Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary Information

For

A micellized fluorescence sensor based on amplified quenching for highly sensitive detection of non-transferrin-bound iron in serum

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Experimental

Materials

All chemicals used in the synthesis were of reagent grade and used without further purification. Pyrene, Methyl 11-bromoundecanoate, 4,4'-Di-*tert*-butyl-2,2'-bipyridine, (1,5-Cyclooctadiene)(methoxy)iridium(I) dimer, and Acetonitrile were purchased from Sigma-Aldrich Chemical Company. Bis(pinacolato)diboron, Sodium hydroxide, Methanol, Cyclohexane, Hexane, Dichloromethane, Ethyl aceate were bought from Daejung Company. Ferric hydroxide polymaltose complex was purchased from Ilyang Pharmaceutical Co., Ltd. Silica gel (Merck, 230-400 mesh) was used for chromatographic purification of all of intermediate and target molecules. All other chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, or Acros Chemical Company and used as received.

Instrumentation and Methods

NMR spectra were recorded using a Bruker (AVANCE III 500 and AVANCE NEO 900), operating at 500 MHz for ^{1}H-NMR and at 125, 225 MHz for ^{13}C-NMR. High-resolution mass spectra (HRMS) were obtained by Synapt G2-HDMS mass spectrometer (Waters). Elemental analysis (EA) was measured with Flash 2000D. Infrared spectra were measured with Thermo Fisher Scientific Nicolet iS5 FTIR spectrometer. UV/Vis absorption spectra were recorded using a Sinco Mega-2100 UV-Vis spectrometer. Steady-state fluorescence spectra were obtained with a Shimadzu fluorometer RF-6000. Lifetime measurements were carried out using a PicoQuant FluoTime 200 Compact Fluorescence Lifetime Spectrometer. A 1 cm quartz cuvette was used for all spectral measurements. Dynamic light scattering (DLS) experiments were performed with Zetasizer Nano ZS from Malvern Panalytical. Scanning Electron Microscopy (SEM) images were obtained with a Field Emission Scanning Electron Microscope (FE-SEM).

Stock solution (3.0 mM) of **1** was prepared in DMSO. The solution has been kept at the room temperature for one hour before use. The chloride salts of Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Sn^{2+}, Pb^{2+}, and Fe^{3+} ions (stock solutions = 10.0 mM in H_2O) were tested to evaluate the metal ion binding properties of **1**.

Synthesis

2-Hydroxypyrene (**2**) was prepared in a good yield as described in the literature.[1]

Methyl 11-(pyren-2-yloxy)undecanoate (3). To a solution of 2-hydroxypyrene (**2**) (2.00 g, 9.2 mmol) in dried CH_3CN (100 mL), anhydrous K_2CO_3 (3.80 g, 27.5 mmol) was added. After stirring for 30 min, methyl 11-bromoacetate (2.43 mL, 7.5 mmol) was added to the reaction mixture. The resulting mixture was vigorously stirred at 80 °C for 24 hours under argon gas. After the reaction mixture was cooled to room temperature, the solvent was removed *in vacuo*. The reaction mixture was acidified with 5 % aqueous HCl solution (100 mL), and then extracted with CH_2Cl_2 (200 mL). The organic layer was separated and washed with water (100 mL) and dried over anhydrous $MgSO_4$, and the solvent was evaporated to yield a yellow solid. The pure product was isolated by column chromatography on silica gel using dichloromethane:hexane (1:3) as the eluent. 84.2% yield.; ^1H NMR (500 MHz, CD_2Cl_2): δ 8.05 (d, 2H, *J* = 7.6 Hz), 7.95 (d, 2H, *J* = 9.0 Hz), 7.98 (d, 2H, *J* = 9.0 Hz), 7.83 (t, 1H, *J* = 7.6 Hz), 7.61 (s, 2H), 4.14 (t, 2H, *J* = 6.6 Hz), 3.53 (s, 3H), 2.18 (t, 2H, *J* = 7.7 Hz), 1.81 (m, 2H), 1.47 (m, 4H), 1.27 (m, 10H); ^{13}C NMR (125 MHz, CD_2Cl_2): δ 174.0, 157.5, 132.6, 130.14, 127.90, 126.80, 125.20, 124.90, 124.60, 119.80, 111.0, 68.5, 51.5, 34.0, 29.5, 29.4, 29.3, 29.1, 26.1, 25.0; ESI(+) HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{28}H_{32}NaO^{3+}$, 439.2244; found, 439.2245.

Sodium 11-(pyren-2-yloxy)undecanoate (1). To a solution of methyl 11-(pyren-2-yloxy)undecanoate (**3**) (1.00 g, 2.4 mmol) in $MeOH/H_2O$ (30 mL, 2:3, *v/v*), NaOH (0.29 g, 7.3

mmol) in water (1 mL) was added dropwise. The reaction mixture was vigorously stirred at 90 ^{o}C for 24 hours. The reaction mixture was cooled to room temperature, and then poured into a solution of methanol (300 mL) and diethyl ether (100 mL) to give the fine white solid **1** precipitate. 76% yield.; ^{1}H NMR (500 MHz; DMSO-d_6): δ 8.25 (d, 2H, J = 7.7 Hz), 8.15 (d, 2H, J = 9.0 Hz), 8.11 (d, 2H, J = 9.0 Hz), 7.99 (t, 1H, J = 7.6 Hz), 7.90 (s, 2H), 4.28 (t, 2H, J = 6.6 Hz), 1.87 (m, 2H), 1.80 (t, 2H, J = 7.3 Hz), 1.53 (m, 2H), 1.40 (m, 4H), 1.24 (m, 8H); ^{13}C NMR (225 MHz, DMSO-d_6): δ 175.7, 157.1, 132.2, 129.6, 127.8, 126.8, 125.2, 125.1, 123.8, 119.0, 111.0, 68.0, 40.0, 39.8, 39.7, 39.6, 39.5, 39.4, 39.3, 39.2, 29.5, 29.2, 29.1, 28.9, 26.6, 25.6; ESI(+) HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{27}H_{30}O_3Na$, 425.2087; found, 425.2090; Anal. calcd. for $C_{27}H_{29}O_3Na$: C, 76.39; H, 6.89; O, 11.31. found; C, 73.09; H, 6.85; O, 10.91.

Serum lipid extraction with organic solvent. Extraction with a chloroform-methanol mixture was based on the methodology of Folch et al.[2] 4 mL of normal human serum was added to 10 mL of chloroform-methanol (2:1, *v/v*). The mixture was agitated manually for 1 minute and centrifuged at 4000 rpm for 10 minutes at room temperature. After centrifugation, the aqueous phase was collected and repeated once more with hexane.[3]

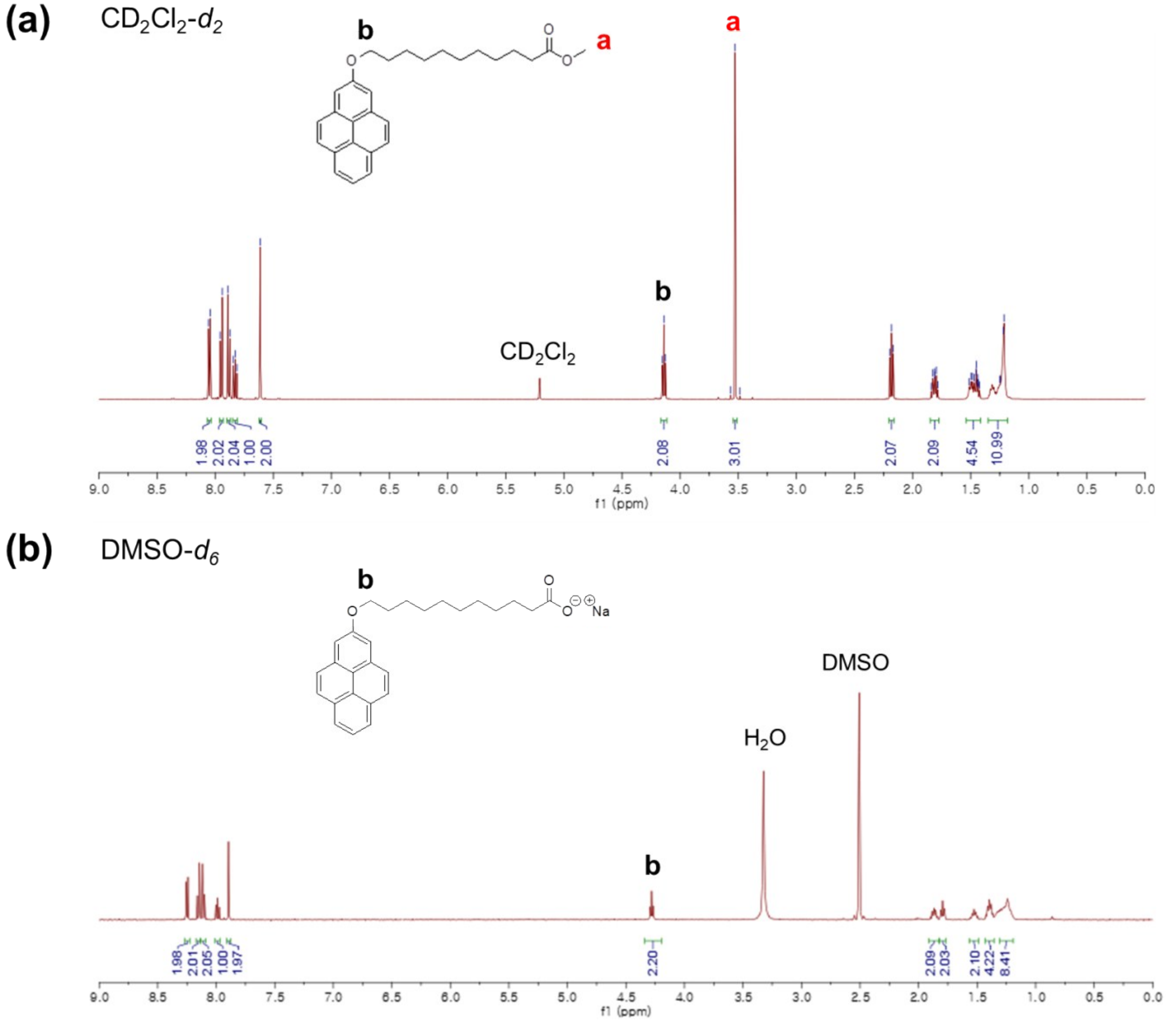

Fig. S1 ^{1}H NMR spectra of precursor **3** in CD_2Cl_2 and an amphiphilic monomer **1** in DMSO.

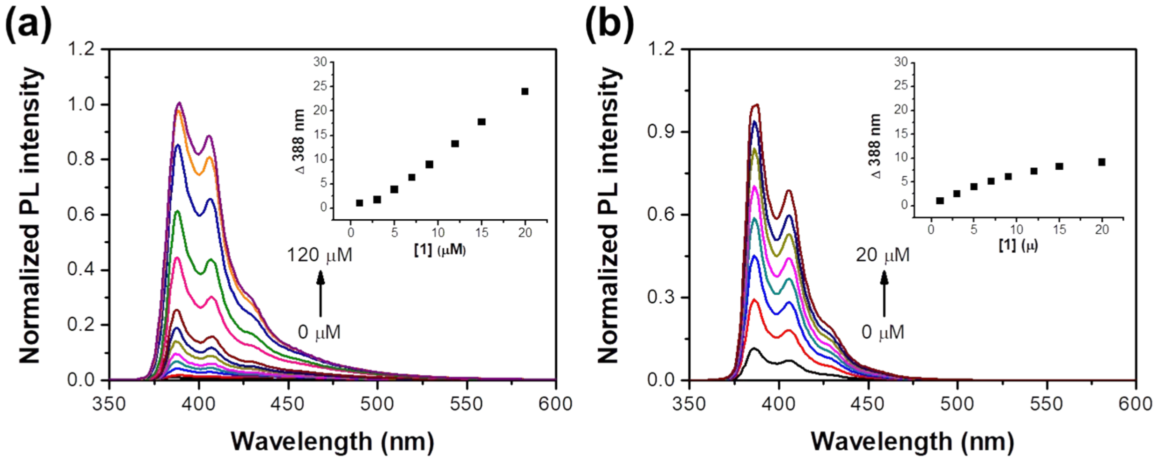

Fig. S2 Fluorescence emission spectra of **1** with increasing concentration (1~120 × 10^{-6} M) in (a) H_2O and (b) DMSO.

Table S1 Fluorescence quantum yields of **1** upon the addition of Fe^{3+} or Fe^{2+} ions.

	1	**1**+Fe^{3+}	**1**+Fe^{2+}
Φ_{FL}	5.0%	0.45%	5.1%

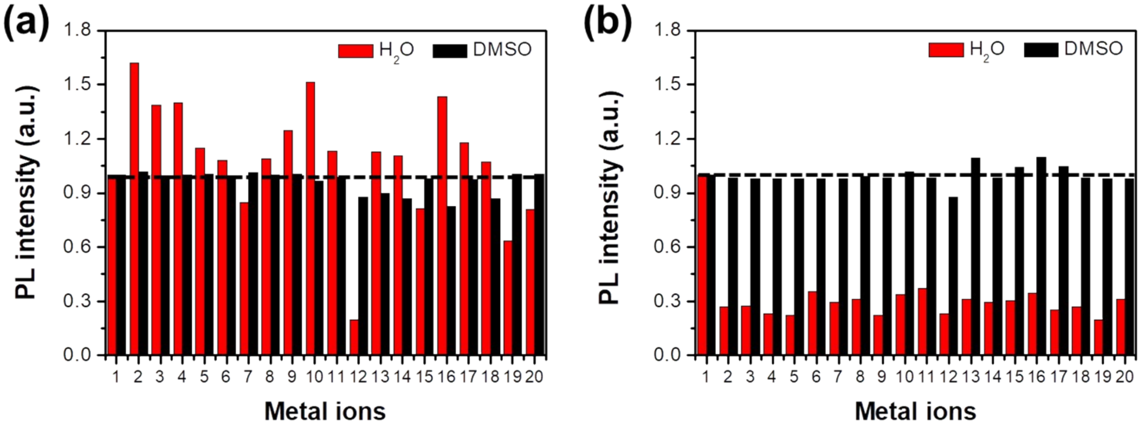

Fig. S3 (a) Fluorescence intensity changes of **1** (5.0×10^{-6} M) in H_2O and DMSO upon the addition of various metal ions (3.0×10^{-6} M; (b) Fluorescence intensity changes of **1** (5.0×10^{-6} M) in H_2O and DMSO toward Fe^{3+} ions in the presence of other metal ions (5.0×10^{-6} M). 1: blank, the chloride salts of metal ions: 2: Li^+, 3: Na^+, 4: K^+, 5: Cs^+, 6: Mg^{2+}, 7: Ca^{2+}, 8: Sr^{2+}, 9: Ba^{2+}, 10: Mn^{2+}, 11: Fe^{2+}, 12: Fe^{3+}, 13: Co^{2+}, 14: Ni^{2+}, 15: Cu^{2+}, 16: Ag^+, 17: Zn^{2+}, 18: Cd^{2+}, 19: Sn^{2+}, 20: Pb^{2+}. Excitation at 329 nm/H_2O, 341 nm/DMSO; fluorescence intensity was monitored at 388 nm; $[Na^+]=[K^+]=[Ca^{2+}]=1.0 \times 10^{-3}$ M.

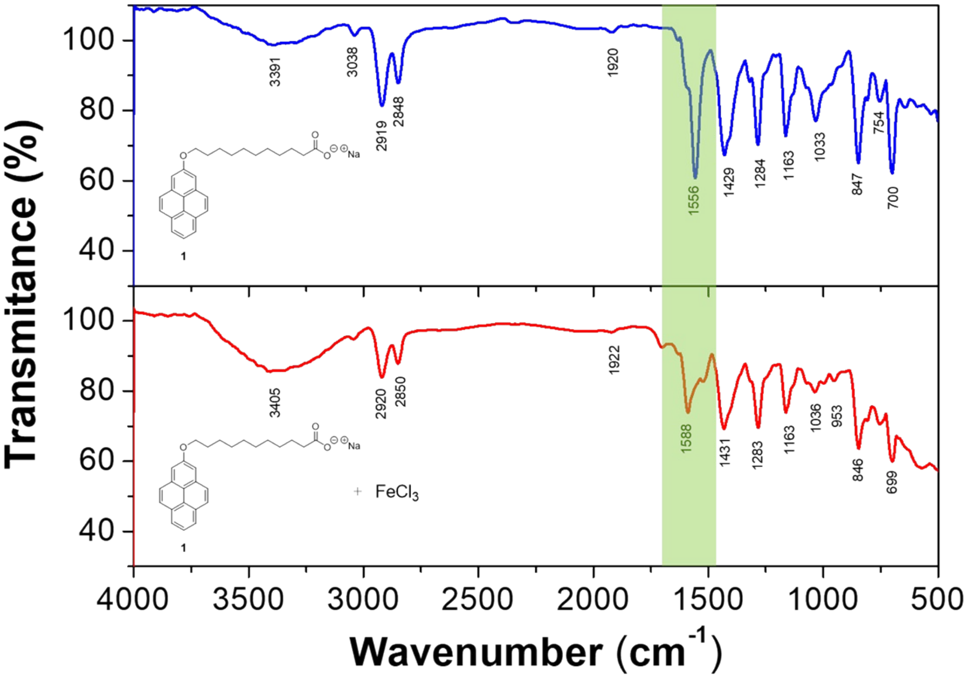

Fig. S4 IR spectra of **1** and **1**-Fe^{3+} complex.

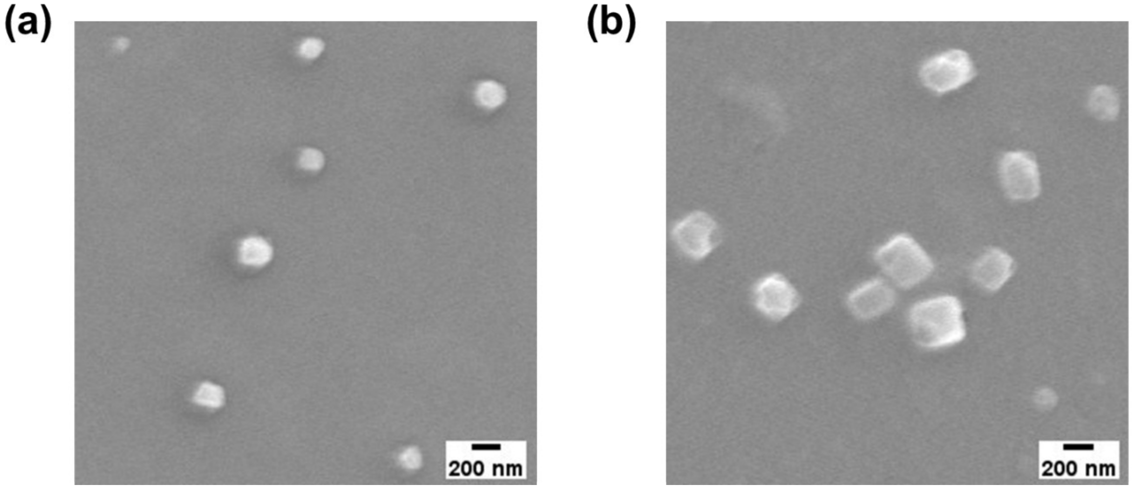

Fig. S5 SEM image of **1** upon the addition of Fe^{3+} ions in H_2O: (a) **1** and (b) **1**-Fe^{3+} ions.

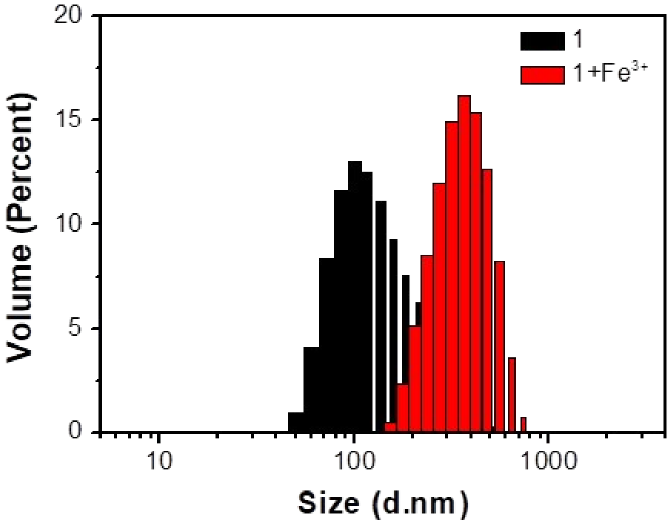

Fig. S6 Hydrodynamic radii obtained from DLS for **1** (5.0×10^{-6} M) upon the addition of Fe^{3+} ions (3.0×10^{-6} M).

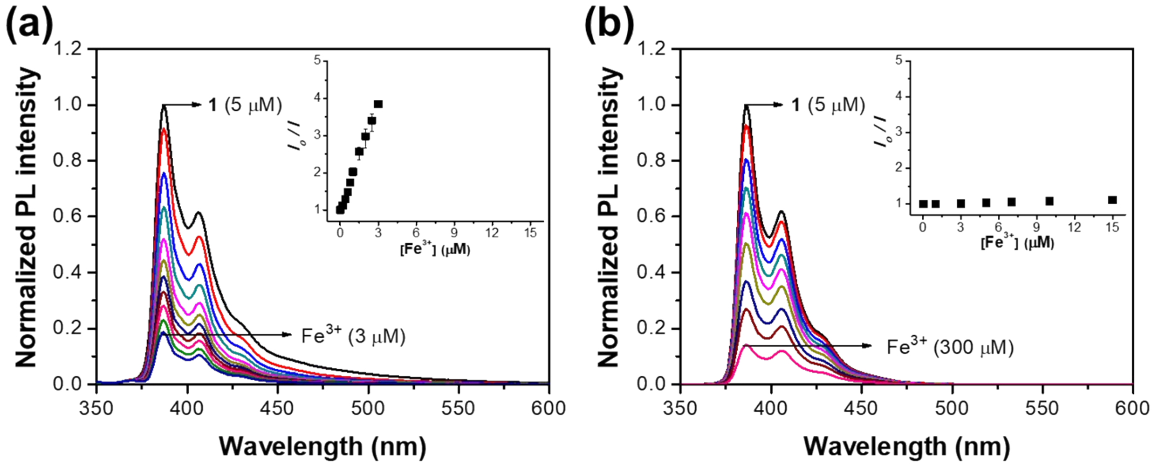

Fig. S7 Fluorescence intensity changes and Stern-Volmer (SV) plots (inset) of **1** (5.0×10^{-6} M) upon the addition of various amounts of Fe^{3+} ions in (a) H_2O and (b) DMSO. Excitation at 329 nm/H_2O, 341 nm/DMSO.

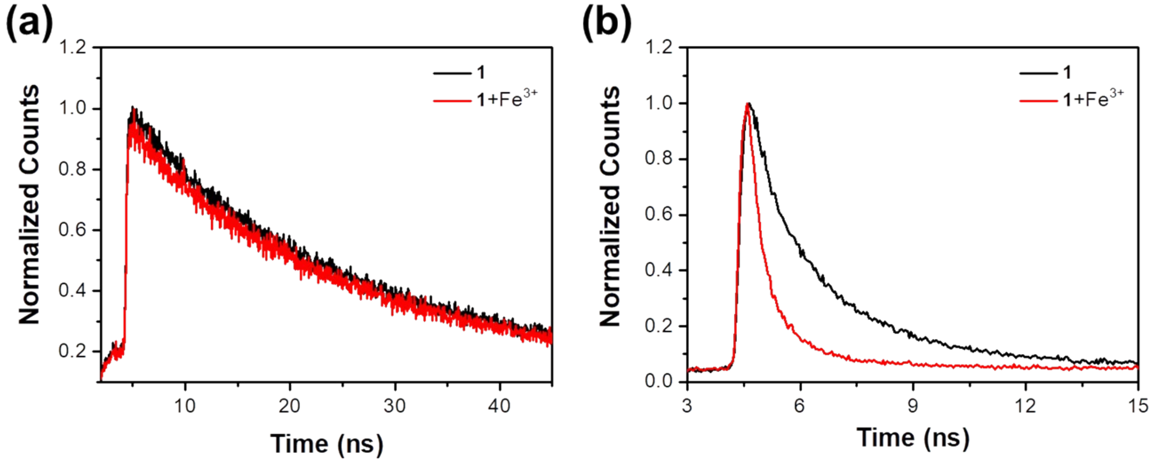

Fig. S8 Time-resolved Fluorescence decay of **1** (5.0×10^{-6} M) upon the addition of Fe^{3+} in DMSO and aqueous solution. Excitation at 370 nm; lifetime decay was monitored at 390 nm.

Table S2 Comparison of **1** with recently reported Fe^{3+} sensors.

Sensors	Response Type	LOD (μM)	Reaction Media	Rf
Dansyl Based derivative	Fluorescence	6.2×10^{-1}	$EtOH/H_2O$ (1:1, *v/v*)	4
Poly Electrospun Nanofiber Hydrogel	Color	61.5	H_2O	5
Anthracene Based derivative	Color	4.4×10^{-1}	DMF	6
Rhodamin derivation	Fluorescence	7.4×10^{-1}	CH_3CN/H_2O (1:1, *v/v*)	7
Nitrogen-doped carbon dots	Color	5.7×10^{-1}	CDs/PVA films	8
Our Works	**Fluorescence**	$\mathbf{7.0 \times 10^{-2}}$	$\mathbf{H_2O}$	

Table S3 Fluorescence lifetimes (τ_i, ns) and relative amplitudes (RA, %) for **1** and **1**-Fe^{3+} in H_2O.

Condition	Samples	τ_1 (ns)	RA (%)	τ_2 (ns)	RA (%)
$\mathbf{H_2O}$	**1**	0.97	97.44	3.15	2.55
	1-Fe^{3+}	0.35	>99	1.19	trace
DMSO	**1**	20	100%	-	-
	1-Fe^{3+}	-	-	-	-

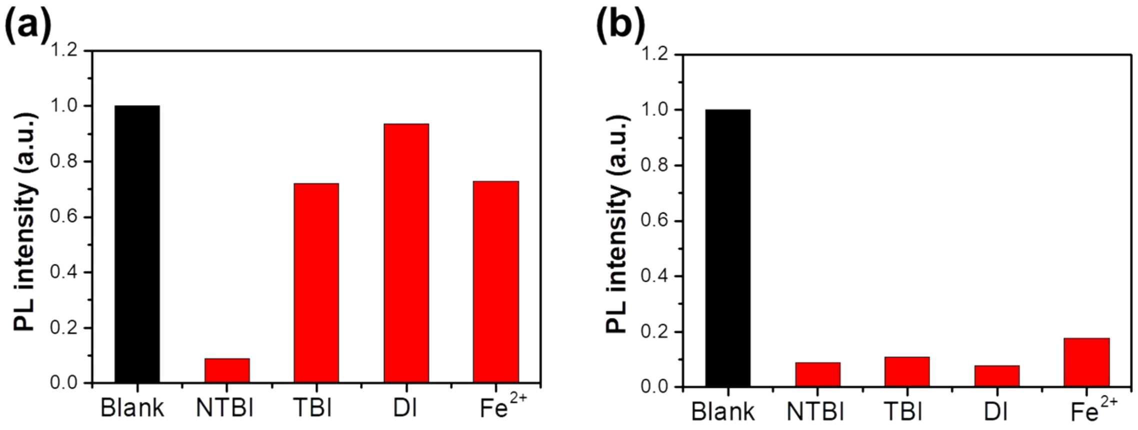

Fig. S9 (a) Fluorescence intensity changes of **1** (5.0×10^{-6} M) in diluted serum (10%) upon the addition of NTBI, TBI, DI, and Fe^{2+} (2.0×10^{-5} M); (b) Fluorescence intensity changes of **1** (5.0×10^{-6} M) in diluted serum (10%) toward Fe^{3+} ions in the presence of other competitive substances; Excitation at 329 nm; fluorescence intensity was monitored at 388 nm.

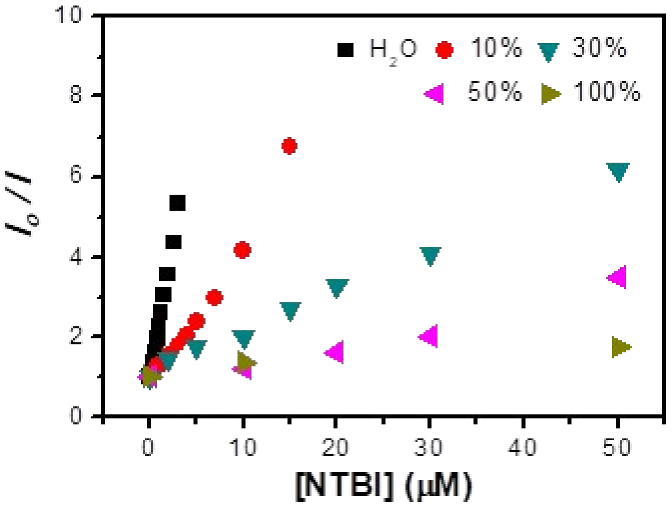

Fig. S10 Fluorescence intensity changes of **1** (5.0×10^{-6} M) in diluted serum (10%) upon the addition of NTBI, TBI, DI, and Fe^{2+} (2.0×10^{-5} M). Excitation at 329 nm; fluorescence intensity was monitored at 388 nm.

Table S3 Comparison of **1** with recently reported Fe^{3+} sensors.

Sensors	**Response Type**	**LOD (μM)**	**Reaction Media**	**Rf**
Dansyl Based derivative	Fluorescence	6.2×10^{-1}	$EtOH/H_2O$ (1:1, *v/v*)	4
Poly Electrospun Nanofiber Hydrogel	Color	61.5	H_2O	5
Anthracene Based derivative	Color	4.4×10^{-1}	DMF	6
Rhodamin derivation	Fluorescence	7.4×10^{-1}	CH_3CN/H_2O (1:1, *v/v*)	7
Nitrogen-doped carbon dots	Color	5.7×10^{-1}	CDs/PVA films	8
Our Works	**Fluorescence**	$\mathbf{7.0 \times 10^{-2}}$	$\mathbf{H_2O}$	

Table S4 Elemental analysis of **1**

Standard		**BBOT**				
Element	No.	Nitrogen	Carbon	Hydrogen	Sulphur	Oxygen
Sample 1 : Compound **1**	1	0.041	73.090	6.852	0.143	10.910
	2	0.037	73.129	6.984	0.080	10.788
	3	0.038	73.252	6.989	0.000	10.710
	Average	0.039	73.157	6.942	0.074	10.803

w/w %

References

(1) A. G. Crawford, Z. Liu, I. A. I. Mkhalid, M.–H. Thibault, N. Schwarz, G. Alcaraz, A. Steffen, J. C. Collings, A. S. Batsanov, J. A. K. Howard, T. B. Marder, *Chem.: Eur. J.*, 2012, **18**, 5022.

(2) J. Folch, M. Lees, GMS. Stanley, *J. Biol. Chem.*, 1957, **467**, 226.

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(4) Y. W. Choi, G. J. Park, Y. J. Na, H. Y. Jo, S. A. Lee, G. R. You, *Sens. Actuators B: Chem.,* 2014, **194**, 343.

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(8) X. Wu, C. Ma, J. Liu, Y. Liu, S. Luo, M. Xu, P. Wu, W. Li, S. Liu, *ACS Sustainable Chemistry & Engineering*., 2019, **7**, 18801.

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DS	2
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FIDRES	0.152588 Hz
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RG	144
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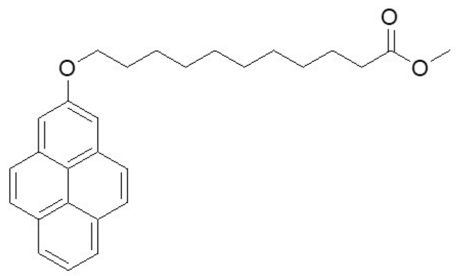
DE	6.50 usec
TE	297.6 K
D1	1.00000000 sec
TD0	1



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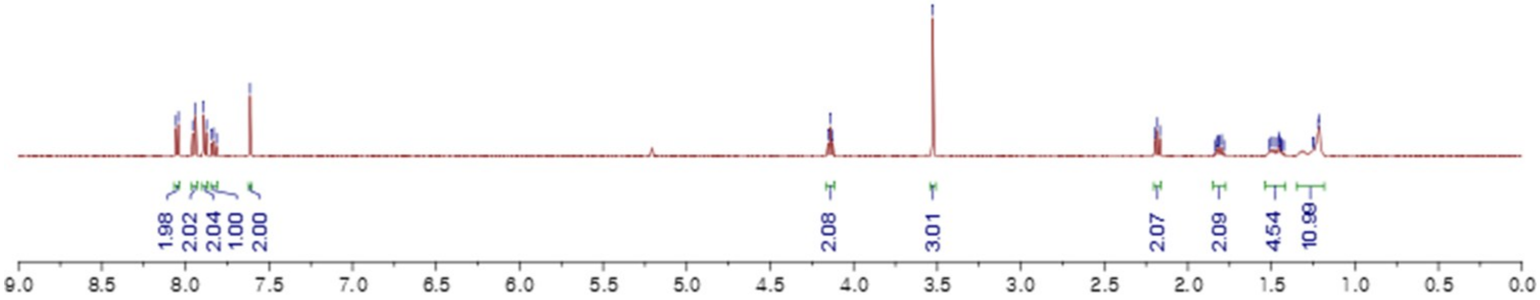

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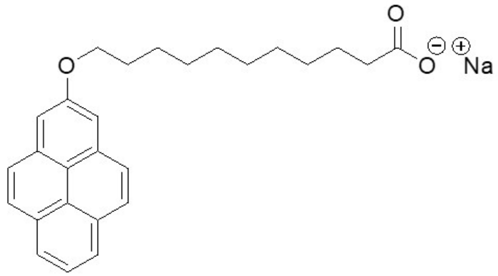
D1 1.00000000 sec
TD0 1



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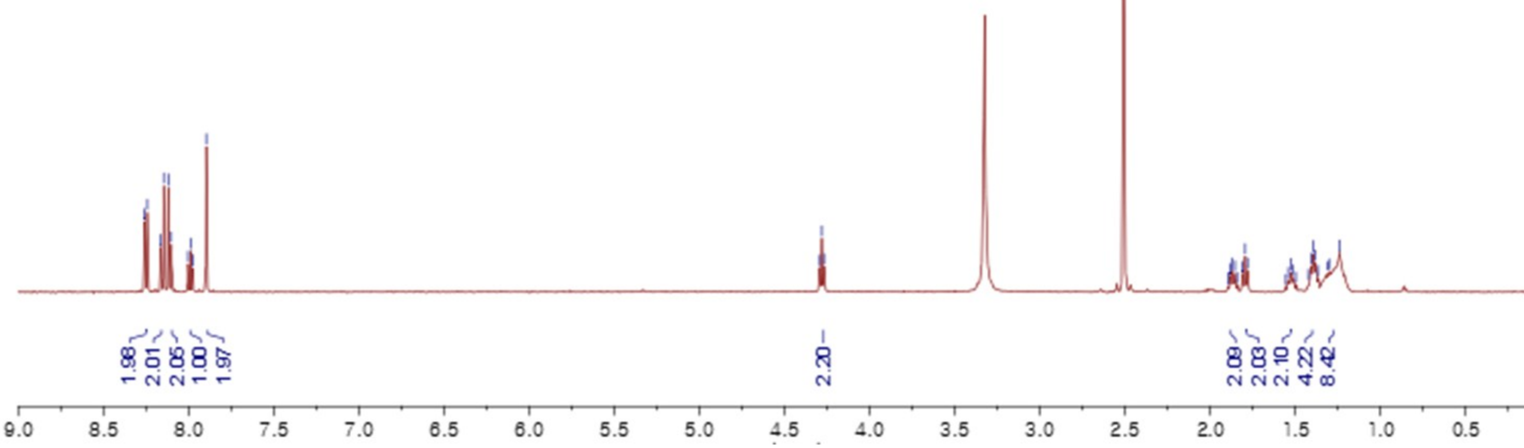

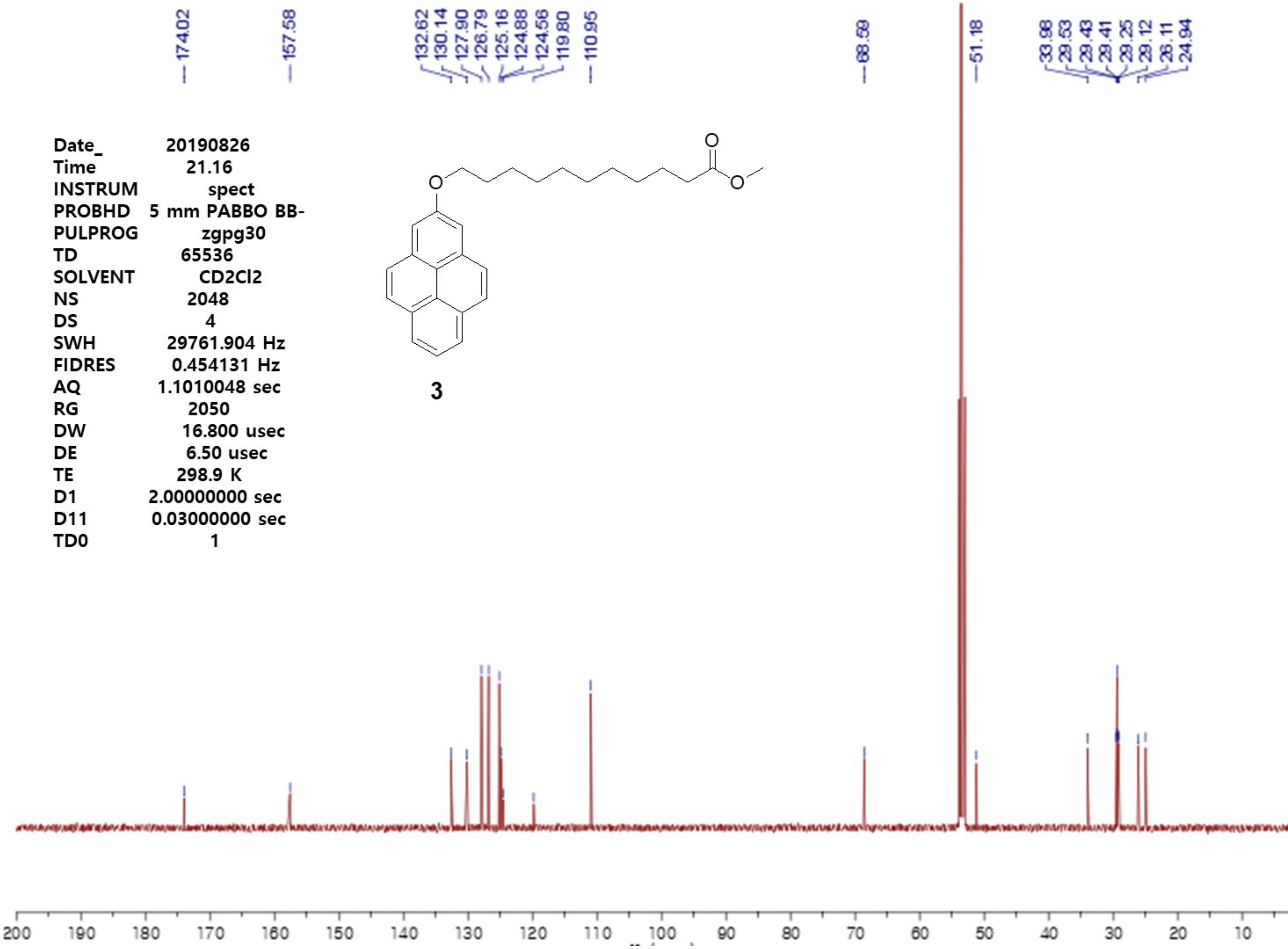

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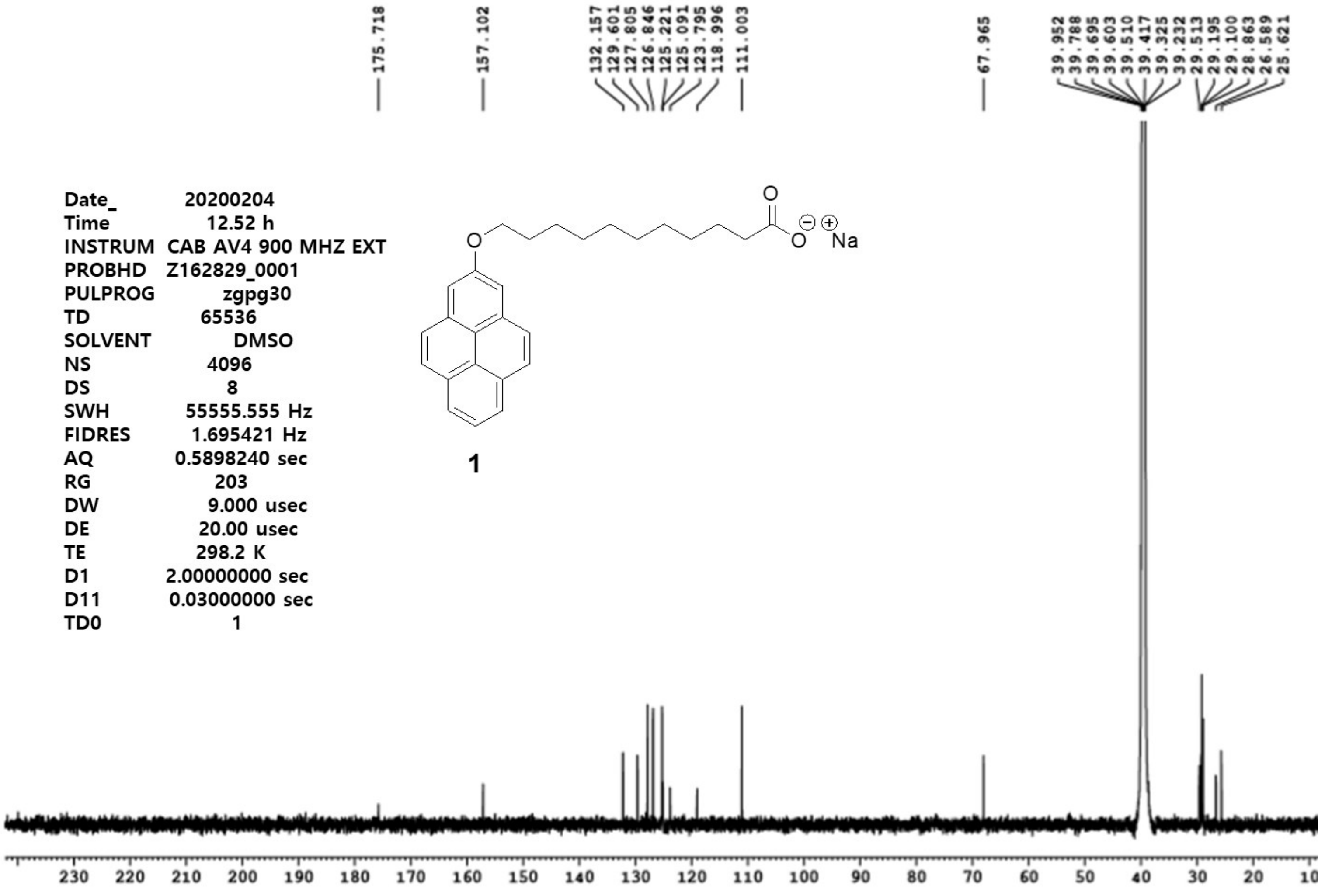
3



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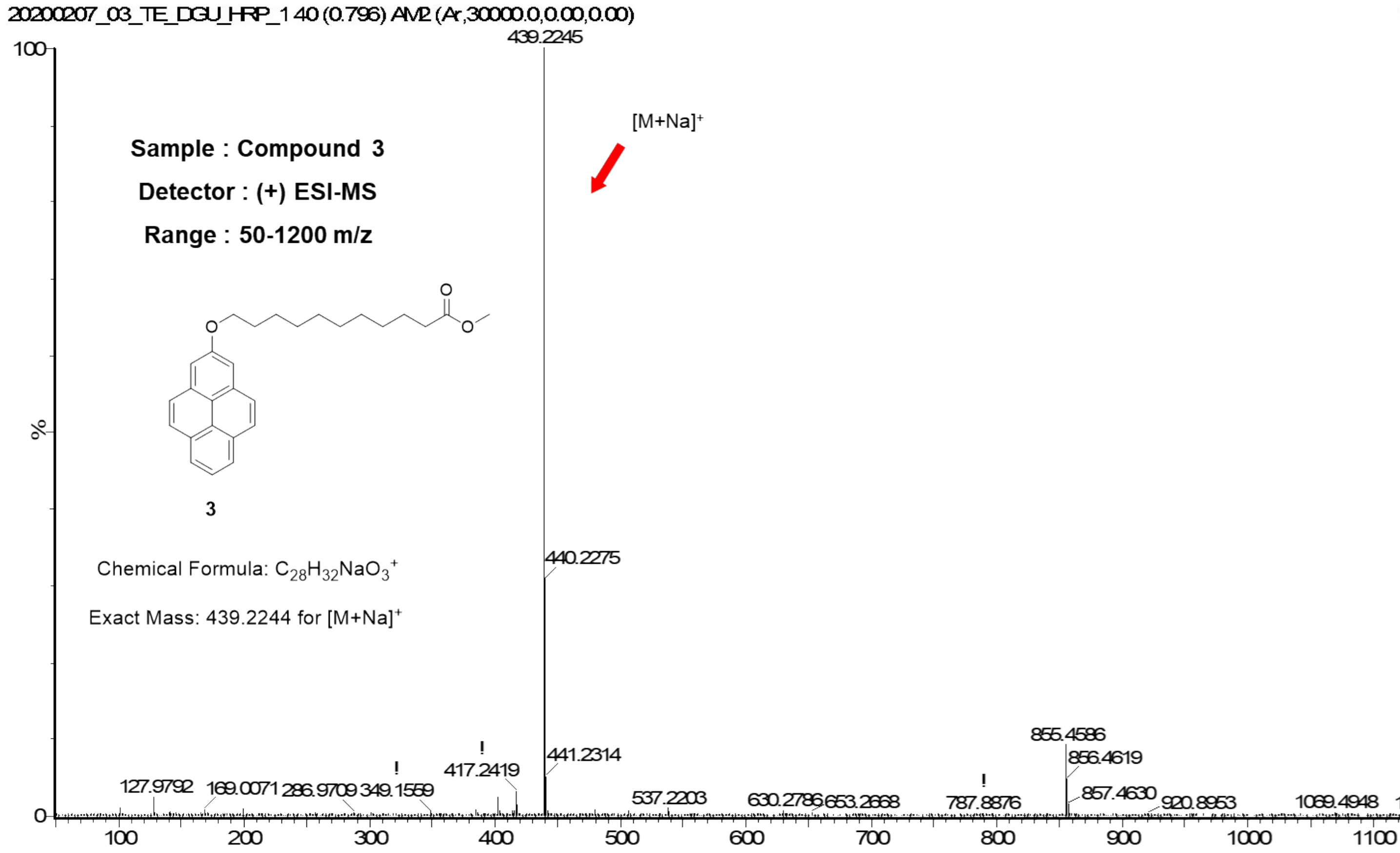
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O
O
3
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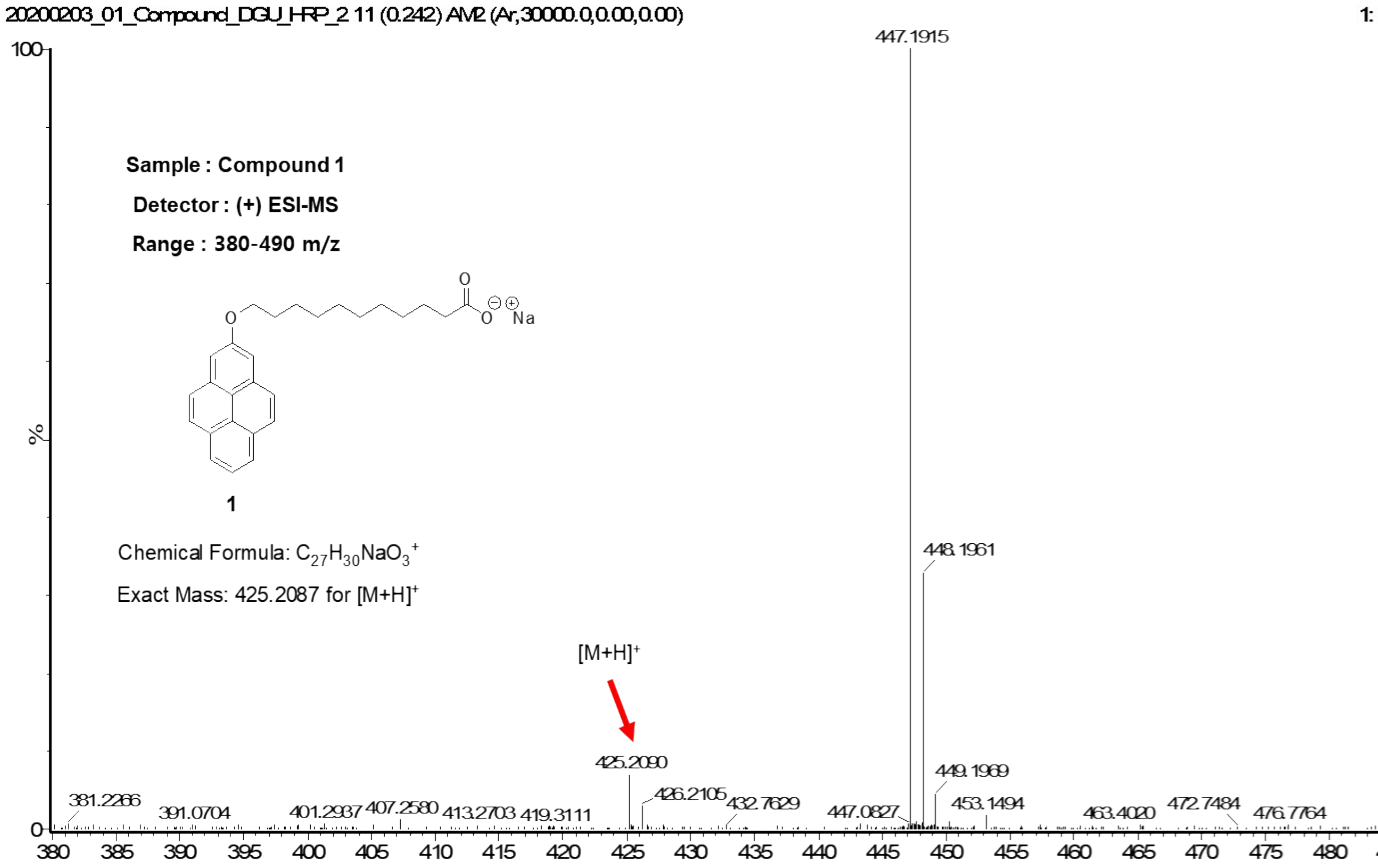
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