

**Identification of the first selective bioluminescent probe for real-time monitoring
of carboxylesterase 2 *in vitro* and *in vivo***

Yuhao Chen,^{a,b,†} Tiantian Zhao,^{a,†} Zhuang Miao,^{a,†} Tianguang Huang,^a Meiyuan Chen,^a
Yi Zhao,^a Ao Hai,^a Qingrong Qi,^b Ping Feng,^{c,*} Minyong Li,^{d,*} and Bowen Ke^{a,*}

^aDepartment of Anesthesiology, Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, Frontiers Science Center for Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China.

^bWest China School of Pharmacy, Sichuan University, Chengdu, Sichuan 610041, China.

^cInstitute of Clinical Trials, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China.

^dDepartment of Medicinal Chemistry, Key Laboratory of Chemical Biology (MOE), School of Pharmacy, Shandong University, Jinan, Shandong 250012, China.

[†] These authors contributed equally.

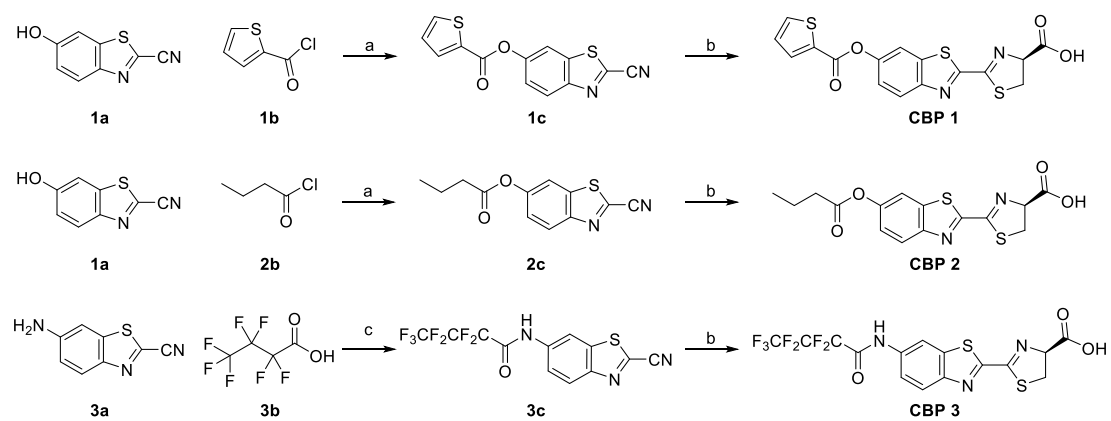
Corresponding Authors

*E-mail: bowenke@scu.edu.cn (B.K.).

*E-mail: mli@sdu.edu.cn (M.L.).

*E-mail: pfyq@yahoo.com (P.F.).

Synthetic routes



Scheme S1. The syntheses of the designed probes. a) NEt_3 , CH_2Cl_2 , 98% for **1c**, and 61% for **2c**; b) K_2CO_3 , D-cysteine·HCl, $\text{H}_2\text{O}/\text{MeOH}$, 36% for **CBP 1**, 26% for **CBP 2** and 85% for **CBP 3**; c) EDCI, DMAP, CH_2Cl_2 , 43%.

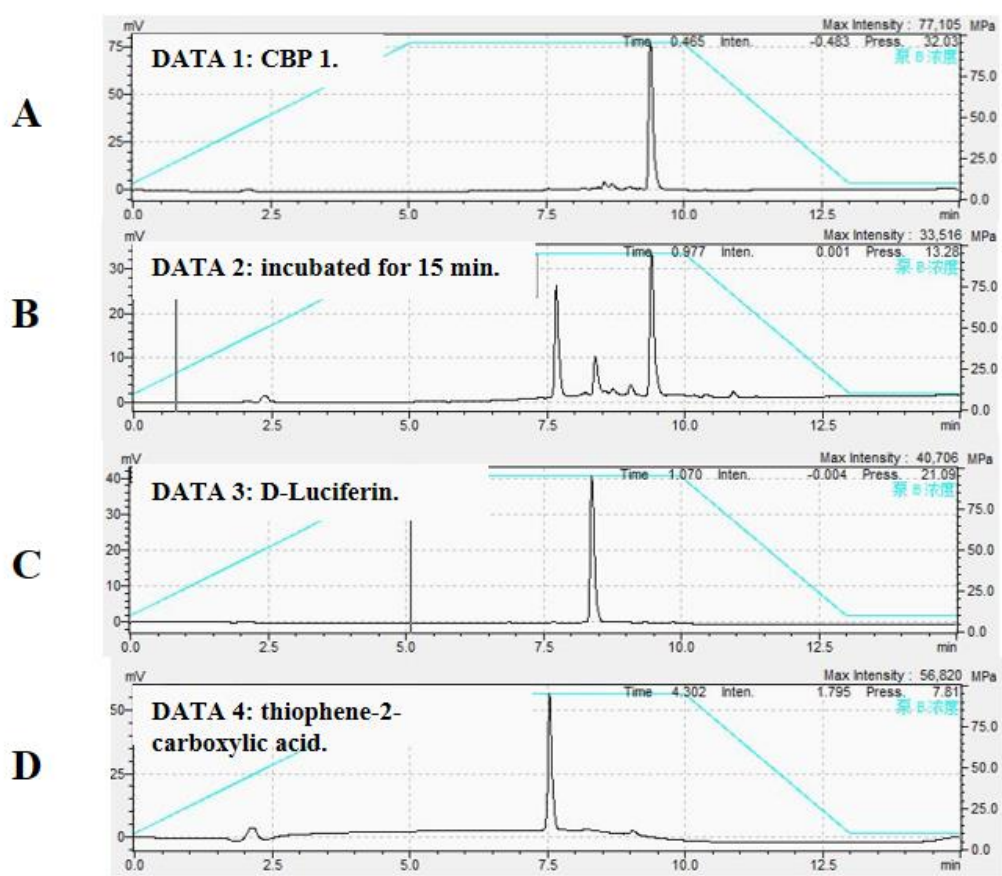


Figure S1. The HPLC analyses of **CBP 1** with CES2: **(A)** **CBP 1** (10 mM); **(B)** **CBP 1** (10 mM, 0.5 mL) with CES2 (5 μ g/mL, 0.5 mL); **(C)** Luciferin (10 mM); **(D)** Thiophene-2-carboxylic acid (10 mM).

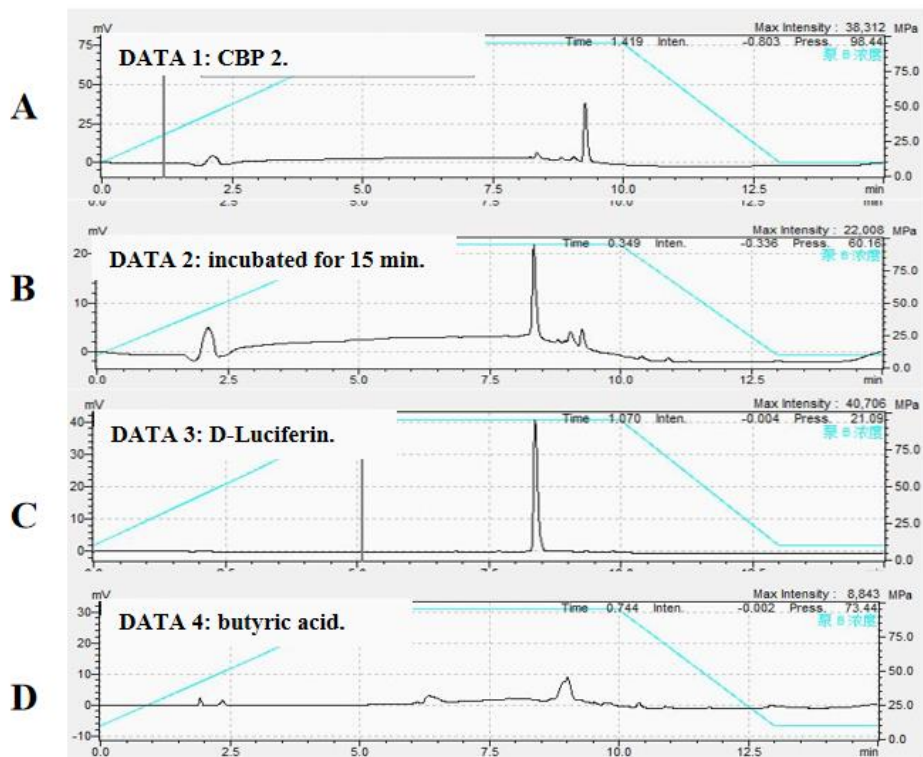


Figure S2. The HPLC analyses of **CBP 2** with CES2: **(A) CBP 2** (10 mM); **(B) CBP 2** (10 mM, 0.5 mL) with CES2 (5 μ g/mL, 0.5 mL); **(C)** Luciferin (10 mM); **(D)** Butyric acid (10 mM).

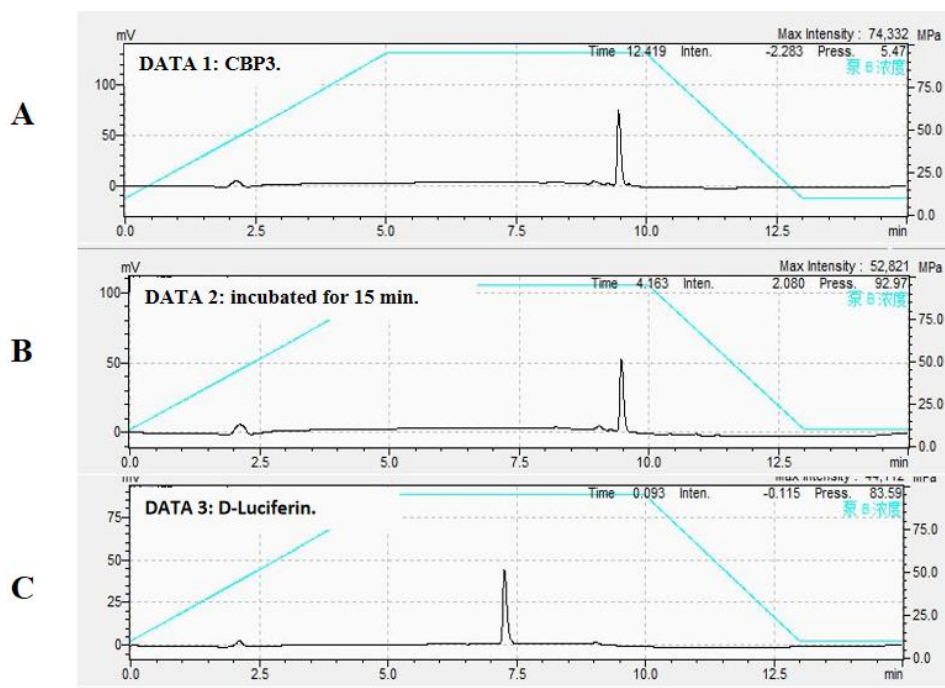


Figure S3. The HPLC analyses of **CBP 3** with CES2: (A) **CBP 3** (10 mM); (B) **CBP 3** (10 mM, 0.5 mL) with CES2 (5 $\mu\text{g}/\text{mL}$, 0.5 mL); (C) Luciferin (10 mM).

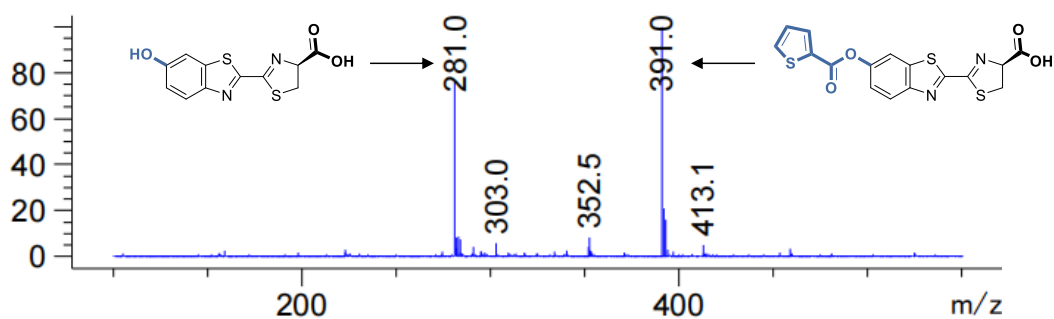


Figure S4. The ESI-MS spectrum of **CBP 1** (10 mM) upon incubation with CES2 (5 $\mu\text{g}/\text{mL}$).

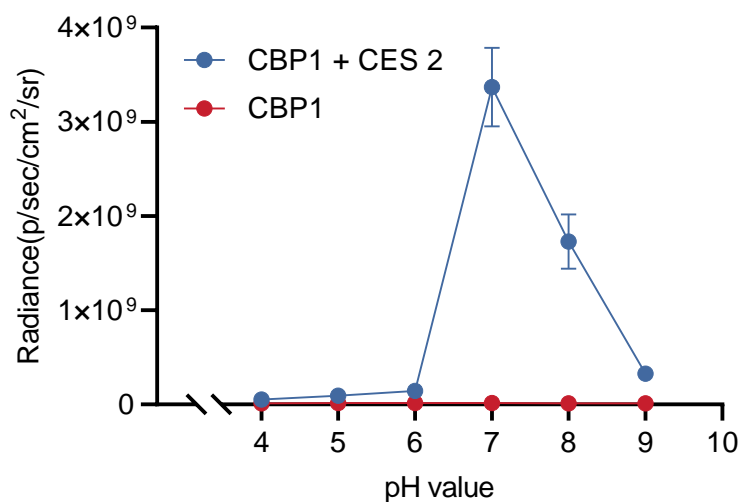


Figure S5. The influence of pH value on the CES2 detection by **CBP 1** (n = 3). Each value was represented as mean ± SEM.

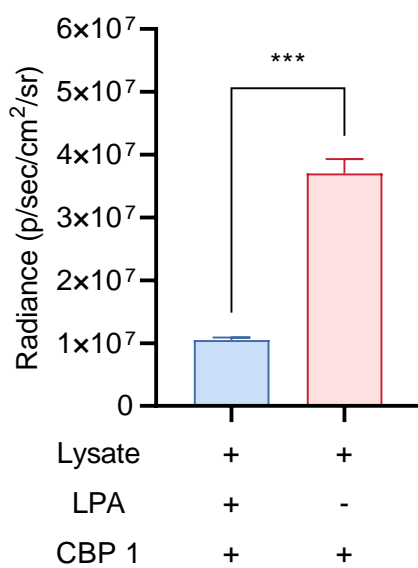


Figure S6. The investigation of the CES2 specificity of **CBP 1** by using the cell lysate (n = 3). *** $P < 0.001$, according to unpaired t test. Each value was represented as mean ± SEM.

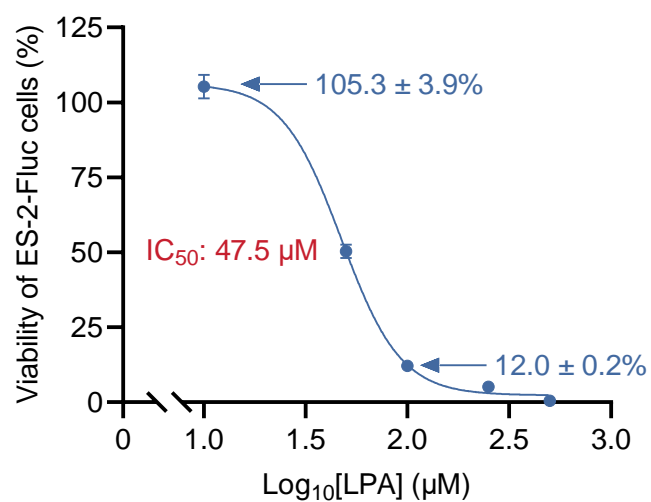
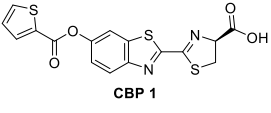
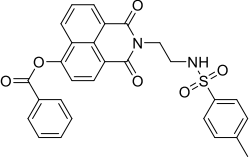
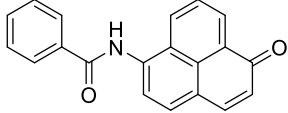
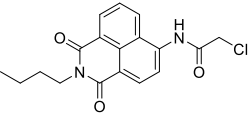
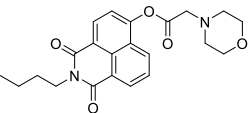
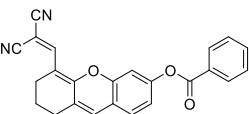
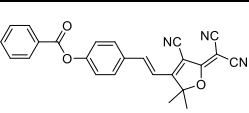
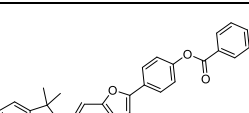
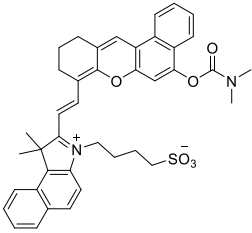
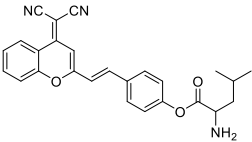
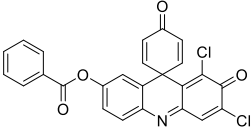
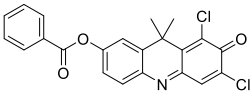
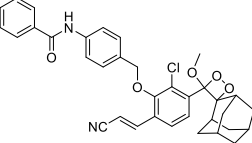


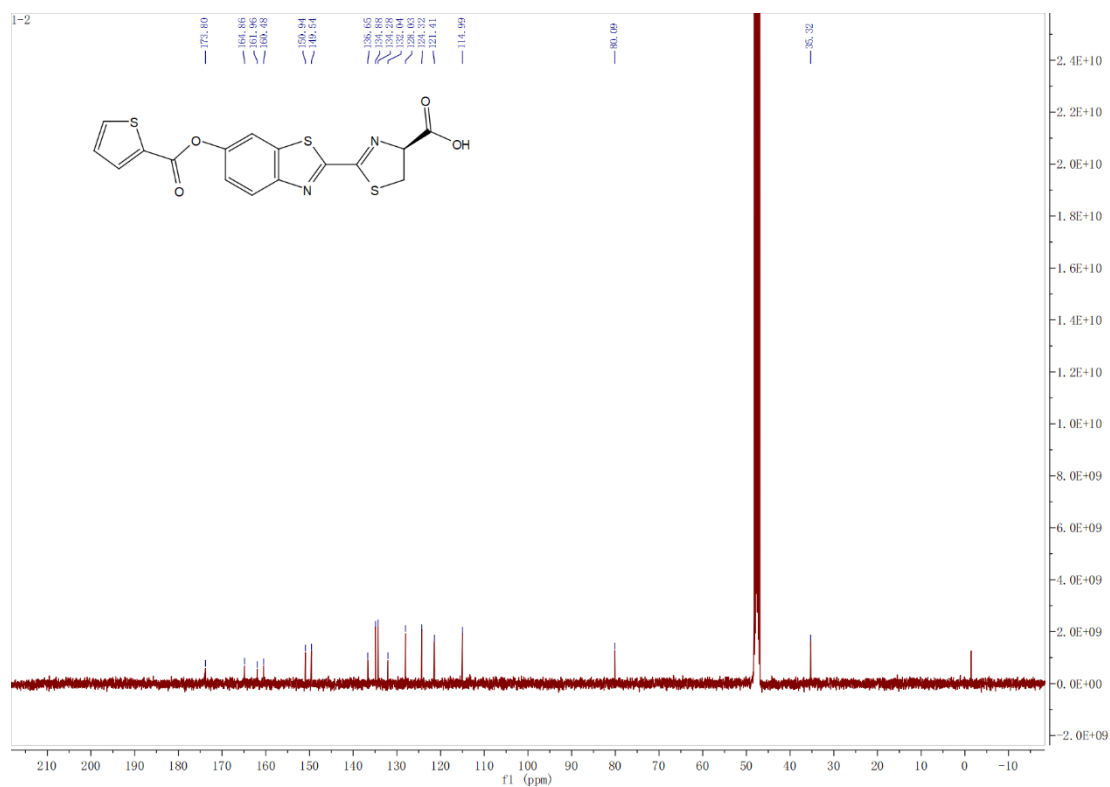
Figure S7. The viability of ES-2-Fluc cells after incubating with different concentrations of LPA (n = 3). Each value was represented as mean ± SEM, and the IC₅₀ value was fitted by GraphPad Prism 8.

Table S1. Representative studies for CES2 detection

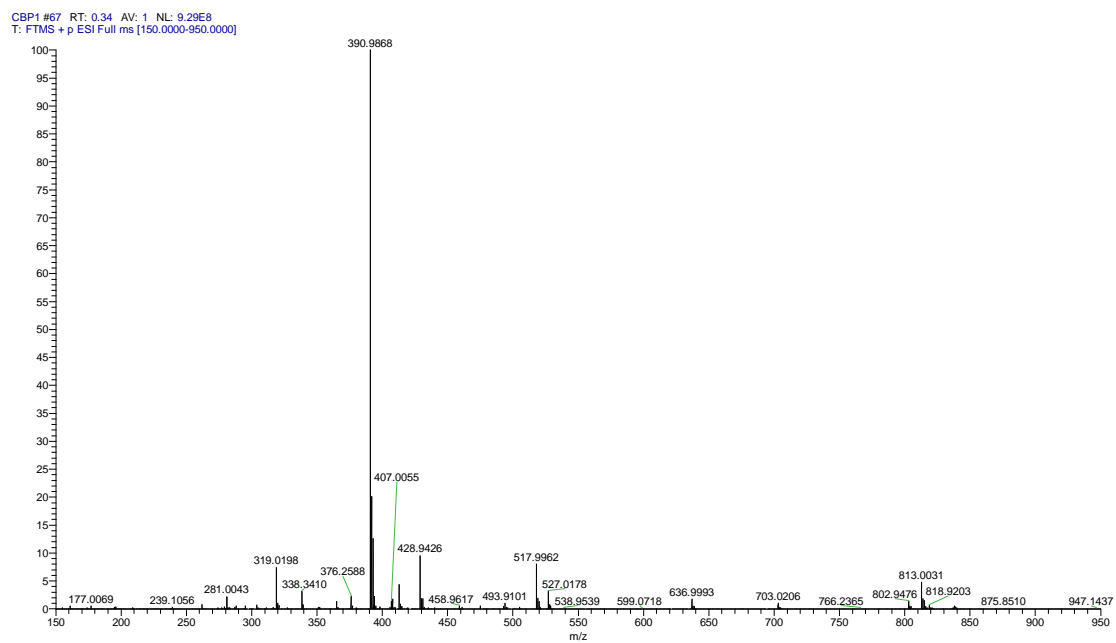
Chemical structure (Strategy)	λ_{ex}/em (nm)	K_m (μM)	LOD	Reference
 CBP 1 (Bioluminescence)	/	5.80	0.0045 $\mu g/mL$	This work
 (Fluorescence)	380/560	3.48 ± 0.61	/	1
 (Fluorescence)	548/605	6.60 ± 3.70	/	2
 (Fluorescence)	430/542	8.58 ± 0.55	0.012 $\mu g/mL$	3
 (Fluorescence)	450/560	21.72	1.986 $\mu g/mL$	4
 (Fluorescence)	590/640	0.93	0.017 $\mu g/mL$	5
 (Fluorescence)	560/612	3.06 ± 0.57	0.460 $\mu g/mL$	6
 (Fluorescence)	498/660	/	0.165 ng/mL	7

 (Fluorescence)	675/850	< 5.00	0.023 µg/mL	8
 (Fluorescence)	830/685	33.43	0.087 µg/mL	9
 (Fluorescence)	630/678	/	0.030 µg/mL	10
 (Fluorescence)	600/662	1.92 ± 0.31	0.070 µg/mL	11
 (Chemiluminescence)	/	/	0.0082 µg/mL	12
Capillary Electrophoresis	/	/	0.827 mg/mL	13

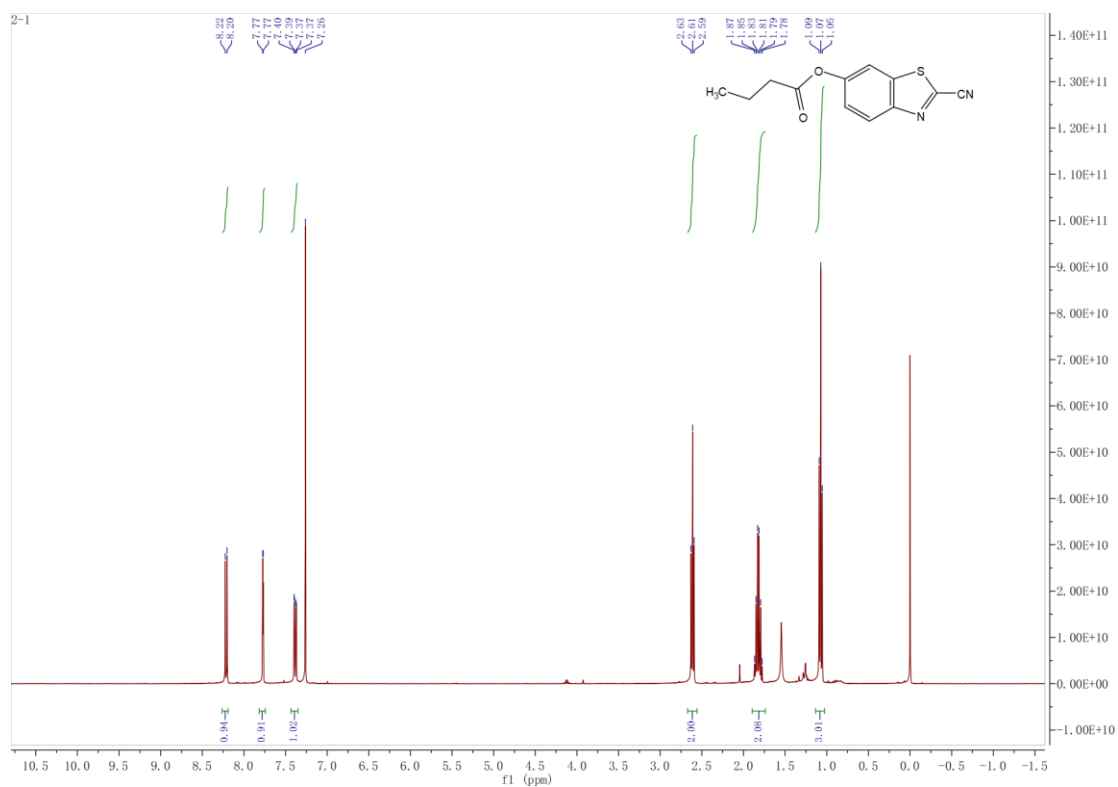
¹³C-NMR spectra of CBP 1



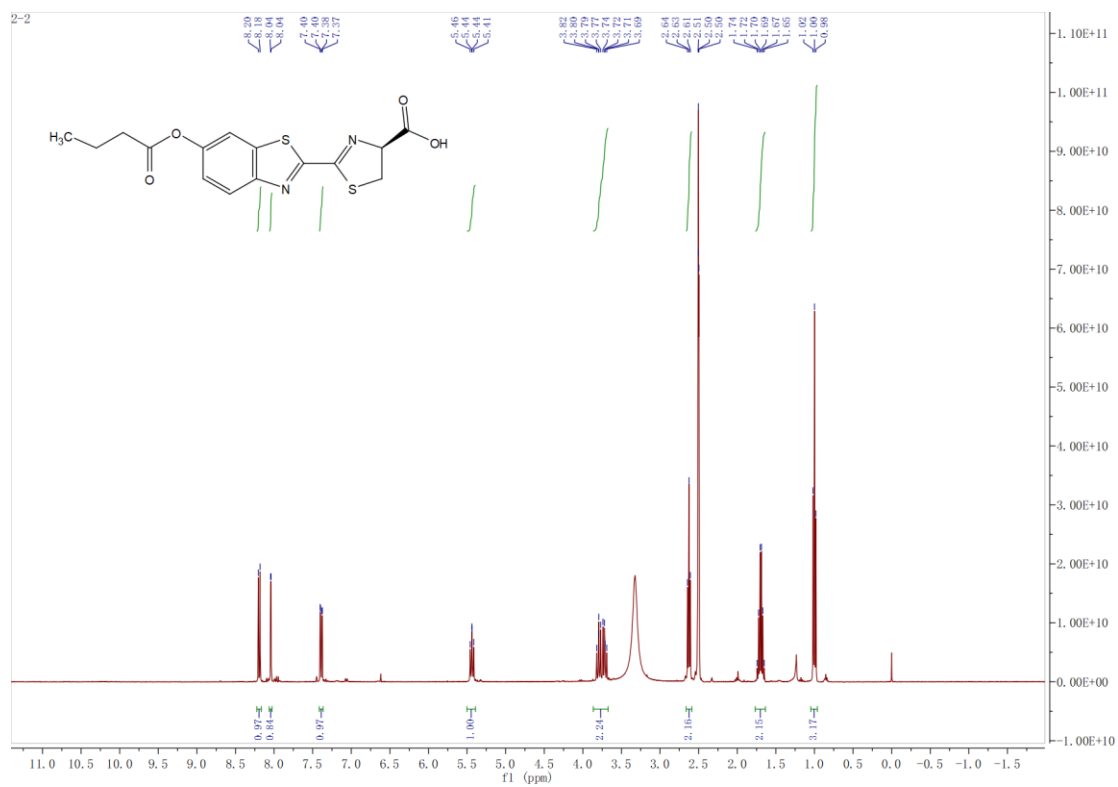
HRMS spectra of CBP 1



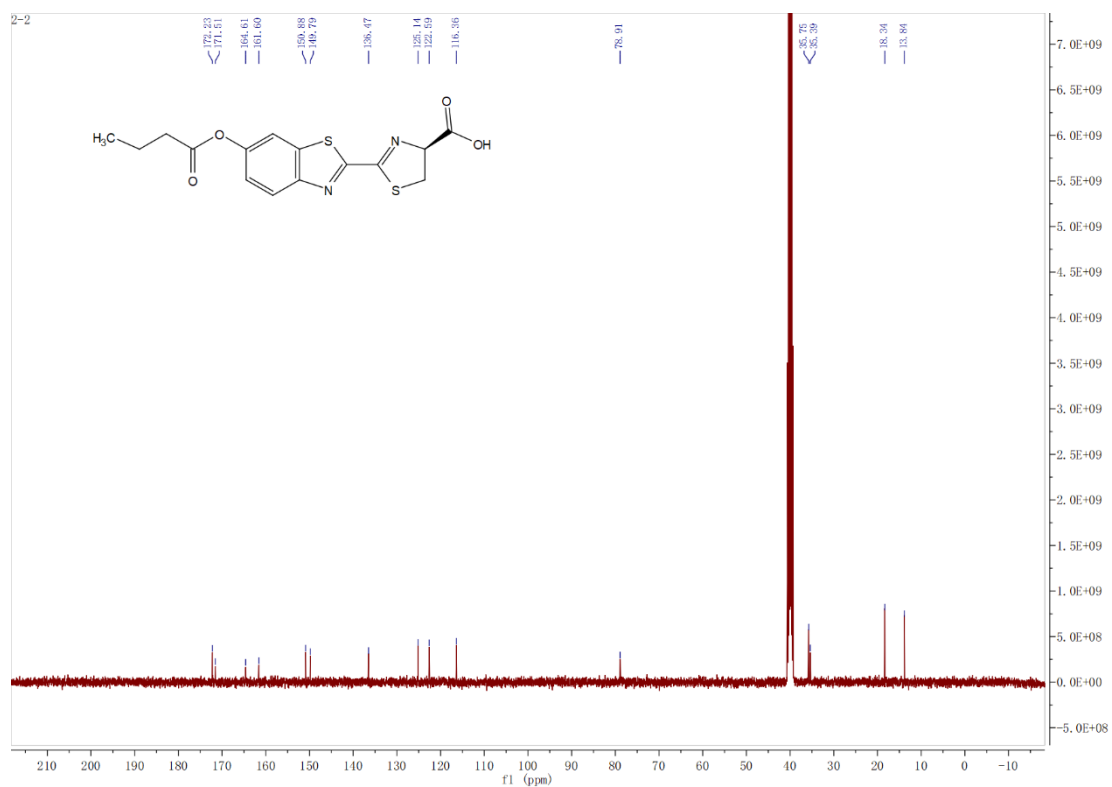
¹H-NMR spectra of compound 2c



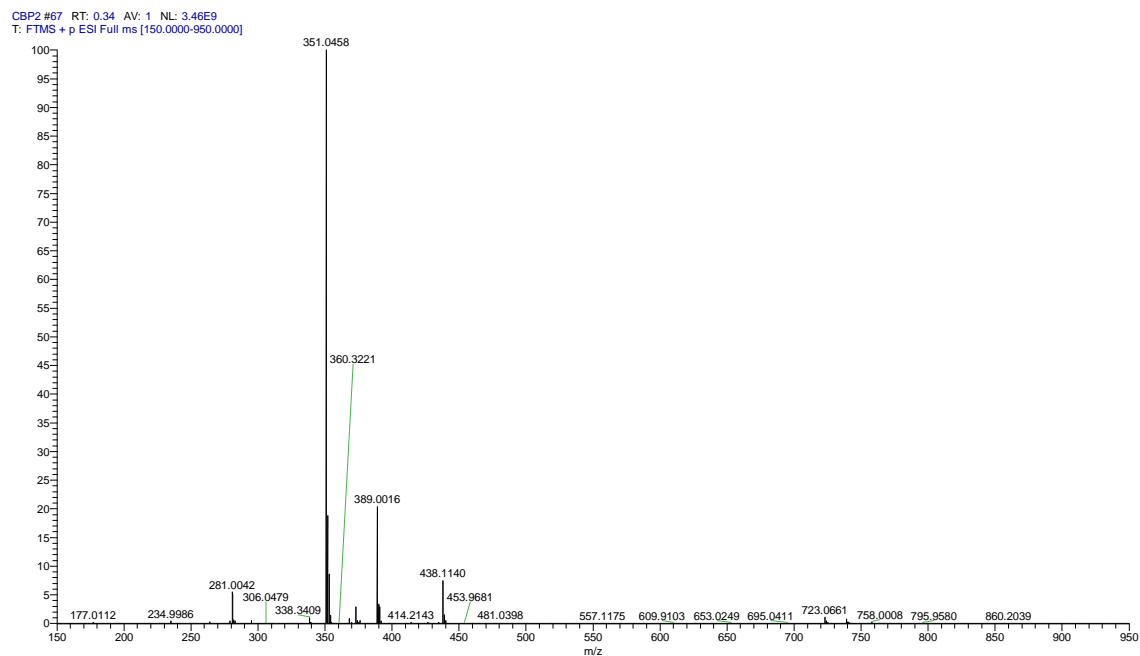
¹H-NMR spectra of CBP 2



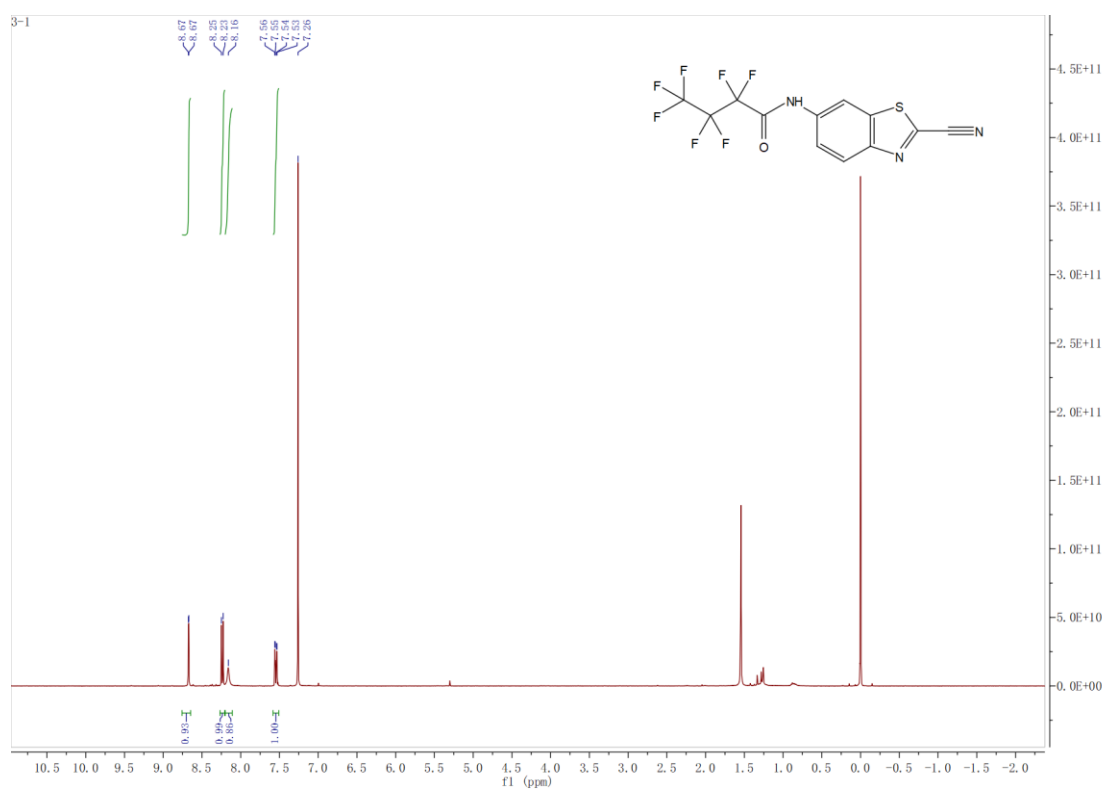
¹³C-NMR spectra of CBP 2



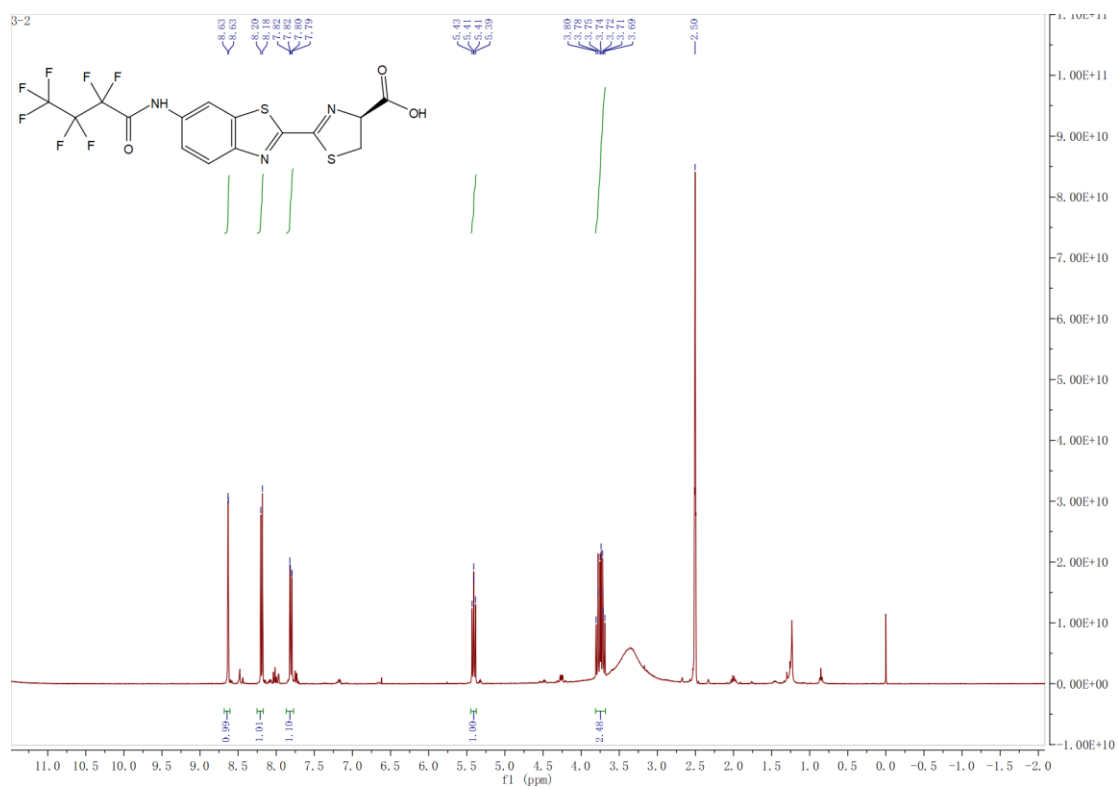
HRMS spectra of CBP 2



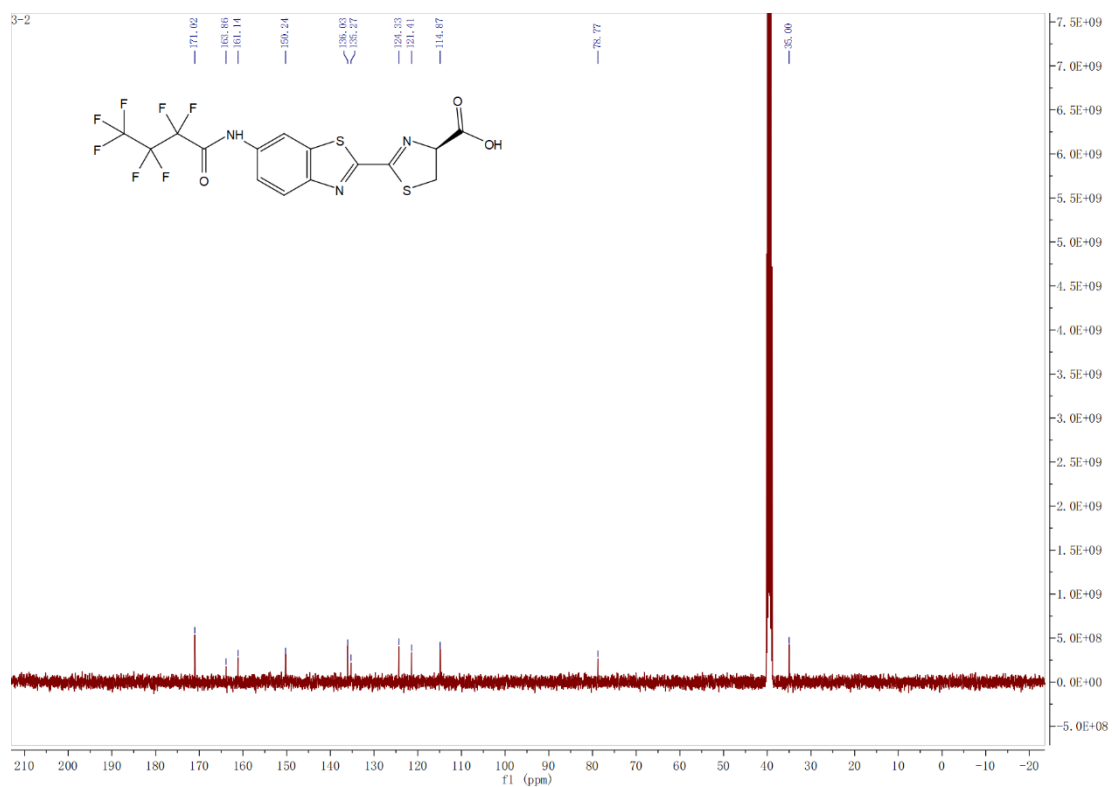
¹H-NMR spectra of compound 3c



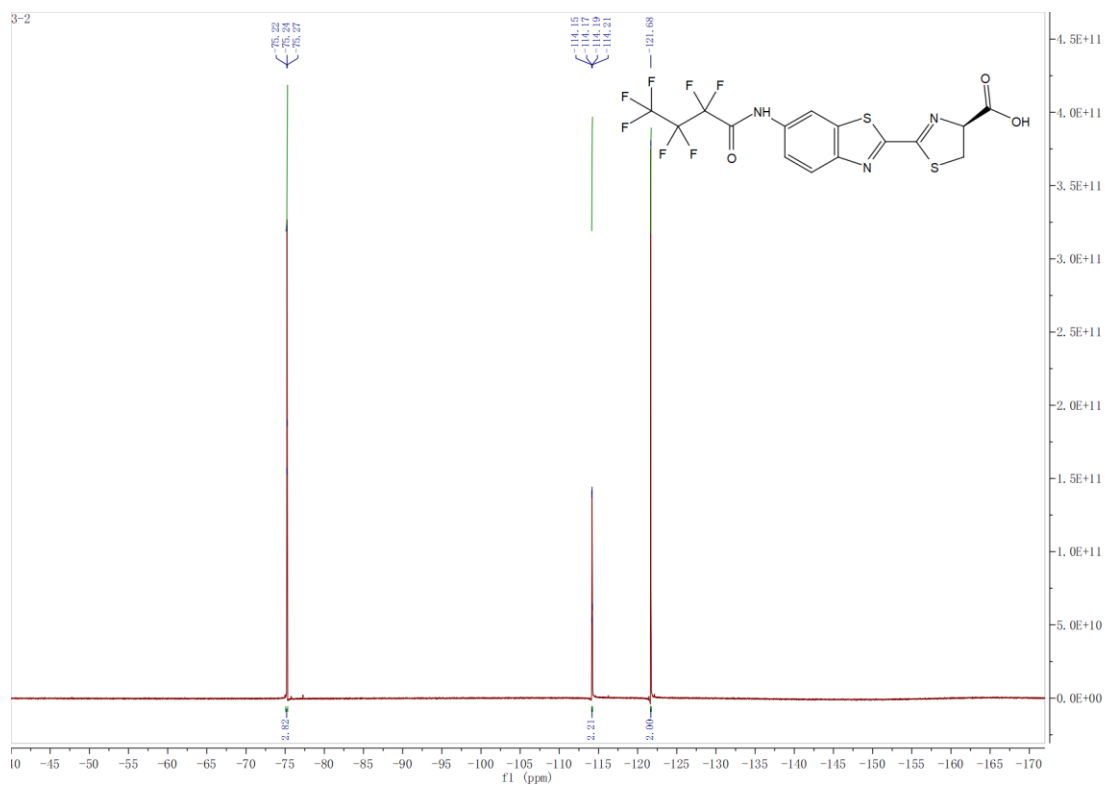
¹H-NMR spectra of CBP 3



¹³C-NMR spectra of CBP 3

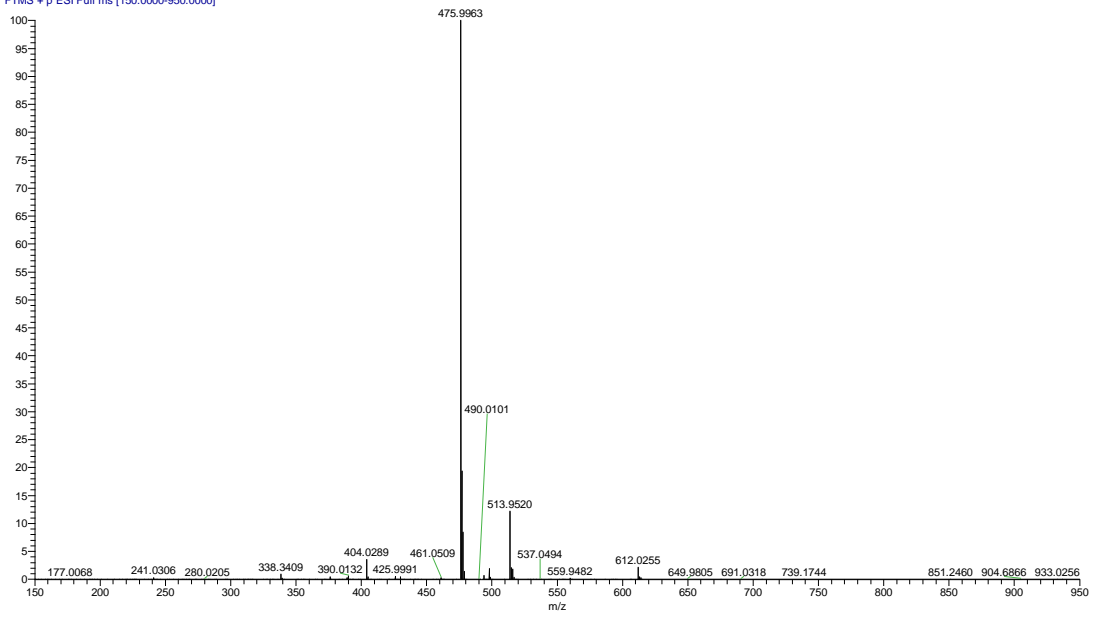


¹⁹F-NMR spectra of CBP 3



HRMS spectra of CBP 3

CBP3 #75 RT: 0.38 AV: 1 NL: 2.63E9
T: FTMS + p ESI Full ms [150.0000-950.0000]



References

1. X. Tian, F. Yan, J. Zheng, X. Cui, L. Feng, S. Li, L. Jin, T. D. James and X. Ma, *Anal. Chem.*, 2019, **91**, 15840-15845.
2. K. Kailass, O. Sadowski, M. Capello, Y. Kang, J. B. Fleming, S. M. Hanash and A. A. Beharry, *Chem. Sci.*, 2019, **10**, 8428-8437.
3. Q. Jin, L. Feng, D. D. Wang, Z. R. Dai, P. Wang, L. W. Zou, Z. H. Liu, J. Y. Wang, Y. Yu, G. B. Ge, J. N. Cui and L. Yang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 28474-28481.
4. Z. Meng meng, L. Peng, H. Feng and J. Yan, *Chem. Phys. Lett.*, 2021, **785**, 139143-139148.
5. S. Y. Liu, X. Zou, Y. Guo and X. Gao, *Anal. Chim. Acta*, 2022, **1221**, 340126-340134.
6. L. Feng, Z. M. Liu, L. Xu, X. Lv, J. Ning, J. Hou, G. B. Ge, J. N. Cui and L. Yang, *Chem. Commun.*, 2014, **50**, 14519-14522.
7. J. Li, J. Cao, W. Wu, L. Xu, S. Zhang, P. Ma, Q. Wu and D. Song, *Sens. Actuators, B*, 2023, **377**, 133122-133129.
8. Z. Chen, J. Yu, K. Sun, J. Song, L. Chen, Y. Jiang and Z. Wang, *Analyst*, 2023, **148**, 876-887.
9. Y. Wang, F. Yu, X. Luo, M. Li, L. Zhao and F. Yu, *Chem. Commun.*, 2020, **56**, 4412-4415.
10. X. Y. Zhang, T. T. Liu, J. H. Liang, X. G. Tian, B. J. Zhang, H. L. Huang, X. C. Ma, L. Feng and C. P. Sun, *J. Mater. Chem. B*, 2021, **9**, 2457-2461.
11. Q. Jin, L. Feng, D. D. Wang, J. J. Wu, J. Hou, Z. R. Dai, S. G. Sun, J. Y. Wang, G. B. Ge, J. N. Cui and L. Yang, *Biosens. Bioelectron.*, 2016, **83**, 193-199.
12. C. Ozsan, K. Kailass, E. M. Digby, T. Alammadov, A. A. Beharry and S. Kolemen, *Chemical Communications*, 2022, **58**, 10929-10932.
13. J. Lamego, A. S. Coroadinha and A. L. Simplicio, *Anal Chem*, 2011, **83**, 881-887.