Supporting Information

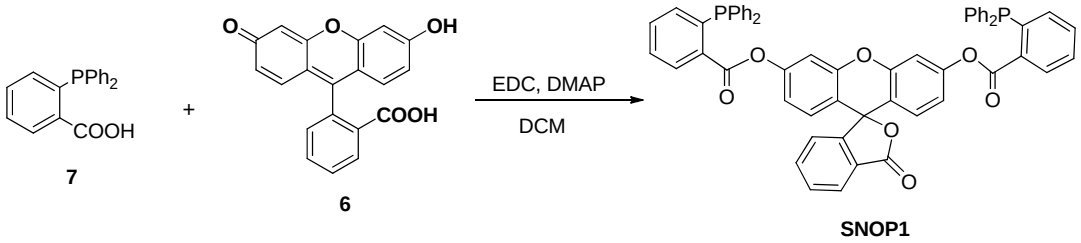
A Reductive Ligation Based Fluorescent Probe for S-Nitrosothiols

Di Zhang‡[ab], Wei Chen‡[b], Zhengrui Miao[c], Yong Ye*[b], Yufen Zhao[b], S. Bruce King[c] and Ming Xian*[a]

[a]*Department of Chemistry, Washington State University, Pullman, WA 99164, U.S.A*.[b]*College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450052, China.* [c]*Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, U.S.A.*

Materials and Methods: All solvents were reagent grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-13 NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported relative to chloroform (δ 7.26) for ^{1}H NMR and chloroform (δ 77.0) for ^{13}C NMR. Fluorescence excitation and emission spectra were measured on Cary Eclipse fluorescence spectro- photometer.

Chemical Synthesis

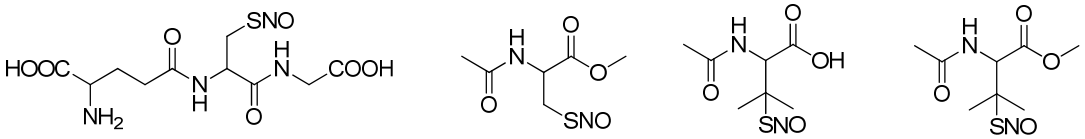


Scheme S1. Synthesis of **SNOP1**

2-(Diphenylphosphino)benzoic acid **7** (918.9 mg, 3 mmol), EDC (574.25mg, 3 mmol) and DMAP (122 mg, 1 mmol) were combined and dissolved in DCM (50 mL). The reaction mixture was stirred for 10 min at room temperature followed by the addition of fluorescein **6** (332.3 mg, 1 mmol) in DCM (10 mL). The reaction mixture was stirred at room temperature over night and then no remaining SM was observed by TLC. The solution was washed with

water and brine, dried over $MgSO_4$. Purification by flash column chromatography on silica gel (EA/Hex = 1/3, v/v) provided the product as a white solid (54% yield). ^{31}P NMR (122 MHz, $CDCl_3$) δ -2.88; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.62-6.63 (d, *J* = 3.0 Hz, 1 H), 6.65-6.66 (d, *J* = 3.0 Hz, 1 H), 6.74 (s, 1 H), 6.77 (s, 1 H), 6.91-6.92 (d, *J* = 3.0 Hz, 2 H), 7.0 (m, 2 H), 7.12-7.15 (t, *J* = 4.5 Hz, 1 H), 7.30 (m, 20 H), 7.47 (m, 4 H), 7.64 (m, 2 H), 8.01 (m, 1 H), 8.25 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 164.9, 164.9, 153.3, 152.1, 151.1, 151.6, 142.0, 141.6, 137.6, 134.7, 134.4, 134.1, 133.0, 129.1, 129.1, 128.9, 128.8, 128.6, 118.0, 116.6, 110.7, 81.9. MS (ESI) m/z calcd for $C_{58}H_{38}O_7P_2$ 908.209, found 909.3 $[M+H^+]^+$, 931.3 $[M+Na^+]^+$. M.P. 125 – 127 ^oC.

Preparation of SNO



GSNO **8** **9** **10**

Scheme S2. Structures of SNO used in this study

Four SNO compounds (GSNO, **8**, **9**, **10**) were used in this study. These compounds were freshly prepared before use, following known procedures. Their NMR data were conformed by literature data.[1]

Angeli's salt (trioxodinitrate)

Angeli's salt was prepared at >98% purity from butyl nitrate and hydroxylamine hydrochloride by the method of Smith and Hein.[2]

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[1] a) M. Xian, X. Chen, Z. Liu, K. Wang and P. G. Wang. *J. Biol. Chem.* 2000, **27**, 20467. b) K. Artur and B. Wojciech *Chem. Res. Toxicol*. 2004, **17**, 392. c) J. Ramirez, L. Yu, J. Li, P. G. Braunschweiger, P. G. Wang, *Bioorg. Med. Chem. Lett.* 1996, **6**, 2575.

[2] Smith, P. A.; Hein, G. E. *J. Am. Chem. Soc.* 1960, **21**, 5731.

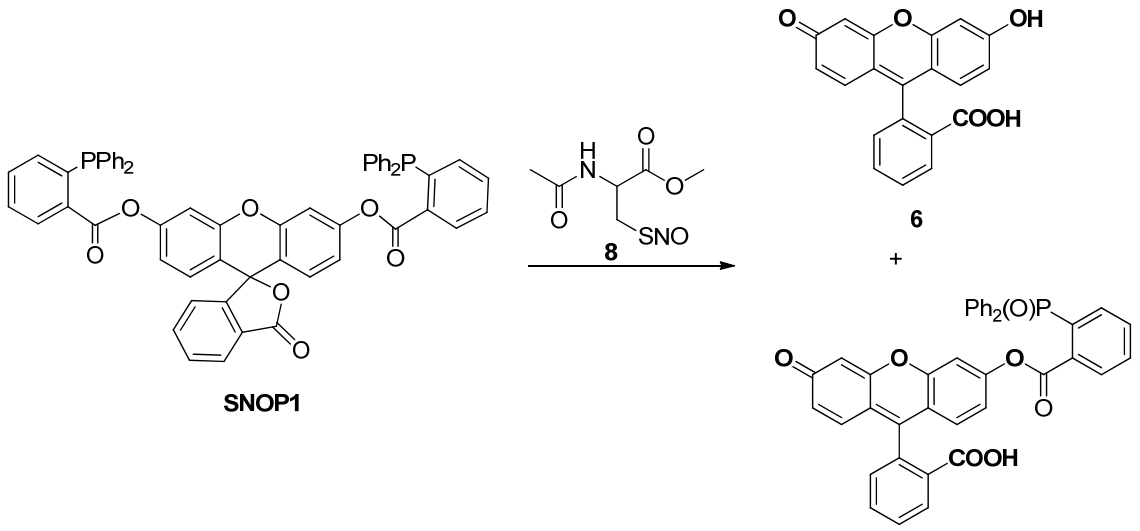

Figure S1. The reaction between the probe and SNO model compound.

The probe (27 mg) and SNO **8** (2 eq) were mixed in a mixture CH_3CN/DMSO/Tris-HCl buffer (10 mL). The reaction was incubated at 37 ^oC for 2 hours. Both free fluorescein and mono-acylated fluorescein were observed as the products, at ~30% yields respectively.

Preparation of the solutions and fluorescence measurements

The stock solution of **SNOP1** (2 mM) was prepared in DMSO. The solutions of various testing species were prepared from Angeli's salt, GSNO, cysteine (Cys), GSH, homocysteine (Hcy), glutathione disulfide (GSSG), Na_2S, $Na_2S_2O_3$, Na_2SO_3, H_2O_2, NaClO, $NaNO_2$ in 50 mM Tris-HCl buffer (pH 7.4). The solutions of various testing SNO (**8**, **9**, **10**) were prepared in DMSO. All the test solutions need to be freshly prepared.

Unless otherwise noted, all the measurements were carried out for 45 min at 37 ^oC in 50 mM Tris-HCl buffer (pH 7.4) with 1% DMSO according to the following procedure. In a test tube, 3.9 mL of 50 mM Tris-HCl buffer (pH 7.4) was first added, and then a requisite volume of testing species sample solution was added. The resulting solution was mixed well, followed by the addition of 20 μL of the stock solution of **SNOP1**. The final volume of the reaction solution was adjusted to 4 mL with 50 mM Tris-HCl buffer (pH 7.4) with 1% DMSO. After mixing and then standing for 45 min at 37 ^oC, a portion of the reaction solution was transferred into a 1-cm quartz cell to measure fluorescence with λ_{ex} = 490 nm. PMT detector voltage = 550 V. In the meantime, a blank solution containing no testing species sample was prepared and measured under the same conditions for comparison.

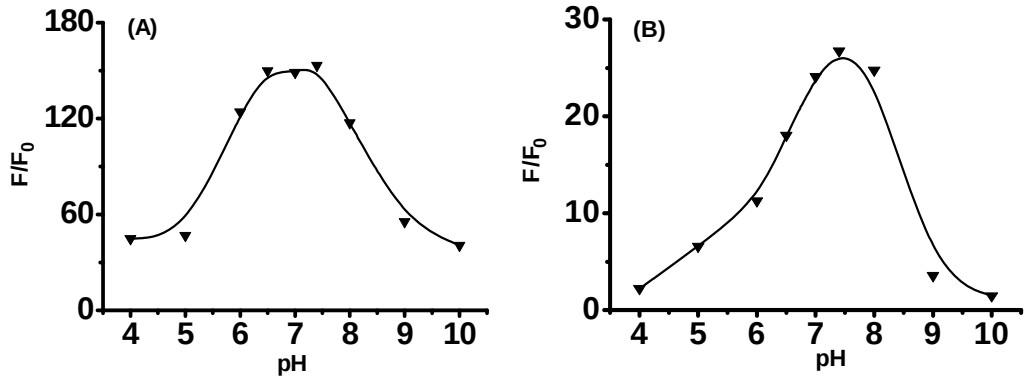

Figure S2. pH-dependent fluorescence intensity change of probe **SNOP1** (10 μM) in the presence of **8** (50 μM) (A) and **GSNO** (50 μM) (B). The reactions were carried out for 45 min at 37℃ in Tris-HCl buffer (50 mM, pH = 4, 5, 6, 6.5, 7, 7.4, 8, 9, 10) with 1% DMSO ($\lambda_{ex/em}$ = 490/518 nm).

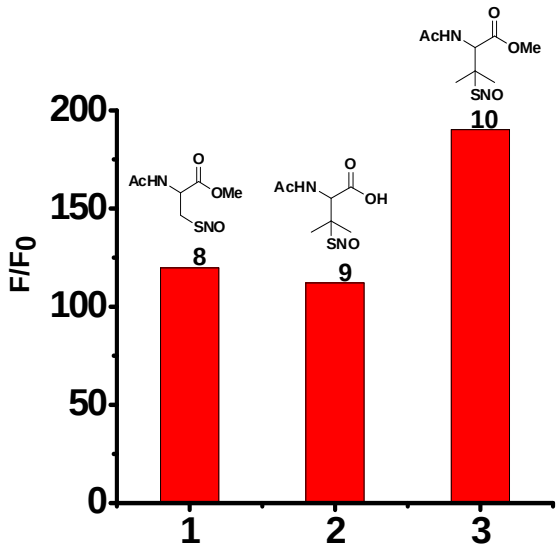

Figure S3 Fluorescence enhancement of **SNOP1** (10 μM) to tertiary SNO **9** and **10**. The reactions were carried out for 45 min in Tris-HCl buffer (50 mM, pH = 7.4) with 1% DMSO at 37 ^{o}C. (1) 50 μM **8**; (2) 50 μM **9**; (3) 50 μM **10**.

SNO detection in bovine plasma

The stock solution of **SNOP1** (1 mM) was prepared in DMSO. The stock solution of GSNO (1 mM) was prepared in Tris-HCl buffer. 1 mL commercially available bovinee

plasma was deproteined by adding 2 mL cold pure ethanol (-20 °C) and then centrifuging for 25 min at 2 °C. The separated supernatant was collected to use for analysis of deproteinized bovine plasma.

In a test tube, 3 mL of 50 mM Tris-HCl buffer (pH 7.4) and 600 μL deproteinized bovine plasma was added, followed by addition of a requisite volume of GSNO solution, the final volume of the reaction solution was adjusted to 3.96 mL with 50 mM Tris-HCl buffer (pH 7.4). Then a 40 μL of 1 mM the stock solution of **SNOP1** (10 μM) was added. The solution was mixed and kept for 45 min at 37 °C. In the meantime, a blank solution containing no GSNO was prepared and measured under the same conditions for comparison. The results are shown in Fig S4.

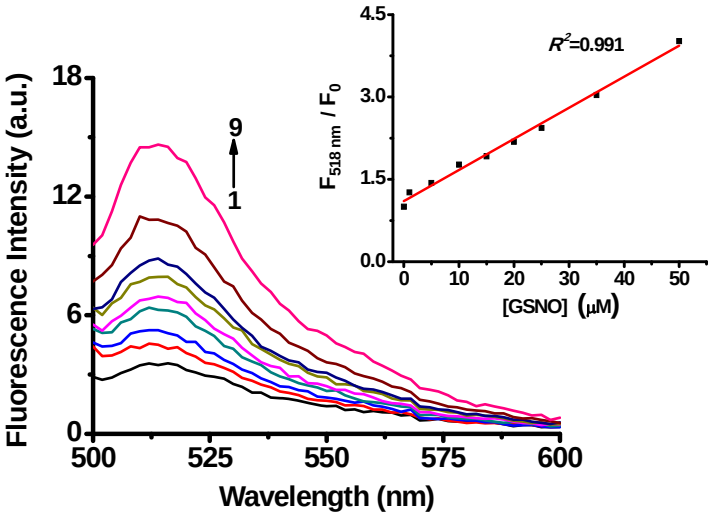

Figure S4 Fluorescence emission spectra of **SNOP1** (10 μM) with varied concentrations of GSNO (0, 1, 5, 10, 15, 20, 25, 35, 50 μM for curves 1-9, respectively). The reactions were carried out for 45 min at 37 °C in diluted deproteinized bovine plasma. The inset depicts the plot of relative fluorescence intensity ($F_{518\ nm}/F_0$) of the reaction system at $\lambda_{ex/em}$ = 490/518 nm against the corresponding reagent blank (without GSNO).

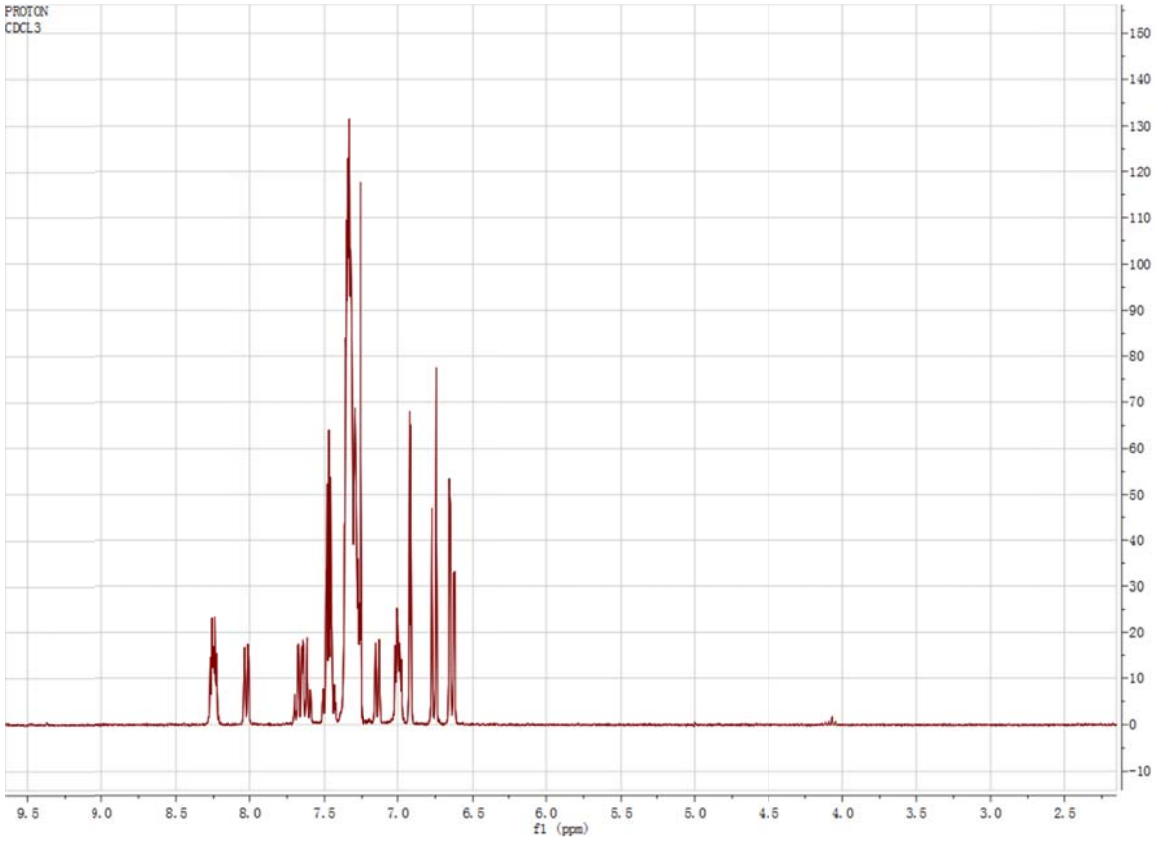

Figure S5 ^{1}H NMR (300 MHz, CD_3Cl) of **SNOP1**

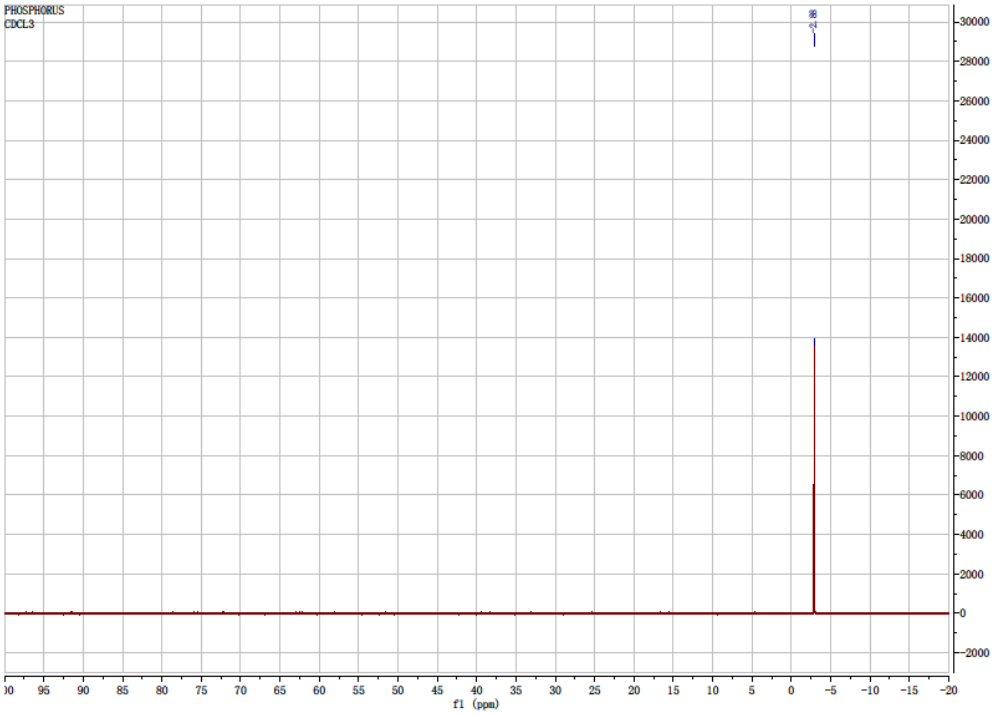

Figure S6 ^{31}P NMR (122 MHz, $CDCl_3$) of **SNOP1**

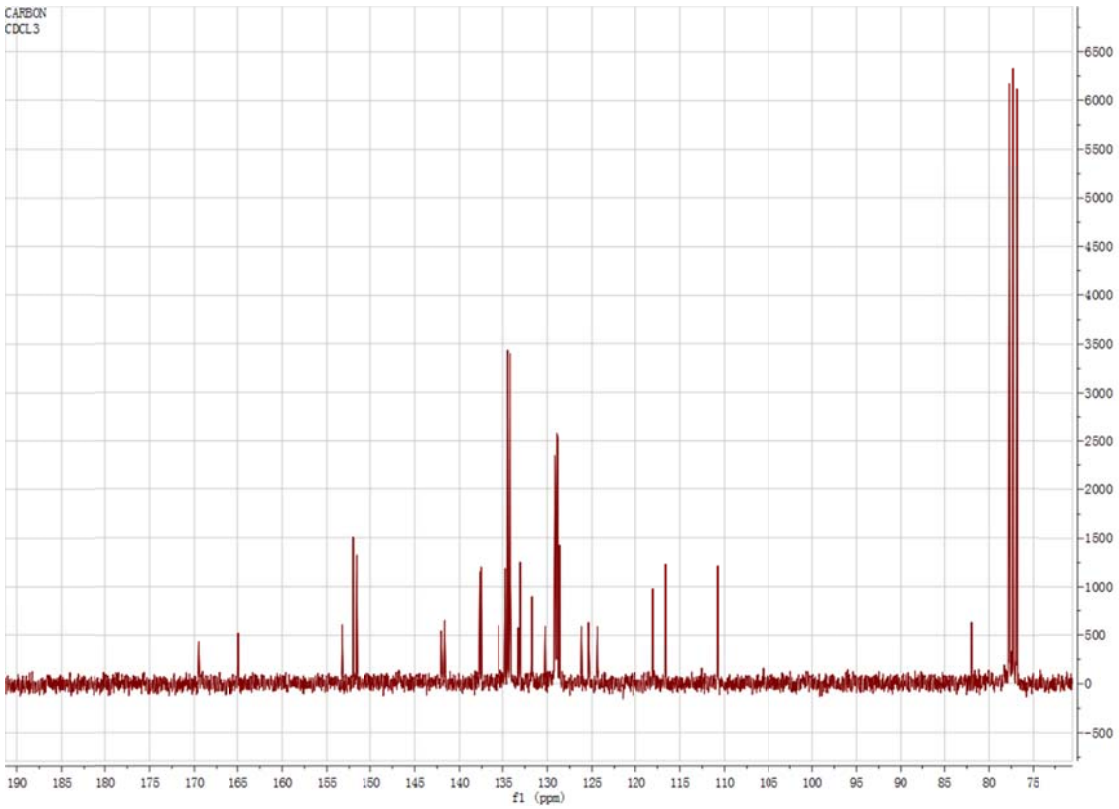

Figure S7 ^{13}C NMR (75 MHz, $CDCl_3$) of **SNOP1**

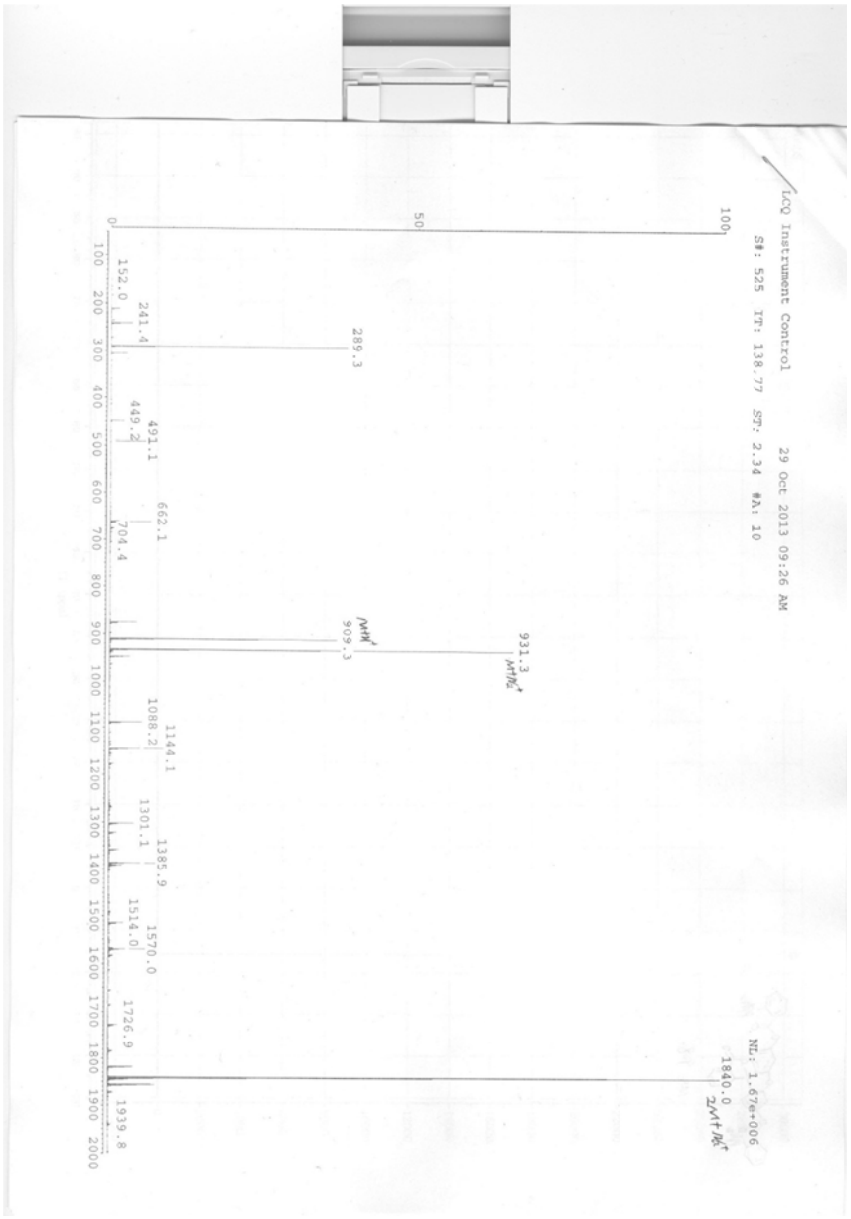

Figure S8 MS (ESI, MeOH) of **SNOP1**