Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Real-time visualization of butyrylcholinesterase activity using a highly selective and sensitive chemiluminescent probe

Alperen Acari,[a1] Toghrul Almammadov,[b,c1] Musa Dirak,[b] Goktug Gulsoy,[b] and Safacan Kolemen[a,b,d*]

[a]Koç University Research Center for Translational Medicine (KUTTAM), 34450 Istanbul, Türkiye

[b]Koç University, Department of Chemistry, 34450 Istanbul, Türkiye

[c]Univesity of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich, Switzerland

[d]Koç University Surface Science and Technology Center (KUYTAM), 34450 Istanbul, Türkiye

[1]*These authors contributed equally.*

1. Materials and Instruments

All commercially available reagents were used without further purification unless otherwise noted. Butyrylcholinesterase from horse serum (C7512) 9-Amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate (Tacrine – A79922) were purchased from Sigma-Aldrich. Anhydrous solvents were obtained through 4 Å molecular sieve, anhydrous THF was obtained through the Mbraun MBSPS5 solvent drying system. The inert atmosphere was obtained by argon. ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on Bruker Avance III Ultrashield (500 MHz) spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. Coupling constants (J values) are given in Hz and chemical shifts are reported as parts per million (ppm). Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). NMR spectra were processed with MestReNova program. Column chromatography was performed by using thick-walled glass columns and silica Gel 60 (Merck 230-400 mesh). Thin layer chromatography (TLC Merck Silica Gel 60 F254) was performed by using commercially prepared 0.25 mm silica gel plates and visualization was provided by UV lamp. Mass analyses were performed with Waters Vion QTOF mass spectrometer. The relative proportions of solvents in chromatography solvent mixtures refer to the volume:volume ratio. Fluorescence spectra were acquired on Agilent Cary Eclipse spectrophotometer. Luminescence measurements were collected by a Biotek Synergy H1 MF microplate reader. Confocal microscopy images were taken by Leica DMI8 SP8 Inverted Confocal Microscope. *In vivo* imaging was performed by using Perkin Elmer IVIS Lumina Series III imaging system.

2. Optical Measurements

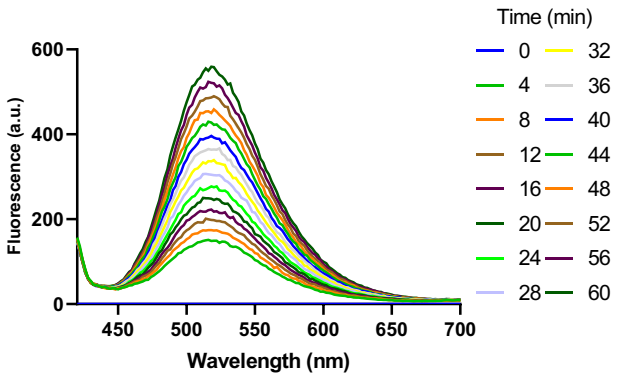

Figure S1. Time-dependent fluorescence spectra of **BCC** (10 μM) upon addition of 20 U/mL BChE in PBS (1% DMSO, pH 7.4) (λ_{ex}= 400 nm, slit width 10/10).

3. HR-MS spectrum of BCC and the resulting benzoate

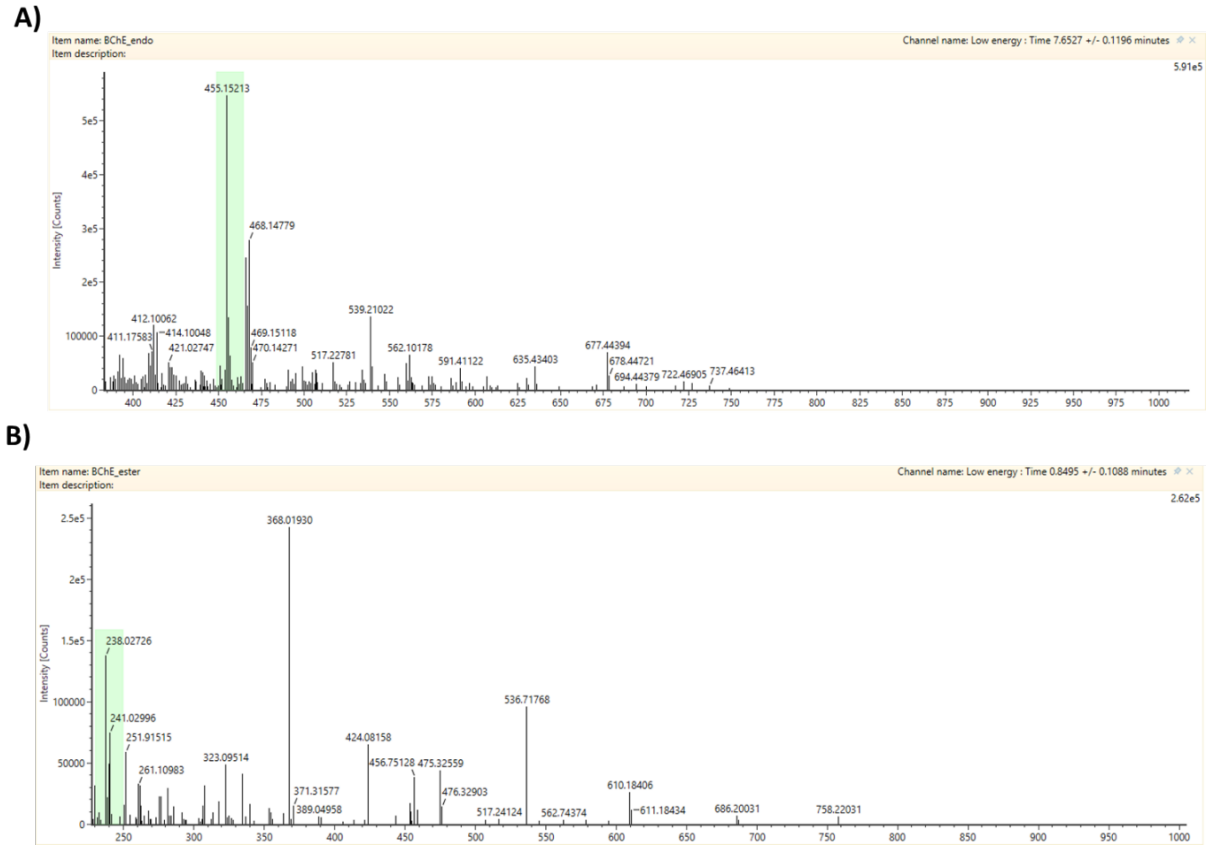

Figure S2. Mass Spectroscopy of (A) **BCC** and (B) the resulting (benzoate) upon addition of BChE. Calculated: 238.02710, found: 238.02726 $[M+H]^+$.

4. Detection of the Chemiluminescence Signal in Solution

All measurements were done in 1% DMSO PBS at pH 7.4 by using Biotek Synergy H1 MF microplate reader.

Detection Limit:

The detection limit was calculated by using the "3 * σ / k" formula, where σ is the standard deviation of the chemiluminescent signal of **BCC** (n=6) and the k is the slope of the enzymatic activity calibration curve (Figure S3).

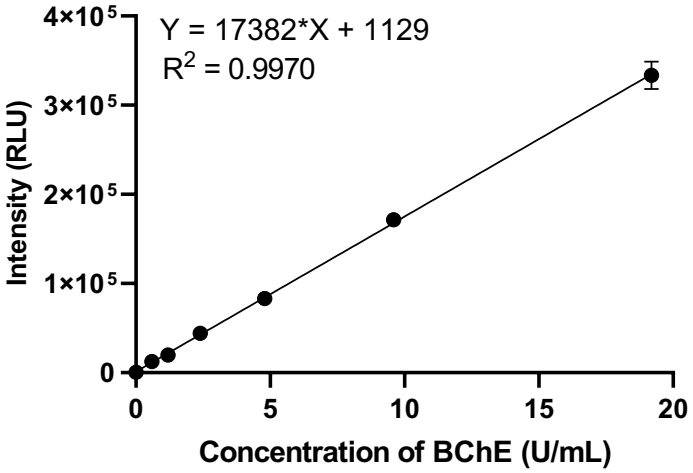

Figure S3. Calibration curve of the chemiluminescent signal intensity (at t= 9 min).

Inhibition efficacy and inhibition coefficient:

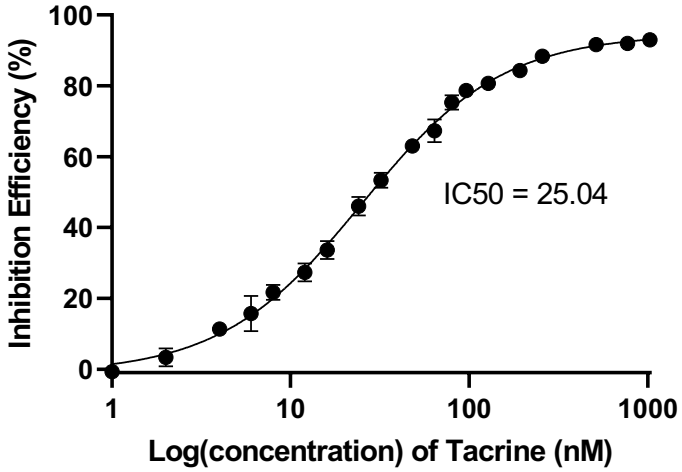

Figure S4. Tacrine concentration dependent inhibition of 2.4 U/mL BChE when treated with 10 μM **BCC** in PBS (1% DMSO, pH 7.4).

5. Cell culture Studies

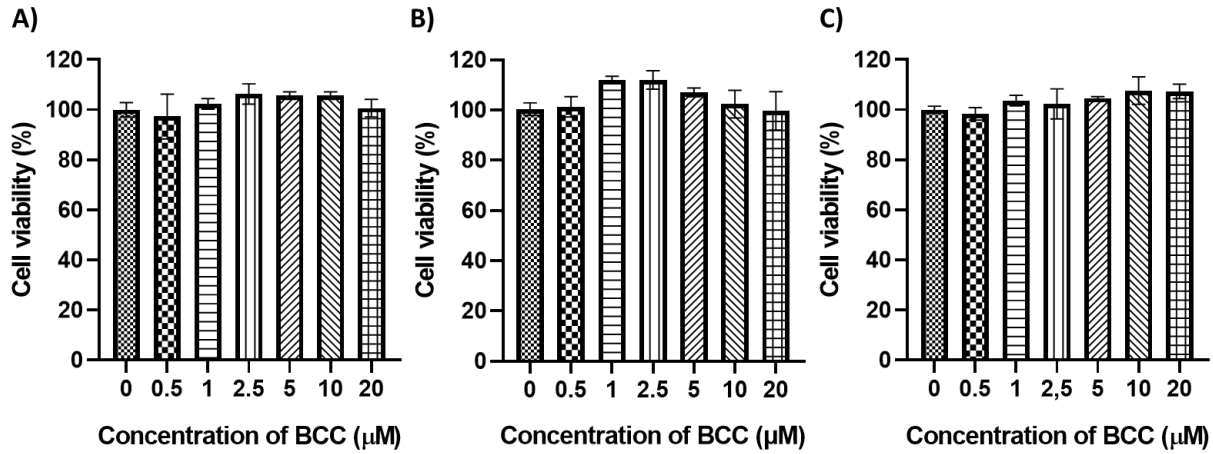

Figure S5. Cell viability of A) HEK293T, B) HepG2, C) SH-SY5Y upon incubating with **BCC**. Results were obtained from CellTiter-Glo® Luminescent Cell Viability Assay.

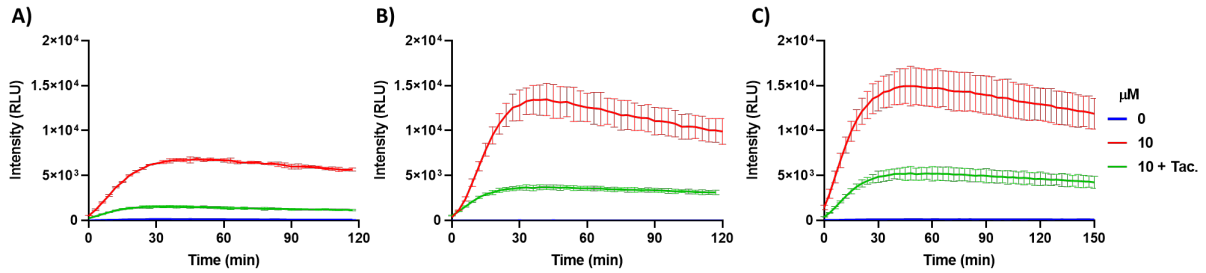

Figure S6. Time- and concentration-dependent luminescence intensity of **BCC** before and after Tacrine (50 μM) incubation for 1 hour. A) HEK293T, B) HepG2, C) SH-SY5Y.

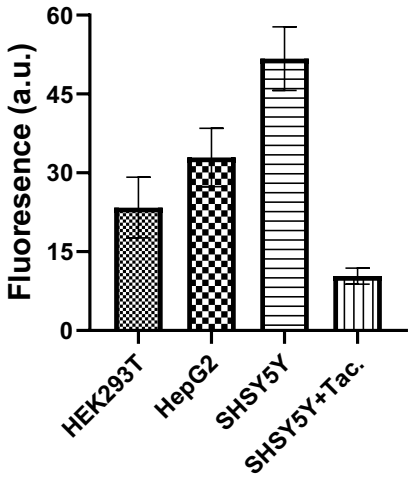

Figure S7. The fluorescence intensity of **BCC** (10 μM) in different cell lines upon 2 hours of incubation.

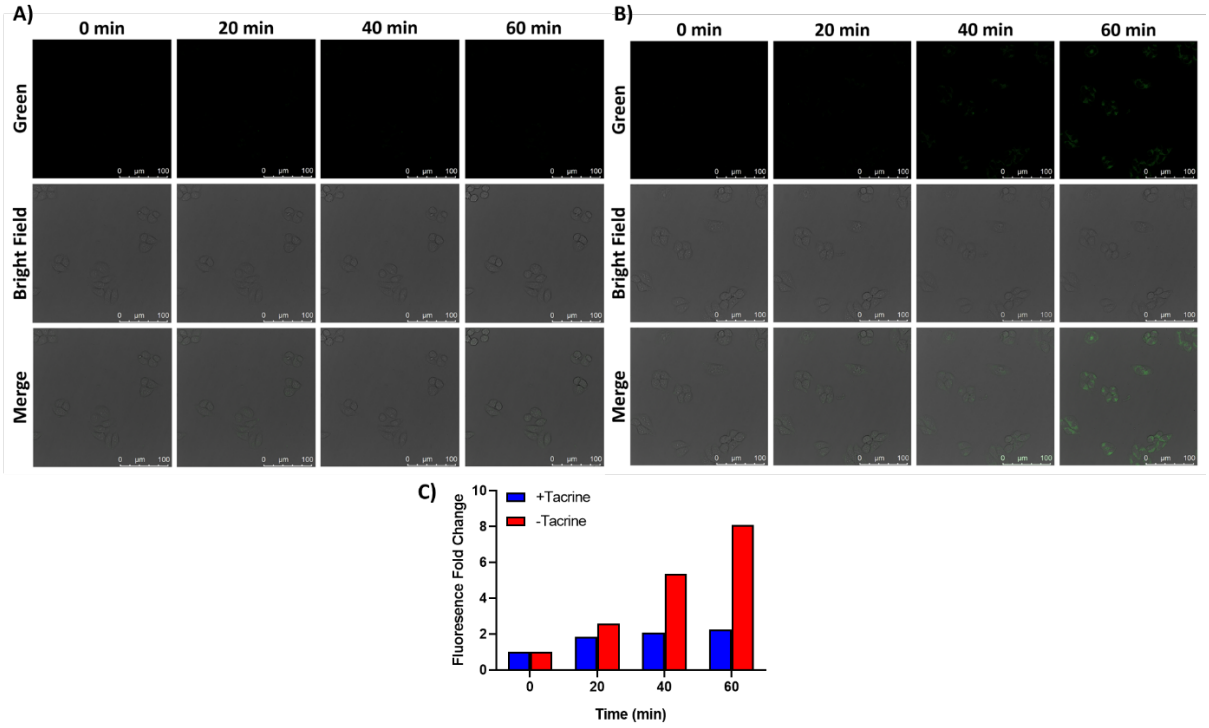

Figure S8. Time-dependent confocal images of SH-SY5Y cells treated with **BCC** (10 μM): (A) with Tacrine (50 μM) incubation for 1 hour and (B) without Tacrine. (C) Fold change of fluorescence signal. λ_{ex}= 405 nm / λ_{em}= 500-600 nm.

6. Previous Literature:

Table S1. A comparison of published fluorescence probes for the detection of BChE.

Name	Detection Limit	Type	*In vivo* Applicability	References
BChE-FP	N/A	Fluorophore	N/A	Chem.Commun. **2017**, 53, 3952
BChE-NIRFP	N/A	Fluorophore	Zebra Fish Model Alzheimer's disease model via prefrontal cortex injection	ACS. Sens. **2018**, 3, 2118
DCPDA	0.06 U/mL	Fluorophore	N/A	Analyst. **2019**, 144, 559
DSC	0.0012 U/mL	Fluorophore	N/A	Chem.Commun. **2019**, 55, 14574
IPAN	0.0117 U/mL	Fluorophore	Zebra Fish Model	Anal. Chem. **2020**, 92, 13405
CyCICP	0.00375 U/mL	Fluorophore	N/A	Talanta. **2020**, 219, 121278
BChE-NBD	29 ng/mL	Fluorophore	Mouse Liver Imaging	Sensors and Actuators B: Chemical. **2021**, 330, 129348

P1	0.8 U/mL	Fluorophore	Healthy Mouse Brain Imaging via prefrontal cortex injection	ACS. Sens. **2021**, 6, 1138
P1	1080 ng/mL	Fluorophore	Alzheimer's disease model via prefrontal cortex injection	Anal. Chem. **2021**, 93, 11337
W1	77 ng/mL	Ratiometric Fluorophore (ACQ)	N/A	Dyes Pigm. **2022**, 197, 109874.
TB-BChE	39.24 ng/mL	Ratiometric Fluorophore (AIE)	Nonalcoholic Fatty Liver Model Healthy Mouse Model	J. Mater. Chem. B, **2022**, 10, 4254
DX-2	0.08 U/mL	Fluorophore	Tumor Model	Anal. Chim. Acta, **2022**, 1235, 340540
Chy-1	0.12 ng/mL	Fluorophore	Alzheimer's disease model via prefrontal cortex injection Healthy Mouse Model Tumor Model	Anal. Chem. **2022**, 94, 39, 13498–13506
HBT-BChE	0,000754 U/mL	Fluorophore (ESIPT-AIEE)	Zebra Fish Model	Spectrochim. Acta - A: Mol. Biomol. Spectrosc **2023**, 287 122044
BCC	**0.015 U/mL**	**Chemiluminescence**	**Healthy Mouse Model Tumor Model**	**This Work**

7. NMR Spectra

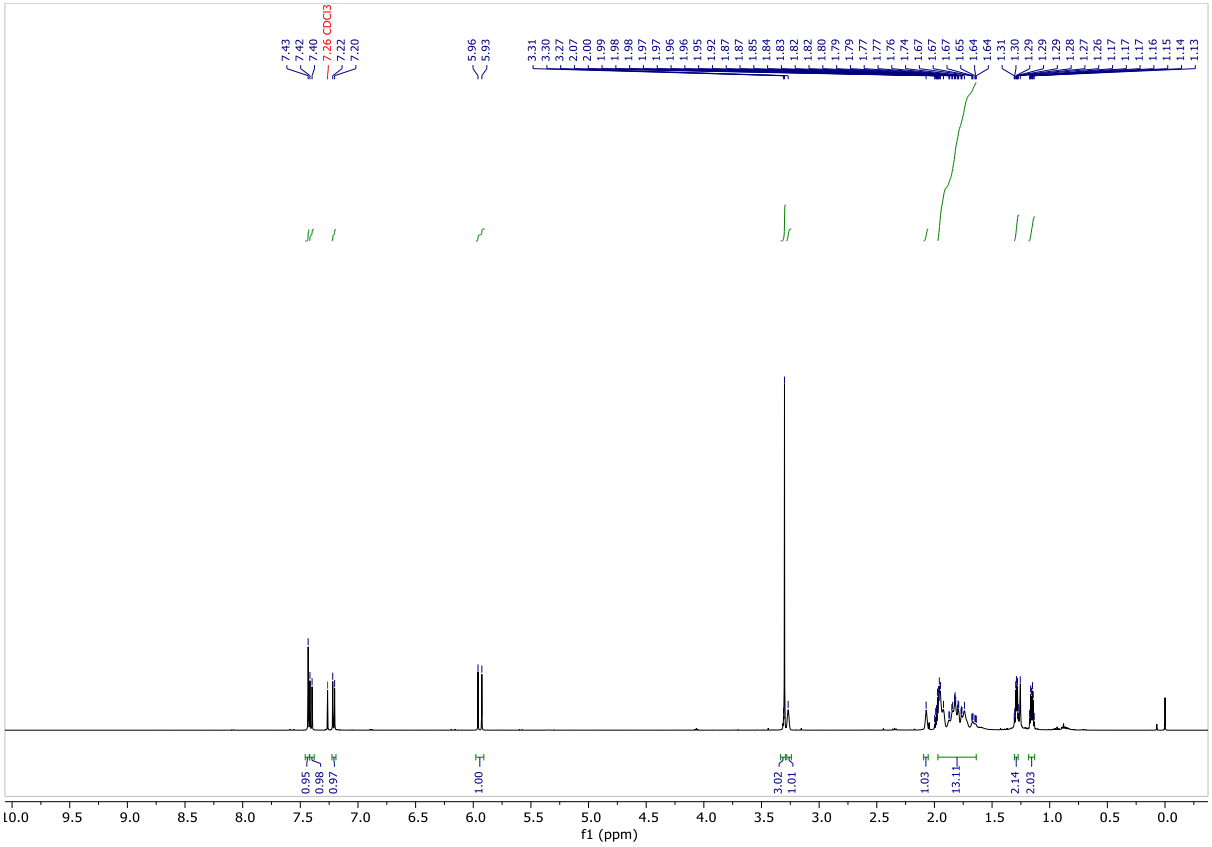

Figure S9. ^{1}H NMR spectrum of **2**.

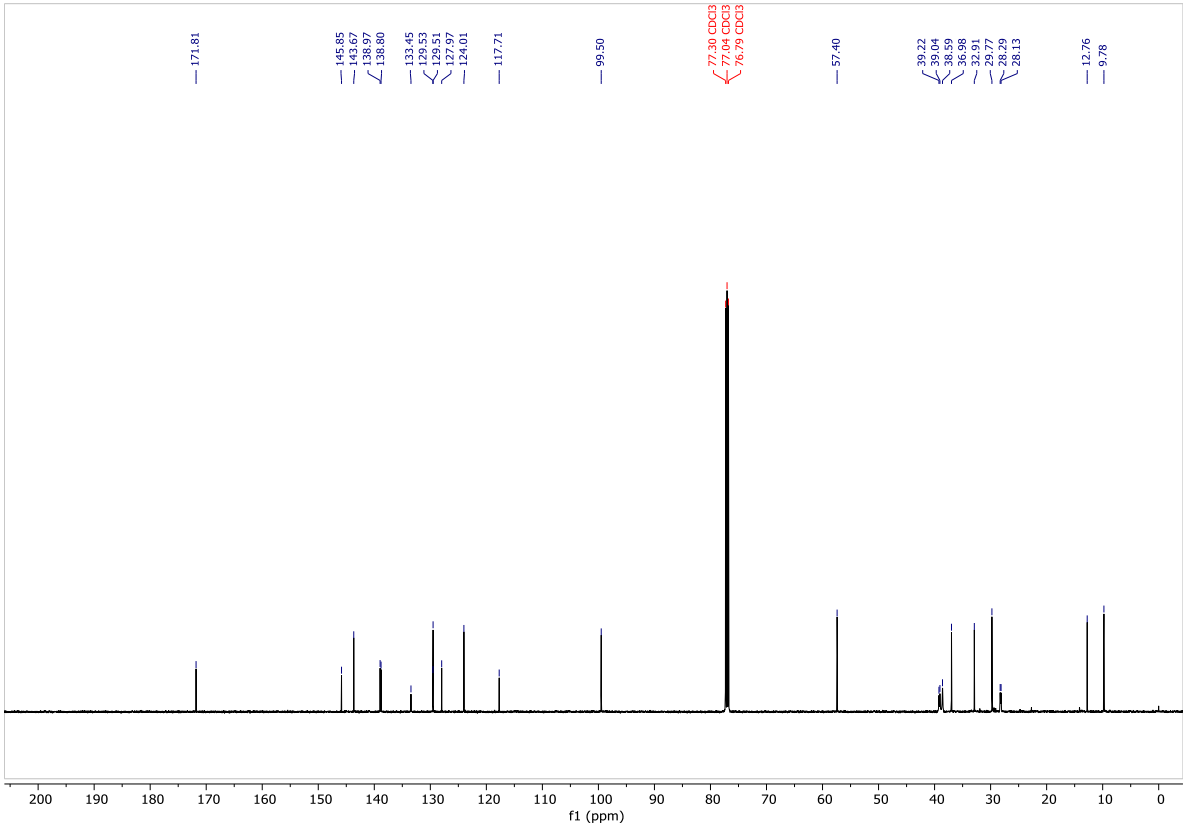

Figure S10. ^{13}C NMR spectrum of **2**.

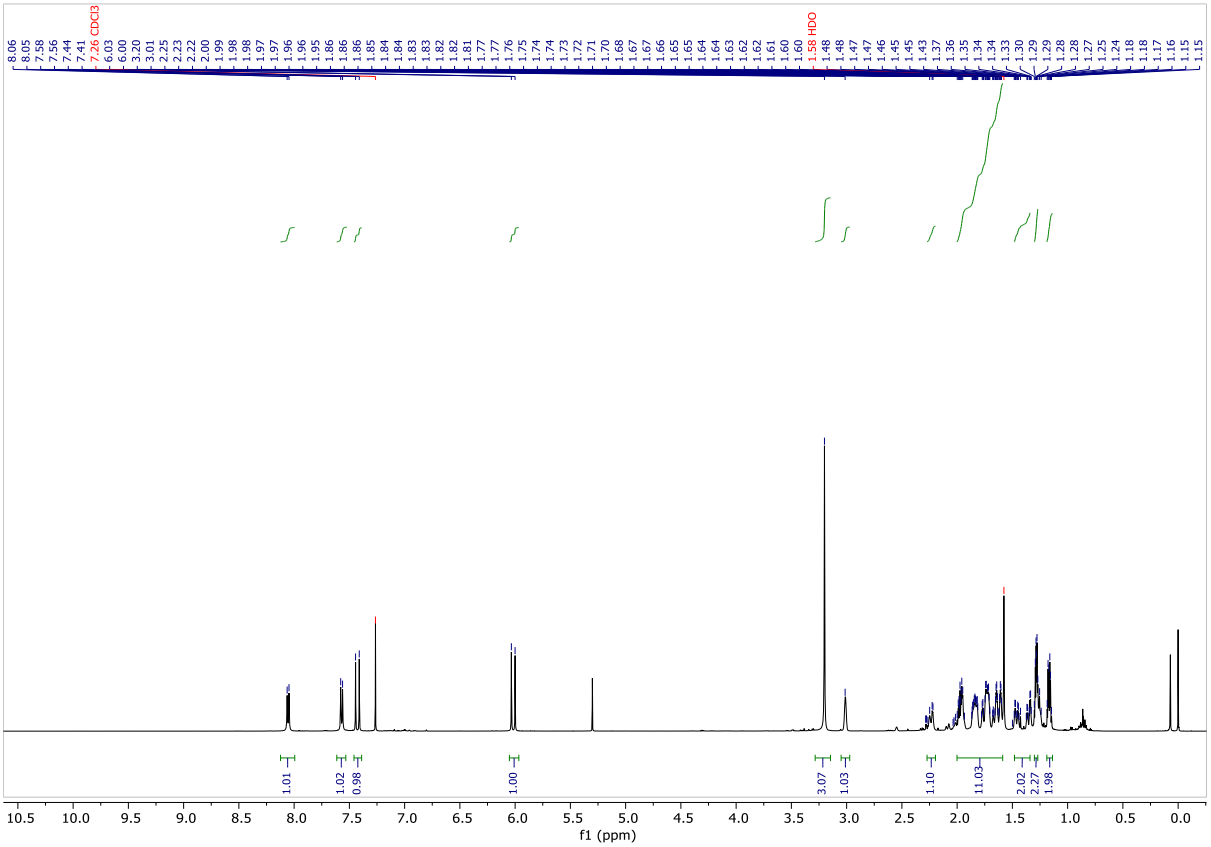

Figure S11. ^{1}H NMR spectrum of **BCC**.

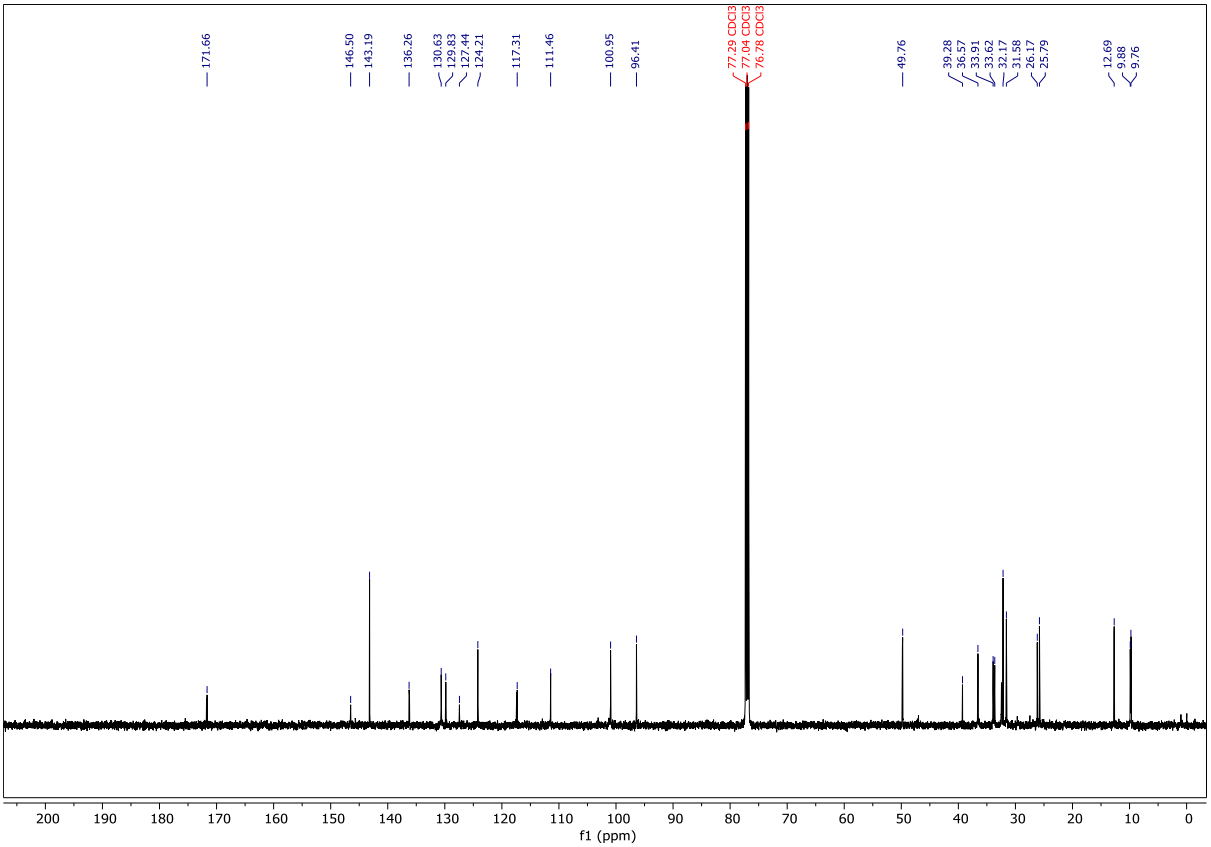

Figure S12. ^{13}C NMR spectrum of **BCC**.

8. Mass Spectra

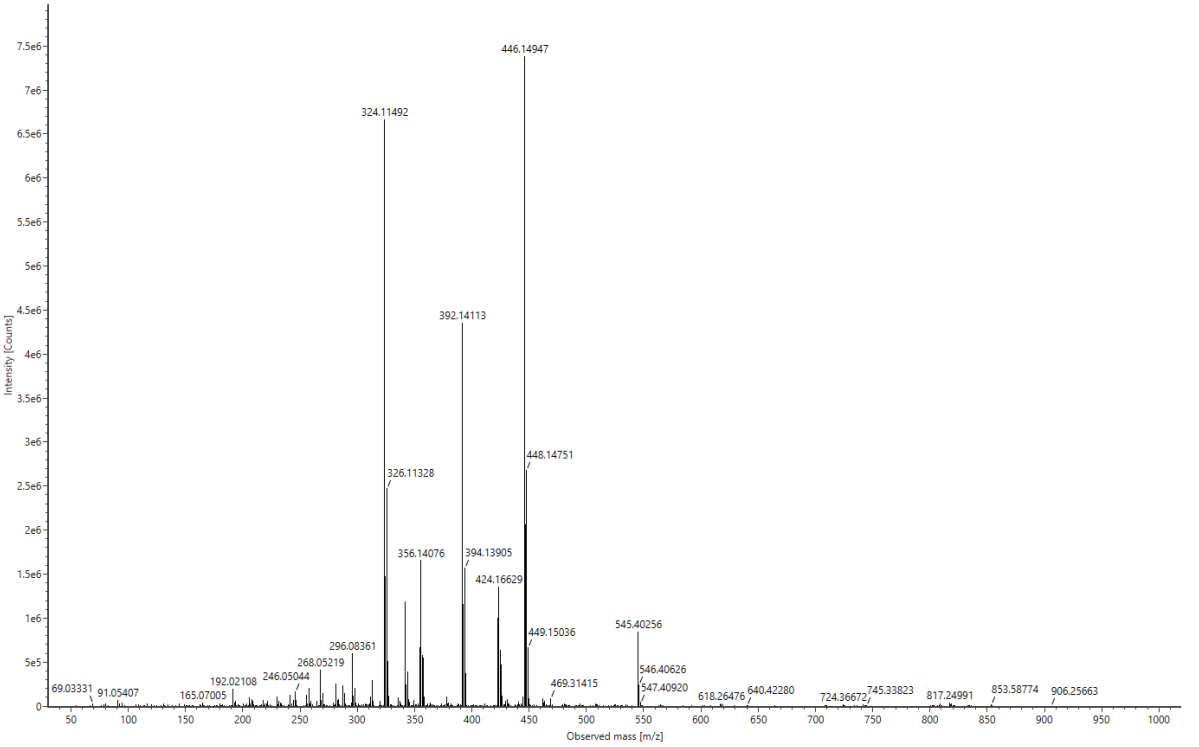

Figure S13. HR-MS spectrum of **2**.