

Supporting Information

A near-infrared fluorescent probe selectively recognizing cysteine to release H₂S and its applications

Xuan Wang^a, Nianwei Wang^a, Yikun Ren^a, Jicheng Wang^a, Jiao Bai^{a*}, Huiming Hua^a, Dahong Li^{a*}

^aKey Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, and School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, P. R. China

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*Corresponding authors. E-mail addresses: lidahong0203@163.com (D. Li); baijiao@hotmail.com (J. Bai).

1. Synthesis section

1.1 Synthesis of TMN-ONCS:

TMN-OH (290 mg, 1 mmol) and Br-Ar-NCS (342 mg, 1.5 mmol) were dissolved in anhydrous acetonitrile, K₂CO₃ (17 mg, 0.1 mmol) and KI (3 mg, 0.02 mmol) were added, then heated with stirring under nitrogen at 60 °C for 2 h. The reaction was monitored by TLC until completion, filtered after cooling and the filtrate concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether: dichloromethane = 2:1) to give TMN-ONCS as an orange solid (197 mg, yield 45%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.67 (d, *J* = 8.8 Hz, 2H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 6.7 Hz, 2H), 7.06 (d, *J* = 8.8 Hz, 2H), 6.84 (s, 1H), 5.19 (s, 2H), 2.61 (s, 2H), 2.55 – 2.52 (m, 2H), 1.02 (s, 6H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 170.82, 159.90, 156.87, 138.02, 137.24, 134.01, 130.09, 129.96, 129.56, 129.51, 129.49, 127.96, 126.55, 122.39, 115.78, 114.51, 113.69, 75.87, 68.98, 42.78, 40.53, 38.65, 32.15, 27.92. HRMS: *m/z* calcd for C₂₇H₂₃ON₃SNa [M + Na]⁺: 460.1460, found: 460.1460.

2.2 Synthesis of TMN-OH

(3,5,5-Trimethyl-2-cyclohexen-1-ylidene) propanedinitrile (466 mg, 2.5 mmol) and *p*-hydroxybenzaldehyde (370 mg, 3 mmol) were dissolved in 6 mL of anhydrous ethanol. After complete dissolution, 150 μL of piperidine was added. The reaction mixture was then refluxed overnight at 80-85 °C under a nitrogen atmosphere. Upon confirming the completion of the reaction by TLC, after the reaction was confirmed by thin layer chromatography, the solvent was evaporated under reduced pressure and purified by silica gel column chromatography to obtain orange powder (472 mg, yield 65%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.98 (s, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.26 – 7.15 (m, 2H), 6.79 (d, *J* = 8.6 Hz, 3H), 2.60 (s, 2H), 2.53 (s, 2H), 1.01 (s, 6H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 170.80, 159.81, 157.25, 138.80, 130.36, 127.60, 126.74, 121.84, 116.35, 114.62, 113.80, 75.24, 42.80, 40.53, 38.66, 32.15, 27.92. HRMS: *m/z* calcd for C₁₉H₁₉ON₂ [M + H]⁺: 291.1497, found: 291.1500.

2.3 Synthesis of Br-Ar-NCS

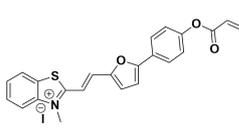
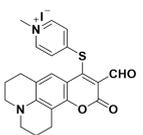
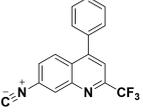
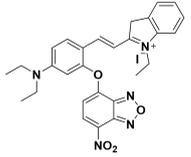
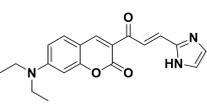
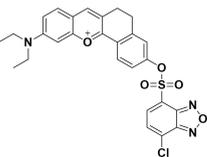
N-bromosuccinic acid imide (1.79 g, 10 mmol) and *p*-tolyl isothiocyanate (1.49 g, 10 mmol) were dissolved in 20 mL of anhydrous carbon tetrachloride. The catalytic amount of benzoyl peroxide (24.2 mg, 0.1 mmol) was then added and stirred for 12 h at 80 °C reflux. After the

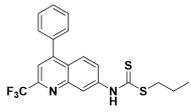
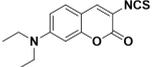
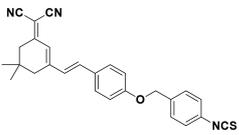
reaction was confirmed by thin layer chromatography, the reaction mixture was filtered, the filtrate was evaporated under reduced pressure, and the white crystals of **Br-Ar-NCS** (1.6 g, yield 55%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.54 – 7.51 (m, 2H), 7.44 – 7.40 (m, 2H), 4.73 (s, 2H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ 138.45, 134.35, 131.29, 130.24, 126.76, 40.53, 33.80. HRMS: *m/z* calcd for C₈H₆BrNSNa [M + Na]⁺: 294.9302, found: 294.9304.

2.4 Synthesis of AzMC¹

The synthesis of **AzMC** followed the reported procedure, and its structure was confirmed by NMR (Fig. S12 and S13) and HRMS (Fig. S14).

2. Comparative analysis and additional analytical data

Table S1. The comparison of the reported fluorescent probes for Cys detection.						
Probe	E _x /E _m (nm)	Distinguish objects	Detection limited	H ₂ S Release function	Applications	Ref.
	461/603	Cys	0.19 μM	-	Cells	2
	400/518 505/566	Cys, Hcy, GSH	132 nM 105 nM 62 nM	-	Cells, zebrafish	3
	350/515	Cys	4.8 μM	-	Cells	4
	450, 543/592	Cys, Hcy	2.33 μM, 2.88 μM	-	Cells	5
	452/510	Cys, Hcy	3.94 μM, 11.01 μM	-	Cells	6
	468/550	Cys, GSH	250 nm, 370 nm	-	Cells, zebrafish	7

	390/518	Cys	105 nM	Yes	Cells, zebrafish	8
	380/504	Cys	25 nM	Yes	Cells, plants	9
	545/670	Cys	1 μM	Yes	Cells	This work

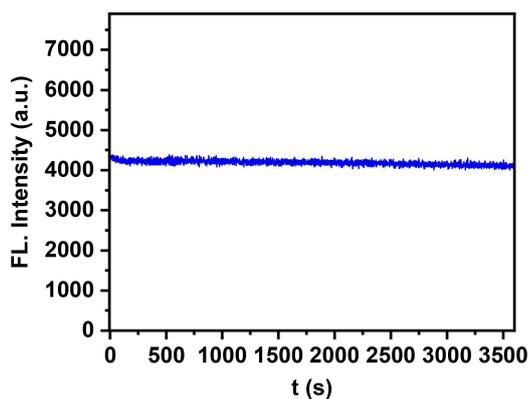


Fig. S1. Photostability of TMN-ONCS (25 μM) after reacting with Cys (250 μM) for 1 h at the plateau phase. Testing conditions: λ_{ex} = 545 nm, slit width: 10/10 nm, PBS buffer solution (10 mM, pH = 7.4, 50% DMSO) at 37 °C.

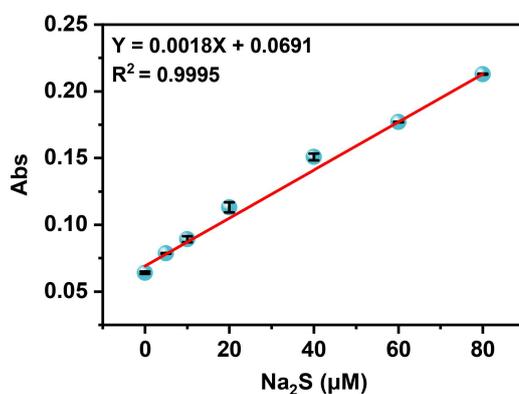


Fig. S2. Calibration curve for H₂S determined by the MB method. Testing conditions: absorbance

measured at 670 nm in PBS buffer solution (10 mM, pH 7.4, 50% DMSO) at 37 °C.

Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

366 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 19-19 H: 19-19 N: 0-100 O: 0-100 Na: 0-1

14

241127-7-297-4-TMN-OH 8 (0.079)

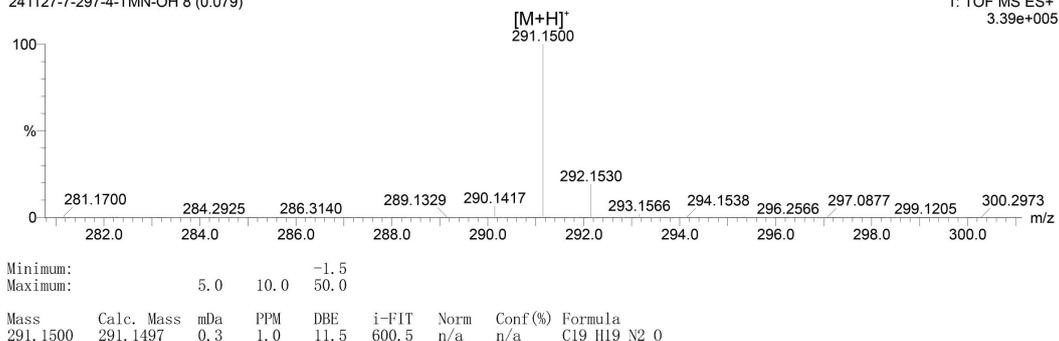
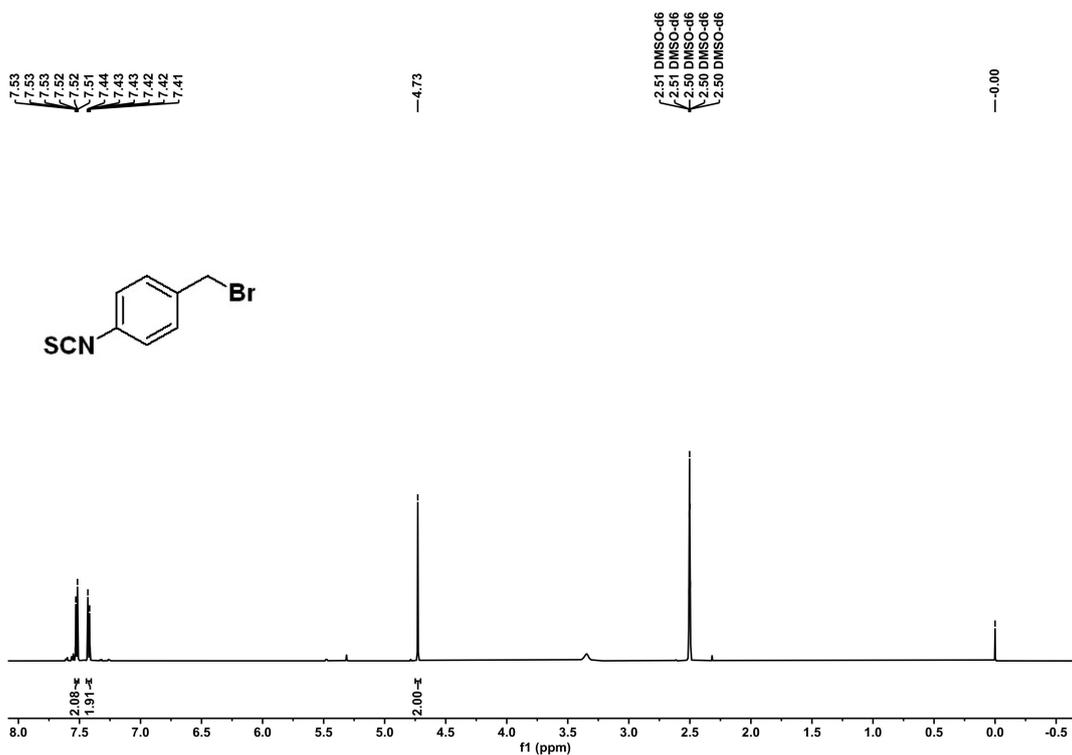


Fig. S5. HRMS of TMN-OH

Fig. S6. ^1H NMR spectrum of Br-Ar-NCS in DMSO- d_6 (600 MHz)

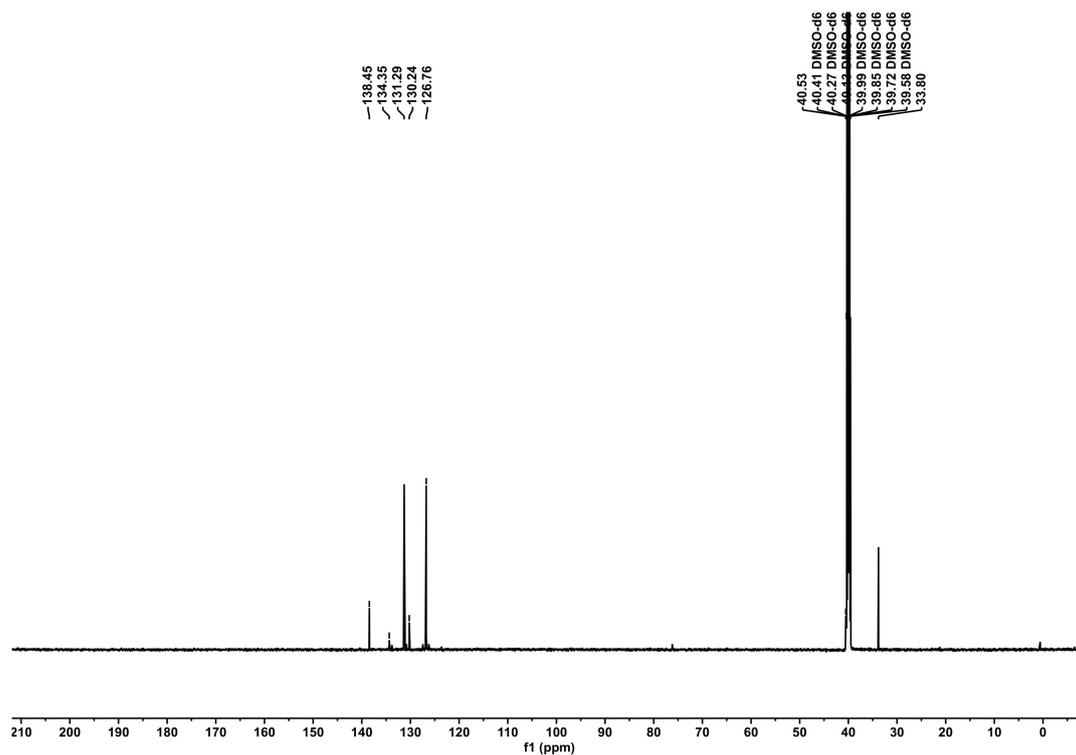


Fig. S7. ^{13}C NMR spectrum of **Br-Ar-NCS** in $\text{DMSO-}d_6$ (150 MHz)

Elemental Composition Report

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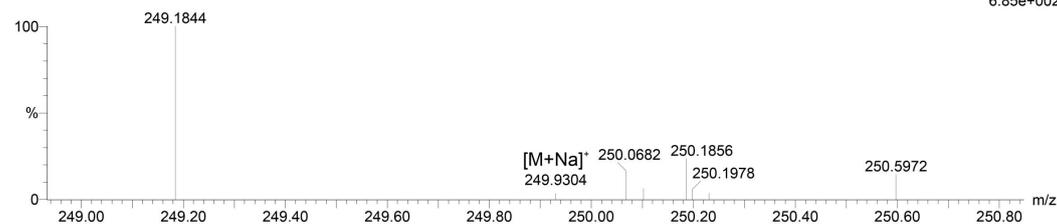
Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
 Element prediction: Off
 Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
 99 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
 Elements Used:
 C: 8-8 H: 6-6 N: 0-100 O: 0-100 Na: 0-1 S: 1-1 Br: 1-4

14
 241127-7-297-4-Br-Ar-NCS 59 (0.366)

1: TOF MS ES+
 6.85e+002



Minimum: -1.5
 Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
249.9304	249.9302	0.2	0.8	5.5	64.9	n/a	n/a	C8 H6 N Na S Br

Fig. S8. HRMS of **Br-Ar-NCS**

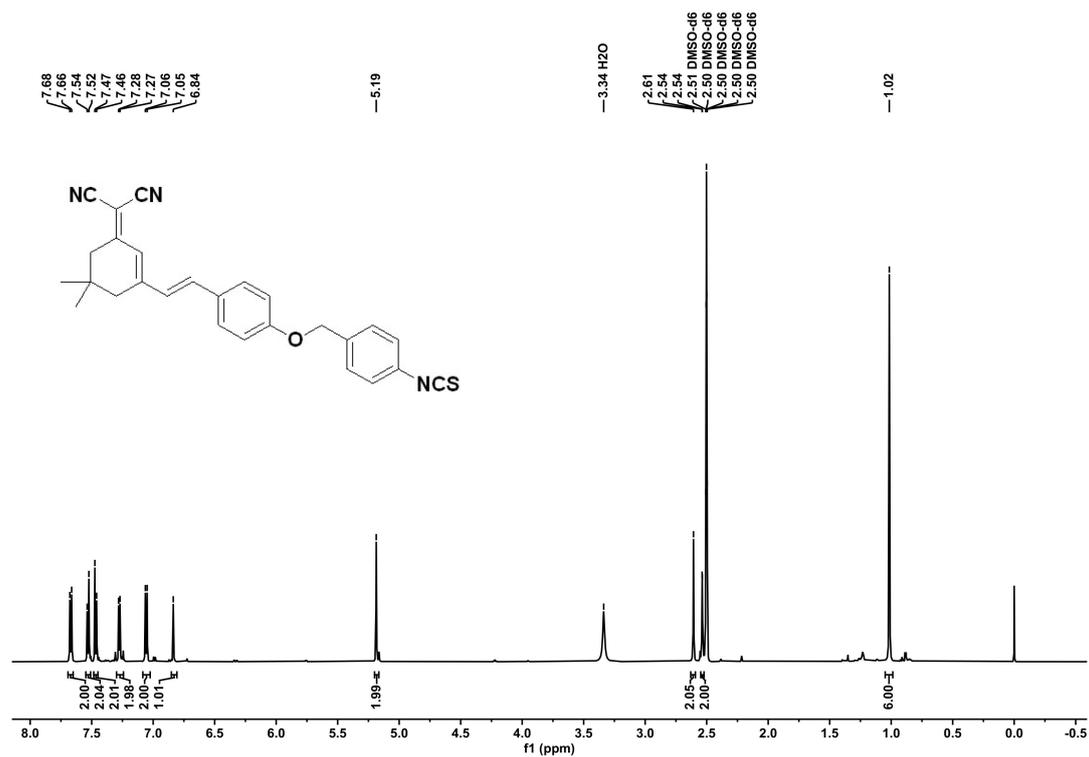


Fig. S9. ¹H NMR spectrum of TMN-ONCS in DMSO-*d*₆ (600 MHz)

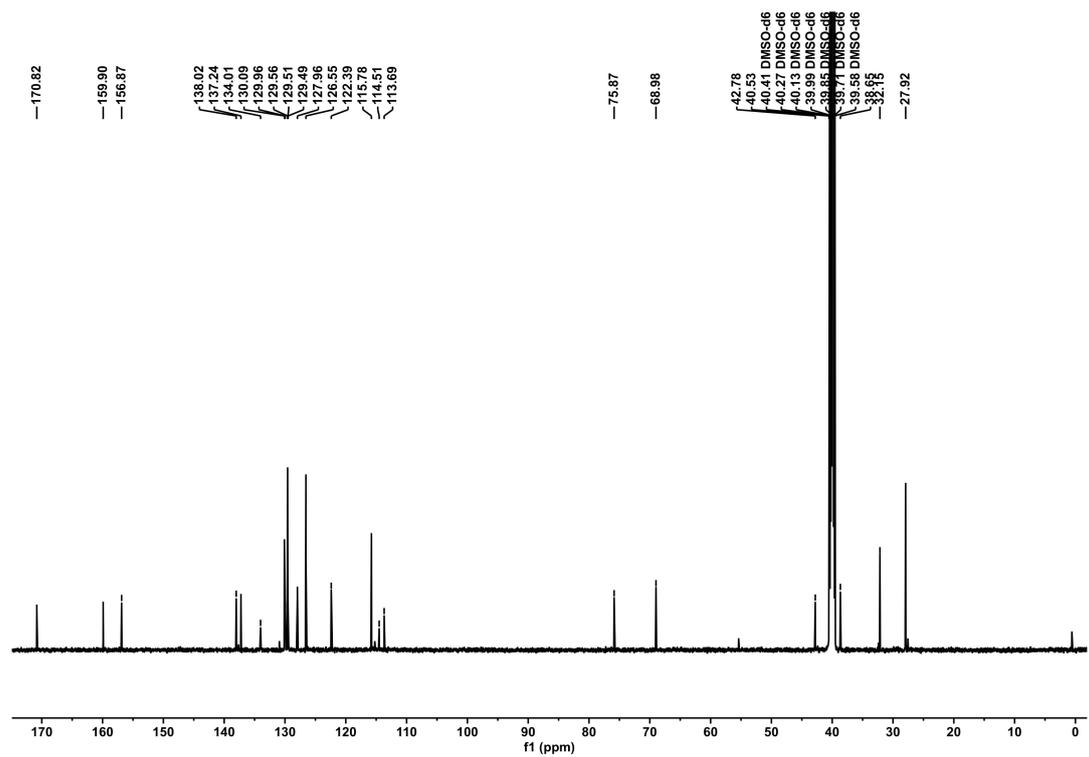


Fig. S10. ¹³C NMR spectrum of TMN-ONCS in DMSO-*d*₆ (150 MHz)

Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

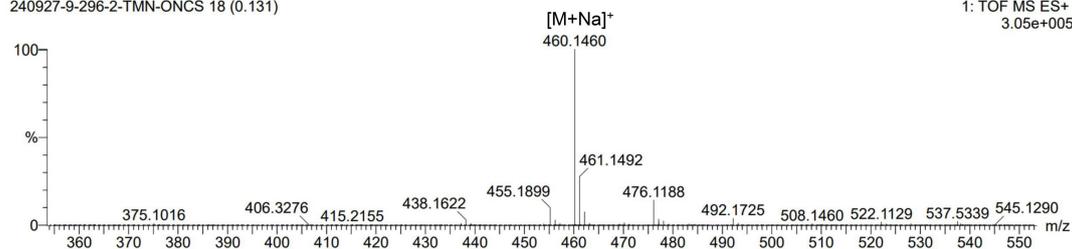
2057 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 27-27 H: 23-23 N: 0-100 O: 0-100 Na: 0-1 S: 1-3

8-P-N

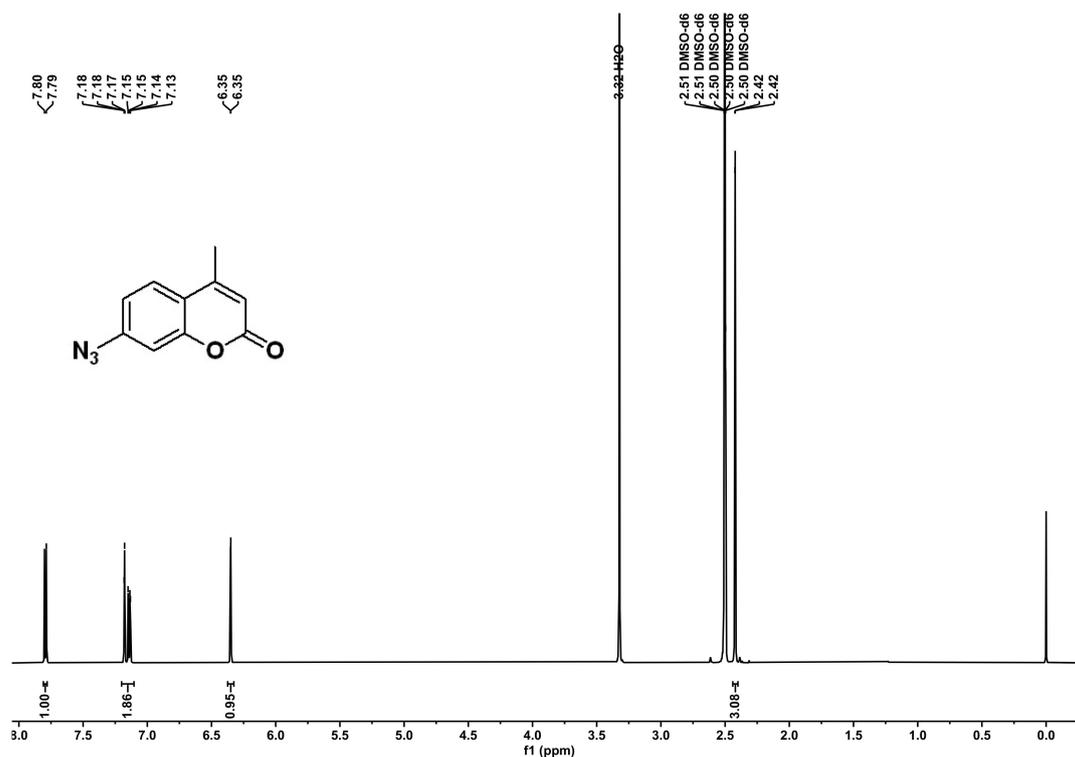
240927-9-296-2-TMN-ONCS 18 (0.131)

1: TOF MS ES+
3.05e+005

Minimum: -1.5
Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
460.1460	460.1460	0.0	0.0	17.5	665.7	n/a	n/a	C27 H23 N3 O Na S

Fig. S11. HRMS of TMN-ONCS

Fig. S12. ¹H NMR spectrum of AzMC in DMSO-*d*₆ (600 MHz)

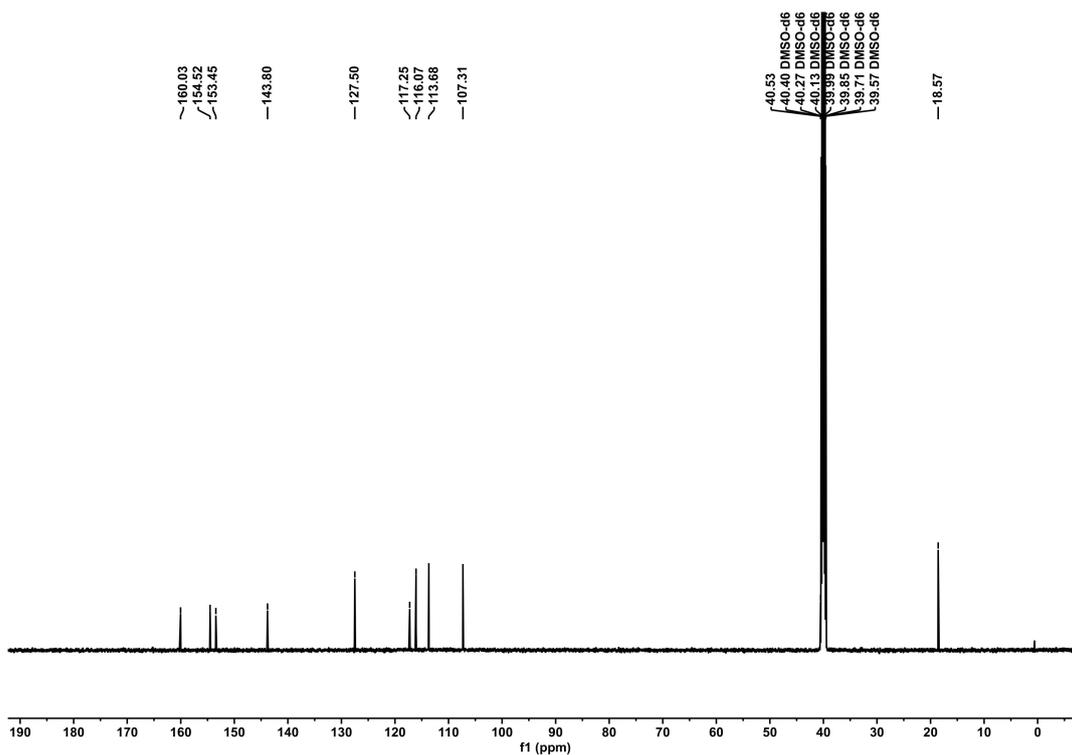


Fig. S13. ^{13}C NMR spectrum of AzMC in $\text{DMSO-}d_6$ (150 MHz)

Elemental Composition Report

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Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
 Element prediction: Off
 Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

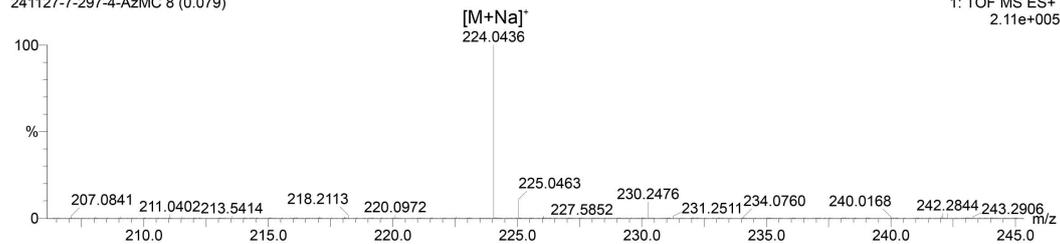
199 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 10-10 H: 7-7 N: 0-100 O: 0-100 Na: 0-1

14

241127-7-297-4-AzMC 8 (0.079)



1: TOF MS ES+
2.11e+005

Minimum: -1.5
 Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
224.0436	224.0436	0.0	0.0	8.5	624.0	n/a	n/a	C10 H7 N3 O2 Na

Fig. S14. HRMS of AzMC

4. References

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