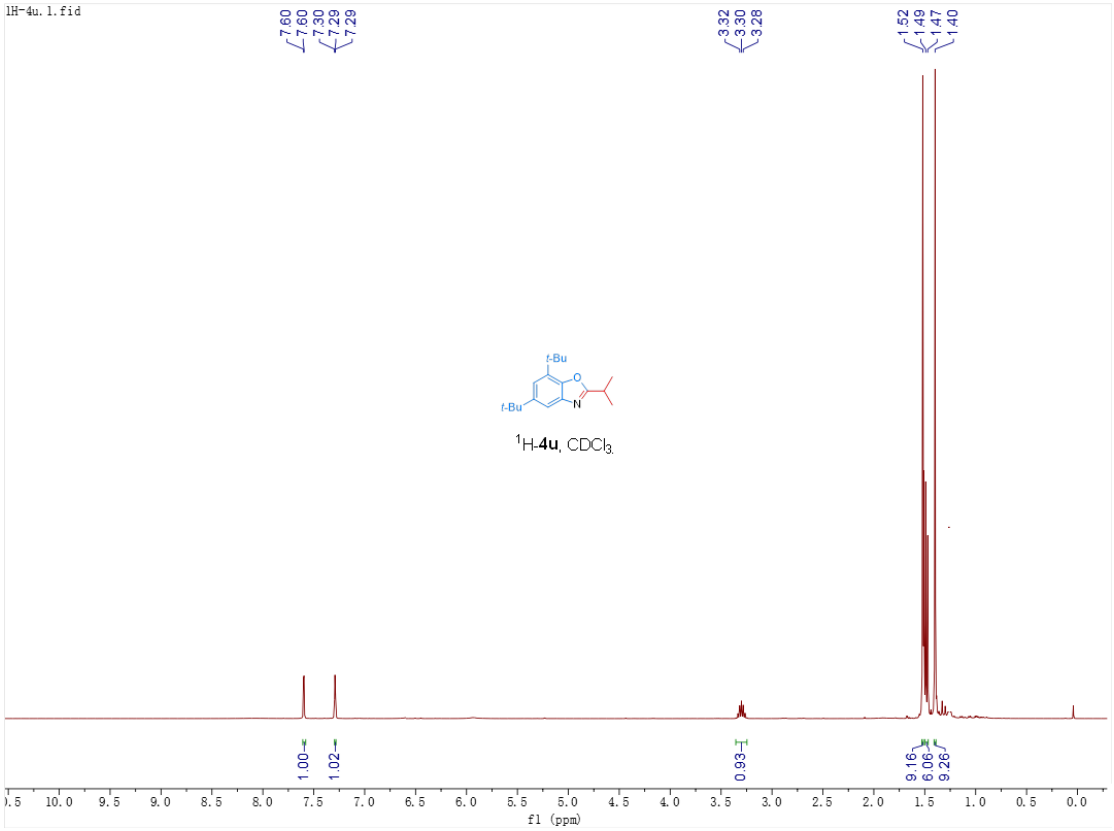
^{1}H-**4r**, $CDCl_3$



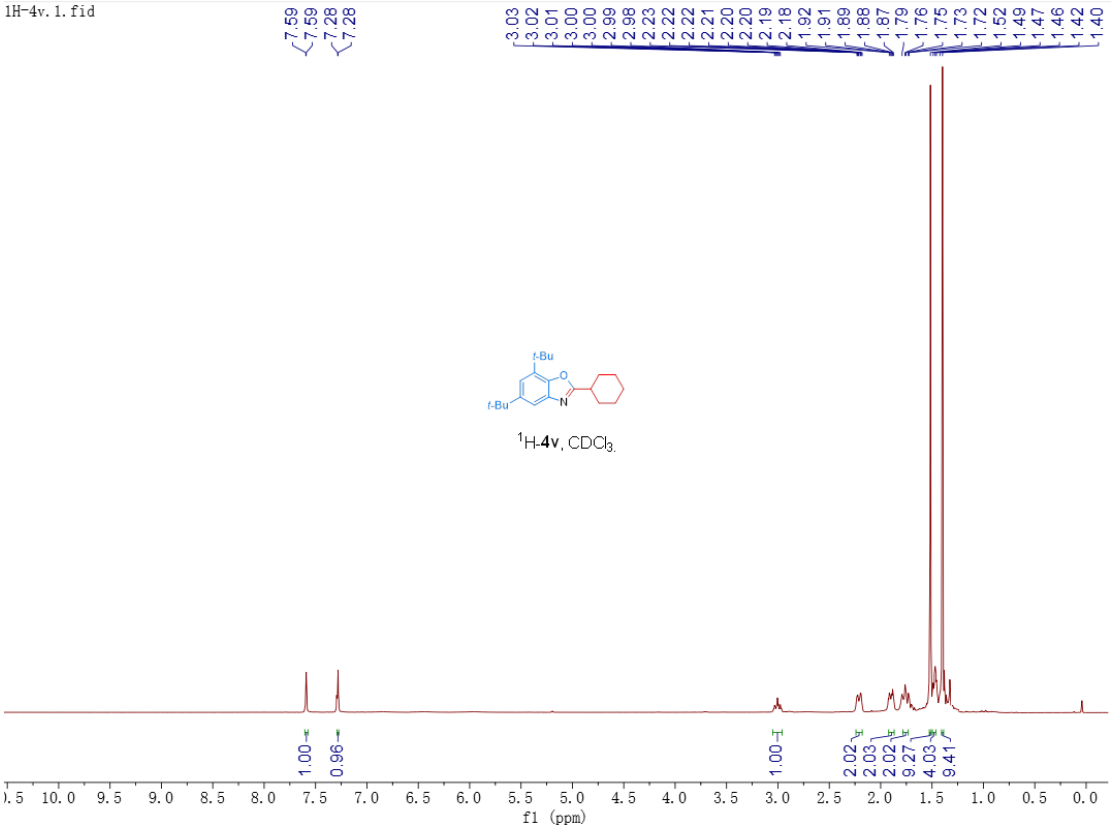
^{1}H-**4s**, $CDCl_3$.



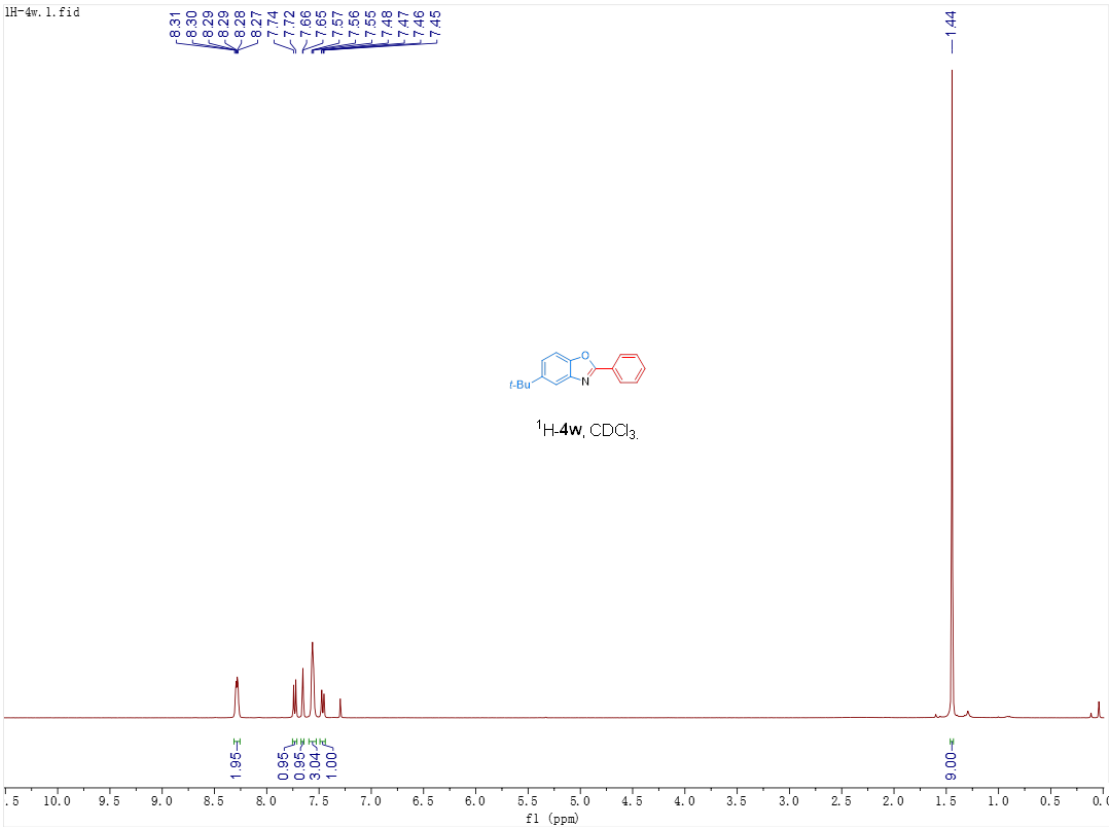
^{1}H-**4t**, $CDCl_3$.



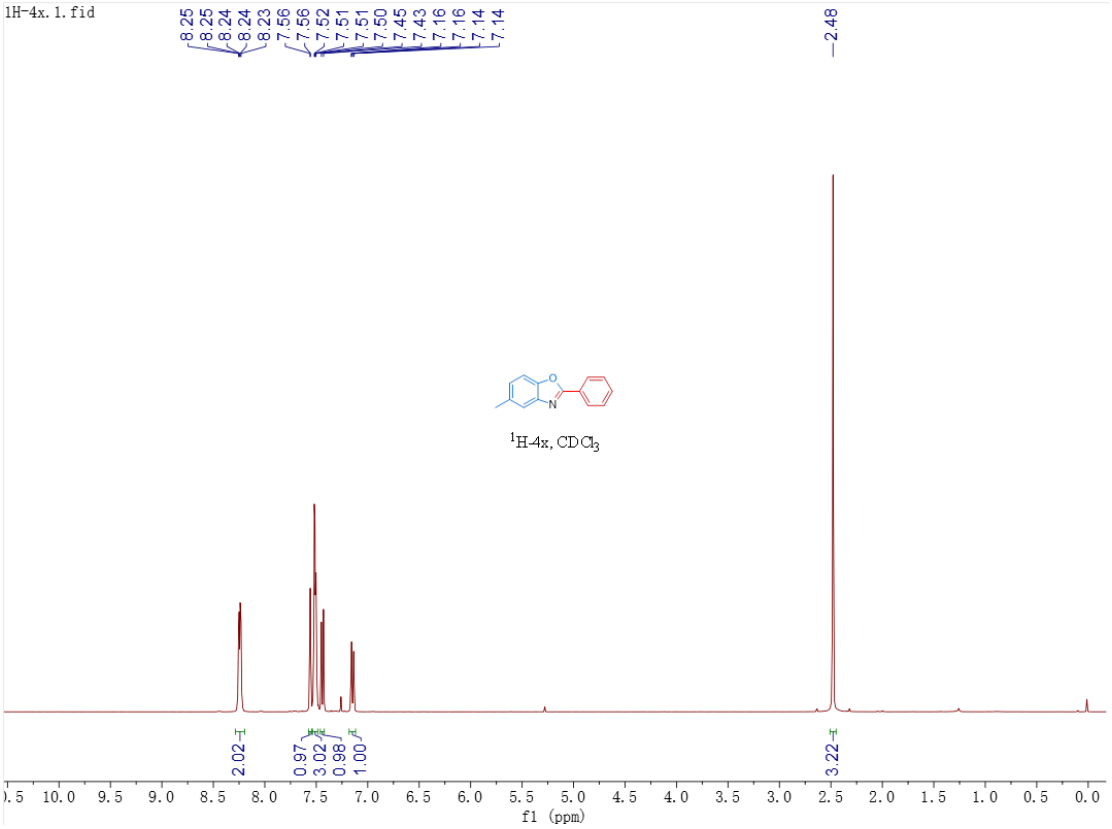
^{1}H-**4u**, $CDCl_3$



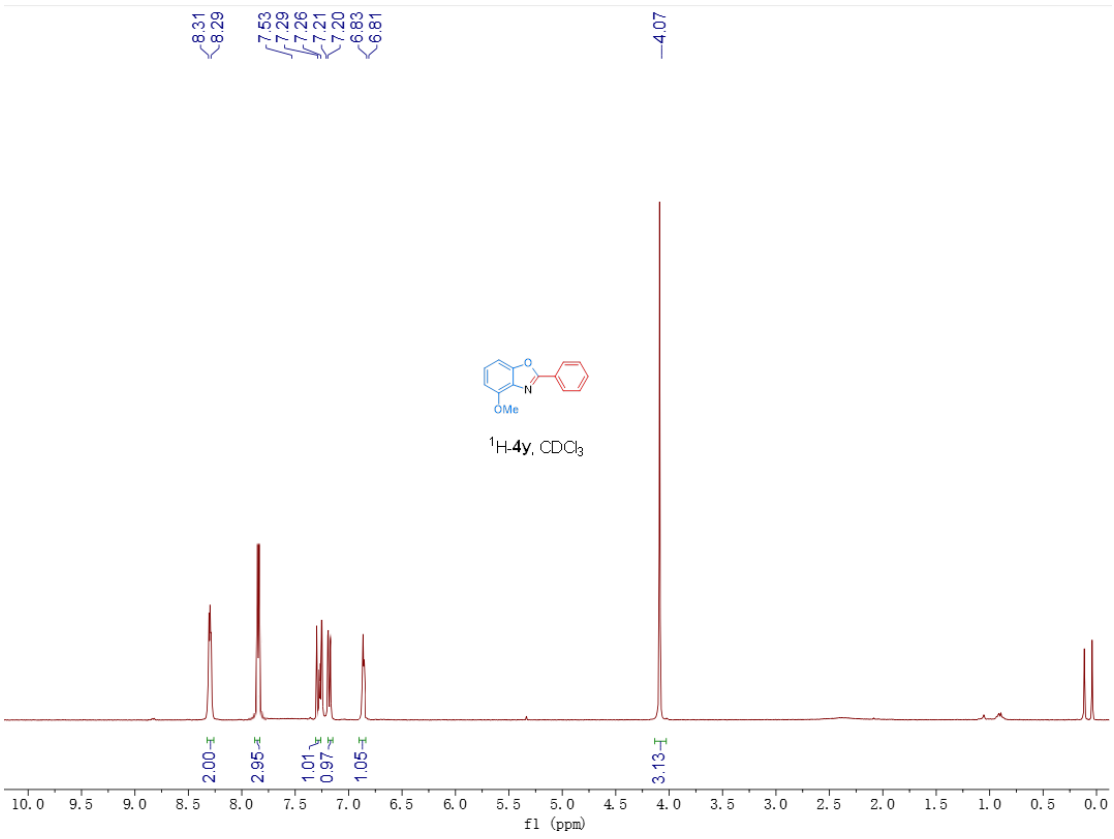
^{1}H-**4v**, $CDCl_3$

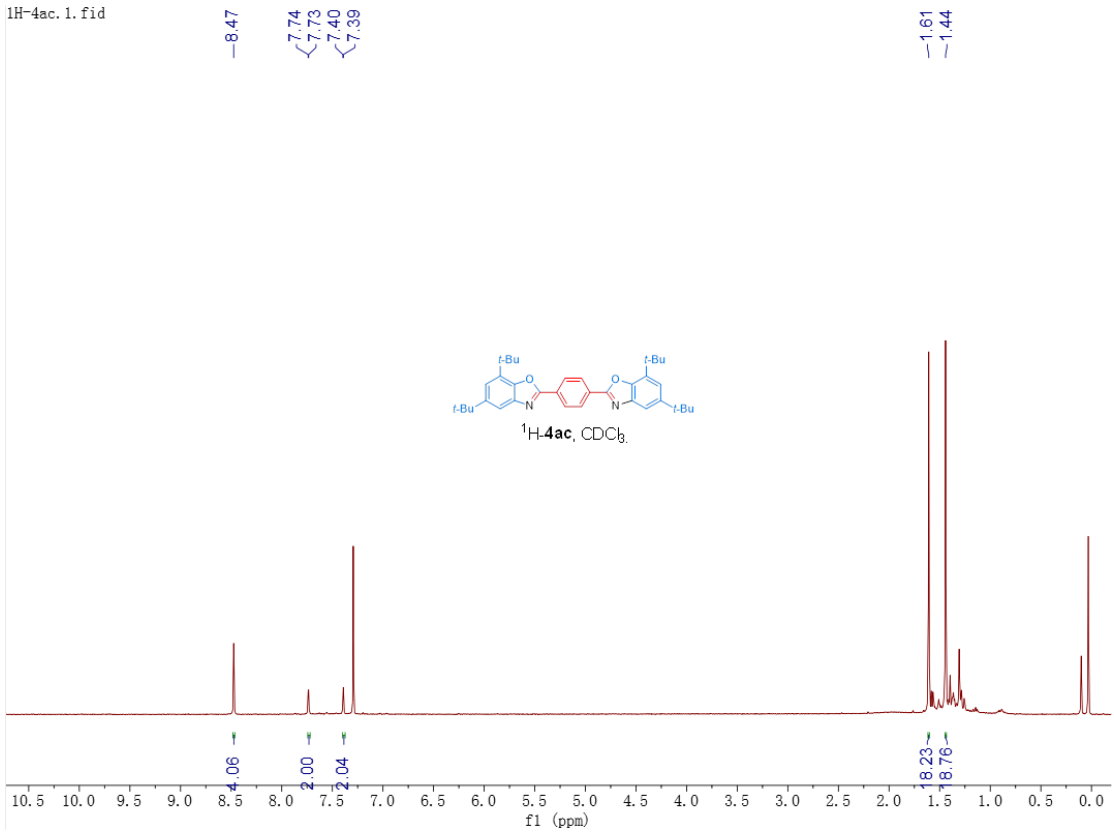


^{1}H-**4w**, $CDCl_3$



^{1}H-4x, $CDCl_3$


8.31
8.29
7.53
7.29
7.26
7.21
7.20
6.83
6.81
4.07
OMe
^{1}H-**4y**, $CDCl_3$
2.00
2.95
1.01
0.97
1.05
3.13
f1 (ppm)


1H-4ac.1.fid
8.47
7.74
7.73
7.40
7.39
1.61
1.44
t-Bu
^{1}H-**4ac**, $CDCl_3$
4.06
2.00
2.04
18.23
8.76
f1 (ppm)

Nucleotide and amino acid sequences of VHb variants

Nucleotide sequence of VHb(Q53H)

ATGTTAGACCAGCAAACCATTAACATCATCAAAGCCACTGTTCCTGTATTGAAGGAG
CATGGCGTTACCATTACCACGACTTTTTATAAAAACTTGTTTGCCAAACACCCTGAAG
TACGTCCTTTGTTTGATATGGGTCGCCAAGAATCTTTGGAGCACCCTAAGGCTTTGGC
GATGACGGTATTGGCGGCAGCGCAAAACATTGAAAATTTGCCAGCTATTTTGCCTGC
GGTCAAAAAAATTGCAGTCAAACATTGTCAAGCAGGCGTGGCAGCAGCGCATTATCC
GATTGTCGGTCAAGAATTGTTGGGTGCGATTAAAGAAGTATTGGGCGATGCCGCAAC
CGATGACATTTTGGACGCGTGGGGCAAGGCTTATGGCGTGATTGCAGATGTGTTTATT
CAAGTGGAAGCAGATTTGTACGCTCAAGCGGTTGAACATCATCATCATCATCATTAA

Amino acid sequence of VHb(Q53H)

MLDQQTINIIKATVPVLKEHGVTITTTFYKNLFAKHPEVRPLFDMGRQESLEHPKALAMT
VLAAAQNIENLPAILPAVKKIAVKHCQAGVAAAHYPIVGQELLGAIKEVLGDAATDDILD
AWGKAYGVIADVFIQVEADLYAQAVEHHHHHH*

Nucleotide sequence of VHb(P54C)

ATGTTAGACCAGCAAACCATTAACATCATCAAAGCCACTGTTCCTGTATTGAAGGAG
CATGGCGTTACCATTACCACGACTTTTTATAAAAACTTGTTTGCCAAACACCCTGAAG
TACGTCCTTTGTTTGATATGGGTCGCCAAGAATCTTTGGAGCAGTGTAAGGCTTTGGC
GATGACGGTATTGGCGGCAGCGCAAAACATTGAAAATTTGCCAGCTATTTTGCCTGC
GGTCAAAAAAATTGCAGTCAAACATTGTCAAGCAGGCGTGGCAGCAGCGCATTATCC
GATTGTCGGTCAAGAATTGTTGGGTGCGATTAAAGAAGTATTGGGCGATGCCGCAAC
CGATGACATTTTGGACGCGTGGGGCAAGGCTTATGGCGTGATTGCAGATGTGTTTATT
CAAGTGGAAGCAGATTTGTACGCTCAAGCGGTTGAACATCATCATCATCATCATTAA

Amino acid sequence of VHb(P54C)

MLDQQTINIIKATVPVLKEHGVTITTTFYKNLFAKHPEVRPLFDMGRQESLEQCKALAMT
VLAAAQNIENLPAILPAVKKIAVKHCQAGVAAAHYPIVGQELLGAIKEVLGDAATDDILD
AWGKAYGVIADVFIQVEADLYAQAVEHHHHHH*

Nucleotide sequence of VHb(Q53H, P54C)

ATGTTAGACCAGCAAACCATTAACATCATCAAAGCCACTGTTCCTGTATTGAAGGAG
CATGGCGTTACCATTACCACGACTTTTTATAAAAACTTGTTTGCCAAACACCCTGAAG
TACGTCCTTTGTTTGATATGGGTCGCCAAGAATCTTTGGAGCACTGTAAGGCTTTGGC
GATGACGGTATTGGCGGCAGCGCAAAACATTGAAAATTTGCCAGCTATTTTGCCTGC
GGTCAAAAAAATTGCAGTCAAACATTGTCAAGCAGGCGTGGCAGCAGCGCATTATCC
GATTGTCGGTCAAGAATTGTTGGGTGCGATTAAAGAAGTATTGGGCGATGCCGCAAC
CGATGACATTTTGGACGCGTGGGGCAAGGCTTATGGCGTGATTGCAGATGTGTTTATT
CAAGTGGAAGCAGATTTGTACGCTCAAGCGGTTGAACATCATCATCATCATCATTAA

Amino acid sequence of VHb(Q53H, P54C)

MLDQQTINIIKATVPVLKEHGVTITTTFYKNLFAKHPEVRPLFDMGRQESLEHCKALAMT

VLAAAQNIENLPAILPAVKKIAVKHCQAGVAAAHYPIVGQELLGAIKEVLGDAATDDILD
AWGKAYGVIADVFIQVEADLYAQAVEHHHHHH*

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