

Problem Session

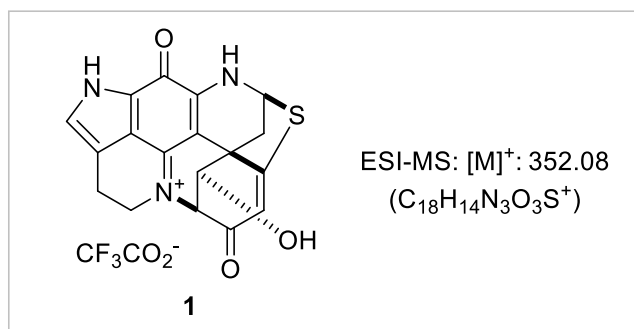
Mar. 2, 2024

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In the process of isolation from crude extracts from sponge containing known natural product **1**, new compound **X** was detected. Spectroscopic data of **X** were highly similar with those of **1**.

isolation process
(steps 1-5 were repeated **multiple times**)
1. HPLC using MeOH/H₂O, 0.1% CF₃CO₂H
2. dried under N₂
3. NMR measurements using DMSO-*d*₆
4. washing the NMR tube with MeOH
5. The combined solution
(DMSO-*d*₆/MeOH) was dried under N₂

crude extracts (40.0 g)
from sponge
containing **1** → **1** (48.2 mg) + **X** (1.1 mg)



Please propose the structure of **X** and the possible mechanism of formation of **X** based on the data set (see below A–D). It is not necessary to use all data set for providing a solution.

Note: There should be contradiction between the correct structure and the reported NMR data shown in the boxes in the next page. Please explain the reasonable factors.

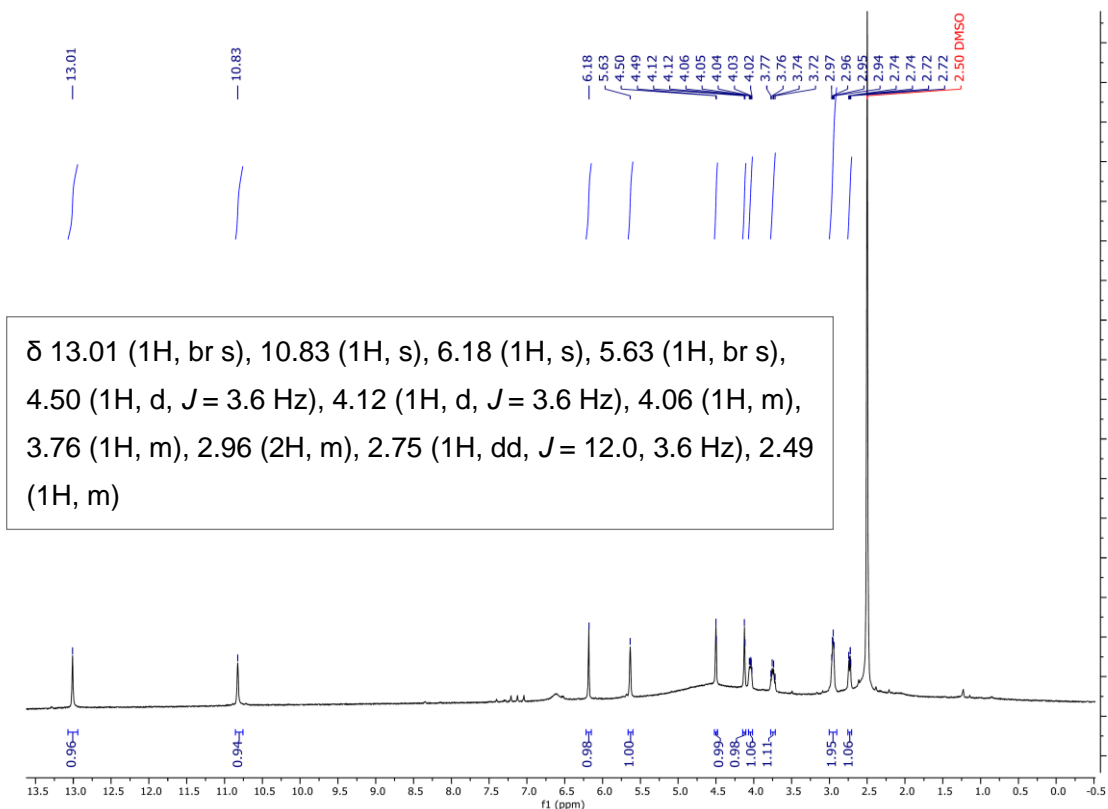
Data set

A. ESI-MS data

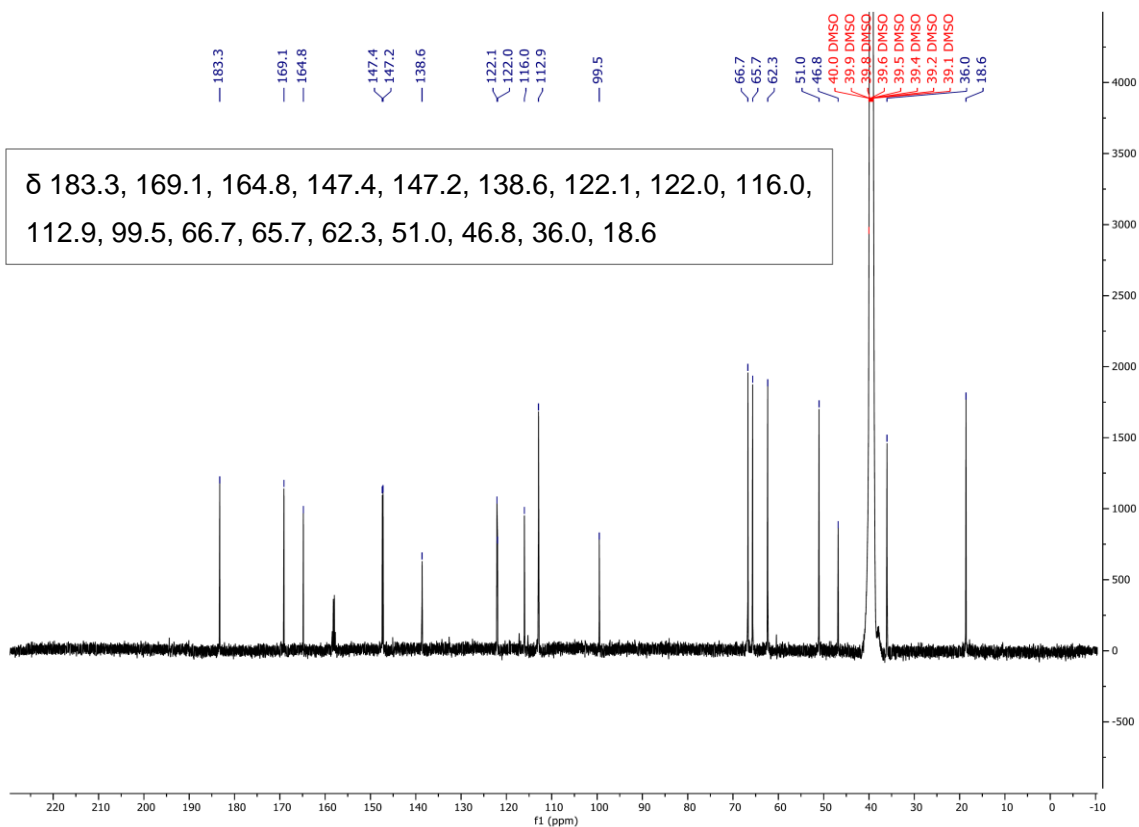
$m/z = 369.11$ (+17 u from **1**)

B. Reported NMR chemical shifts (see boxes, the spectra are also shown)

^1H NMR (DMSO- d_6 , 600 MHz)



^{13}C NMR (DMSO- d_6 , 150 MHz)



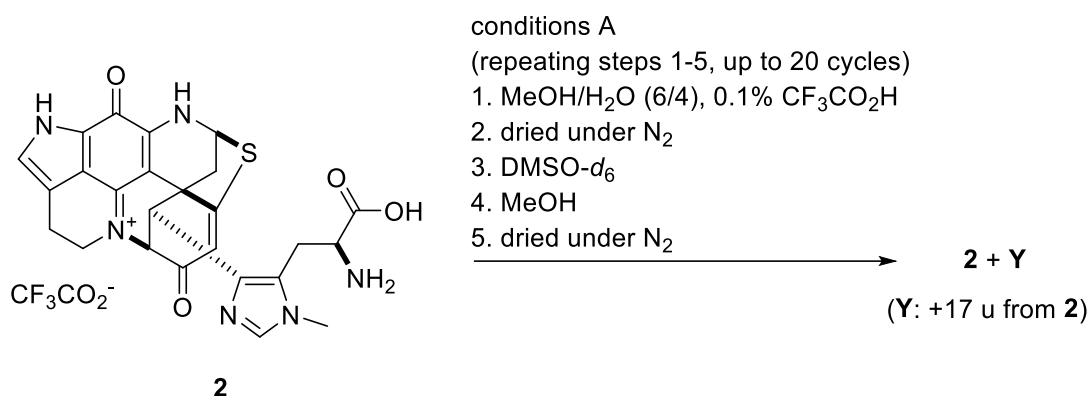
C. ICP-MS analysis

Results of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis of element concentration in the solvents that were used in the experiments ($\mu\text{g/mL}$, semiquantitative analysis, $\pm 30\%$)

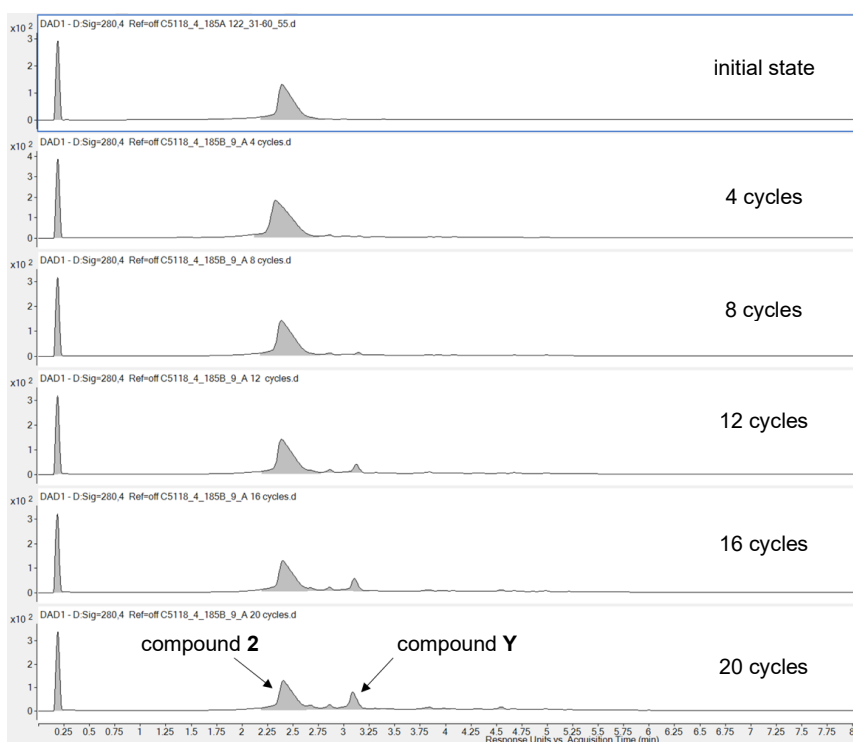
solvent	Na	Mg	Al	K	Ca	Fe
H ₂ O	1.5945	0.0059	0.0056	0.0524	0.2324	0.0015
DMSO- <i>d</i> ₆	10.7043	0.1108	1.6995	0.3858	9.7627	0.1340
MeOH	1.4591	0.0374	0.0050	0.3214	0.9298	0.0285
CF ₃ CO ₂ H	4.6675	0.2586	0.0248	0.6821	0.4649	0.2814

D. Additional information

New compound **Y** was detected when pure **2** that shares the substructure with **1** was treated with conditions A. Compound **Y** also showed 17 u larger mass from **2**.



Data of LC-MS analysis (RPHPLC)



Natural Product Artifacts: Case study

1. Background

- Discorhabdin L (**1**) is one of discorhabdins isolated from sponge genus *Latrunculia*.^{1,2}
(For isolations of other discorhabdins, see references^{3,4,5,6,7,8,9,10,11,12,13,14})

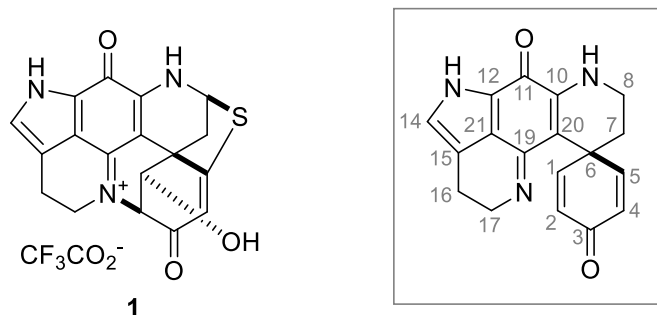


Figure 1. Structure of discorhabdin L (**1**) and general structure of discorhabdins and atom numbering.

- Natural product isolation could be good lesson for dealing with the complex compounds properly.
- The unusual reaction during isolation of discorhabdins is featured in this session.¹⁵

2. Structure of X

- Compound **X** was generated via **trideuteromethylation at C14**.

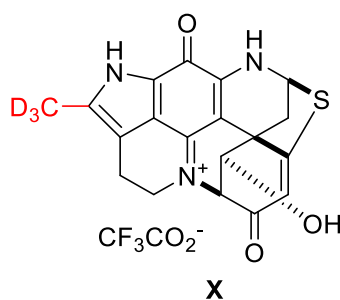
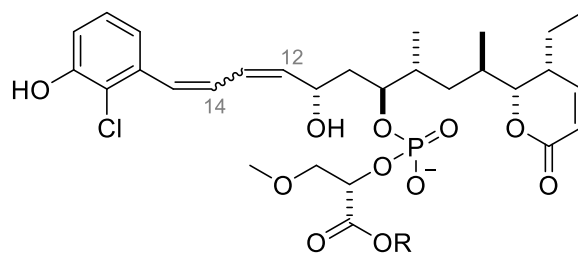


Figure 2. Structure of **X**.

- Note: For structure determination, it is necessary to utilize extensive data such as 2D NMR spectra (for details of structure elucidation, see the original paper).

- Artifacts of trideuteromethyl group (CD₃) have not been frequently reported. Several papers regarding trideuteromethylation indicated that the source of CD₃ group was CD₃OD used in the NMR experiments. In these cases, mechanisms of formation are relatively more obvious, because these reactions occurred by intrinsic nucleophilicity of MeOH (hemiacetal formation, esterification etc.).



franklinolide A (**3**): 12Z, 14Z, R = H
franklinolide A methyl ester (**4**): 12Z, 14Z, R = CH₃
franklinolide A deuteromethyl ester (**5**): 12Z, 14Z, R = CD₃
franklinolide B (**6**): 12E, 14E, R = H
franklinolide C (**7**): 12E, 14Z, R = H

Figure 3. Isolated franklinolides and trideuteromethylated **5**.¹⁶

• For a review article of formation of natural product artifacts, see reference 17.

MS analysis:

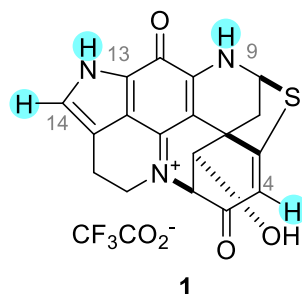
The results of ESI-MS analysis (+17 u from **1**) strongly indicated that introduction of a trideuteromethyl group.

NMR analysis:

*The ¹H signals of **1** that are expected to appear at downfield (>6 ppm)*

- H13 and H9 (two NH protons in DMSO-*d*₆)
- H14 (pyrrole: typical signal appears at 6–7 ppm)
- H4 (enone: typical signal appears around 6 ppm)

*The observed broad peak around 6.7 ppm might be a signal of the OH group



→The peak corresponding to pyrrole (H14) is missing.

c.f. reported H14 in CD₃OD (7.10, s)

*The chemical shifts of **1** in DMSO-*d*₆ have not been reported.

The contradiction of reported ^{13}C NMR signals

- Only 18 signals of ^{13}C NMR were detectable in the obtained compound (note: signal around 157 ppm probably corresponds to the carbon of carbonyl group of trifluoroacetic acid), indicating introduction of a trideuteromethyl group to the C14 due to the J_{CD} (septet) coupling and long T_1 , causing low S/N ratio.
- The corresponding signal is expected to be around 11 ppm based on the methyl (non-deuterated) version of **X** (compound **17** in page A6).

Additional support

- In the case of compound **Y**, obviously, the chemical shift of C14 was largely shifted.

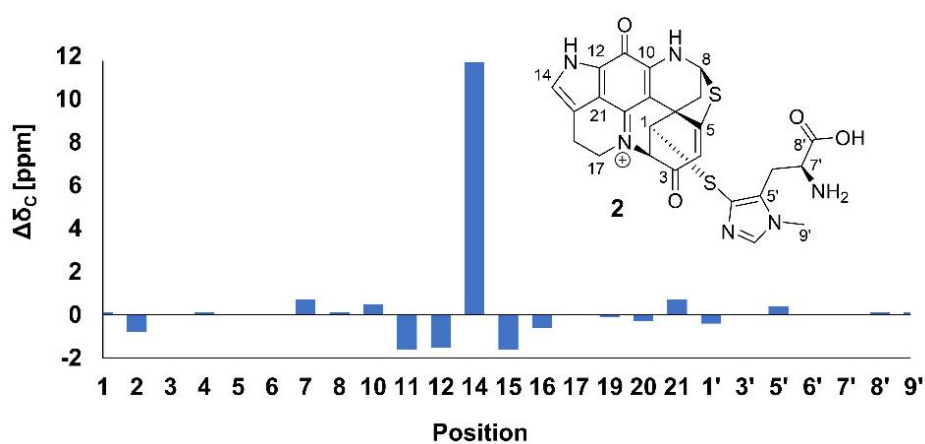


Figure 4. ^{13}C NMR chemical shift difference between natural product **2** and trideuteromethylated **Y**. (note: the previously reported ^1H NMR data of **1** and **2** was recorded in CD_3OD)

- Regarding LC-MS analysis, the more hydrophobic nature of **Y** than **2** is consistent with the C14-trideuteromethylation.

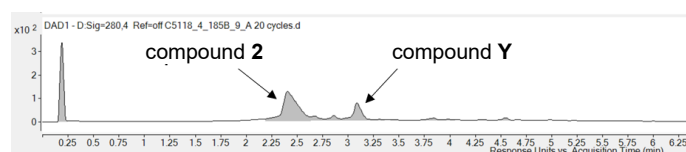


Figure 5. Reversed-phase LC-MS analysis (UV 280 nm) of **Y** formation after 20 cycles of dissolution of **2** in $\text{DMSO-}d_6$ and drying under N_2 .

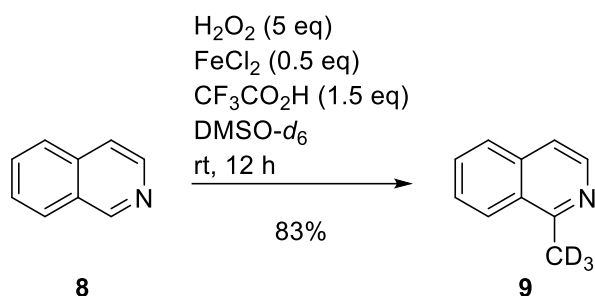
Direct evidence of existence of a trideuteromethyl group

- Based on these observations, authors conducted ^2H NMR experiments (90 MHz, MeOH): 2.62 ppm for **X**, 2.60 ppm for **Y**

3. How does trideuteromethylation occur?

• The source of the CD₃ group is obviously DMSO-*d*₆. Because of DMSO is a well-known methyl radical source in the presence of [•]OH, the authors proposed that [•]CD₃ species was generated from DMSO-*d*₆ and a [•]OH via Fenton reaction. The source of H₂O₂/[•]OH will be discussed later.

• Authors hypothesized that the trace amount of iron species catalyzed the reaction. Similar catalytic (trideutero) methylation of aromatic ring using DMSO (-*d*₆) was reported (**8**→**9**).^{18,19}

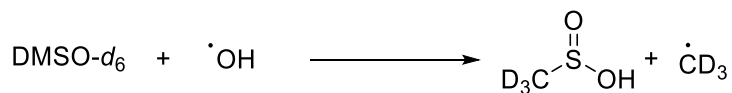
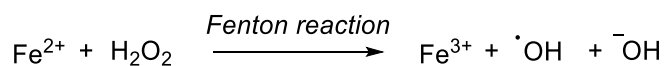


• To investigate the trace amount of metal element, ICP-MS analysis of the solvents was conducted. Authors reported that these trace amount of iron species in DMSO-*d*₆ and CF₃CO₂H could be responsible for the reaction because the iron species could be concentrated by repetitive NMR analysis and evaporation of each fraction.

• Considering the reported isolation procedure (approx. 1100 mL total HPLC solvent containing 0.1% CF₃CO₂H, 8.5 mL DMSO-*d*₆ for analyzing fractions), the maximal amount of solvent-derived iron species could be 0.01 equivalent to the generated **X**. If the iron is relevant with the reaction, other sources (e.g. labware/HPLC) might exist.

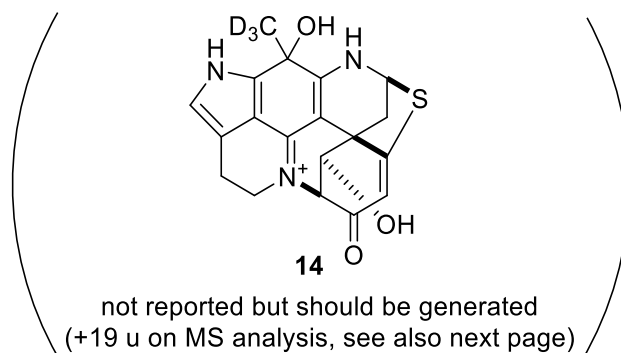
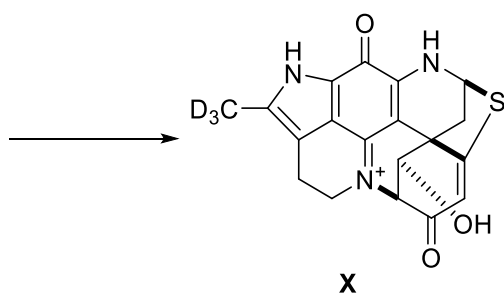
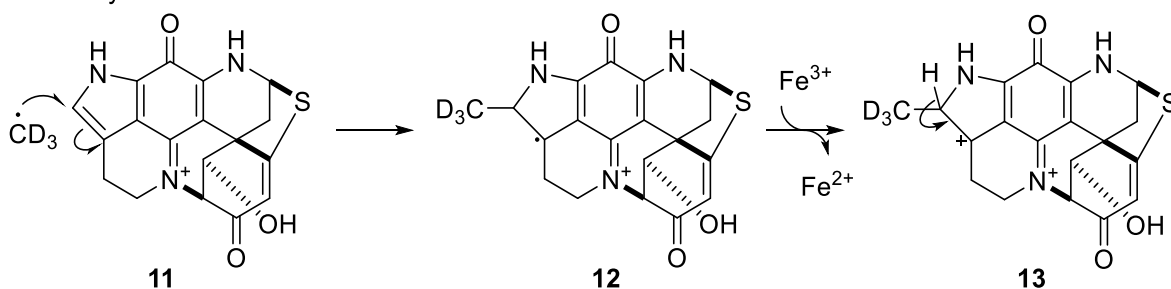
Table 1. Extracted data of ICP-MS analysis (iron, µg/mL, semiquantitative analysis, ±30%)

solvent	Fe
H ₂ O	0.0015
DMSO- <i>d</i> ₆	0.1340
MeOH	0.0285
CF ₃ CO ₂ H	0.2814



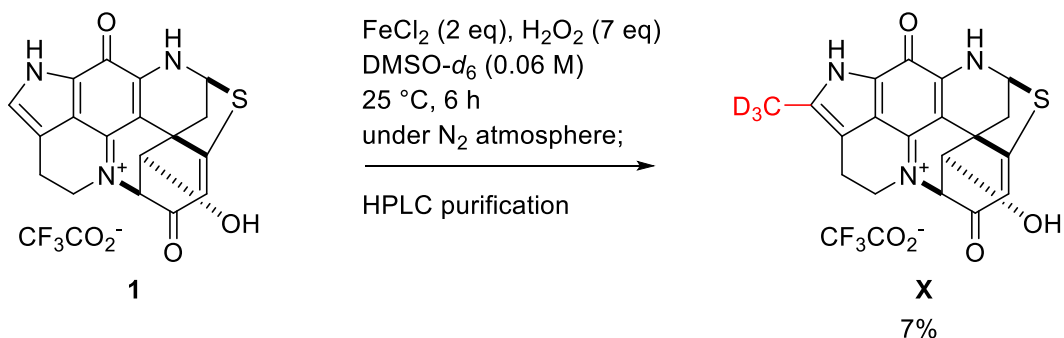
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electron deficient/
sterically unhindered site

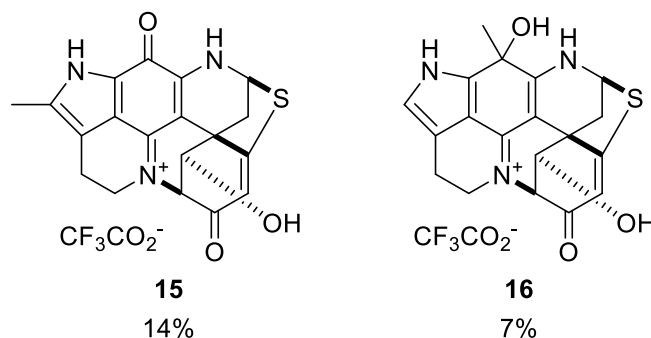


• To investigate the feasibility of the above hypothetical reactions, authors conducted the following semisynthetic experiments (see top of the next page). In this investigation, substantial amounts of trideuteromethylated **X** and **Y** were obtained from **1** and **2**, respectively. These results supported the formation of trideuteromethyl radical species from DMSO-*d*₆ during the NMR-guided isolation process of **1** and **2**.

• Since the end of the reaction was reported to be confirmed by LC-MS analysis, it can be ruled out that **X** and **Y** formed from residual **1** and **2** during these purification processes.



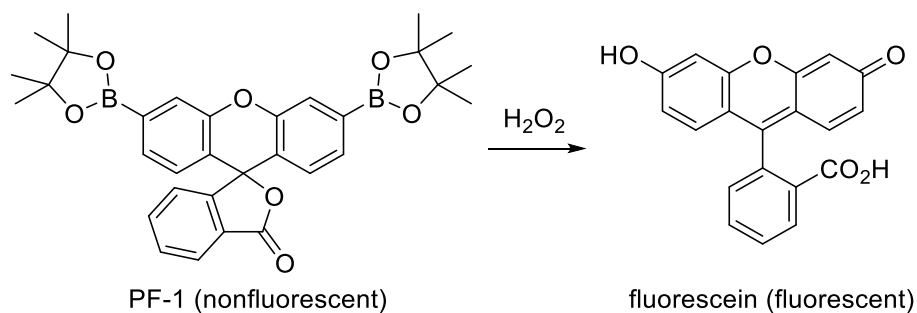
In the case of nondeuterated DMSO, **16** was also reported.



- In this semisynthetic investigation, excess amounts of reagents were used to accelerate the reaction. There was no investigation for evaluating feasibility that contaminated small amount of iron species could catalyze this reaction.

4. What is the origin of H_2O_2 (or $\cdot\text{OH}$)?

- In the main text, authors mention the possibility of $\cdot\text{OH}$ and H_2O_2 (formed by coupling of $\cdot\text{OH}$) generation from microdroplets, which is a controversial topic.^{20,21,22}
- The original report claims *spontaneous* H_2O_2 formation (reported H_2O_2 concentration: $\sim 30\ \mu\text{M}$) at the air-water interface of water microdroplets by pneumatic spray.^{23,24} H_2O_2 was initially detected by using a following established fluorescent probe.²⁵



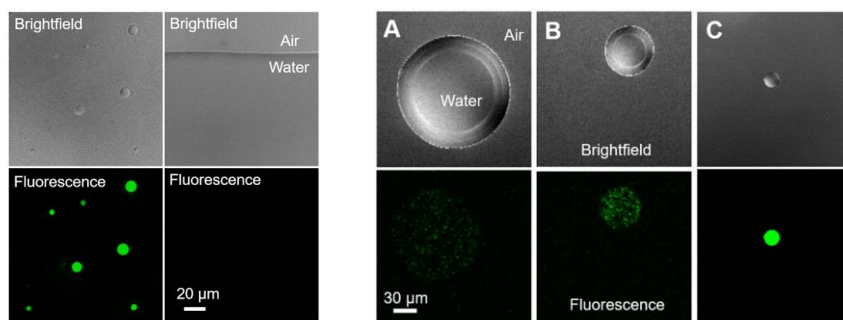
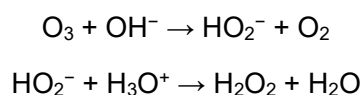
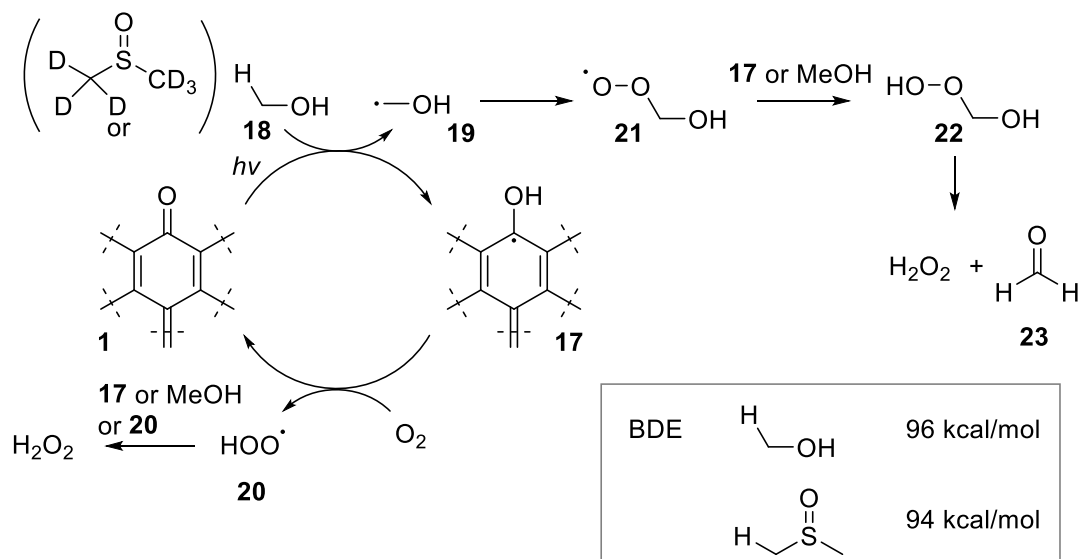


Figure 6. Detection of H₂O₂ in microdroplets by confocal microscopy PF-1 as a fluorescent probe in ref. 23.

• Other research group reported that atmospherically relevant O₃ concentration (10-100 ppb) is necessary for formation of 2–30 μM H₂O₂ in the water microdroplets.^{26,27}



• Considering the quinone structure of discorhabdins, photocatalytic production of H₂O₂ like anthraquinone might be plausible, while intentional light irradiation was not conducted.



• It is unclear that the reported procedure can supply sufficient amount of O₂. The purity of N₂ gas for drying process is not mentioned (although the drying instrument was not mentioned, it should be a rapid evaporation system by combination of N₂ flow and vacuum, which is able to evaporate DMSO).

• $\cdot\text{OOH}$ is reported to generate methyl radical from DMSO.²⁸ This reaction could be an additional source of methyl radical, which does not require the iron (II).

References

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