Scavenging 4-Oxo-2-nonenal

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Supporting Information

GENERAL METHODS

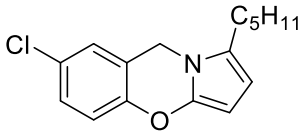
Pyridoxamine dihydrochloride was from commercial sources. HNE[1] and ONE[2] were prepared and stored as 1 M solutions in dichloromethane and acetonitrile, respectively. Salicylamine (SA), 5-chlorosalicylamine (ClSA), 5-ethylsalicylamine (EtSA), 5′-*O*-pentylpyridoxamine (PnPM) and 5′-*O*-hexylpyridoxamine (HxPM) as acetic acid salts,[3,4] and 4-hydroxybenzylamine (4-HOBA) and 1-(aminomethyl)-2-naphthalenol[5] as hydrochloride salts were synthesized using previous methods.

Detection of Pyrrole

Reaction mixture containing **1** (30 μL of 50 mM solution), 0.2 M sodium phosphate dibasic (30 μL), water (140 μL), 0.5 M phosphate buffer, pH 7.4 (100 μL), acetonitrile (170 μL) and ONE (30 μL of 50 mM solution in acetonitrile) was mixed at 37 ºC. At various time points, aliquot of 50 μL was heated with 450 μL of water and 500 μL of Ehrlich reagent (80 mM 4-dimethylaminobenzaldehyde in 3:2 1 M HCl-methanol) at 65 ºC for 2 min, cooled, and uv spectrum in the range 610 to 500 nm was acquired.

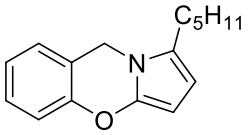
Synthesis of benzopyrrolooxazines

7-Chloro-1-pentyl-9*H*-benzo[*e*]pyrrolo[2,1-*b*][1,3]oxazine (**11Bc**)



ClSA.AcOH (66 mg, 0.3 mmol) was taken in acetonitrile (15 mL) and ONE (0.3 mL of 1 M solution) was added. The mixture was stirred at room temperature under Ar for 2 h and evaporated. The residue was purified by column chromatography (silica; 6:1 hexanes-ethyl acetate) to get a pale yellow solid; 45 mg (54%); MS (by flow injection of solution in 60/40 acetonitrile-water containing 5 mM formic acid on Quantum mass spectrometer in positive ion mode (+ESI)): *m/z* 278 and 276 ($M + H^+$), 183 ($M - C_4H_9 - Cl$); ^{1}H NMR (600 mHz) δ 0.85 (t, 3H, J = 7.2 Hz), 1.1.-1.5 (m, 6H), 2.50 (t, 2H, J = 7.8 Hz), 4.88 (s, 2H), 5.39 (d, 1H, J = 3.6 Hz), 5.74 (d, 1H, J = 3.6 Hz), 6.73 (d, 1H, J = 7.8 Hz), 6.94 (d, 1H, J = 7.8 Hz), 7.12 (m, 1H); ^{13}C NMR δ 13.8, 22.4, 25.9, 28.8, 31.5, 41.4, 85.0, 103.3, 118.0, 119.2, 119.3, 126.6, 126.7, 128.6, 128.8, 146.9.

1-Pentyl-9*H*-benzo[*e*]pyrrolo[2,1-*b*][1,3]oxazine (**11Ba**)[6]



Starting with the free base of SA and ONE, **11Ba** was similarly prepared and purified by column chromatography (silica; 9:1 hexanes-ethyl acetate); white solid, mp 60-61 ºC; MS: *m/z* 242.06 ($M + H^+$), 184 ($M - C_4H_9$); ^{1}H NMR δ 0.85 (t, 3H, *J* = 7.2 Hz), 1.1.-1.5 (m, 6H), 2.51 (t, 2H, *J* = 7.5 Hz), 4.92 (s, 2H), 5.39 (d, 1H, *J* = 3.6 Hz), 5.74 (d, 1H, *J* = 3.6 Hz), 6.99 (m, 2H), 7.10 (d, 1H, *J* = 7.2 Hz), 7.18 (m, 1H); ^{13}C NMR δ 14.1, 22.2, 25.6, 28.7, 31.5, 41.6, 84.6, 103.0, 116.6, 122.6, 127.0, 128.6.

Scavenging of ONE by SA

ONE (1 mM) was mixed with or without 3 mM SA in 3:2 phosphate buffer-acetonitrile at 37 ºC. ONE was separated by LC on a Kinetex 2.6 μm C8 column (2.1 × 75 mm) using isocratic elution (1:1 acetonitrile-water) and measured at 230 nm. The concentrations of ONE in the two solutions were compared.

Rates of Reaction

The reaction solution containing 3 mM **1**, 3 mM ONE, and 1.5 mM pentylpyridoxine (internal standard) in 3:2 phosphate buffer (pH 7.4)-acetonitrile was incubated at 37 ºC. At various time points, including at the start of the reaction, an aliquot of 10 μL was diluted to 100 μL of solvent A and analyzed by Shimadzu liquid chromatograph 10A. The column (Polarity C18, 4.6 × 150 mm, 3 μm) was eluted at 0.7 mL/min. Solvent system A (20% acetonitrile in 5 mM formic acid) was held for 1 min, changed linearly to solvent system B (100% acetonitrile containing 5 mM formic acid) in 10 min, held at system B for 6 min, brought to 100% A in 1 min and held for 6 min before the next injection. The peak areas of PM and PM derivative at 325 nm and the peak areas of SA, EtSA, ClSA, 1-(aminomethyl)-2-naphthalenol, and the internal standard at 280 nm were measured. From the ratios of the reactant to the internal standard the concentration of **1** was obtained for calculating rate constants.

Competing Experiments

When EtSA was treated with ONE (3 mM each) in 3:2 phosphate buffer (pH 7.4)-acetonitrile, 51% of EtSA was found to have reacted in 1 h at 37 °C. Including 3 mM *N*-α-acetyllysine did not reduce the yield of the reaction. The inability of lysine to compete effectively with EtSA in reacting with ONE was also shown in another experiment. Under the same reaction conditions described above, ONE was treated with equivalent amount of *N*-acetylcysteine before adding EtSA. Again, whether *N*-α-acetyllysine was present or not, within 5 min 78% of EtSA reacted with the Michael adduct of *N*-acetylcysteine on ONE to form pyrroles.

Reaction with HNE

The reaction mixture given for measuring the rate of reaction was altered by having HNE instead of ONE. The samples were analyzed by LC using the same conditions given above.

References

(1) Kurangi, R., Tilve, S. G., and Blair, I. A. (2006) Convenient and efficient syntheses of 4-hydroxy-2(*E*)-nonenal and 4-oxo-2(*E*)-nonenal. *Lipids 41*, 877-880.

(2) Zhang, W., Liu, J., Xu, G., Yuan, Q., and Sayre, L. M. (2003) Model studies on protein side chain modification by 4-oxo-2-nonenal. *Chem. Res. Toxicol. 16*, 512-523.

(3) Zagol-Ikapitte,I., Amarnath, V., Bala, M., Roberts, L. J., Oates, J. A., and Boutaud, O. (2010) Characterization of scavengers of γ-ketoaldehydes that do not inhibit prostaglandin biosynthesis. *Chem. Res.Toxicol. 23*, 240-250.

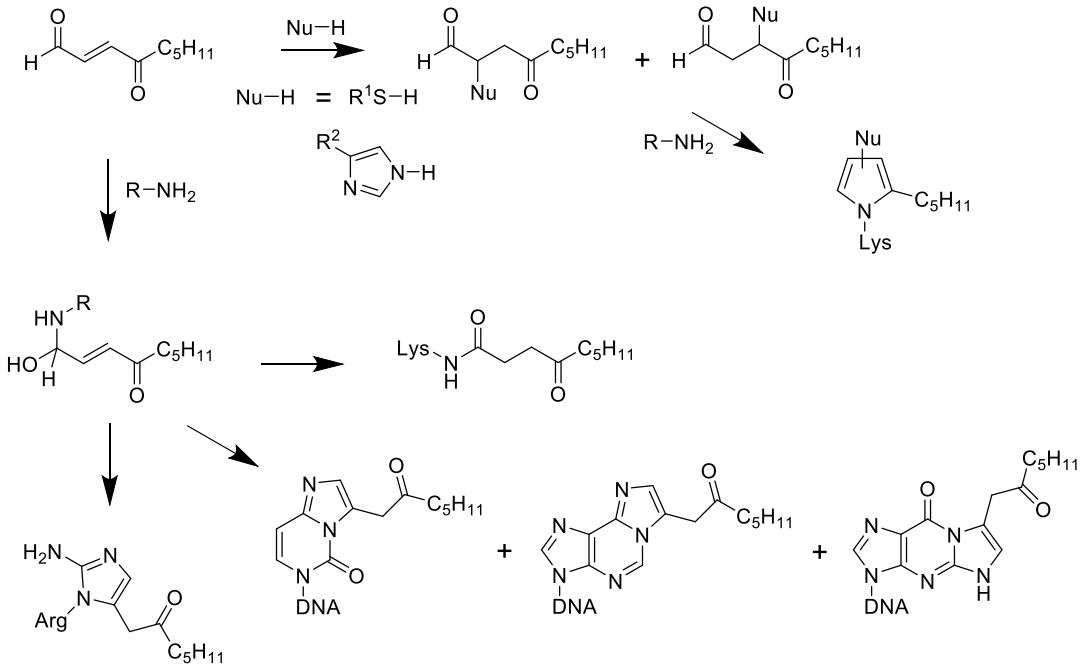
(4) Amarnath, V., Amarnath, K., Avance, J., Stec, D. F., and Voziyan, P. (2015) 5'-*O*-Alkylpyridoxamines: lipophilic analogs of pyridoxamine are potent scavengers of 1,2-dicarbonyls. *Chem. Res.Toxicol. 28*, 1469-1475.

(5) 4-Hydroxybenzaldehyde was converted to the oxime ($NH_2OH.HCl$ and CH_3CO_2Na in ethanol) and reduced with zinc powder and acetic acid to the amine. In a similar way, 1-

(aminomethyl)-2-naphtnalenol was prepared from 2-hydroxynaphthalene-1-carboxaldehyde.

(6) Yadav, V. D., Dighe, S. U., and Batra, S. (2014) Synthesis of *N*-alkyl pyrroles *via* decarboxylation/dehydration in neutral ionic liquid under catalyst-free conditions. *RSC Adv. 4*, 57587-57590.

Scheme S1. Reactions of ONE and HNE in biological systems.



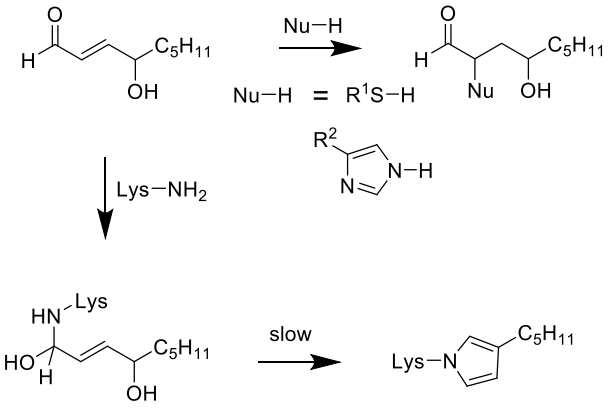


Figure S1. Detection of pyrrole in the reactions of 2-aminomethylphenols with ONE

The level of pyrrole was measured by the absorbance (at 532 nm for SA, PM, and 4-HOBA and at 570 nm for 1-(aminomethyl)naphthalenol) of its adduct with Ehrlich reagent: SA (black square), PM (black triangle), 1-(aminomethyl)-2-naphthalenol (black diamond) or 4-HOBA (black circle).

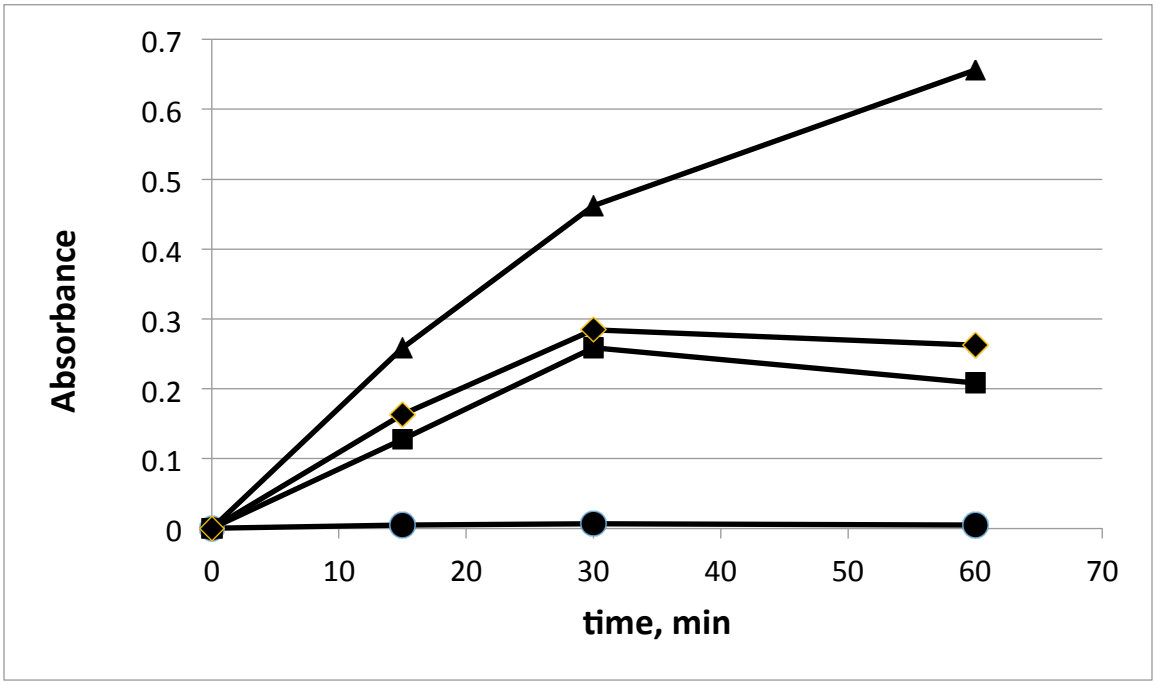


Figure S2. The chromatogram of Michael adduct of *N*-acetylcysteine on ONE treated with PnPM.

ONE and AcCySH (3 mM each) were mixed for 5 min and equimolar PnPM was added. The chromatogram was obtained after 15 min. The peak at 4.8 min is unreacted PnPM and the one at 11.5 min is the internal standard pentylpyridoxine. The peaks at 14.0 and 14.5 min are obtained after the addition of PnPM to the Michael adduct and are ascribed to the resulting pyrroles.

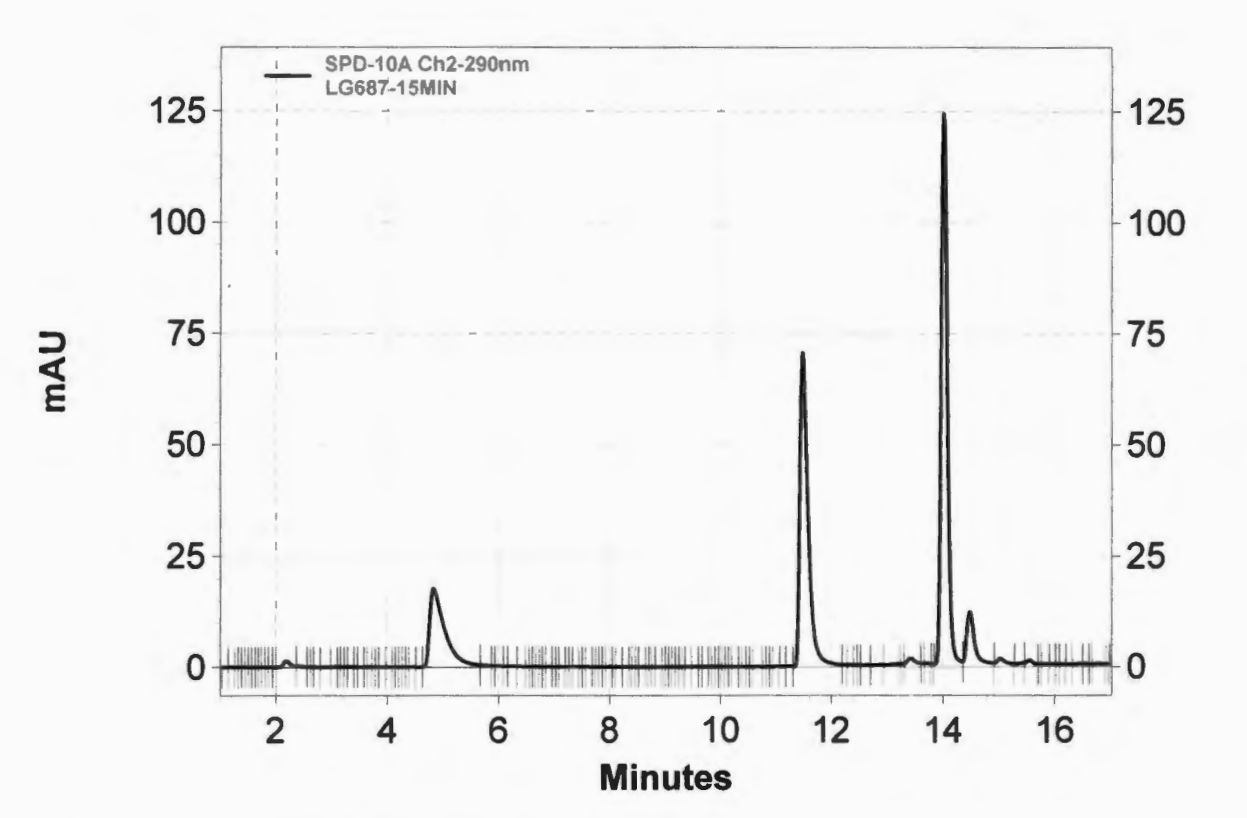
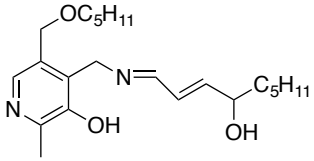


Figure S3. The chromatogram of the reaction mixture containing PnPM with HNE.

The chromatogram obtained after mixing at 37 ºC for 2 h exhibited a peak at 4.75 min for PnPM and peaks at 12.50 and 12.75 min. The reaction mixture was extracted with ethyl acetate. The extract was concentrated, purified by column chromatography (20% methanol-ethyl acetate), and the MS of the product (*m/z* 377 ($M + H^+$), 289 ($M - C_5H_{11}O$)) suggested the Schiff base as its structure.



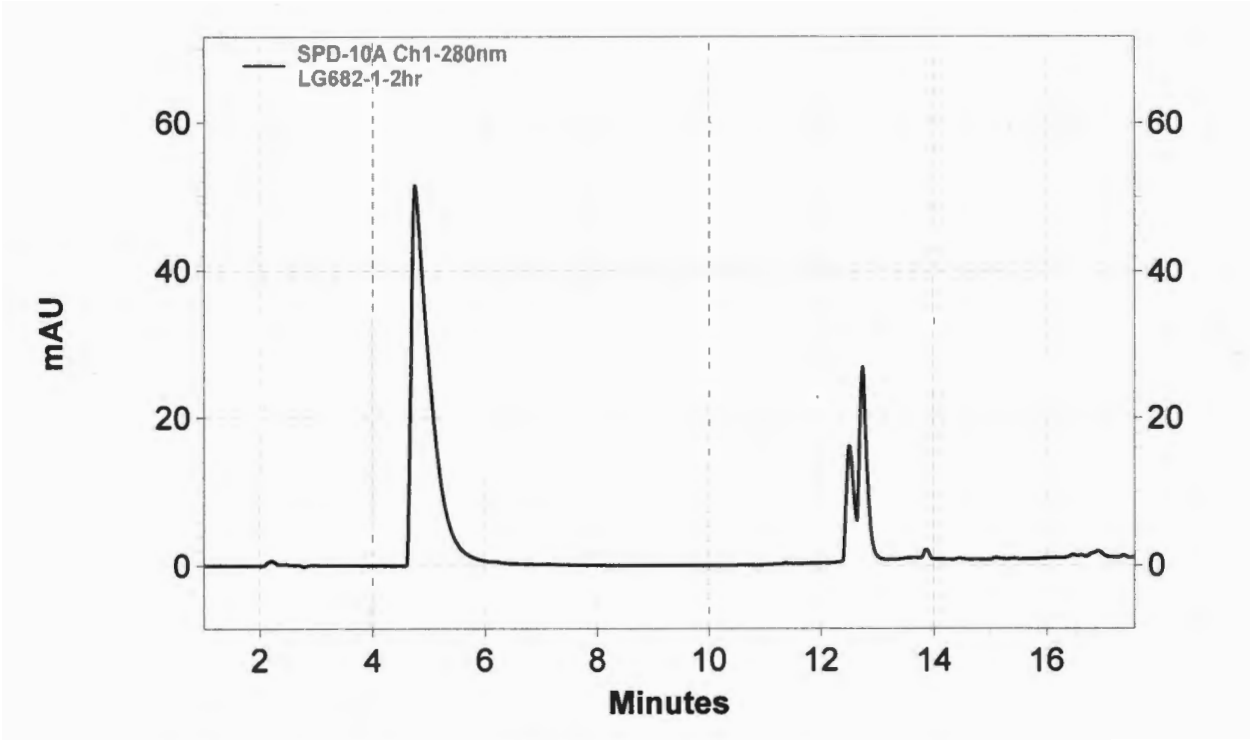
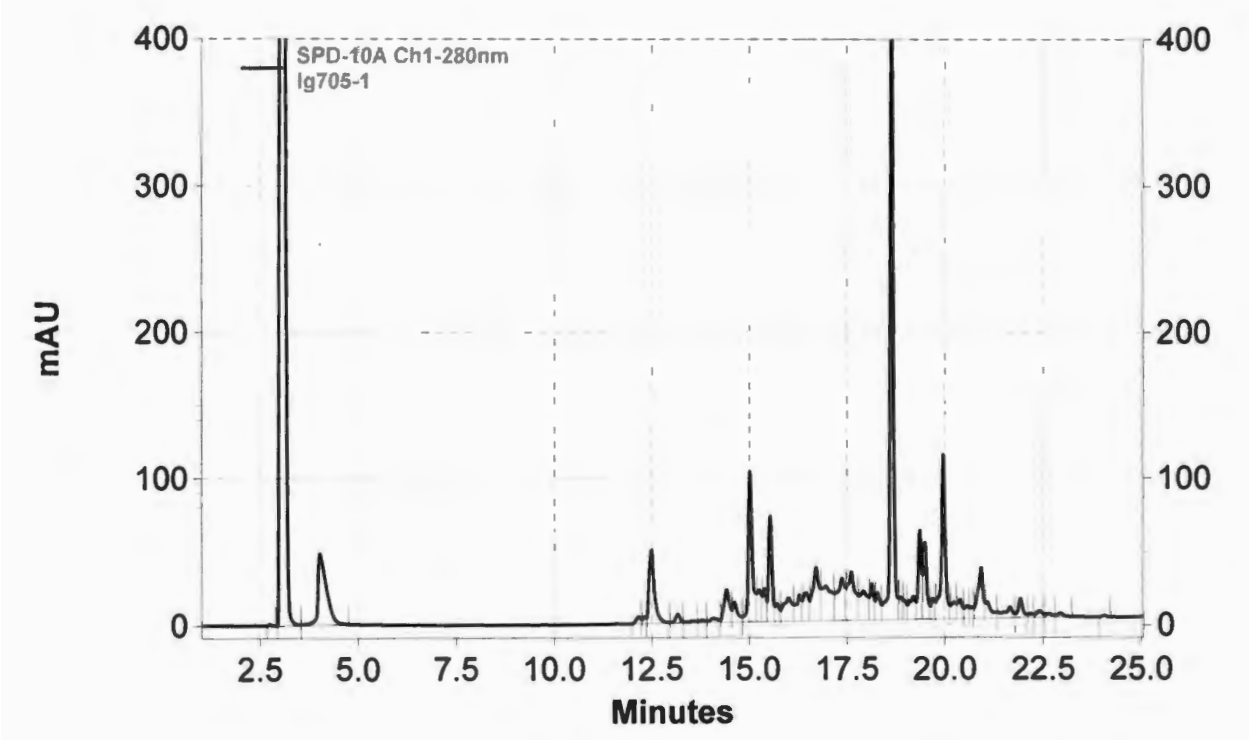
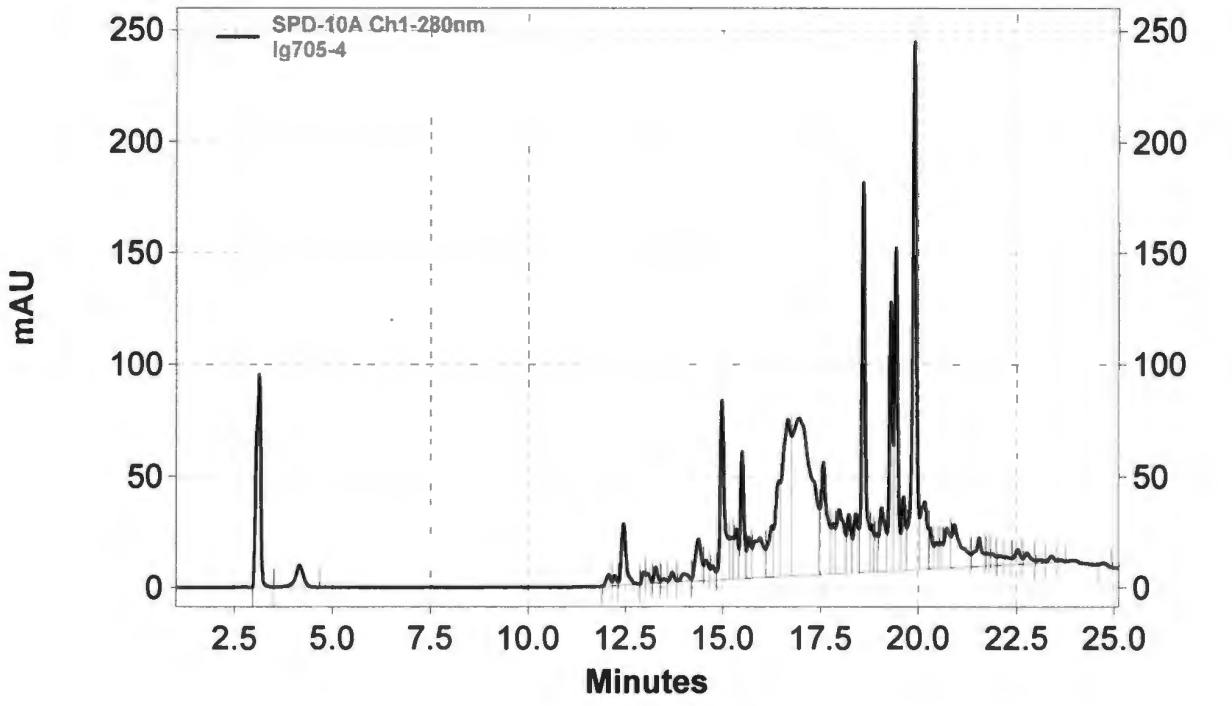


Figure S4. The progression of the reaction between ONE and ClSA.

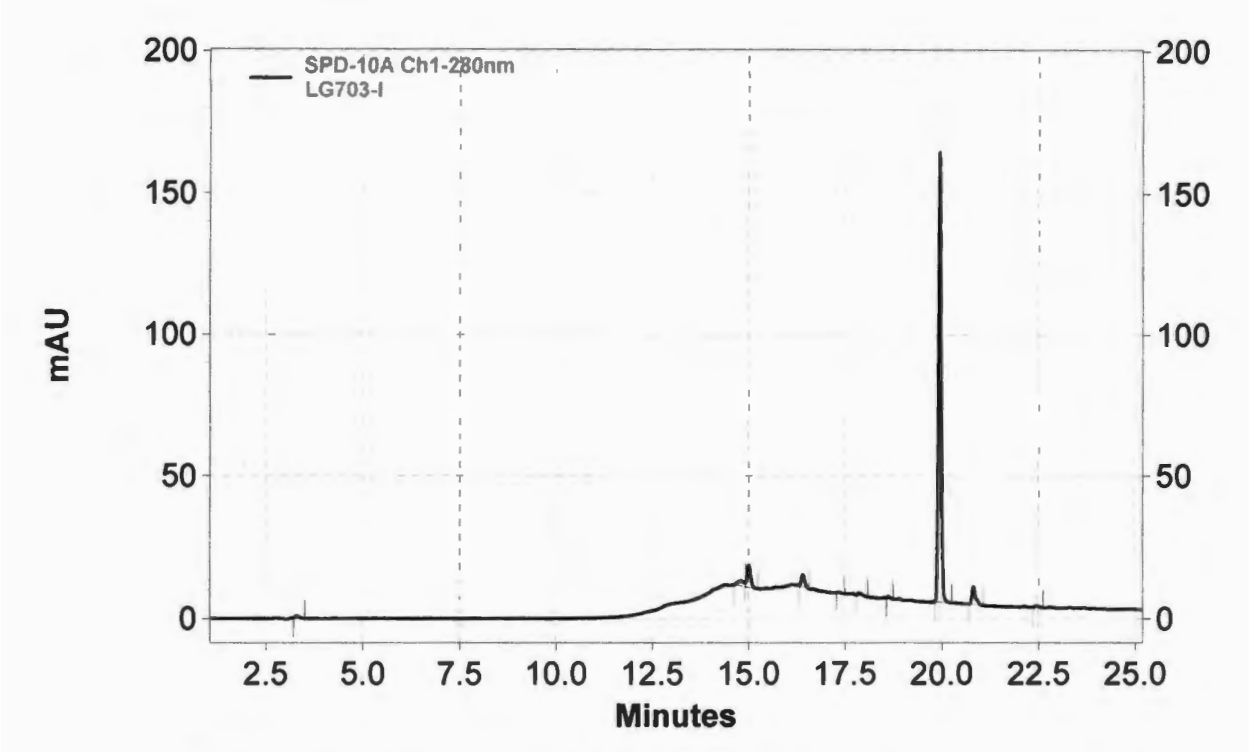
A



B



C



ClSA (5 mM) and ONE (5 mM) in phosphate buffer (pH 7.4) and acetonitrile (7:3, 0.5 mL) were mixed at 37 ºC. After 10, 30, 50, and 80 min the reaction solution was extracted with ice-cold ethyl acetate (200 μL). The extract was evaporated under a stream of N_2 and the residue was dissolved in 150 μL of 1:1 5 mM formic acid-acetonitrile for LC analysis. After 10 min of reaction, the major peak (A) was at 18.63 min. The compound corresponding to this peak was isolated in a separate larger scale experiment (column purification with 3:1 hexane-ethyl acetate); MS *m/z* 294 and 296 ($M + H^+$), 277 ($M - H_2O$); ^{1}H NMR indicated a mixture of products; the absence of benzylic protons at around δ 2.6 ppm and the presence of two singlets at δ 3.64 and 3.79 ppm suggested that imine **7** had cyclized to **8**. As time progressed, the peak at 19.95 min grew at the expense of the peak at 18.63 min and after 80 min, it was the highest peak (B). It was identified as 7-chloro-1-pentyl-9*H*-benzo[*e*]pyrrolo[2,1-*b*][1,3]oxazine (**11Bc**) by comparison with the chromatogram (C) of the sample characterized by MS and NMR.