Electronic Supplementary Material (ESI) for Analyst.


**SUPPORTING INFORMATION**

# "Lighting up" fluoride: cellular imaging and zebrafish model interrogations using a simple ESIPT based mycophenolic acid precursor-based probe

Neha Jain, *[a] Prasad M. Sonawane, *[a] Haoyan Liu,[b] Arkaprava Roychaudhury,[c] Youngseob Lee,[d] Jongkeol An,[a] Donghyeon Kim,[a] Dongwook Kim,[e] Yunsu Kim,[f] Yeu–Chun Kim,[b] Kyung-Bin Cho,[d] Hee–Sung Park,[f] Cheol–Hee Kim,*[c] David G. Churchill*[a,b]

[a.] *Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea. E-mail: dchurchill@kaist.ac.kr*
[b.] *Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea*
[c.] *Department of Biology, Chungnam National University, Daejeon 34134, Republic of Korea*
[d.] *Department of Chemistry, Jeonbuk National University and Research Institute for Physics and Chemistry, Jeonju 54896, Republic of Korea*
[e.] *Center for Catalytic Hydrocarbon Functionalizations, Institute for Basic Science (**IBS**), Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea.*
[f.] *KAIST Institute for Health Science and Technology (KIHST) (Therapeutic Bioengineering Section), Daejeon 34141, Republic of Korea*

Corresponding Authors

*Email: zebrakim@cnu.ac.kr (C. H. Kim)

*Email: dchurchill@kaist.ac.kr (D. G. Churchill)

**Table of Contents**

**General Information**

All chemical reagents and solvents were purchased from the commercial suppliers (Aldrich, Tokyo Chemical Industry etc.), and used without further purification. Reactions were monitored by thin-layer chromatography (TLC). Column chromatography separations were conducted with silica gel (200–300 mesh). $^{1}H$, $^{13}C$, COSY, HMBC, HSQC, and NOESY NMR spectra were conducted on a Bruker Avance 400MHz instrument. TMS was used as an external solvent. Chemical shifts are reported in the standard notation ($\delta$) of ppm relative to the residual solvent peak at 7.26 ($CDCl_3$) for $^{1}H$ and 77.16 ($CDCl_3$) for $^{13}C$ as an internal solvent. The signal multiplicities were denoted by the abbreviations as s (singlet), d (doublet), t (triplet) and m (multiplet) etc. A Bruker Alpha ATR FTIR Spectrometer was used to collect infrared (IR) spectra. The frequency units are reciprocal centimeters ($cm^{-1}$). Bands can be depicted as broad (br), strong (s), medium (m), or weak (w). The electrospray ionization mass spectrometry (ESI-MS) was performed on a BRUKER micrOTOF-Q Ⅱ by the research support staff at KAIST. A Time-of-Flight mass spectrometer was measured at a resolution of 20 000. Absorption spectra were measured using a JASCO V-530 spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF-5301pc spectrofluorophotometer.

**Experimental Section**

**Synthesis of Myco-F probe**

The **Myco-OH** was prepared by published literature methods (see reference 25 in the main text).

Myco-OH (0.500 g, 1.85 mmol) was dissolved in DMF (15 mL) in the $N_2$ environment and stirred for 5 minutes. Then, this solution was treated with imidazole (0.277 g, 4.00 mmol) and stirred for an additional 15 minutes to afford a clear solution. After that time, tert-butyldimethylsilyl chloride (0.474 g, 3.00 mmol) was added slowly at 0$^{o}$ C. This mixture was stirred for 6 h at room temperature. The reaction was monitored by TLC. After the reaction was determined to be finished by TLC, water was added into the reaction mixture; the organic layer was then extracted by diethyl ether (3 × 25 mL). The combined organic layer was washed with 25 mL saturated NaCl solution and was then dried over $Na_2SO_4$ and concentrated. The residue was purified by silica gel flash chromatography using n-hexane/EtOAc (16:1) as an eluent to afford **Myco-F** as a white solid (0.51 g, 71.7% yield, M. P. 135-136$^{o}$ C). $^{1}H$ NMR (400 MHz, $CDCl_3$): δ = 7.28 (s, 1H, $H_6$), 3.11 (s, 3H, $H_2$), 2.68 (s, 3H, $H_{10}$), 1.05 (s, 9H, $H_{13}$), 0.27 (s, 6H, $H_{12}$), $^{13}C$ NMR (100 MHz, $CDCl_3$): 168.18 ($C_3$), 166.13 ($C_1$), 150.94 ($C_7$), 133.14 ($C_5$), 131.14 ($C_9$), 130.87 ($C_8$), 130.40 ($C_6$), 119.65 ($C_4$), 30.95 ($C_{12}$), 25.51 ($C_{13}$), 23.74 ($C_2$), 18.31 ($C_{10}$), 16.39 ($C_{11}$). MS/EI m/z C16H22BrNO3Si M+Na, 406.0449, found 406.0449.

**Spectral analysis measurements**

A stock solution of **Myco-F** (10 mM) was prepared by dissolving the probe (3.8 mg) in 1 mL of THF, and was further diluted in PBS (pH 7.4) solution with various concentrations to allow for reduced probe concentrations for further studies. All the absorbance and emission spectra were examined in PBS (pH 7.4) solution at an excitation wavelength of 398 nm. Further, the stock solution of analytes was prepared in PBS (7.4 pH). For determining the limit of detection, the fluorescent titration intensity of **Myco-F** (518 nm) was used to obtain the value of the slope. The detection limit was calculated with the following equation

Detection limit = $3\sigma/k$

In which, $\sigma$ is the standard deviation of the 10 control (**Myco-F** without addition of the fluoride ion) and k is the absolute value of the slope between the fluorescence intensity of **Myco-F** versus the fluoride ion concentration.

**DFT calculations**

All calculations in this research used Density Functional Theory[a] through the Gaussian 16 package[b] with unrestricted B3LYP functional.[c] The Def2-SVP basis set[d] was used for structure optimizations and frequency calculations. The Def2-TZVPP basis set[d] was used for energy calculations. Solvent (water) was included in the geometry optimizations through the CPCM scheme[e] implemented in Gaussian 16. In the text, we use electronic energies rather than Gibb's free energies, as it is our *repeated and consistent* experience that the electronic energies are more in agreement with experimental results than Gibb's free energies, probably due to the fact that parametrization of B3LYP as well as the CPCM modelling is with respect to experiments, so certain free energy effects are already included.

**Cell cultures and cell imaging**

HeLa cells were seeded in a cell culture flask with cell culture media (DMEM medium supplemented with 10% FBS and 1% AAS), and were cultured in a humidified incubator at 37°C under 5% $CO_2$. Then, cells were trypsinized and subcultured after reaching 80% confluency. For cell imaging experiments, cells were seeded in the ibidi µ-Slide 8 well and cultured overnight, then washed with DPBS once. After that, **Myco-F** (20 µM) in opti-mem was confined to the vessel containing cells for 1 h in an incubator at 37°C. After the cells were washed with DPBS once, and fixed with 4% paraformaldehyde in DPBS for 15 min in 5% $CO_2$ incubator at 37°C. Then, cells were washed with DPBS once again followed by 0, 0.1, 1, 5, 8 mM NaF in DPBS treatment for 15 min incubation in 5% $CO_2$ incubator at 37°C. Finally, NaF solution with 70 µL of DPBS and observed cells under a confocal Laser Scanning Microscope instrument (Zeiss, LSM-880).

**Cell viability assay**

1. Seeding of 6000 cells was carried out per a well in a 96-wells plate and incubated overnight under conditions of 5% $CO_2$ and 37°C. 2. The cell culture media (DMEM+ 10% FBS+ 1% AAS) was removed followed by rinsing of the cells by 100 µL PBS once. Then, the cells were treated with 100 µL sample (997 µL DMEM + 3 µL THF to serve as a control; 5 µM: 997 µL DMEM + 0.5 µL **Myco-F** + 2.5 µL THF; 10 µM: 997 µL DMEM + 1 µL Myco-F + 2 µL THF) and incubated in 5% $CO_2$ incubator at 37°C for 12 h and 24 h. 3. After that, add 20 µL of MTT (Thiazolyl Blue Tetrazolium Bromide) (5 mg/ml, dissolved in PBS) in each well of media for the incubation of 4 h in 5% $CO_2$ incubator at 37°C. 4. Then, the media gently solubilized formazan crystals by 150 µL DMSO. 5. The plate was shaken for 15 s prior to reading the absorbance at 570 nm.

**Animal Care**

Adult wild types of zebrafish were housed in a zebrafish facility with optimum conditions of 14:10 hours light: dark in 28°C. Wild type and transgenic males and females were crossed and their embryos were kept in egg water at 28.5°C until experiments were conducted.

**Exposure**

To identify the lethality of the **Myco-F** probe, 6dpf zebrafish were used. Five different concentrations (10, 20, 40, 80 and 100 µM) were prepared with 0.1% DMSO. Control (0.1% DMSO) was prepared separately. 6 dpf zebrafish larvae were exposed to control and five different concentrations in 24 well-plates; lethality was calculated using the Kruskal Wallis One Way ANOVA post-test. Similarly, *Tg(huc:egfp)* and *Tg(flk1:egfp*) transgenic zebrafish lines were also exposed to three different concentrations (10, 20 and 40 µM) to identify any abnormalities in neuronal development and vasculature development, respectively.

**Determination of morphological abnormalities:**

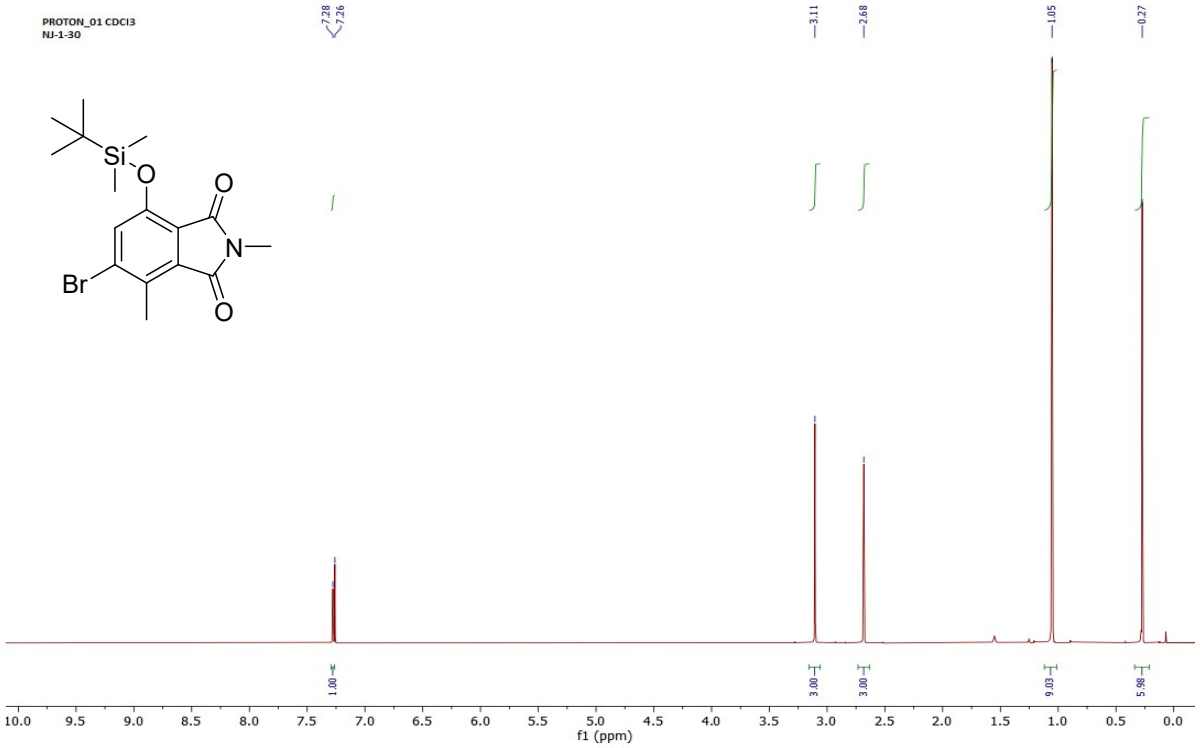
Post 24-hour exposure, 6 dpf zebrafish were analyzed for morphological abnormalities. We checked the status of heartbeat rate, eye movement, eye size, pigment formation, pericardial edema, swim bladder development and overall morphological defects. Similarly, *Tg(huc:egfp)* and *Tg(flk1:egfp*) transgenic zebrafish were used to determine neuronal development and vasculature development in larval zebrafish. Each larva was then anaesthetized using Tricaine (MS-222) and used for imaging.

**Imaging**

6 dpf zebrafish larvae were mounted using 3% methyl cellulose (Sigma Aldrich, M0512); imaging was performed through the use of the Leica DFC50C instrument; fluorescence images were acquired using a Logos Biosystems Celena S instrument.

**Statistical Analysis**

Lethal concentrations were made using a Kaplan-Meier estimate. Fluorescence intensity was measured using a Kruskal-Wallis Post hoc test.


PROTON_01 CDCl3
NJ-1-30
7.28
7.26
3.11
2.68
1.05
0.27
Si
O
O
N
Br
O
1.00
3.00
3.00
9.03
5.98
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

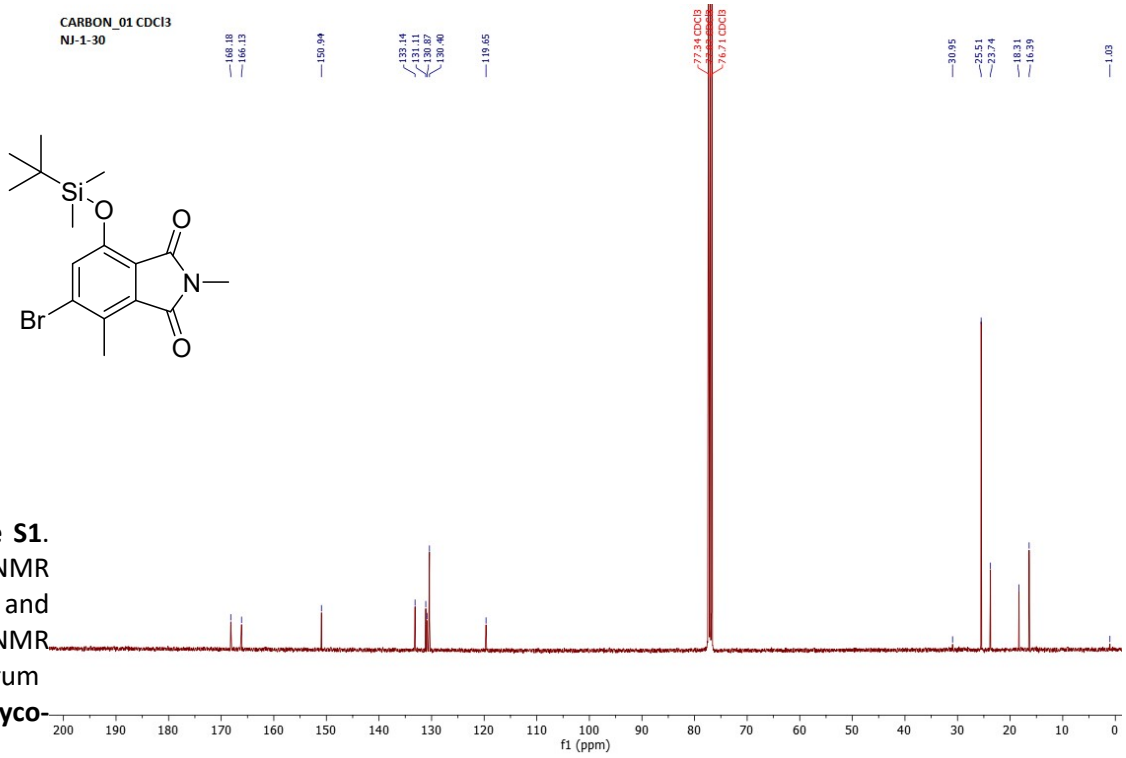



**Figure S1**. $^{1}H$ NMR (top) and $^{13}C$ NMR spectrum of **Myco-F**.

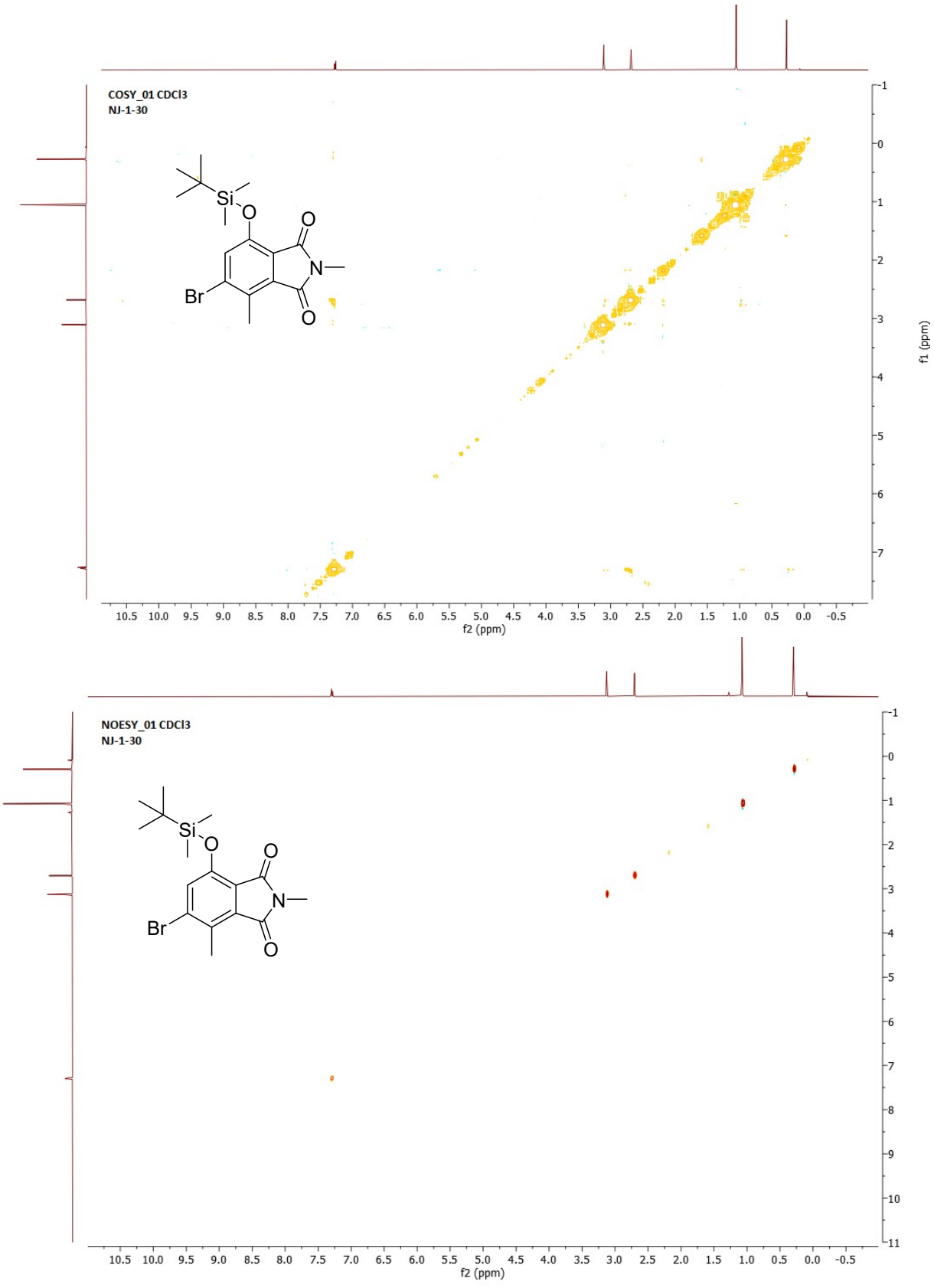
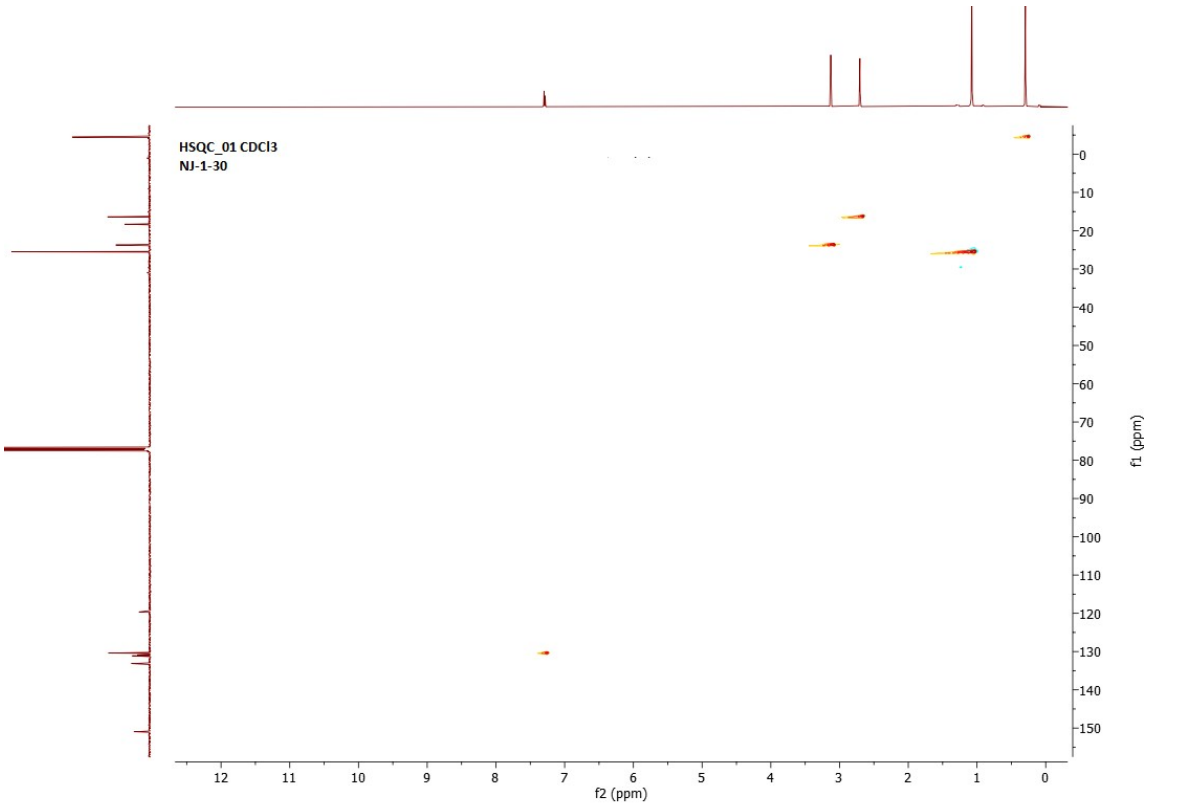



**Figure S2.** (top) $^{1}$H- $^{1}$H COSY and (bottom) $^{1}$H - $^{1}$H NOESY NMR spectrum of **Myco-F**.



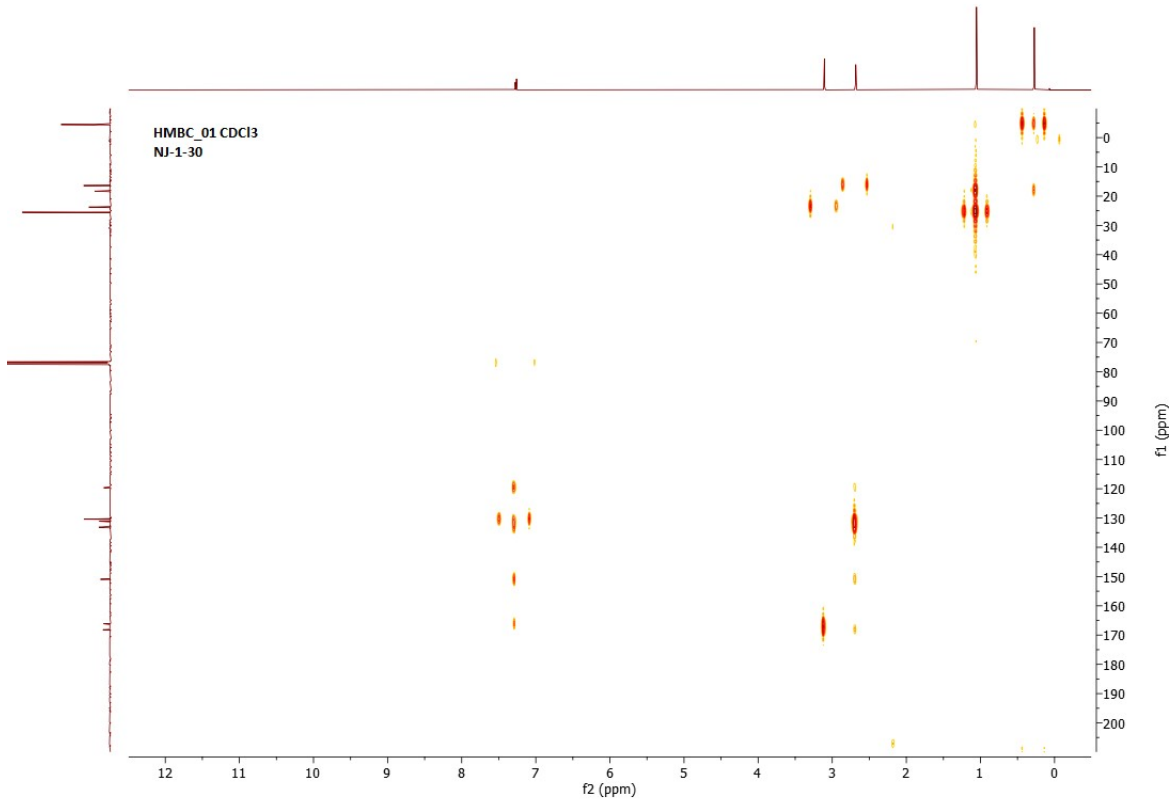
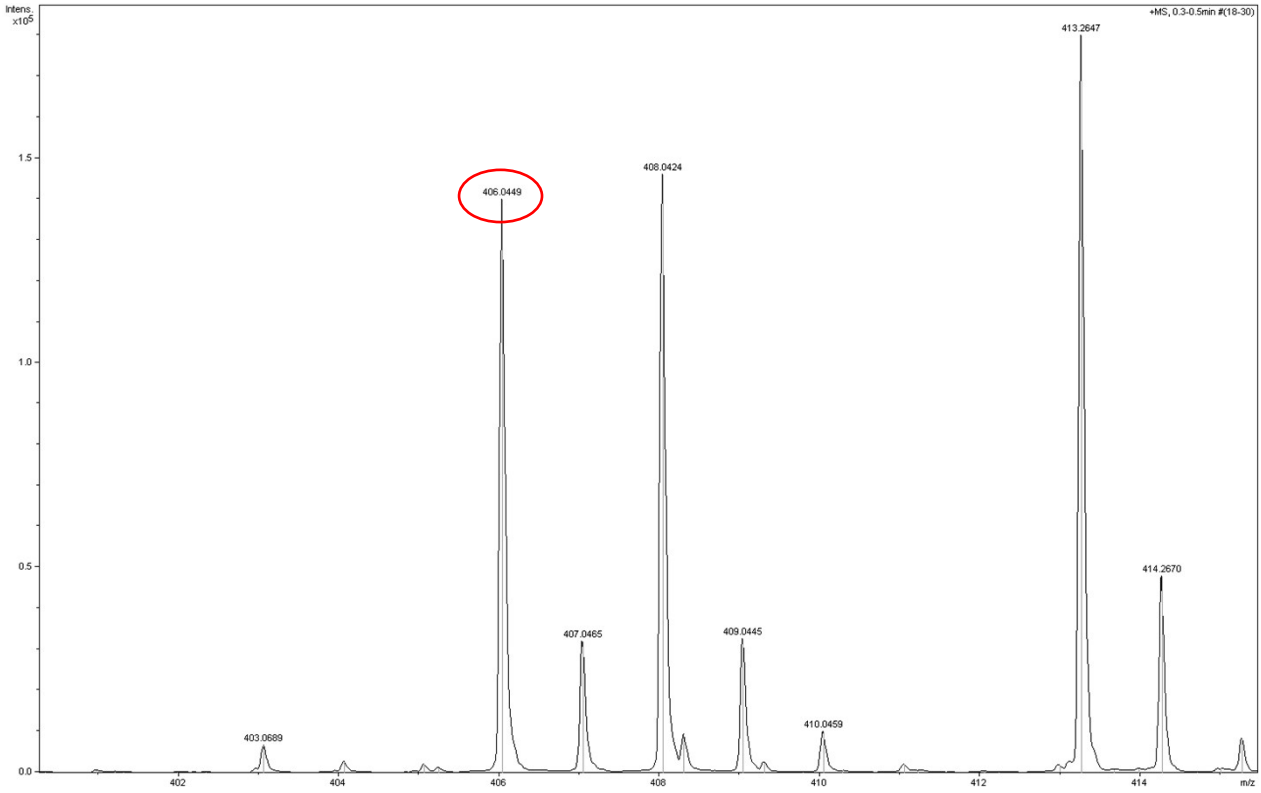
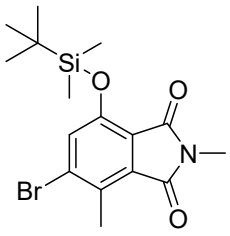




**Figure S3.** (top) $^{1}$H-




Found: M + $Na^+$ = 406.0449

Calculated: M + $Na^+$ = 406.0449

Bromine isotope: 406.0449, 408.0424

| # | m/z | I |
|---|---|---|
| 177 | 399.2536 | 807 |
| 178 | 400.1944 | 354 |
| 179 | 400.3438 | 288 |
| 180 | 400.9878 | 718 |
| 181 | 401.2155 | 315 |
| 182 | 401.3147 | 281 |
| 183 | 401.9891 | 284 |
| 184 | 402.3440 | 219 |
| 185 | 403.0689 | 6776 |
| 186 | 404.0705 | 2767 |
| 187 | 405.0686 | 2009 |
| 188 | 405.2457 | 1318 |
| 189 | 406.0449 | 140007 |
| 190 | 407.0465 | 32041 |
| 191 | 408.0424 | 145972 |
| 192 | 408.3045 | 9430 |
| 193 | 409.0445 | 32540 |
| 194 | 409.3062 | 2567 |
| 195 | 409.7905 | 209 |
| 196 | 410.0459 | 10042 |
| 197 | 410.2901 | 529 |

**Figure S4.** ESI-MS spectrum of **Myco-F**.

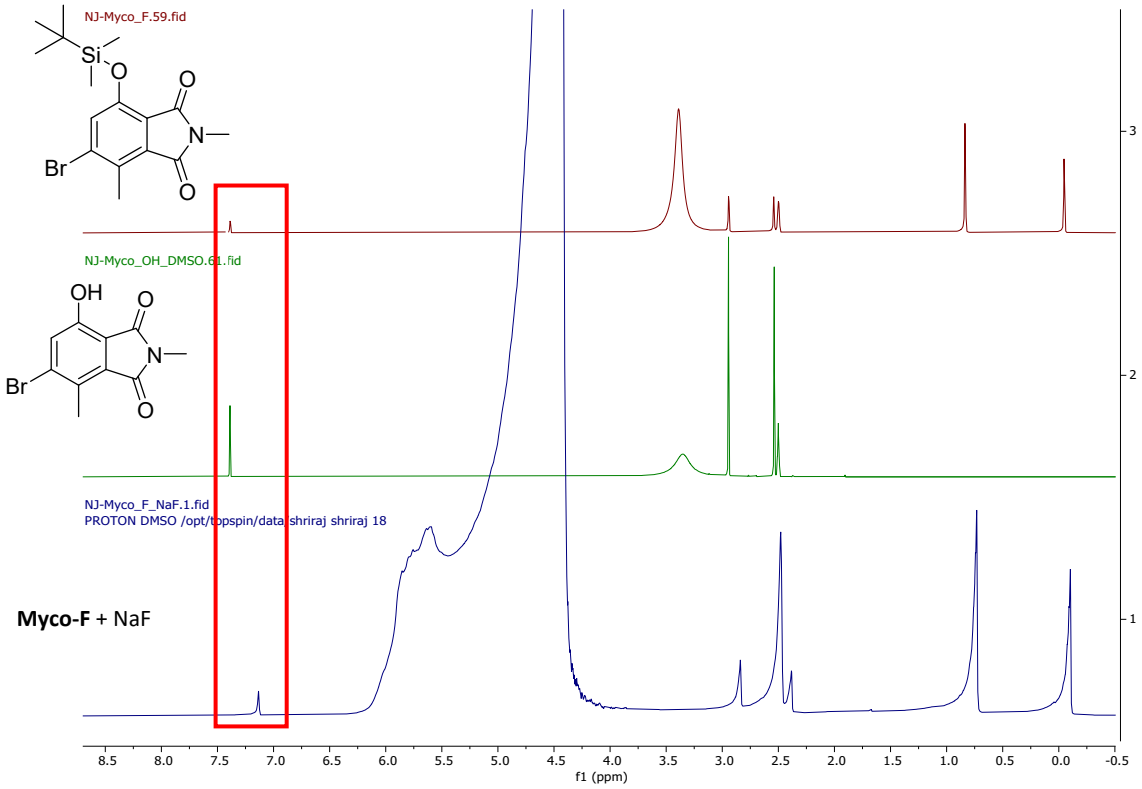



**Figure S5.** $^{1}$H NMR spectrum of **Myco-F**, **Myco-OH** and **Myco-F** and NaF.

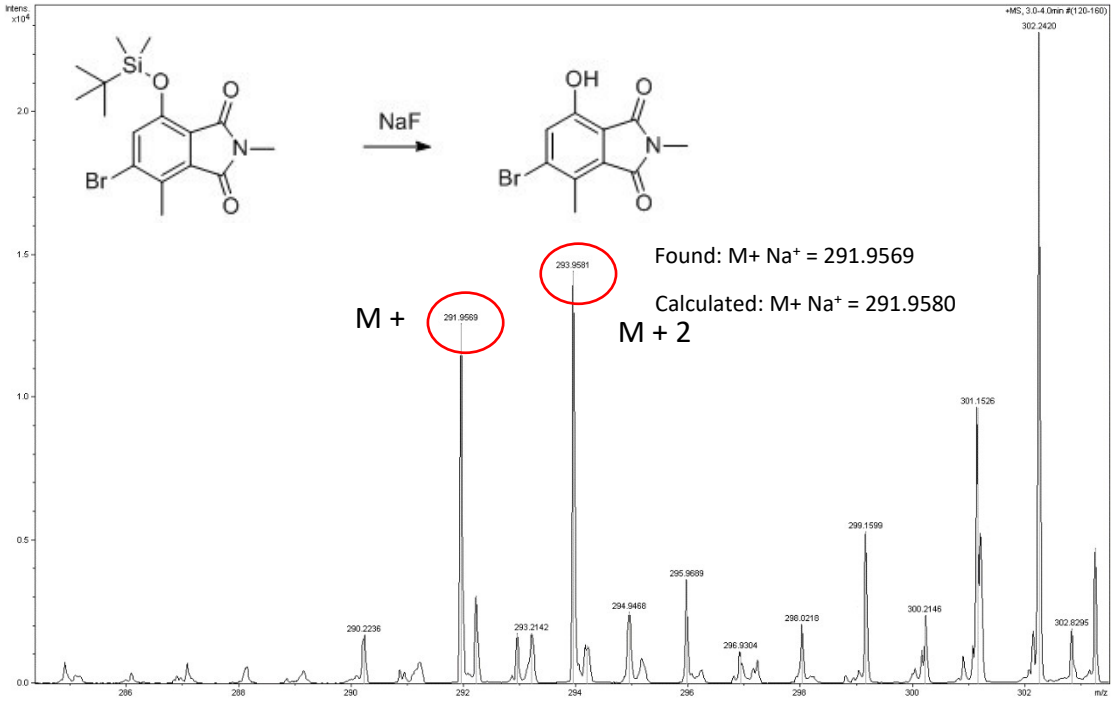
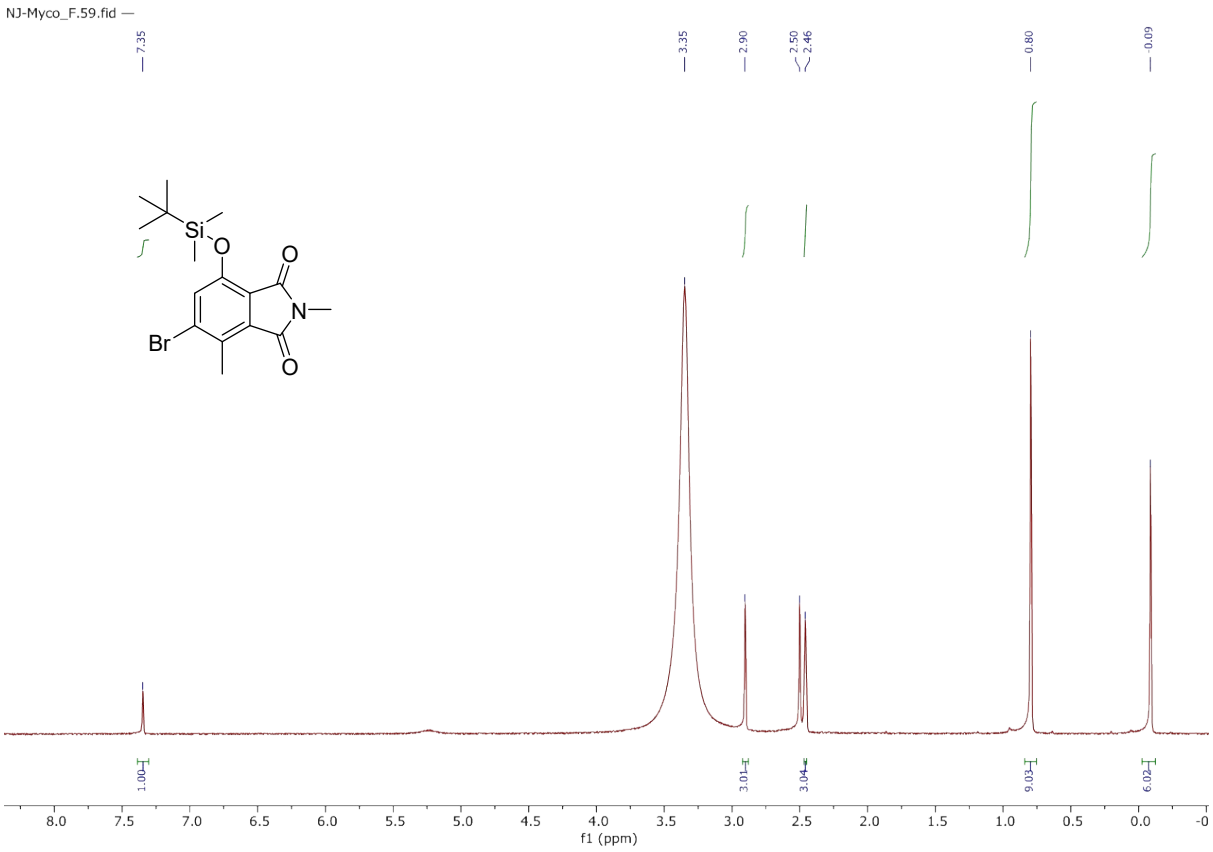
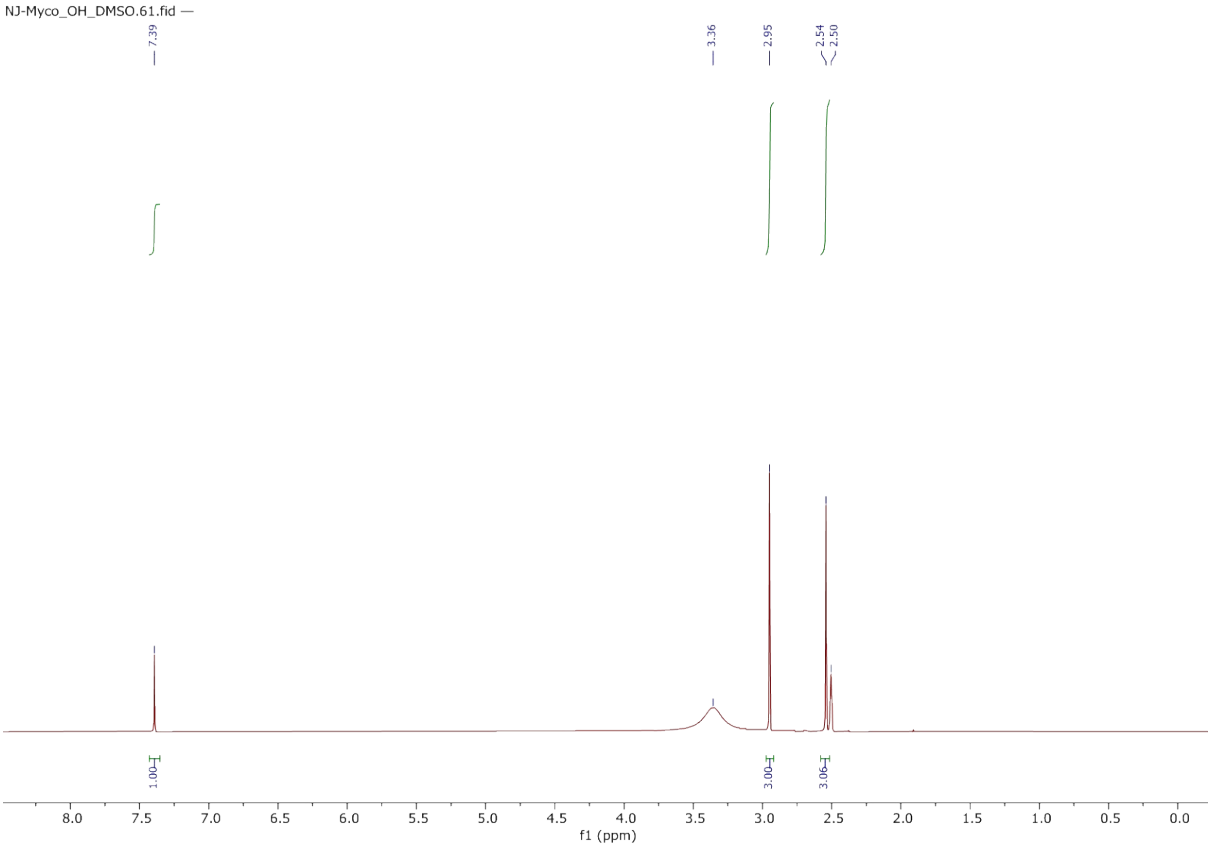



**Figure S6**. ESI-MS spectrum of the reaction product of **Myco-F** and NaF.


NJ-Myco_F.59.fid
7.35
3.35
2.90
2.50
2.46
0.80
-0.09
Si
O
O
N
Br
O
1.00
3.01
3.04
9.03
6.02
f1 (ppm)


NJ-Myco_OH_DMSO.61.fid
7.39
3.36
2.95
2.54
2.50
1.00
3.00
3.06
f1 (ppm)

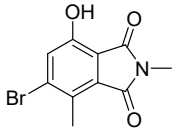
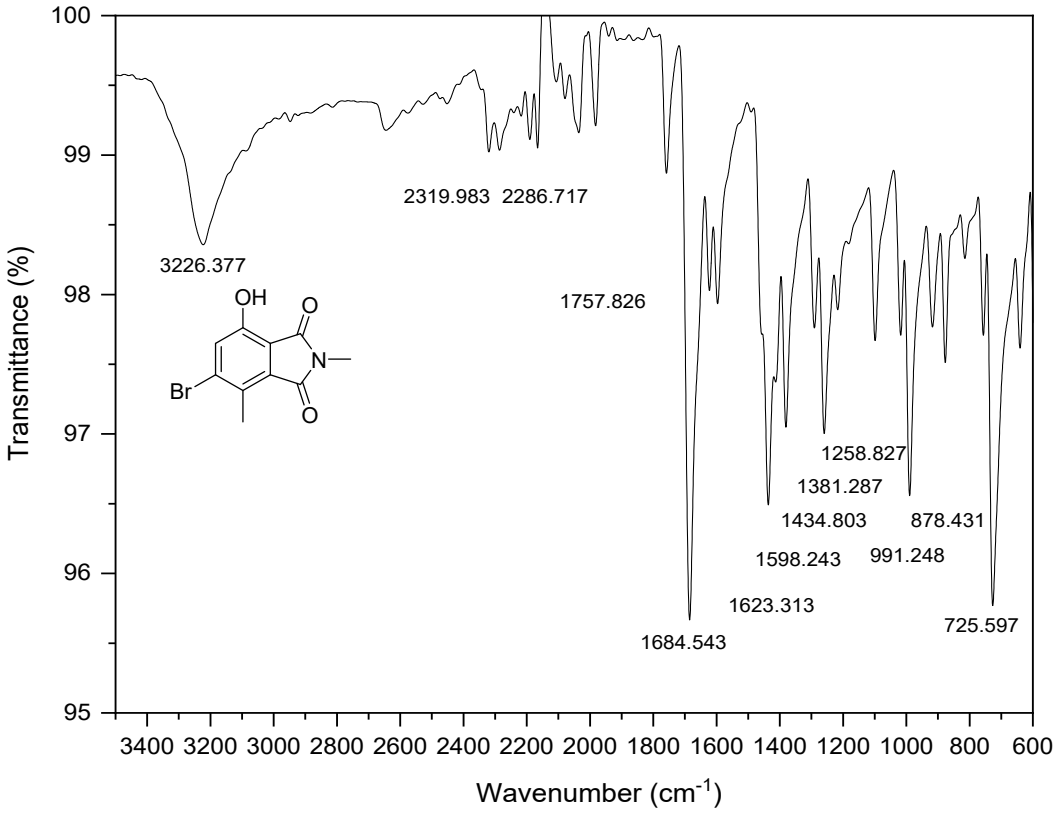
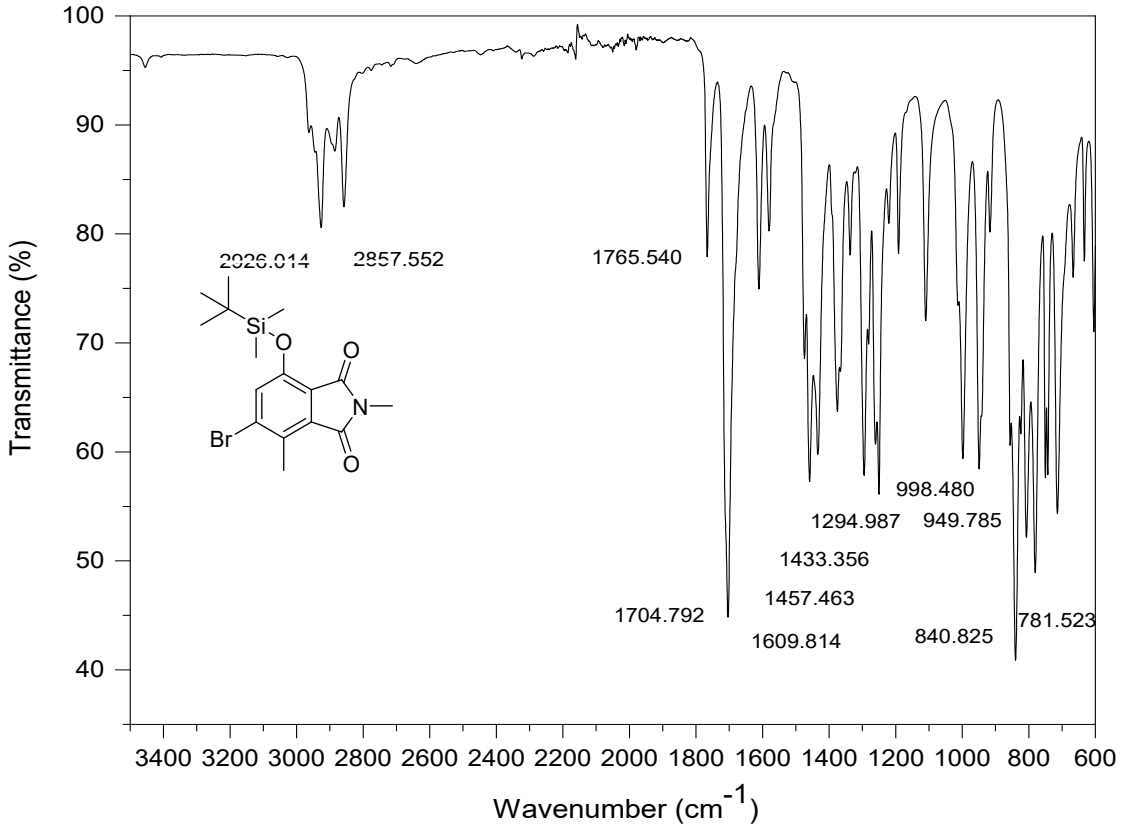

OH
O
N
Br
O

**Figure S7**. $^{1}H$ NMR spectrum of **Myco-OH** (top) and **Myco-F** (bottom) in DMSO-$d_6$.


100
99
98
97
96
95
Transmittance (%)
3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000 800 600
Wavenumber (cm-1)
3226.377
2319.983
2286.717
1757.826
1684.543
1623.313
1598.243
1434.803
1381.287
1258.827
991.248
878.431
725.597
OH
O
N
Br
O


Transmittance (%)
Wavenumber (cm-1)
2926.014
2857.552
1765.540
1704.792
1609.814
1457.463
1433.356
1294.987
998.480
949.785
840.825
781.523
Si
O
N
Br

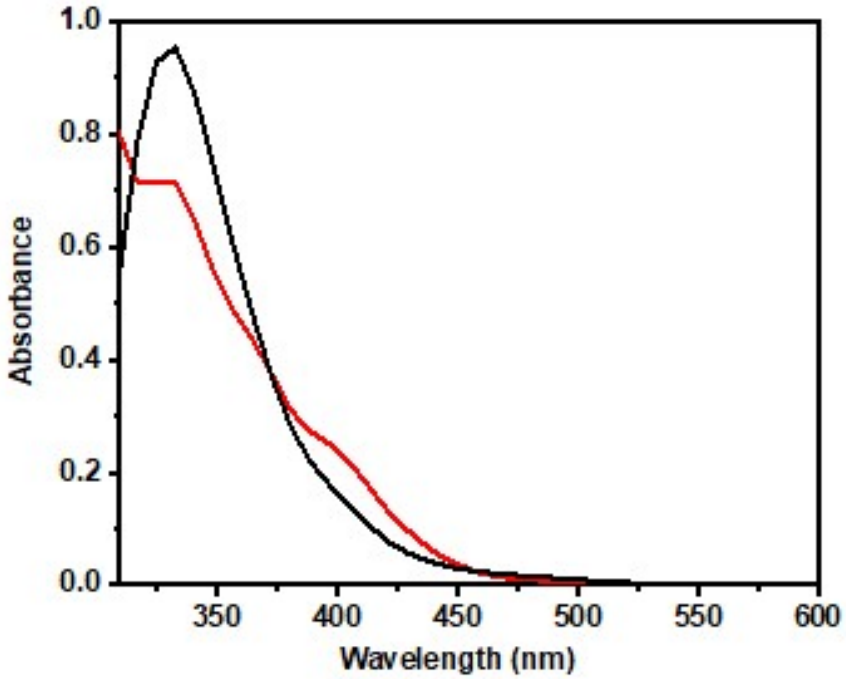



**Figure S8**. IR spectrum of **Myco-OH** (top) and **Myco-F** (bottom).

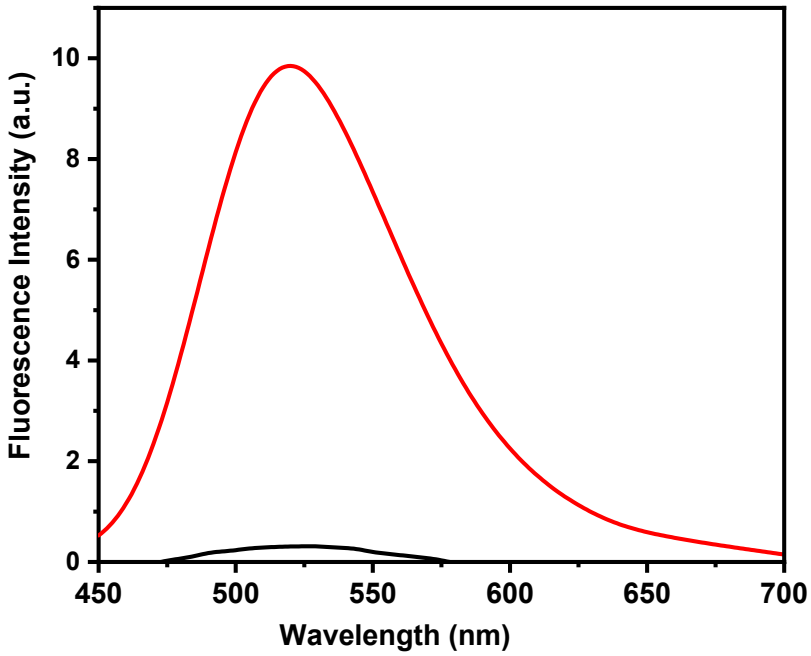



**Figure S9.** UV- absorption spectra of **Myco-F** (10.0 μM) in the absence (black) and presence (red) of $F^-$ (8 mM) in the solution of PBS (10 mM, 7.4 pH) ($\lambda_{ex}$ = 398 nm, $\lambda_{em}$ = 518 nm; slit width = 10 nm/10 nm at RT.

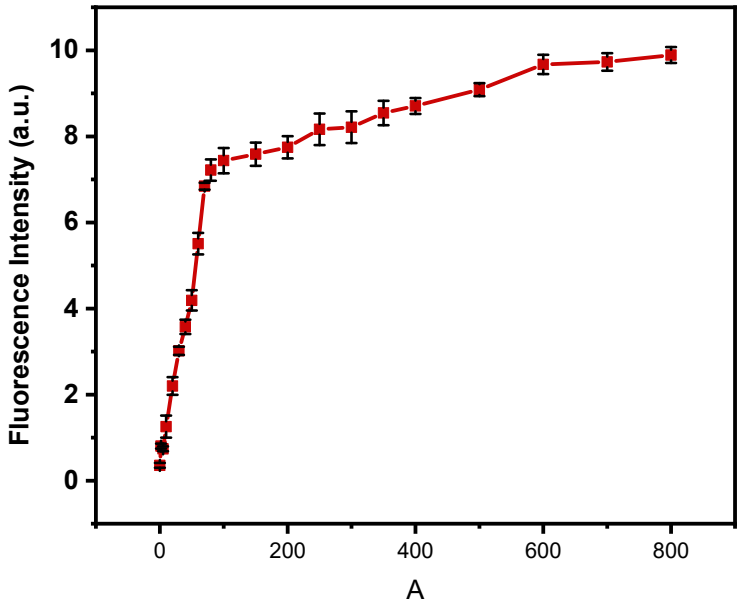



**Figure S10.** Fluorescence intensity of probe **Myco-F** (10.0 μM) in the absence (black) and presence (red) of $F^-$ (8 mM) in the solution of PBS (10 mM, 7.4 pH) ($\lambda_{ex}$ = 398 nm, $\lambda_{em}$ = 518 nm; slit width = 10 nm/10 nm at RT.

**Figure S11**. Plot of fluorescence intensity of **Myco-F** (10.0 μM) with increasing concentration of $F^-$ (0-8 mM) in the PBS (10 mM, 7.4 pH) ($\lambda_{ex}$ = 398 nm, $\lambda_{em}$ = 518 nm; slit width = 10 nm/10 nm at RT.


**Figure S12.** Time dependent fluorescence spectra of **Myco-F** (10.0 μM) in the absence of $F^-$ at pH 7.4 (PBS 10 mM) ($\lambda_{ex}$ = 398 nm, $\lambda_{em}$ = 518 nm; slit width = 10 nm/10 nm at RT.

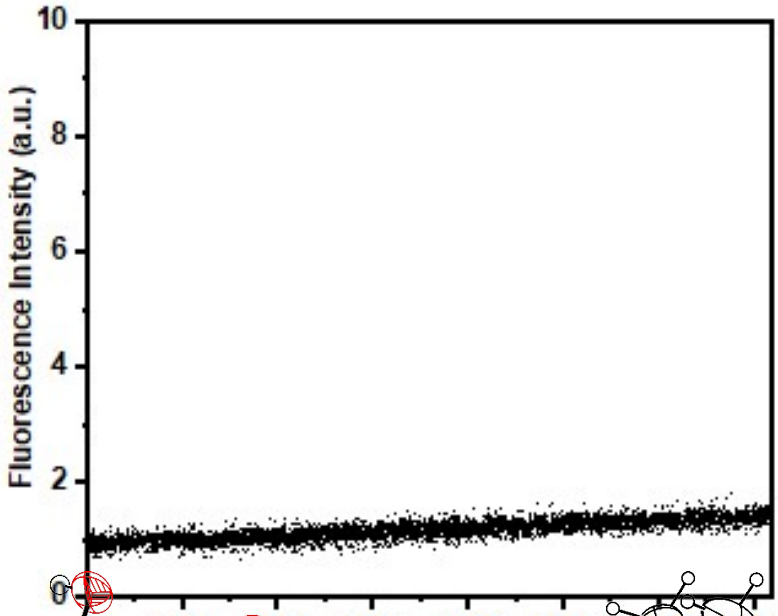



**Figure S13.** Selectivity study of **Myco-F** with different analytes at pH 7.4 (PBS 10mM).

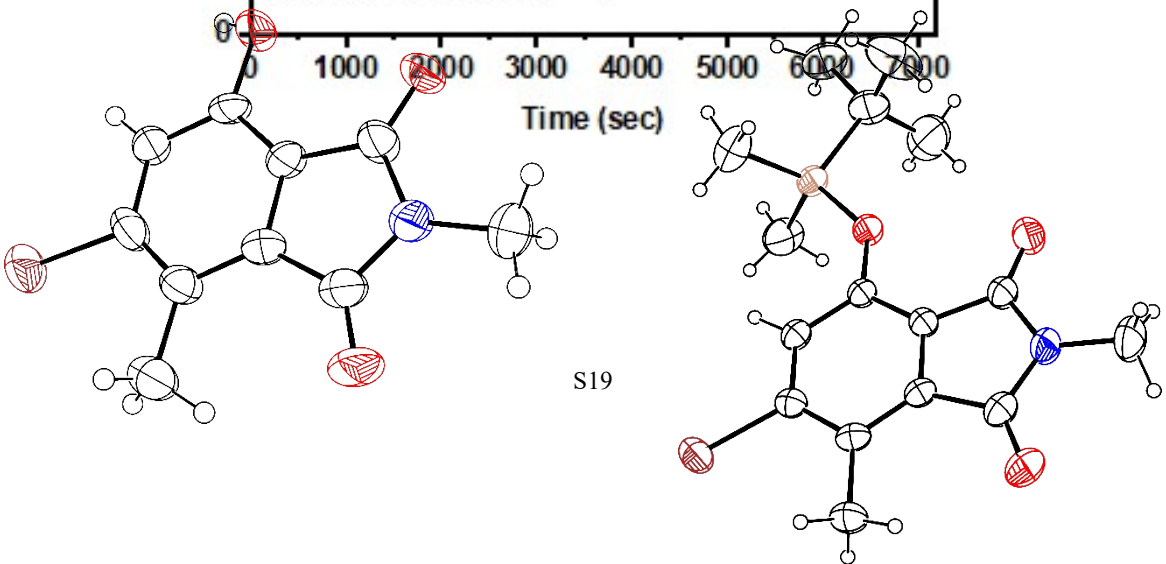


**Figure S14**. Crystal structure of **Myco- OH** and **Myco- F**.

**Table S1**. Crystal data and structure refinement for **A22108O_169_Myco-OH**.

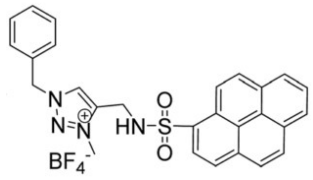
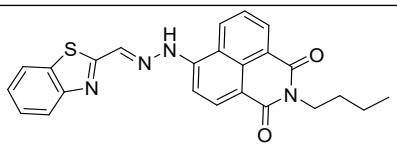
| | | |
|---|---|---|
| Empirical formula | $C_{10}$ $H_8$ Br N $O_3$ | |
| Formula weight | 270.08 | |
| Temperature | 296(2) K | |
| Wavelength | 0.71073 Å | |
| Crystal system | Hexagonal | |
| Space group | $P6_1$ | |
| Unit cell dimensions | a = 15.9925(13) Å | α = 90° |
| | b = 15.9925(13) Å | β = 90° |
| | c = 20.794(3) Å | γ = 120° |
| Volume | 4605.7(10) $Å^3$ | |
| Z | 18 | |
| Density (calculated) | 1.753 $Mg/m^3$ | |
| Absorption coefficient | 4.001 $mm^{-1}$ | |
| F(000) | 2412 | |
| Crystal size | 0.326 × 0.141 × 0.124 $mm^3$ | |
| Theta range for data collection | 2.450 to 27.682°. | |
| Index ranges | –20<=h<=20, –20<=k<=20, –24<=l<=27 | |
| Reflections collected | 77336 | |
| Independent reflections | 6998 [R(int) = 0.1125] | |
| Completeness to theta = 25.242° | 99.8 % | |
| Absorption correction | Semi-empirical from equivalents | |
| Max. and min. transmission | 0.7456 and 0.2729 | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data / restraints / parameters | 6998 / 1 / 418 | |
| Goodness-of-fit on $F^2$ | 0.988 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0367, wR2 = 0.0779 | |
| R indices (all data) | R1 = 0.0719, wR2 = 0.0877 | |
| Absolute structure parameter | 0.051(14) | |
| Largest diff. peak and hole | 0.344 and –0.374 $e·Å^{-3}$ | |

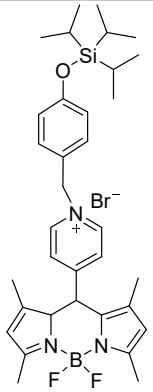
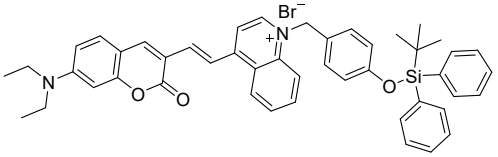
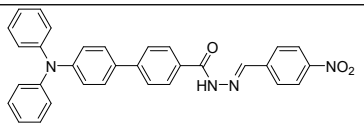
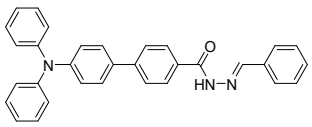
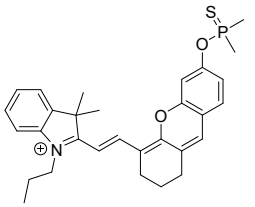
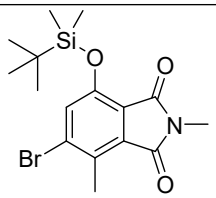
**Table S2**. Crystal data and structure refinement for **A22145O_012_Myco-F**.

| | |
|---|---|
| Empirical formula | $C_{16}$ $H_{22}$ Br N $O_3$ Si |
| Formula weight | 384.34 |
| Temperature | 296(2) K |
| Wavelength | 0.71073 Å |
| Crystal system | Monoclinic |
| Space group | *I*2/*m* |

| | | |
|---|---|---|
| Unit cell dimensions | a = 16.0619(14) Å | α = 90° |
| | b = 7.1033(7) Å | β = 90.135(2)° |
| | c = 32.658(3) Å | γ = 90° |
| Volume | 3726.0(6) $Å^3$ | |
| Z | 8 | |
| Density (calculated) | 1.370 $Mg/m^3$ | |
| Absorption coefficient | 2.281 $mm^{-1}$ | |
| F(000) | 1584 | |
| Crystal size | 0.125 × 0.032 × 0.029 $mm^3$ | |
| Theta range for data collection | 2.495 to 25.353°. | |
| Index ranges | –19<=h<=19, –8<=k<=8, –38<=l<=39 | |
| Reflections collected | 17887 | |
| Independent reflections | 3574 [R(int) = 0.0814] | |
| Completeness to theta = 25.242° | 96.9 % | |
| Absorption correction | Semi-empirical from equivalents | |
| Max. and min. transmission | 0.7452 and 0.6057 | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data / restraints / parameters | 3574 / 218 / 308 | |
| Goodness-of-fit on $F^2$ | 1.026 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0553, wR2 = 0.1070 | |
| R indices (all data) | R1 = 0.1105, wR2 = 0.1274 | |
| Largest diff. peak and hole | 0.288 and –0.276 $e·Å^{-3}$ | |

**Table S3.** Comparison of previously reported fluorescent probes for the detection of $F^-$.

| No. | Probe Structure | Mechanism | Reaction medium | Detection limit |
|---|---|---|---|---|
| **1.** |  | $(C–H)^+\cdots$ anion H-bonding | $CH_3OH/H_2O$ (9:1, *v/v*) | 0.039 μM |
| **2.** |  | H-bonding | $CH_3CN$ | 0.41 μM |

| | | | | |
|---|---|---|---|---|
| **3.** |  | Si-O cleavage | PBS (pH 7.4) | 20 μM |
| **4.** |  | Si-O cleavage | DMSO/ PBS (7:3 v/v) | 0.771 μM |
| **5.** |  and  | H-bonding | DMSO | 1.24 μM<br>15.73 μM |
| **6.** |  | P-O cleavage | $CH_3CN$ | 4.28 μM |
| **7.** |  **This work** | Si-O cleavage | PBS (pH 7.4) | 0.38 μM |

**References**


1 B.-Y.Wang, Z.-Q. Chen, H. Huang, Q.-Y. Cao, *RSC Adv*., 2017, **7**, 43950-43956.

2 X. Chen, T. Leng, C. Wang, Y.-J. Shen, W. Zhu, *Dyes Pigm*., 2017, **141**, 299-305.

3 M. Du, B. Huo, M. Li, A. Shen, X. Bai, Y. Lai, J. Liu, Y. Yang, *Ana. Chemica Acta*., 2018, **1030**, 172-182

4 J. Ji, Z. Zhang, F. Zhan, Q. Wang, G. Zheng, *J. Photochem. Photobio. A. Chem*., 2020, **390**, 112349.

5 H. Shi, H. Chen, X. Li, J. Xing, G. Zhang, R. Zhang, J. Liu, *RSC Adv*., 2021, **11**, 1-6.

6 L. Li, M. Zhang, L. Ding, G. Ren, X. Hou, W. Liu, H. Wang, B. Wang, L. Yan, H. Diao, *Spectrochim. Acta. A Mol. Biomol. Spectrosc*. 2021, **252**, 119518.

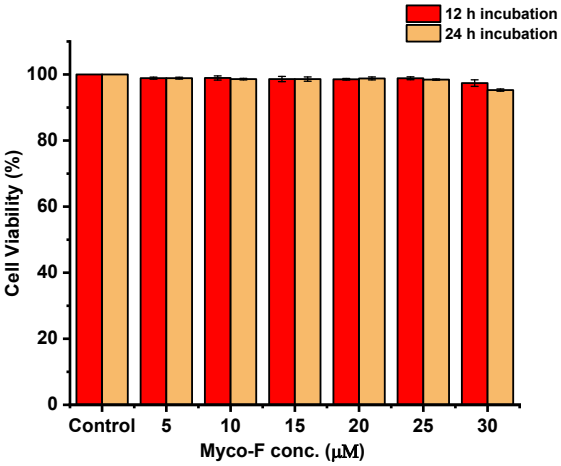
[a] W. Kohn and L. J. Sham, Phys. Rev. A, 1965, 140, 1133.

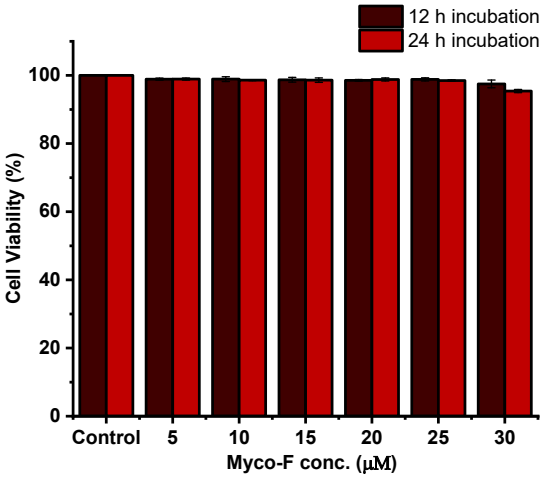
[b] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zarkrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox, *Gaussian 16, Revision B.01*, Gaussian, Inc., Wallingford, CT, 2016.

[c] (a) A.D. Becke, *Phys. Rev. A*, 1988, **38**, 3098; (b) A.D. Becke, *J. Chem. Phys.*, 1993, **98**, 1372; (c) A.D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648; (d) C. Lee, W. T. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785.

[d] (a) P. J. Hay and W. R. Wadt, *J. Chem. Phys.*, 1985, **82**, 270; (b) P. J. Hay and W. R. Wadt, *J. Chem. Phys.*, 1985, **82**, 299.

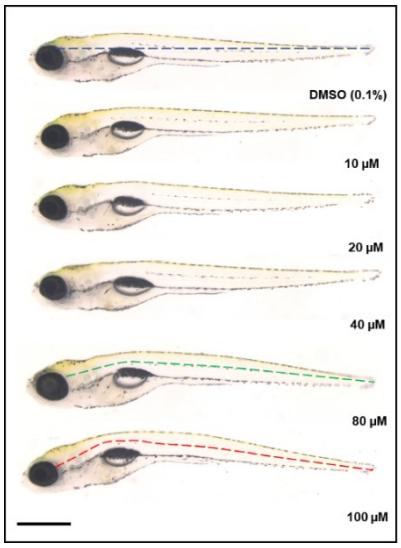
[e] M. Cossi, N. Rega, G. Scalmani and V. Barone, *J. Comput. Chem.*, 2003, **24**, 669.


12 h incubation
24 h incubation
Cell Viability (%)
0
20
40
60
80
100
Control
5
10
15
20
25
30
Myco-F conc. (μM)


12 h incubation
24 h incubation
Cell Viability (%)
0
20
40
60
80
100
Control
5
10
15
20
25
30
Myco-F conc. (μM)

HeLa cells and HEK293 cells for 12 h and 24 h.

**Figure S16**. Investigation of cytotoxicity study on normal development of zebrafish larvae at 6 dpf after 24 h exposure of various concentrations (10, 20, 40, 80 and 100 µM) of Myco-F. Higher concentration of Myco-F induce body curvature phenotype 6dpf zebrafish larvae. Scale bar




(dashed line green and red) in = 100 µm

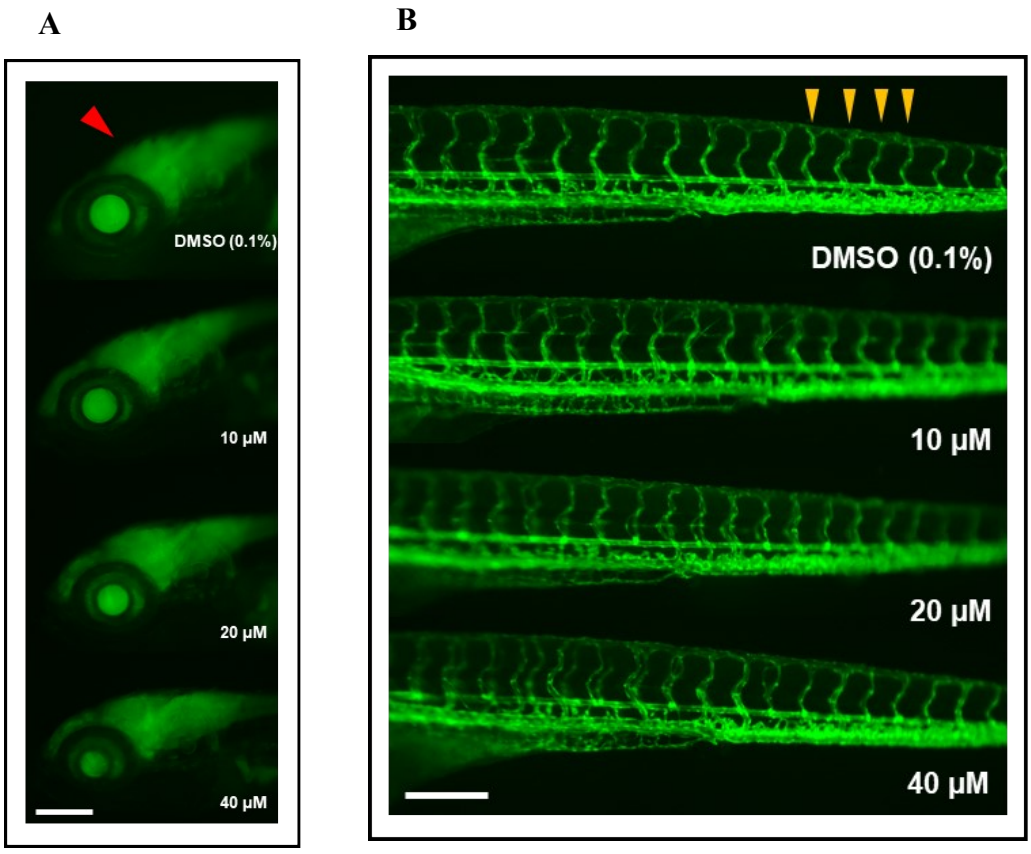

A
B
DMSO (0.1%)
10 μM
20 μM
40 μM
DMSO (0.1%)
10 μM
20 μM
40 μM

**Figure S17. (a)** Effects of Myco-F with different concentrations (10 µM, 20 µM, 40 µM) on development of central nervous system in zebrafish larvae at 6 dpf (n = 48). Fluorescent neurons are monitored in live transgenic zebrafish, *Tg(huc:egfp).* Midbrain region is marked with a red arrowhead. Scale bar = 200 µm; **(b)** Effects of Myco-F with different concentrations (10, 20, 40 µM) on blood vessel development in transgenic zebrafish, *Tg(flk1:egfp)* at 6 dpf (n = 48). Fluorescent blood vessels were monitored in live transgenic zebrafish, *Tg(flk1:egfp).* Intersegmental vessel (ISV) in the trunk region marked with yellow arrowheads. Scale bar = 200 µm

**Table S4.** Relative energies of structures in kcal/mol

| S = 0 | Def2-SVP | Def2-TZVPP | **E(total)**[a] | Z0 | E(thermal)[b] | -TΔS [b] | **ΔG**[c] |
|---|---|---|---|---|---|---|---|
| RC | 3.58 | -0.54 | **3.04** | -0.26 | +0.09 | -0.06 | **2.81** |
| $TS_1$ | 8.79 | -1.15 | **7.64** | -0.99 | -0.10 | +0.20 | **6.76** |
| $IM_1$ | 0.00 | +0.00 | **0.00** | +0.00 | +0.00 | +0.00 | **0.00** |
| $TS_2$ | 34.00 | -1.27 | **32.73** | -1.52 | -0.04 | +0.09 | **31.26** |
| PC | 27.77 | -1.43 | **26.34** | -0.71 | +0.17 | -0.34 | **25.45** |
| S = 1 | | | | | | | |
| RC | 66.56 | -0.97 | **65.59** | -2.30 | +0.29 | -1.11 | **62.47** |
| $TS_1$ | 74.14 | -1.84 | **72.30** | -3.51 | +0.22 | -1.14 | **67.87** |
| $IM_1$ | 61.43 | -0.22 | **61.21** | -2.35 | +0.22 | -1.02 | **58.06** |
| $TS_2$ | 63.77 | +0.85 | **64.62** | -4.74 | -0.14 | -0.35 | **59.39** |
| $IM_2$ | 53.09 | +0.49 | **53.58** | -1.72 | +0.16 | -0.78 | **51.24** |
| $TS_3$ | 62.42 | -0.58 | **61.83** | -2.83 | +0.09 | -0.78 | **58.31** |
| PC | 58.92 | -0.51 | **58.41** | -2.19 | +0.37 | -1.37 | **55.22** |

[a] Electronic energy as sum of the two previous columns. [b] T = 298.15 K. [c] Sum of the four previous columns, but not used in the text due to added uncertainty (see Methods section).

**Table S5**. Relative energies of $S = 1$ single point calculations on the $S = 0$ optimized structure at B3LYP/Def2-TZVPP level, in kcal/mol

| S = 1 | E(total) |
|---|---|
| RC | 71.35 |
| $TS_1$ | 80.89 |
| $IM_1$ | 66.75 |

| | |
|---|---|
| $TS_2$ | 67.43 |
| PC | 63.88 |

**Table S6**. Relative energies of *S* = 0 single point calculations on the *S* = 1 optimized structure at B3LYP/Def2-TZVPP level, in kcal/mol

| S = 0 | E(total) |
|---|---|
| RC | 10.89 |
| $TS_1$ | 17.84 |
| $IM_1$ | 7.68 |
| $TS_2$ | 19.49 |
| $IM_2$ | 24.54 |
| $TS_3$ | 38.54 |
| PC | 32.08 |

**Table S7.** Mulliken spin density distributions of structures at B3LYP/Def2-TZVPP level

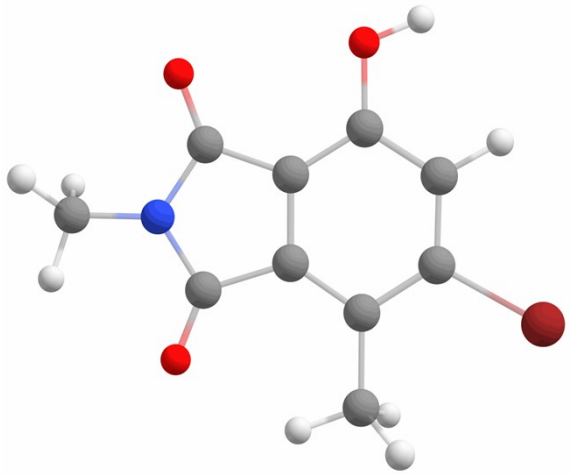
Note: There is no mulliken spin density distributions at S = 0 Multiplicity.

| S = 1 | 6-ring | 5-ring | $O_{top}$ | $O_{bottom}$ | $O_{top\text{-}6ring}$ | Br | Sum |
|---|---|---|---|---|---|---|---|
| RC | 1.42 | 0.08 | 0.19 | 0.13 | 0.11 | 0.08 | 2.00 |
| $TS_1$ | 1.48 | 0.11 | 0.16 | 0.12 | 0.00 | 0.13 | 2.00 |
| $IM_1$ | 1.33 | 0.13 | 0.17 | 0.17 | 0.15 | 0.06 | 2.01 |
| $TS_2$ | 1.21 | 0.20 | 0.12 | 0.24 | 0.24 | 0.01 | 2.01 |
| $IM_2$ | 1.01 | 0.31 | 0.07 | 0.26 | 0.35 | 0.00 | 2.00 |
| $TS_3$ | 0.90 | 0.43 | 0.01 | 0.27 | 0.37 | 0.00 | 1.98 |
| PC | 0.98 | 0.33 | 0.07 | 0.26 | 0.37 | 0.00 | 2.00 |

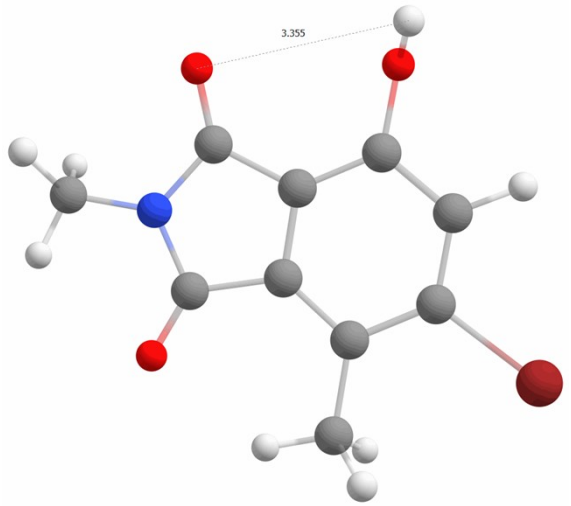
**Table S8.** Mulliken spin density distributions of *S* = 1 single point calculations on the *S* = 0 optimized structures

| S = 1 | 6-ring | 5-ring | $O_{top}$ | $O_{bottom}$ | $O_{top\text{-}6ring}$ | Br | Sum |
|---|---|---|---|---|---|---|---|
| RC | 1.39 | 0.11 | 0.16 | 0.14 | 0.14 | 0.06 | 2.00 |
| $TS_1$ | 1.41 | 0.16 | 0.15 | 0.12 | 0.01 | 0.16 | 2.00 |
| $IM_1$ | 1.36 | 0.13 | 0.16 | 0.15 | 0.15 | 0.05 | 2.00 |
| $TS_2$ | 0.93 | 0.46 | 0.02 | 0.24 | 0.34 | 0.00 | 1.98 |
| PC | 1.00 | 0.36 | 0.08 | 0.22 | 0.34 | 0.00 | 2.00 |

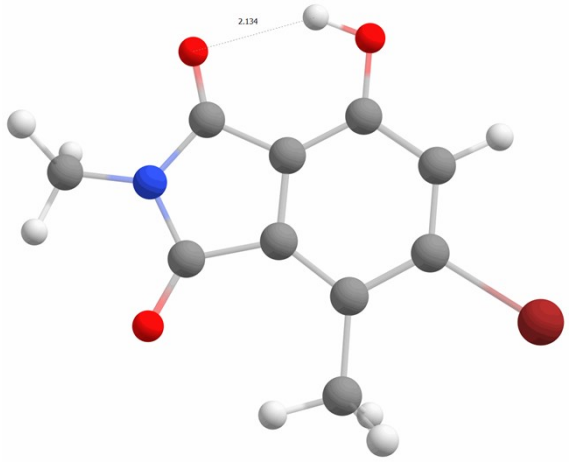
**Figure S17.** DFT optimized structures. $^{1}$RC



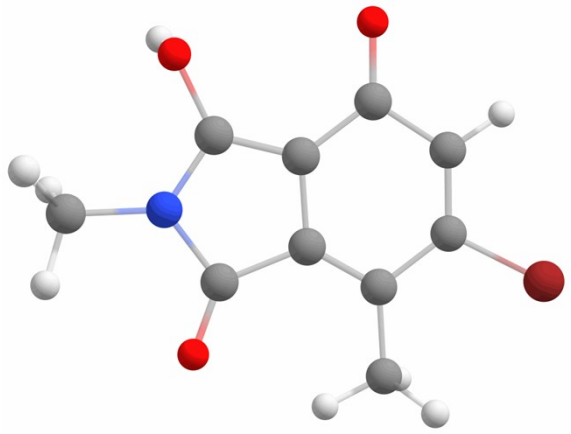
$^{1}TS_{1}$



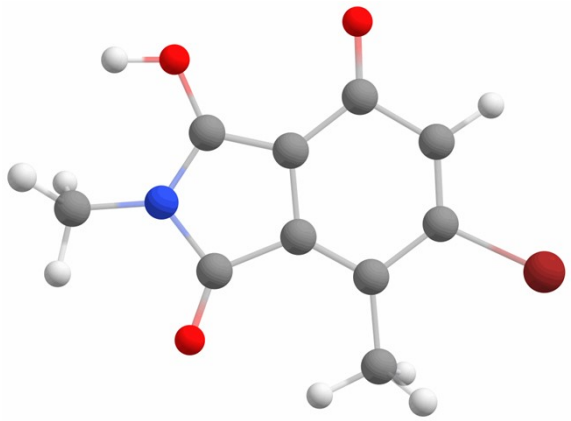

$^{1}IM_{1}$



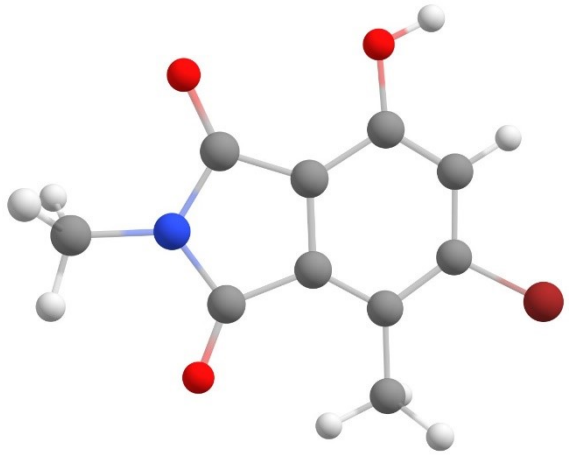
$^{1}TS_{2}$



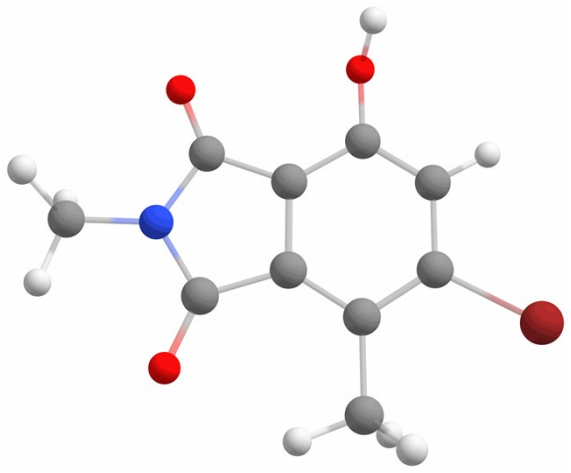
$^{1}PC$



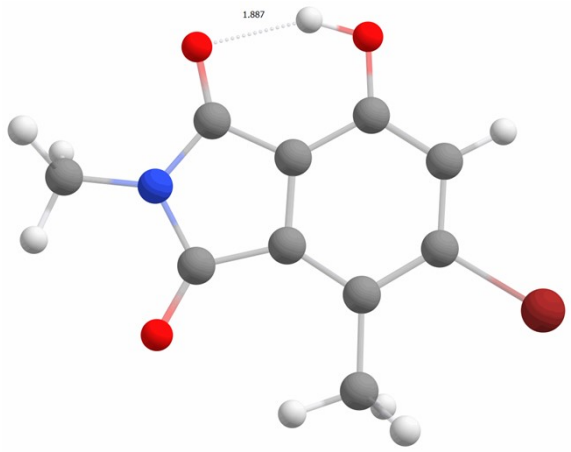
$^{3}RC$



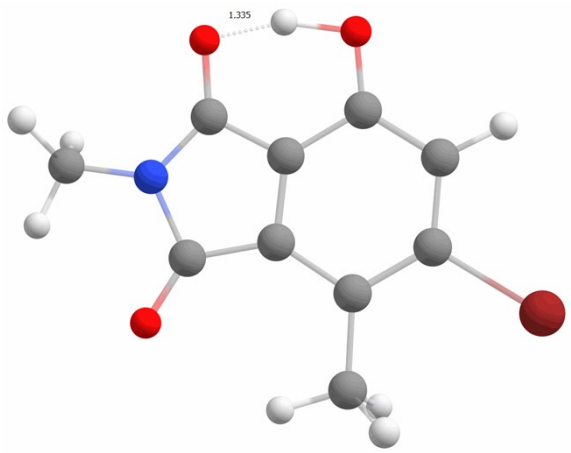
$^3TS_1$



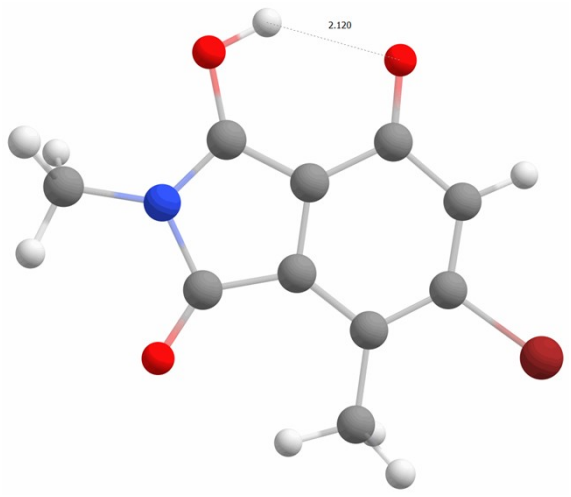
$^3IM_1$



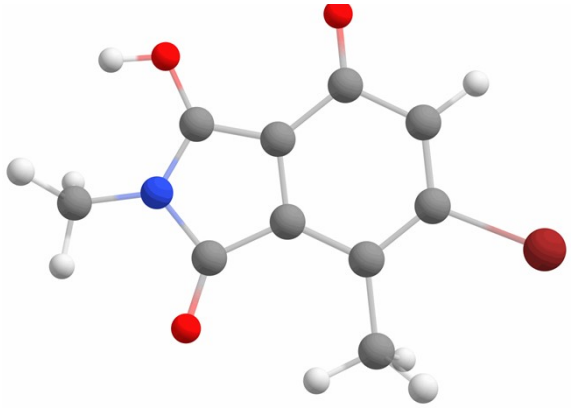

$^3TS_2$



$^3IM_2$




$^3PC$



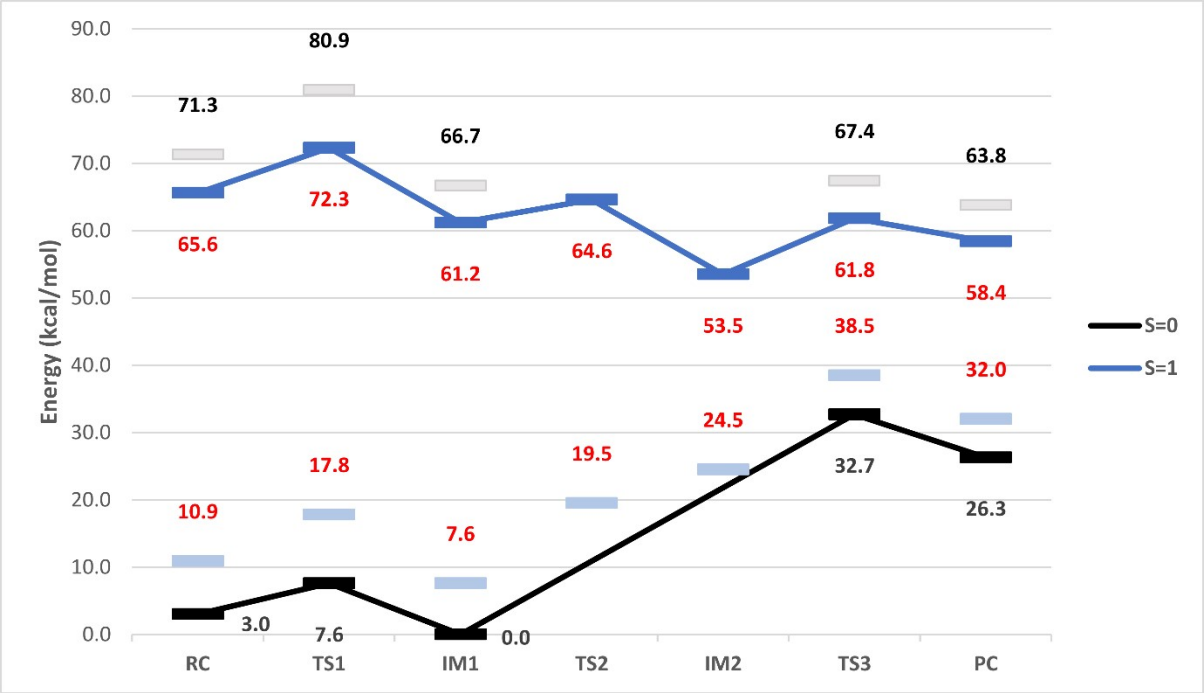



**Figure S18.** Relative Energies of the reaction pathways (see below text).

Figure S18 shows an energy graph of the proposed chemical mechanism of tautomerization. *S*=0 singlet and *S*=1 triplet calculations show that the number of transition states in the different spin states were different. The gray states are triplet single-point calculations on the singlet optimized geometry; the light blue states are singlet single-point calculations on the triplet optimized geometry.

The *S*=0 singlet surface consists of only two steps, each with one transition state. The first step is rotation of the OH on the hexagonal ring to form a hydrogen bond with the opposite side O. The energy of this transition state ($TS_1$) was found to be 4.6 kcal/mol compared to the energy of the reactant. The second step is the H atom transfer in which the H-atom bonded to O on the hexagonal ring moves to the O atom of the pentagonal ring and the resulting OH concertedly rotates to point away from the hexagonal oxygen. The activation barrier of this $TS_2$ was found to be 32.7 kcal/mol. The Mulliken spin density distributions was 0 for all *S*=0 structures meaning that the surface depicts a closed shell surface.

The *S*=1 triplet surface consists of three steps. Like *S*=0, the first step is rotation of the OH of the hexagonal ring to form a hydrogen bond with the opposite side O. The *S*=1 reactant energy was 65.6 kcal/mol compared to the *S*=0 lowest point, and the energy of the triplet $TS_1$ was 72.3 kcal/mol. The second transition state is the transfer of H from the hexagonal ring to the pentagonal ring O. The energy of this $TS_2$ was 64.6 kcal/mol. The third transition state is the rotation of OH on the pentagonal ring, and the energy of this $TS_3$ was 61.8 kcal/mol.

The *S*=0 lowest point Reactant (0.0 kcal/mol) structure became 71.4 kcal/mol in energy when used in a *S*=1 single-point calculation. In addition, the two energies not found in the singlet surface ($^3TS_2$, $^3IM_2$), single-point *S*=0 calculations on the *S*=1 geometries resulted in energies of 19.5 kcal/mol and 24.5 kcal/mol, respectively, which are lower than the energy of $^1TS_2$, so it can be seen that they are on the downhill side of $^1TS_2$.

# Cartesian Coordinates of DFT geometries

$^{1}$RC
C -0.251784 1.870930 -0.000083
C 1.118716 1.539323 -0.000103
C 1.529930 0.207830 -0.000064
C 0.633831 -0.891738 -0.000232
C -0.713814 -0.523694 -0.000287
C -1.159162 0.809047 -0.000282
H 1.862521 2.338588 -0.000303
Br 3.412491 -0.119800 0.000170
C 1.103119 -2.321128 -0.000341
O -0.691569 3.139609 0.000059
H 0.055075 3.757950 0.000053
H 1.726846 -2.526440 0.884691
H 1.730486 -2.525072 -0.883054
H 0.251662 -3.009648 -0.002360
C -2.645740 0.816450 -0.000222
C -1.939520 -1.397175 -0.000098
N -3.032176 -0.530511 -0.000172
C -4.415848 -0.962085 0.000548
H -4.427669 -2.058772 -0.000091
H -4.939247 -0.589367 -0.892622
H -4.938057 -0.590404 0.894859
O -3.423366 1.747477 0.000025
O -2.021559 -2.609188 0.000051

$^{1}$TS1
C -0.254431 1.865603 -0.015127
C 1.114707 1.543296 -0.011437
C 1.535279 0.213085 -0.001744
C 0.640652 -0.887087 0.000842
C -0.710797 -0.528133 -0.003579
C -1.156487 0.803828 -0.008325
H 1.841209 2.356791 -0.023670
Br 3.418758 -0.110625 0.002328
C 1.113161 -2.314723 0.006643
O -0.655072 3.166892 -0.091653
H -0.718262 3.554266 0.794826
H 1.739649 -2.511637 0.891485
H 1.740882 -2.518489 -0.875800
H 0.264577 -3.006695 0.008581
C -2.648335 0.807261 -0.002566
C -1.933250 -1.404355 -0.001445
N -3.029417 -0.538948 -0.002714
C -4.411992 -0.974982 0.003209
H -4.420583 -2.071609 0.010606
H -4.937484 -0.610124 -0.891940
H -4.933210 -0.597712 0.895660
O -3.425719 1.737266 0.005987
O -2.014011 -2.615788 0.001782

$^{1}$IM1
C -0.270766 1.850247 0.000025
C 1.106969 1.551070 -0.000024
C 1.532310 0.222097 0.000009
C 0.657576 -0.901466 0.000062
C -0.695955 -0.562069 0.000211
C -1.141090 0.764639 0.000260
H 1.828990 2.368354 -0.000111
Br 3.420057 -0.079600 -0.000020
C 1.150100 -2.322277 0.000052
O -0.695339 3.122448 -0.000172
H -1.673385 3.120489 -0.000095
H 1.776974 -2.519498 0.884628
H 1.778714 -2.518954 -0.883382
H 0.307824 -3.023159 -0.000916
C -2.610763 0.792640 0.000129
C -1.933142 -1.420958 0.000019
N -3.026637 -0.534290 -0.000001
C -4.415313 -0.953116 -0.000035
H -4.436434 -2.049641 -0.009008
H -4.935522 -0.569768 -0.890337
H -4.931861 -0.584882 0.898851
O -3.332959 1.778548 0.000023
O -2.035501 -2.628713 -0.000246

$^{1}$TS2
C 0.278108 1.962989 0.001305
C -1.145313 1.568960 -0.003597
C -1.553406 0.266835 -0.000521
C -0.647695 -0.875904 0.005276
C 0.685245 -0.538988 0.003451
C 1.167920 0.814798 -0.003378
H -1.869579 2.385361 -0.006368
Br -3.439686 -0.081359 -0.003078
C -1.150531 -2.288903 0.011160
O 0.624424 3.149798 0.012285
H 3.772535 2.038514 0.764713
H -1.782139 -2.480396 -0.871782
H -1.784976 -2.472523 0.893697
H -0.316955 -2.999320 0.015377
C 2.559677 0.750724 -0.007262
C 1.887038 -1.428915 0.005768
N 2.996799 -0.548485 -0.001910
C 4.382564 -0.982739 -0.012430
H 4.386583 -2.065787 -0.184980
H 4.872728 -0.770659 0.950082
H 4.934885 -0.477681 -0.816794
O 3.437266 1.743104 -0.099228
O 1.990398 -2.638362 0.009259

$^{1}$PC
C 0.274255 1.966576 -0.000019
C -1.145038 1.566895 0.000066
C -1.551365 0.261388 0.000045
C -0.649390 -0.875969 -0.000013
C 0.684770 -0.530321 -0.000020
C 1.160038 0.821012 -0.000010
H -1.872912 2.380228 0.000113
Br -3.440430 -0.086416 0.000027
C -1.146196 -2.292424 -0.000128
O 0.620843 3.156285 -0.000034
H 4.309468 1.539428 -0.000105
H -1.777179 -2.485094 -0.883284
H -1.777878 -2.485021 0.882531
H -0.309974 -2.999868 0.000193
C 2.561590 0.760646 -0.000020
C 1.885254 -1.416718 -0.000013
N 3.002333 -0.534282 -0.000014
C 4.381818 -0.990423 0.000184
H 4.355171 -2.087158 -0.000588
H 4.915326 -0.653916 0.902551
H 4.915917 -0.652643 -0.901338
O 3.370001 1.789924 -0.000112
O 1.997448 -2.623132 -0.000024

$^{3}$RC
C -0.200455 1.894861 -0.000273
C 1.141597 1.578961 -0.000244
C 1.549081 0.213655 -0.000160
C 0.619070 -0.896319 -0.000271
C -0.732568 -0.567662 -0.000307
C -1.184500 0.808247 -0.000057
H 1.892212 2.370291 -0.000375
Br 3.402711 -0.127099 0.000266
C 1.089099 -2.315447 -0.000266
O -0.689774 3.128008 -0.000497
H 0.025024 3.788116 -0.001047
H 1.721497 -2.510328 0.882022
H 1.723196 -2.509909 -0.881397
H 0.236270 -3.002535 -0.001140
C -2.634259 0.832433 0.000309
C -1.924670 -1.418102 -0.000228
N -3.010964 -0.524652 0.000149
C -4.393341 -0.944601 0.000346
H -4.414863 -2.041205 -0.001112
H -4.916697 -0.562352 -0.890264
H -4.915814 -0.564851 0.892567
O -3.433036 1.770365 0.000576
O -2.045100 -2.643666 -0.000416

$^{3}$TS1
C -0.223372 1.903609 -0.025935
C 1.104121 1.587180 -0.026858
C 1.539086 0.179458 -0.009345
C 0.638222 -0.925678 -0.007834
C -0.706616 -0.589734 -0.012494
C -1.169613 0.819523 -0.011045
H 1.854768 2.377008 -0.047272
Br 3.388419 -0.100014 0.011208
C 1.117355 -2.349463 -0.000966
O -0.659655 3.191592 -0.100819
H -0.685528 3.593504 0.781081
H 1.740854 -2.544294 0.886457
H 1.747205 -2.550750 -0.882455
H 0.265257 -3.037662 -0.001628
C -2.619998 0.826924 0.004814
C -1.899366 -1.434722 -0.007435
N -2.986235 -0.536348 0.001804
C -4.366377 -0.959430 0.013995
H -4.388381 -2.055667 0.008973
H -4.896427 -0.569371 -0.869421
H -4.878326 -0.577999 0.911769
O -3.430656 1.749346 0.022213
O -2.026073 -2.656669 -0.008117

$^{3}$IM1
C -0.238720 1.850909 -0.000146
C 1.126850 1.588504 -0.000122
C 1.550547 0.235964 -0.000161
C 0.658417 -0.904540 -0.000290
C -0.721116 -0.620827 0.000018
C -1.154632 0.730127 0.000082
H 1.846663 2.405768 -0.000093
Br 3.416300 -0.072997 0.000235
C 1.157431 -2.310419 -0.000547
O -0.763283 3.058964 -0.000469
H -1.757519 2.959713 -0.000099
H 1.793900 -2.494700 0.881574
H 1.797096 -2.493593 -0.880508
H 0.317674 -3.014621 -0.002239
C -2.583505 0.802080 0.000052
C -1.926476 -1.446829 -0.000107
N -3.013369 -0.518724 0.000080
C -4.403726 -0.917320 0.000489
H -4.439496 -2.013702 -0.007014
H -4.922266 -0.525965 -0.888694
H -4.918757 -0.538824 0.897357
O -3.278269 1.841821 0.000119
O -2.081523 -2.668787 -0.000237

$^{3}$TS2
C -0.293377 1.816986 -0.000002
C 1.111810 1.578310 0.000000
C 1.546703 0.239129 0.000000
C 0.696913 -0.912700 -0.000002
C -0.724985 -0.658814 -0.000003
C -1.137591 0.670013 -0.000005
H 1.816964 2.408384 0.000001
Br 3.434123 -0.038634 0.000002
C 1.213882 -2.311623 -0.000003
O -0.901375 2.954542 0.000000
H -2.043275 2.670945 -0.000001
H 1.854062 -2.491595 0.880806
H 1.854059 -2.491595 -0.880815
H 0.383362 -3.027640 -0.000002
C -2.550058 0.779365 -0.000002
C -1.939172 -1.459582 0.000000
N -3.033546 -0.493712 0.000000
C -4.430156 -0.878176 0.000004
H -4.471211 -1.974639 0.000010
H -4.942729 -0.493463 -0.894961
H -4.942727 -0.493453 0.894966
O -3.142098 1.912892 0.000001
O -2.135502 -2.676961 0.000001

$^{3}$IM2
C -0.281932 1.893078 -0.000012
C 1.126747 1.575209 -0.000001
C 1.558458 0.238326 -0.000003
C 0.697140 -0.876725 -0.000010
C -0.720098 -0.581403 0.000005
C -1.165985 0.745984 0.000024
H 1.839807 2.400046 -0.000017
Br 3.453035 -0.055745 0.000007
C 1.181719 -2.292450 -0.000009
O -0.751185 3.057531 -0.000043
H -2.818822 2.590930 0.000121
H 1.815809 -2.486902 0.881635
H 1.815870 -2.486886 -0.881613
H 0.338326 -2.992374 -0.000043
C -2.582234 0.747925 0.000002
C -1.899135 -1.436533 -0.000005
N -3.020711 -0.526076 -0.000005
C -4.406232 -0.957179 0.000006
H -4.404825 -2.053948 0.000100
H -4.928572 -0.590230 -0.895922
H -4.928603 -0.590070 0.895849
O -3.390883 1.791218 0.000019
O -2.044300 -2.660543 -0.000015

$^{3}$TS3
C 0.263083 1.929263 -0.000708
C -1.140576 1.573033 -0.002129
C -1.569177 0.240935 -0.000317
C -0.691694 -0.856013 0.003774
C 0.716718 -0.531016 0.003235
C 1.183623 0.792578 -0.003505
H -1.857016 2.395379 -0.001587
Br -3.461280 -0.069812 -0.001625
C -1.159507 -2.278567 0.006907
O 0.646236 3.116827 0.007610
H 3.825642 2.038032 0.768441
H -1.787847 -2.480883 -0.877074
H -1.796243 -2.474695 0.886148
H -0.311170 -2.971283 0.012778
C 2.609987 0.756441 -0.004102
C 1.882440 -1.414143 0.004803
N 3.008081 -0.541265 -0.002407
C 4.383140 -1.005918 -0.009433
H 4.363788 -2.089615 -0.175434
H 4.874137 -0.796338 0.953276
H 4.947121 -0.512599 -0.813194
O 3.484198 1.761139 -0.099233
O 1.989765 -2.642375 0.007776

$^{3}$PC
C 0.258872 1.933498 0.000078
C -1.145159 1.574044 0.000169
C -1.568861 0.238311 0.000164
C -0.690482 -0.855185 0.000104
C 0.721345 -0.530468 0.000059
C 1.182341 0.799846 0.000043
H -1.863439 2.394547 0.000209
Br -3.461249 -0.076590 -0.000007
C -1.153733 -2.279458 -0.000197
O 0.637708 3.121515 0.000032
H 4.348203 1.561067 -0.000138
H -1.783641 -2.481849 -0.883055
H -1.787167 -2.481262 0.880206
H -0.302619 -2.968990 0.001500
C 2.605992 0.764727 -0.000135
C 1.880184 -1.408627 -0.000094
N 3.013426 -0.526240 -0.000210
C 4.383903 -1.004950 0.000309
H 4.337961 -2.101080 0.000092
H 4.922740 -0.672584 0.901223
H 4.923509 -0.672282 -0.900024
O 3.410781 1.813989 -0.000310
O 2.002482 -2.635963 0.000116