

Supporting Information

Highly sensitive chemiluminescence sensors for the detection and differentiation of chemical warfare agents

Orit Redy Keisar,^{*a} Alexander Pevzner,^b Gil Fridkin,^a Omri Shelef,^c Doron Shabat^{*c} and Nissan Ashkenazi^{*a,d}

^a Department of Organic Chemistry, IIBR-Israel Institute for Biological Research, P.O.Box 19, Ness Ziona, 7410001, Israel. E-Mail: OritR@iibr.gov.il; nissan.ashkenazi@iibr.gov.il

^b Department of Physical Chemistry, IIBR-Israel Institute for Biological Research, P.O.Box 19, Ness Ziona, 7410001, Israel.

^c School of Chemistry, The Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 69978, Israel. E-Mail: Chdoron@tauex.tau.ac.il

^d Department of Chemical Sciences, Ariel University, 4070000 Ariel, Israel

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General methods

All reactions requiring anhydrous conditions were performed under an Argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich. Chemicals and solvents were either A.R. grade or purified by standard techniques. VX and GB were synthesized locally at IIBR (>99% purity).

Warning!! These chemicals are extremely toxic and should be handled only by qualified personal using the adequate protecting gear.

Thin-layer chromatography (TLC): silica gel plates Merck 60 F254: compounds were visualized by irradiation with UV light. Column chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent is detailed in parentheses. Reverse-phase high-pressure liquid chromatography (RP-HPLC) was performed using a C18 5 μ , 250x4.6mm column, the eluent is detailed in parentheses. Preparative RP-HPLC was performed using a C18 5 μ , 250x21mm column, the eluent is detailed in parentheses. $^1\text{H-NMR}$ spectra were measured using Bruker Avance operated at 400MHz. $^{13}\text{C-NMR}$ spectra were measured using Bruker Avance operated at 100 MHz. Chemical shifts were reported in ppm on the δ scale relative to a residual solvent (CDCl_3 : $\delta = 7.26$ for $^1\text{H-NMR}$ and 77.16 for $^{13}\text{C-NMR}$, $\text{DMSO-}d_6$: $\delta = 2.50$ for $^1\text{H-NMR}$ and 39.52 for $^{13}\text{C-NMR}$). Mass spectra were measured on Waters Xevo TQD.

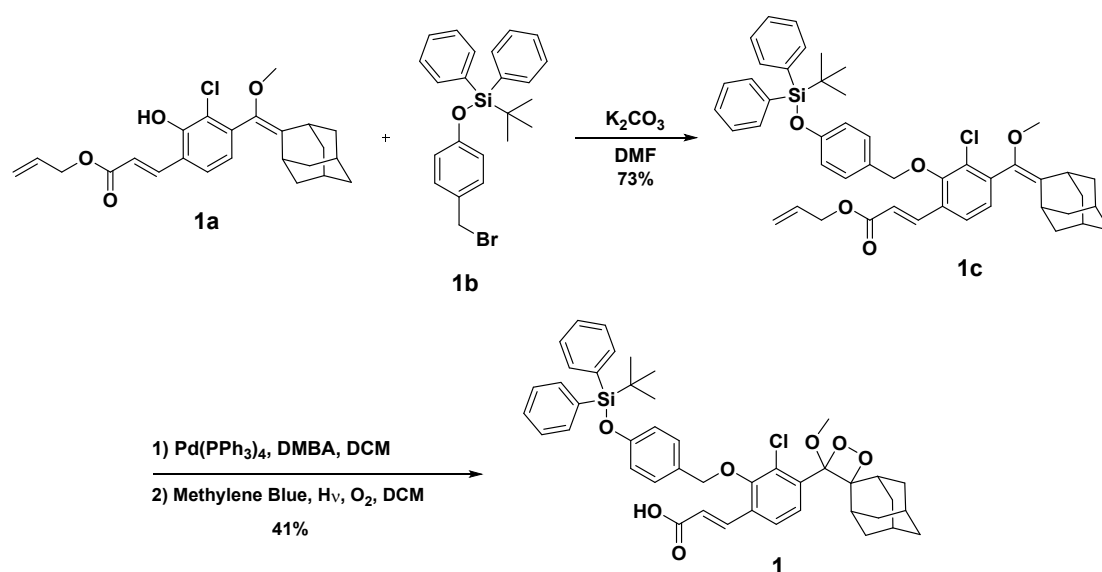
Chemiluminescence was recorded with a Synergy H1 multimode reader (BioTek, USA), $n = 3$ technical replicates. Light irradiation for photochemical reactions: LED PAR38 lamp (19W, 3000K).

Abbreviations

ACN- Acetonitrile, **CHCl_3** - Chloroform, **DCM**- dichloromethane, **DMBA**- Dimethyl barbituric acid, **DMF**- N,N'-Dimethylformamide, **EtOAc**- Ethylacetate, **Hex**- Hexanes, **K_2CO_3** - Potassium carbonate, **MB**- Methylene blue, **MeOH**- Methanol, **NH_4Cl** - ammonium chloride, **NaHCO_3** - Sodium bicarbonate, **Na_2SO_4** - Sodium Sulfate, **$\text{Pd}(\text{PPh}_3)_4$** - Tetrakis(triphenylphosphine)palladium, **THF**- Tetrahydrofuran.

Synthetic Schemes and Experimental Procedures

Compound 1

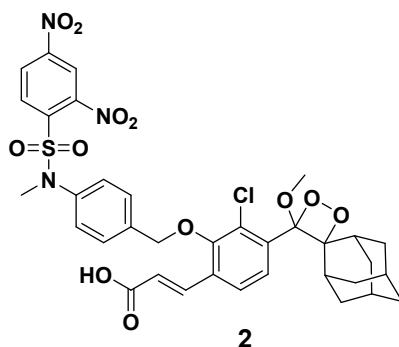


Compound 1c

Phenol enol ether **1a**¹ (51 mg, 0.12 mmol, 1.2 eq) and K_2CO_3 (25 mg, 0.18 mmol, 1.5 eq) were dissolved in DMF (1 mL). The solution was stirred for 5 minutes before adding compound **1b**² (43 mg, 0.12 mmol, 1 eq). The reaction mixture was stirred at room temperature and monitored by TLC (EtOAc/Hex mixture). Upon completion, the reaction mixture was diluted with EtOAc (100 mL) and washed with 0.1M HCl (50 mL) and brine (50 mL). The organic layer was separated, dried over Na_2SO_4 , and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc: Hex gradient). Compound **1c** was obtained as a yellow oil (68 mg, 73% yield). MS (ES⁺): m/z calc. for $C_{47}H_{51}ClO_5Si$: 759.46; found: 781.9 [M+Na]⁺. ¹H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, $J = 16.2$ Hz, 1H), 7.77 – 7.70 (m, 4H), 7.42 (ddd, $J = 12.4, 6.6, 2.4$ Hz, 7H), 7.23 (d, $J = 8.5$ Hz, 2H), 7.07 (d, $J = 8.0$ Hz, 1H), 6.77 (d, $J = 8.5$ Hz, 2H), 6.47 (d, $J = 16.2$ Hz, 1H), 5.97 (ddt, $J = 17.1, 10.5, 5.7$ Hz, 1H), 5.35 (dd, $J = 17.2, 1.5$ Hz, 1H), 5.23 (dd, $J = 10.4, 1.3$ Hz, 1H), 4.89 (d, $J = 5.0$ Hz, 2H), 4.71 (dt, $J = 5.7, 1.3$ Hz, 2H), 3.31 (s, 3H), 3.29 (s, 1H), 2.04 – 1.63 (m, 13H), 1.12 (s, 9H). ¹³C NMR (101 MHz, $CDCl_3$) δ 166.28, 156.00, 153.65, 139.43, 139.13, 138.12, 135.49, 132.77, 132.21, 130.47, 129.94, 129.67, 128.40, 127.82, 127.65, 124.94, 119.80, 119.71, 118.27, 75.94, 65.26, 57.20, 39.27, 39.19, 39.04, 38.61, 37.07, 32.92, 29.70, 28.37, 26.50, 19.47.

Compound 1

Compound **1c** (30 mg, 0.4 mmol, 1 eq.) was dissolved in DCM (2 ml), followed by the addition of DMBA (20 mg, 0.13 mmol, 3 eq.) and tetrakis(triphenylphosphine)palladium (5.1 mg, 0.004 mmol, 0.1 eq.). The reaction was stirred at room temperature and monitored by RP-HPLC. Upon completion, additional DCM was added (8 mL), followed by the addition of a catalytic amount of methylene blue. Then, oxygen was bubbled through the solution while irradiating with yellow light. The reaction was monitored by RP-HPLC. Upon completion, the solvent was concentrated under reduced pressure, and the product was purified by preparative RP-HPLC (gradient of ACN in water). Compound **1** (Fluoride probe) was obtained as an off-white oil (10.7 mg, 41% yield). MS (ES⁻): m/z calc. for C₄₄H₄₇ClO₇Si: 751.39; found: 773.8 [M+Na]⁺. ¹H NMR (400 MHz, CDCl₃) δ 8.00 (d, J = 16.2 Hz, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.73 (dd, J = 7.9, 1.4 Hz, 4H), 7.59 (d, J = 8.4 Hz, 1H), 7.47 – 7.34 (m, 6H), 7.21 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 6.51 (d, J = 16.2 Hz, 1H), 4.86 (s, 2H), 3.22 (s, 3H), 3.04 (s, 1H), 2.33 (d, J = 12.3 Hz, 1H), 2.02 (s, 1H), 1.95 – 1.22 (m, 12H), 1.11 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 171.32, 156.18, 154.31, 140.51, 135.49, 132.69, 131.34, 130.53, 129.97, 128.84, 127.83, 125.43, 120.50, 119.83, 111.76, 96.39, 76.30, 49.72, 36.58, 33.88, 33.58, 32.60, 32.22, 31.59, 31.51, 29.71, 26.49, 26.17, 25.80, 19.46.

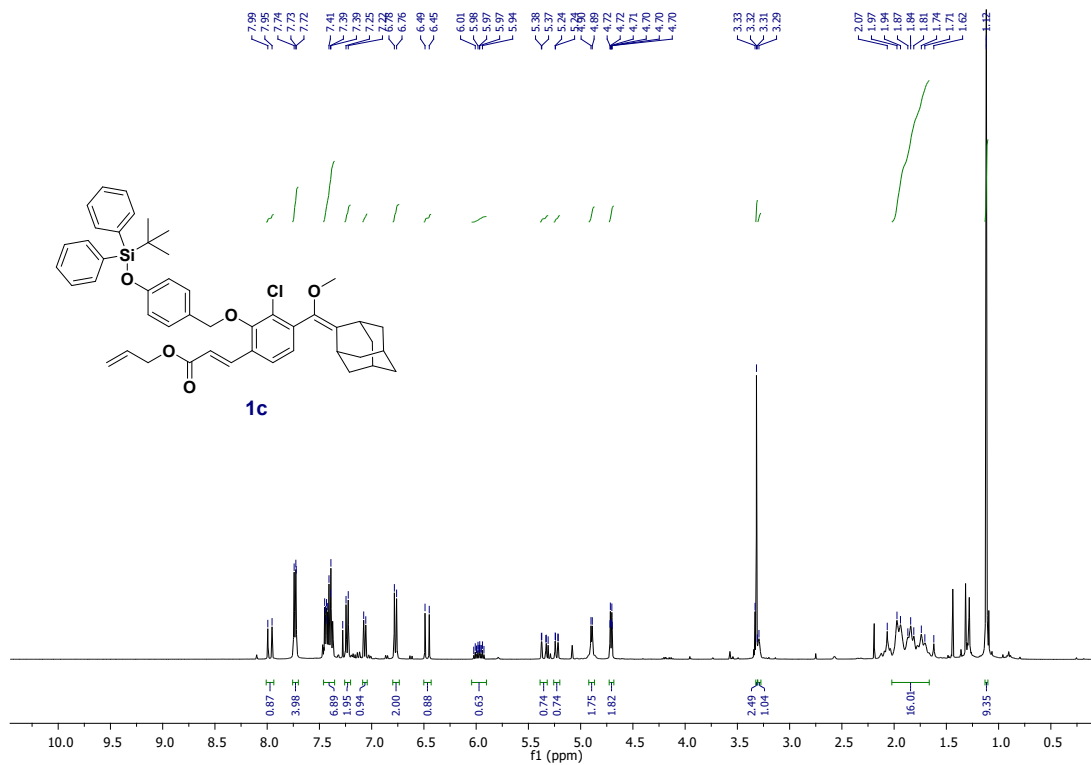


Compound 2

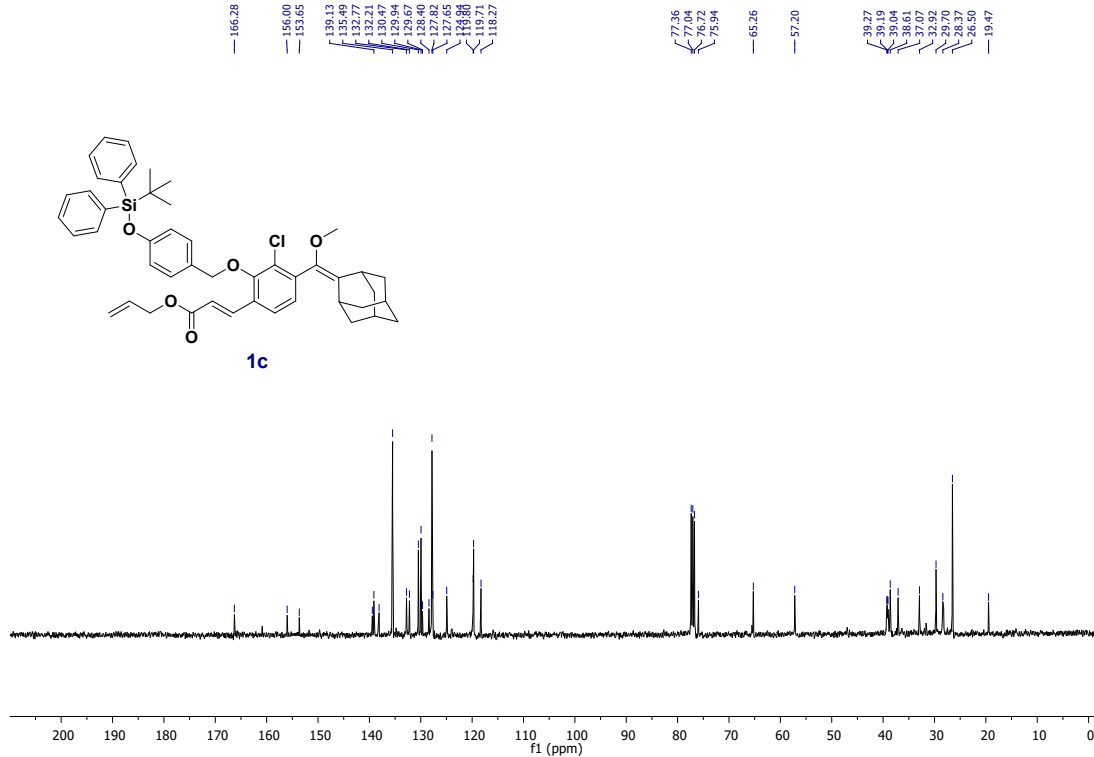
The thiol chemiluminescent probe (**2**) was synthesized according to a known procedure.³

NMR spectra

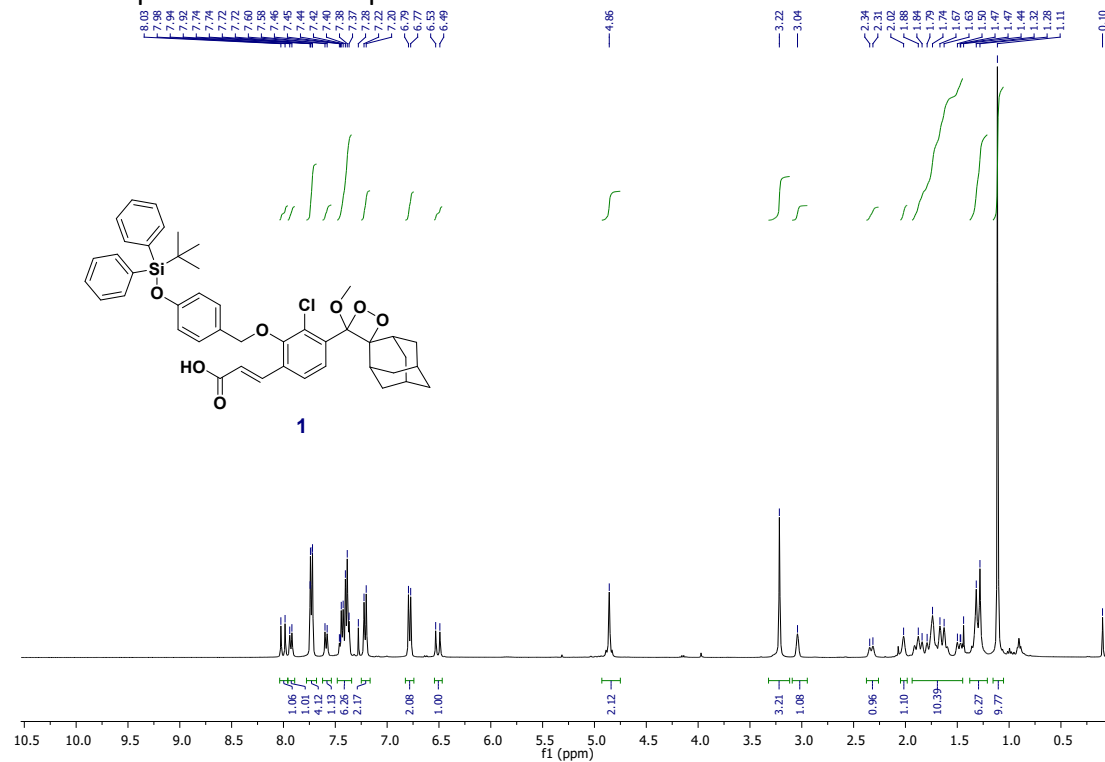
¹H-NMR spectra of compound **1c**



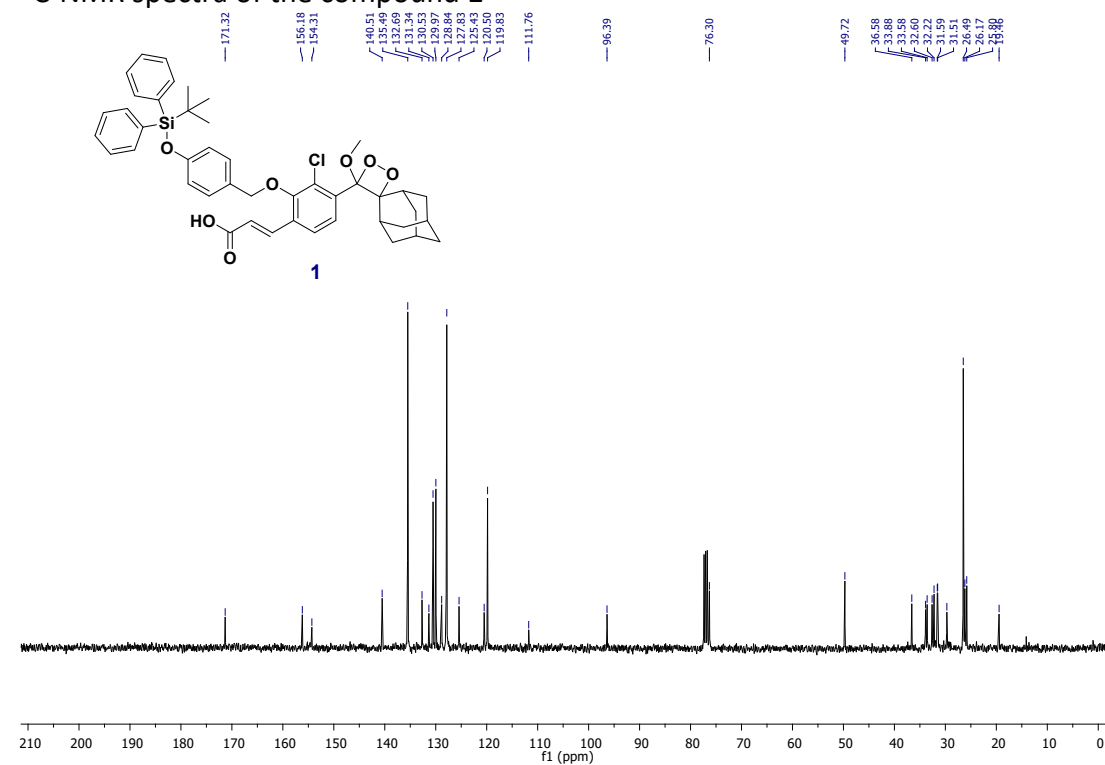
¹³C-NMR spectra of compound **1c**



¹H-NMR spectra of the compound **1**

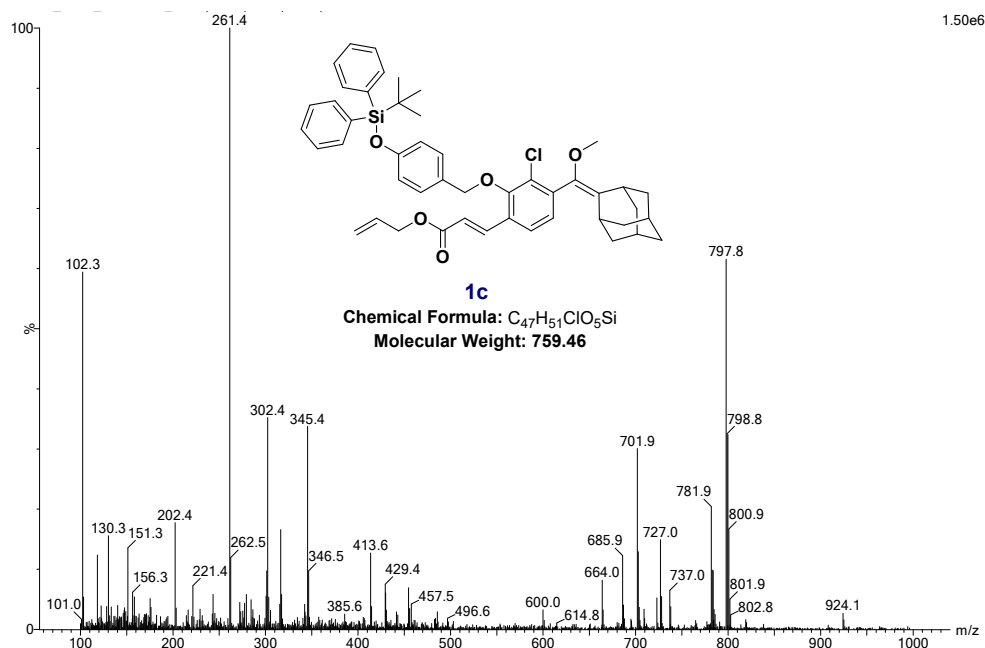


¹³C-NMR spectra of the compound **1**

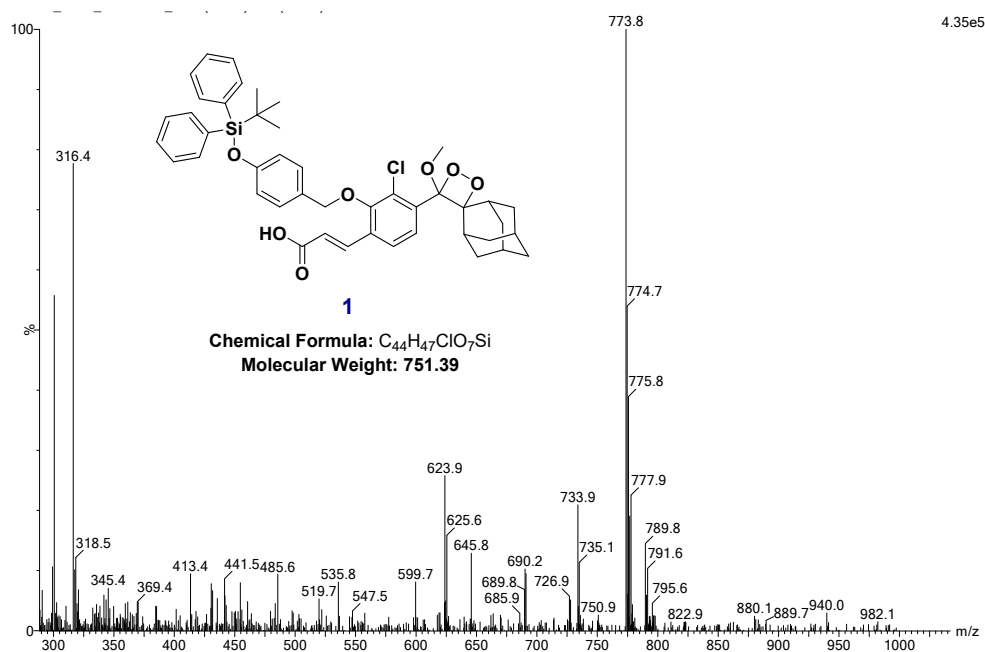


MS and HPLC Spectra

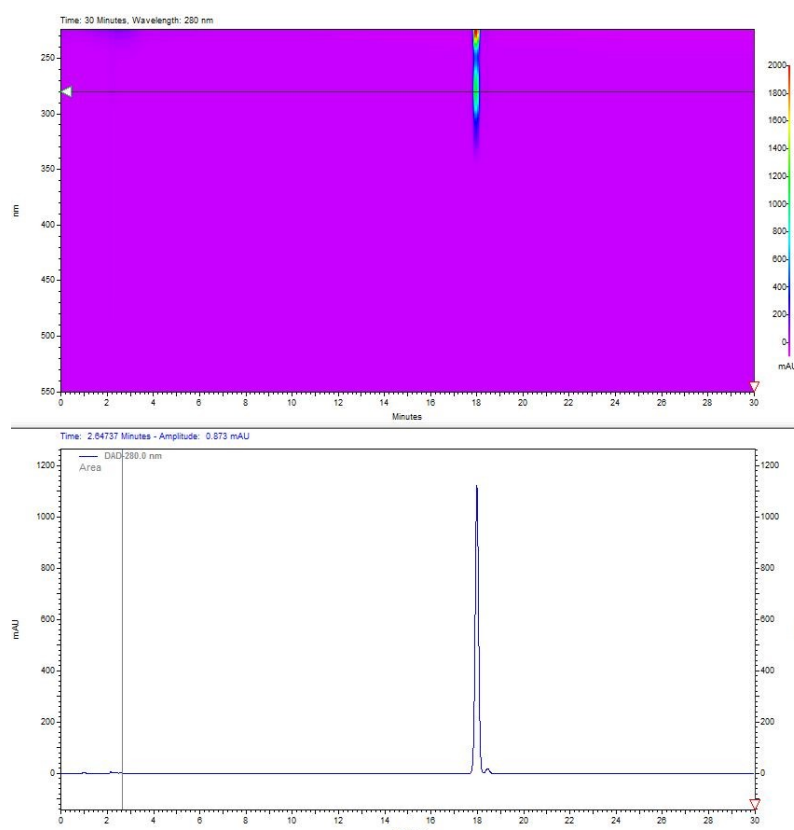
Mass spectra of compound 1c



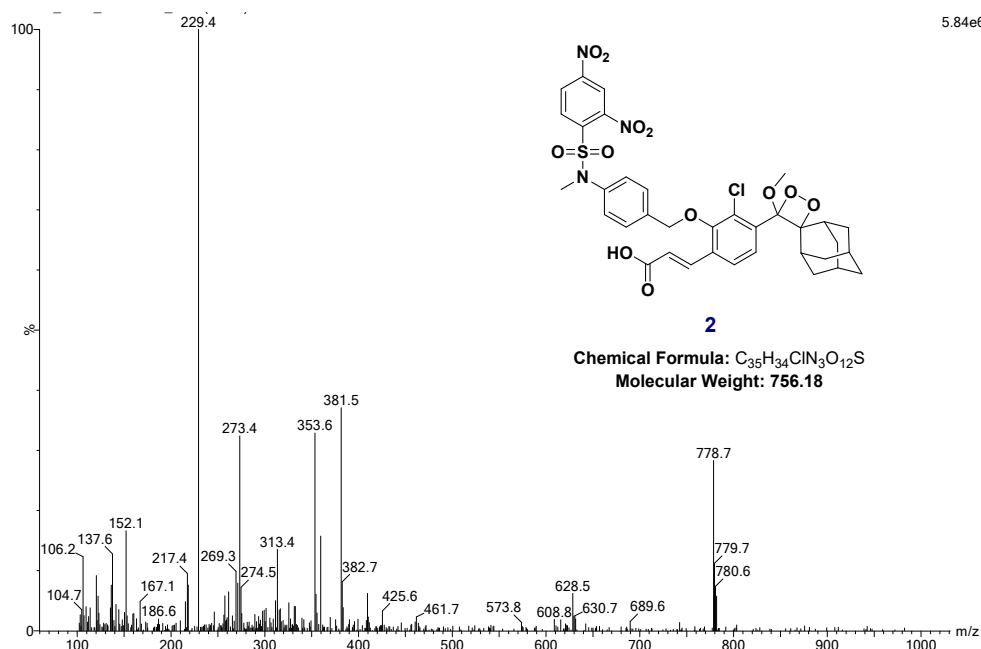
Mass spectra of the compound 1



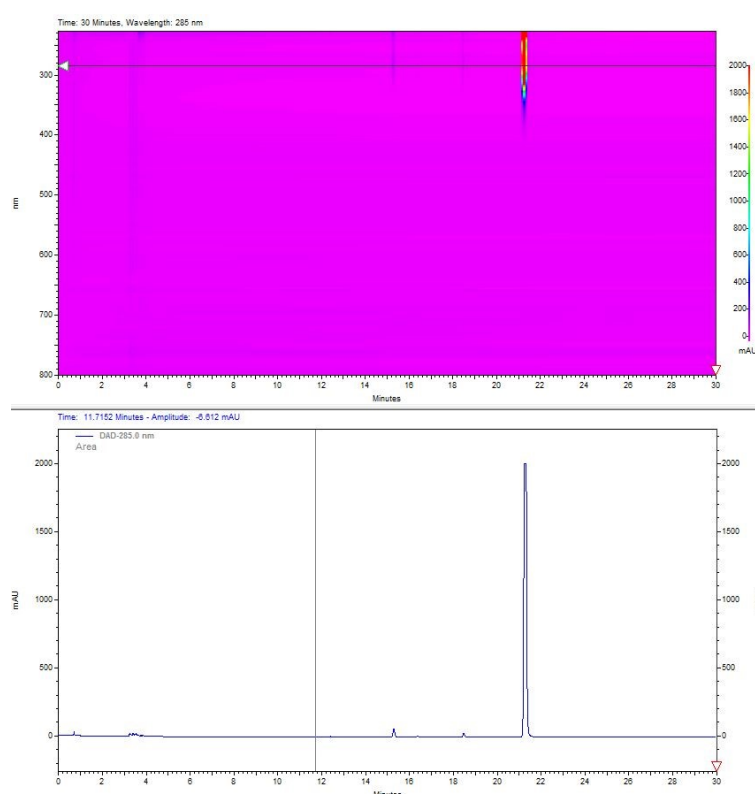
3D and 2D HPLC spectra of compound 1 (2D absorbance measured at 280nm)



Mass spectra of the compound 2



3D and 2D HPLC spectra of compound 2 (2D absorbance measured at 285 nm)



LOD Calculations

The limit of detection (LOD) of the probes for GB and VX was calculated to be 0.07 μM . The LOD was calculated using the following equation: $\text{LOD}=3.3(\sigma/S)$ where σ is the standard deviation of the response, and S is the slope of the calibration curve. [ref (<https://doi.org/10.1016/j.bios.2013.01.021>)]

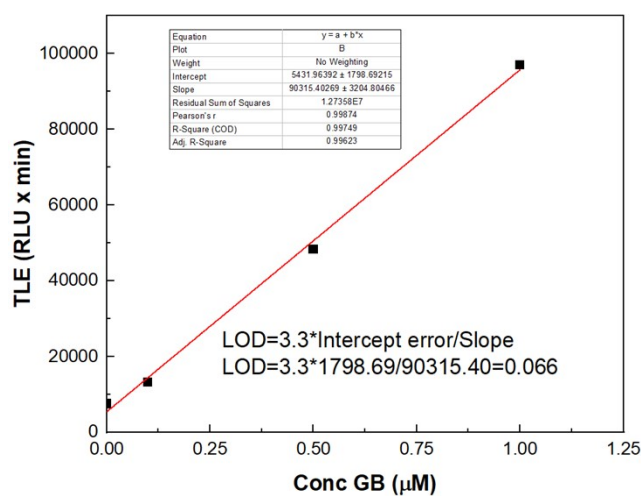


Figure S1. LOD calculation for GB.

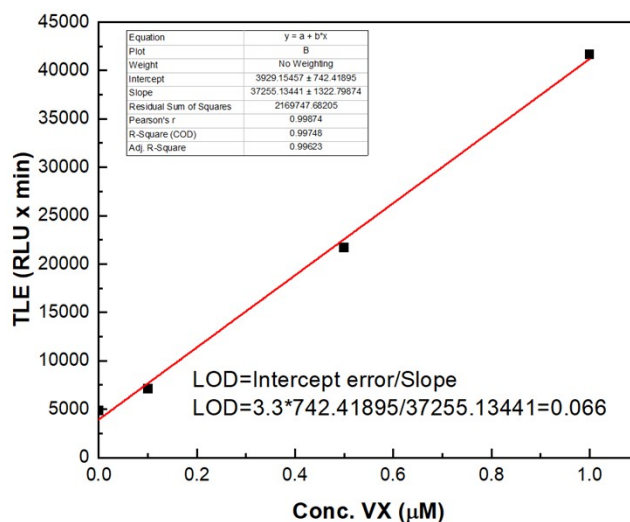


Figure S2. LOD calculation for VX.

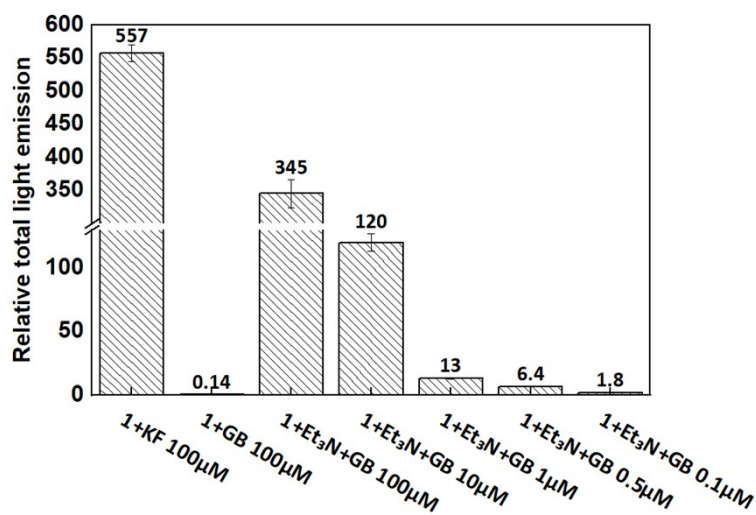


Figure S3. Signal-to-noise ratio values for GB and KF. Probe 1 (10 μM) in a 3:1 DMSO/H₂O mixture in the presence of GB (0.1-100 μM) and triethylamine (10mM) or KF (100 μM).

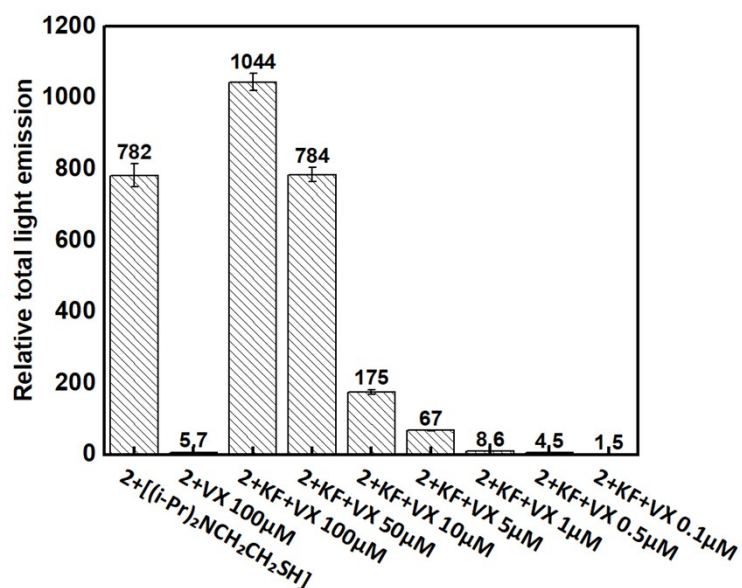


Figure S4. Signal-to-noise ratio values for VX and [(i-Pr)₂NCH₂CH₂SH]. Probe **2** (10 µM) in a (1:9) DMSO:PBS buffer (pH=7.4) mixture in the presence of VX (0.1-100 µM) and KF (80mM) or [(i-Pr)₂NCH₂CH₂SH] (100 µM).

References

- (1) Green, O.; Eilon, T.; Hananya, N.; Gutkin, S.; Bauer, C. R.; Shabat, D. *ACS Cent. Sci.* 2017, **3** (4), 349-358.
- (2) Núñez-Villanueva D, Hunter CA. *Homochiral Chem. Sci.* 2017, **1**;8(1):206-213.
- (3) Sachin Popat Gholap, Chunyan Yao, Ori Green, Matej Babjak, Pavol Jakubec, Tomáš Malatinský, Julian Ihssen, Lukas Wick, Urs Spitz, and Doron Shabat *Bioconjug. Chem.* 2021, **32** (5), 991-1000.