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Supporting Information

Far-Red Fluorogenic Probes for Esterase and Lipase Detection

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Supporting Information

Materials and Methods

Unless otherwise stated, reactions were magnetically stirred in oven-dried glassware under argon atmosphere. Anhydrous solvents were purchased in sure-seal bottles and kept under argon atmosphere. All chemicals were purchased from Sigma-Aldrich or Fisher Scientific and used as received unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) using glass-backed silica gel plates. Flash column chromatography was performed with the indicated solvents using Silicycle SiliaFlash P60 as the stationary phase. Mass spectra were acquired on an electrospray ionization (ESI) spectrometer and obtained by peak matching.

^{1}H-NMR data were obtained at ambient temperature at 400 MHz in $CDCl_3$. ^{13}C-NMR data were obtained at 101 MHz in $CDCl_3$. Chemical shifts are reported as δ values in ppm and were calibrated according to the tetramethylsilane (TMS) peak. Coupling constants (*J*) are reported in Hertz (Hz) and are rounded to the nearest 0.1 Hz. Multiplicities are defined as: s = singlet, d = doublet, m = multiplet, dd = doublet of doublets.

Synthesis of DDAO-AME 1 and 2

DDAO (Synchem OHG, 20 mg, 0.065 mmol), powdered 4-Å molecular sieves (60 mg), and silver(I) oxide (38 mg, 0.16 mmol, 2.5 equiv) were suspended in anhydrous acetonitrile (1.5 mL). Bromomethyl acetate (8 µL, 0.08 mmol, 1.2 equiv) was added, and the mixture was stirred for 24 h at room temperature. The reaction mixture was diluted with dichloromethane (CH_2Cl_2), filtered through a plug of Celite® S, and concentrated by rotary evaporation to give an orange solid. Purification by silica gel flash chromatography (5% ethyl acetate/40% CH_2Cl_2/55% hexanes) yielded two products: one bright orange and UV active (DDAO-AME 1, R_f = 0.26; 14 mg, 57% yield), the other yellow and not UV active (DDAO-AME 2, R_f = 0.14; 6.4 mg, 26% yield). NOESY was used to assign the two regioisomers (Figures S1-S2).

DDAO-AME 1 (Figures S11-S12)
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.65 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.14 (d, *J* = 2.7 Hz, 1H), 7.08 (dd, *J* = 8.7, 2.7 Hz, 1H), 5.86 (s, 2H), 2.17 (s, 3H), 1.89 (s, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 173.2, 169.7, 159.6, 148.5, 140.5, 139.4, 137.0, 136.8, 135.0, 133.9, 114.7, 114.6, 84.5, 39.1, 26.7, 20.9.
ESI-MS $[M+H]^+$ m/z calculated for $C_{18}H_{16}Cl_2NO_4$: 380.0456, found, 380.0459.

DDAO-AME 2 (Figures S13-S14)
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.78 (s, 1H), 7.37 (d, *J* = 10.1 Hz, 1H), 6.70 (m, 2H), 5.80 (s, 2H), 2.16 (s, 3H), 1.81 (s, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 187.3, 170.0, 153.0, 151.7, 148.2, 140.9, 140.5, 133.3, 132.6, 132.3, 129.5, 128.8, 127.9, 88.0, 38.1, 28.8, 20.9.
ESI-MS $[M+H]^+$ m/z calculated for $C_{18}H_{16}Cl_2NO_4$: 380.0456; found, 380.0458.

Synthesis of Res-AME

Res-AME was synthesized on a 100 mg scale according to the published protocol.[1] Following extraction, Res-AME was purified by silica gel flash chromatography (0-10% MeOH in CH_2Cl_2). The product was isolated as a dark yellow solid (53 mg, 40% yield).

Res-AME (Figures S15-S16)
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, *J* = 8.8 Hz, 1H), 7.43 (d, *J* = 9.8 Hz, 1H), 7.05 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.00 (d, *J* = 2.6 Hz, 1H), 6.85 (dd, *J* = 9.8, 2.1 Hz, 1H), 6.34 (d, *J* = 2.0 Hz, 1H), 5.84 (s, 2H), 2.16 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 186.3, 169.6, 160.1, 149.6, 146.7, 145.3, 134.8, 134.6, 131.7, 129.3, 114.3, 107.0, 102.6, 84.6, 20.9.
ESI-MS $[M+H]^+$ m/z calculated for $C_{15}H_{12}NO_5$: 286.0715; found, 286.0714.

pK_a Determination
The pK_a's of DDAO and resorufin were determined by measuring their fluorescence over a range of pH's. The following buffers were prepared at room temperature at the indicated pH: citric acid-disodium phosphate (pH 2.8, 2.9, 3.1, 3.4, 3.8, 4.4, 4.9, 5.3, 5.9, 6.1, 6.4); potassium phosphate (pH 4.7, 5.6, 6.6, 7.2, 7.6); Tris (pH 7.8, 8.0, 8.1, 8.6, 8.8); and sodium carbonate-sodium bicarbonate (pH 9.2, 9.6, 10.3). DDAO (5 mM in DMSO) and resorufin (20 mM in 0.1 N NaOH) were diluted to 10 µM in H_2O. Fluorophores were then further diluted to 1 µM in buffer at each pH (*n* = 3 for each pH). Fluorescence was assessed on a Tecan Infinite M200 Pro microplate reader (DDAO: Ex 635, Em 670; resorufin: Ex 550, Em 600). Data were plotted and fit to a sigmoidal curve. The pK_a was determined as the inflection point of the curve (Figure S3).

Spectral characterization
The spectral properties of DDAO-AME 1, DDAO-AME 2, and Res-AME are summarized in Table S1. Absorbance and emission spectra were acquired on a Tecan Infinite M200 Pro microplate reader in fluorescence-grade quartz cuvettes for absorbance reads (2 nm step size) and in black 96-well plates for fluorescence reads (10 reads per well; 2 nm step size). Compounds were diluted to 1 µM in 10 mM HEPES (pH 7.3).

The extinction coefficient was determined for DDAO, resorufin, DDAO-AME 1, DDAO-AME 2, and Res-AME. Three to four separate stock solutions were prepared in DMSO (5 mM for DDAO, DDAO-AME 1, DDAO-AME 2, and Res-AME) or 0.1 N NaOH (20 mM resorufin). Experiments were performed in a 96-well plate format using a pathlength correction based on the absorbance of water: sample pathlength (cm) = ($Abs977_{water}$ – $Abs900_{water}$)/0.182, where 0.182 = ($Abs977_{water}$ – $Abs900_{water}$) in a 1 cm cuvette. The absorbance at λ_{max} was then divided by the sample pathlength for the pathlength-corrected absorbance (i.e., the absorbance for a 1 cm pathlength). Molar extinction coefficients were calculated as the slope of the absorbance vs. probe concentration using Beer's Law ($A=\varepsilon cl$; A = absorbance, ε = extinction coefficient, c = concentration, l = pathlength). Only pathlength-corrected absorbance values between 0.1 and 1 were used for these calculations.

The relative quantum yields[2] of DDAO-AME 1 and Res-AME were determined using 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran (DCM, Exciton; ϕ = 0.44 in EtOH) as the reference standard. Each sample was excited at 450 nm, and the full fluorescence emission curve was obtained on a Tecan Infinite M200Pro microplate reader. DDAO-AME 1 and Res-AME were evaluated in both DMSO and in 10 mM HEPES (pH 7.3) buffer. DDAO-AME 2 was not evaluated, as it displayed no detectable fluorescence.

Aqueous stability
DDAO-AME 1, DDAO-AME 2, Res-AME, and FDA were diluted to 1 µM in PBS (pH 7.4), DMEM-FBS [Dulbecco's Modified Eagle Medium (Gibco®) supplemented with 10% fetal bovine serum (HyClone™)], or DMEM-FBS (h.i.) (heat-inactivated serum; heated at 56 °C for 40 min). Samples, prepared in triplicate, were incubated at 37 °C, and fluorescence was measured every 5 min for 15 h on a Molecular Devices SpectraMax M5 microplate reader (DDAO: Ex 635 nm, Em 670 nm; resorufin: Ex 550 nm, Em 600 nm; fluorescein: Ex 490, Em 525). Stability curves are shown in

Figure S4. Since samples were completely hydrolyzed within the 15 h time course in DMEM-FBS and DMEM-FBS (h.i.), curves were fit to a one phase decay equation ($Y = [Y_0 – Plateau]*exp(-K*X) + Plateau$, where Y = fluorescence, K = the rate constant, X = time, and the half-life is calculated as ln(2)/K) in GraphPad Prism 6. Since the probes did not completely hydrolyze in PBS, each probe was also diluted into PBS containing 1 µg PLE and allowed to react for the duration of the experiment to obtain the fluorescence value (plateau) of fully hydrolyzed probe. The one phase decay equation was constrained with this value in order to calculate the probe half-life in PBS. Calculated half-lives are reported in Table S2.

Esterase screen

Esterases and lipases were purchased from Sigma-Aldrich. Lipases were purchased as Lipase Basic Kit (product number 62327). The enzymes evaluated are listed in Table S3. Enzymes were prepared as 10 mg/mL stocks and diluted to 5 µg/mL in 10 mM HEPES buffer (pH 7.3). Heat-inactivated PLE was prepared by incubating PLE at 90 °C for 15 min. Alternatively, PLE activity was inhibited by incubation with 50 µM E-600 (Sigma-Aldrich, 36186) at 37 °C for 60 min prior to probe addition. The reactions were initiated with 5 µM DDAO-AME 1, DDAO-AME 2, Res-AME, or FDA. The reactions were incubated at 37 °C before reading on a Tecan Infinite M200Pro microplate reader [fluorescein: Ex 485 nm, Em 530 nm; resorufin: Ex 550 nm, Em 600 nm; DDAO: Ex 635 nm, Em 670 nm] at 10 min (Figure 2).

PLE detection limit

PLE was diluted in 10 mM HEPES (pH 7.3) so that the final amount in each reaction mixture ranged from 0.275 pg to 550 pg for DDAO-AME 1, DDAO-AME 2, Res-AME, and FDA. A larger range, from 1.1 pg to 2750 pg, was used for *p*-NPA owing to the lower sensitivity for detecting *p*-NP absorbance (Figure S5). The reactions were initiated by the addition of substrate at a final concentration of 25 µM for DDAO-AME 1, DDAO-AME 2, Res-AME, and FDA, or 1700 µM for *p*-NPA and incubated at 37 °C. Fluorescence (DDAO, resorufin, and fluorescein) or absorbance (*p*-NP) was measured on a Tecan Infinite M200Pro microplate reader (DDAO: Ex 635 nm, Em 670 nm; resorufin: Ex 550 nm, Em 600 nm; fluorescein: Ex 490 nm, Em 525 nm; *p*-NP: Abs 348 nm).

Kinetics

All kinetic assays were performed in triplicate or sextuplicate in 10 mM HEPES buffer (pH 7.3) at 37 °C. Probe solutions were pre-incubated at 37 °C for 15 min prior to enzyme addition. For each enzyme and probe pair, the amount of enzyme used was optimized to allow an accurate measurement of the initial rate. After enzyme addition, fluorescence was measured every 20 or 30 s on a Molecular Devices SpectraMax M5 microplate reader. Kinetic parameters were calculated using GraphPad Prism 6 software with the method of initial rates.[3] Data were fit to a Michaelis-Menten enzyme kinetics curve, $V = V_{max}[S]/(K_M + [S]$, where V is the reaction rate and S is the substrate concentration.

Porcine liver esterase was evaluated with DDAO-AME 1 and Res-AME at a final concentration of 500 ng/mL enzyme with DDAO-AME 1 and a final concentration of 50 ng/mL enzyme for Res-AME (Figure S6). DDAO-AME 1 was used at a concentration range of 3 to 80 µM, and enzyme-catalyzed hydrolysis was measured by detecting DDAO formation (Ex 635 nm, Em 670 nm). Res-AME was used at a concentration range of 0.4 and 40 µM, and hydrolysis was measured by detecting resorufin formation (Ex 550 nm, Em 600 nm).

B. subtilis esterase was evaluated with DDAO-AME 1 and Res-AME at a final concentration of 500 ng/mL enzyme (Figure S7). DDAO-AME 1 was used at a concentration range of 0.6 to 80 µM, and hydrolysis was measured by detecting DDAO formation (Ex 635 nm, Em 670 nm). Res-AME was

used at a concentration range of 0.4 to 40 μM, and hydrolysis was measured by detecting resorufin formation (Ex 550 nm, Em 600 nm).

C. antarctica lipase was evaluated with DDAO-AME 1 and Res-AME at a final concentration of 10 μg/mL enzyme (Figure S8). DDAO-AME 1 was used at a concentration range of 0.6 to 80 μM, and hydrolysis was measured by detecting DDAO formation (Ex 635 nm, Em 670 nm). Res-AME was used at a concentration range of 0.6 to 80 μM, and hydrolysis was measured by detecting resorufin formation (Ex 550 nm, Em 600 nm).

M. miehei lipase was evaluated with DDAO-AME 1 and Res-AME at a final concentration of 10 μg/mL enzyme for DDAO-AME 1 and 20 μg/mL enzyme for Res-AME (Figure S9). DDAO-AME 1 was used at a concentration range of 0.6 to 80 μM, and hydrolysis was measured by detecting DDAO formation (Ex 635 nm, Em 670 nm). Res-AME was used at a concentration range of 0.6 to 80 μM, and hydrolysis was measured by detecting resorufin formation (Ex 550 nm, Em 600 nm).

Mycobacterial lysate screen

Mycobacteria were cultured, harvested, and lysed as previously described.[4] Each species was handled using appropriate biosafety level 2 or 3 precautions. The species analyzed included *M. smegmatis*, *M. marinum*, *M. flavescens*, *M. nonchromogenicum*, *M. kansasii*, *M. avium*, *M. intracellulare*, *M. bovis* (BCG), *M. africanum*, and three strains of *M. tuberculosis* (Erdman, H37Rv, and CDC1551). Lysates (1 μg of total protein) were evaluated with 5 μM of DDAO-AME 1, DDAO-AME 2, or Res-AME in triplicate in 10 mM HEPES (pH 7.3). Reactions were incubated at 37 °C for 10 min, and hydrolyzed probe fluorescence was measured on a Tecan Infinite M200Pro microplate reader (DDAO: Ex 635 nm, Em 670 nm; resorufin: Ex 550 nm, Em 600 nm). After 10 min, each species displayed significant esterase activity (Figure S10).

In-gel activity assay

Lysates (1 to 8 μg of total protein per lane, adjusted approximately to normalize according to band brightness) were resolved by native gel electrophoresis (10-20% Tris-HCl Criterion gel, Bio-Rad). Gels were run on ice for 95 min at 200 V in 1X Tris-Glycine buffer (Bio-Rad) prepared in deionized water without methanol. NativeMark Unstained Protein Standard (Life Technologies) was included, although it is difficult to accurately estimate molecular weight on a native protein gel. Gels were incubated in 10 mM HEPES (pH 7.3) containing 1-5 μM fluorogenic probe for 5 min before imaging on a fluorescence scanner (Typhoon 9410 Variable Mode Imager, GE Healthcare). DDAO-AME 1 and 2 were evaluated at 1 μM instead of 5 μM due to optimal signal-to-noise at this concentration. DDAO was detected using 633 nm excitation and a 670 nm (bp 30) emission filter. Resorufin was detected using 532 nm excitation and a 580 nm (bp 30) emission filter. The resulting images were analyzed in ImageJ.[6]

Supplemental Figures and Tables

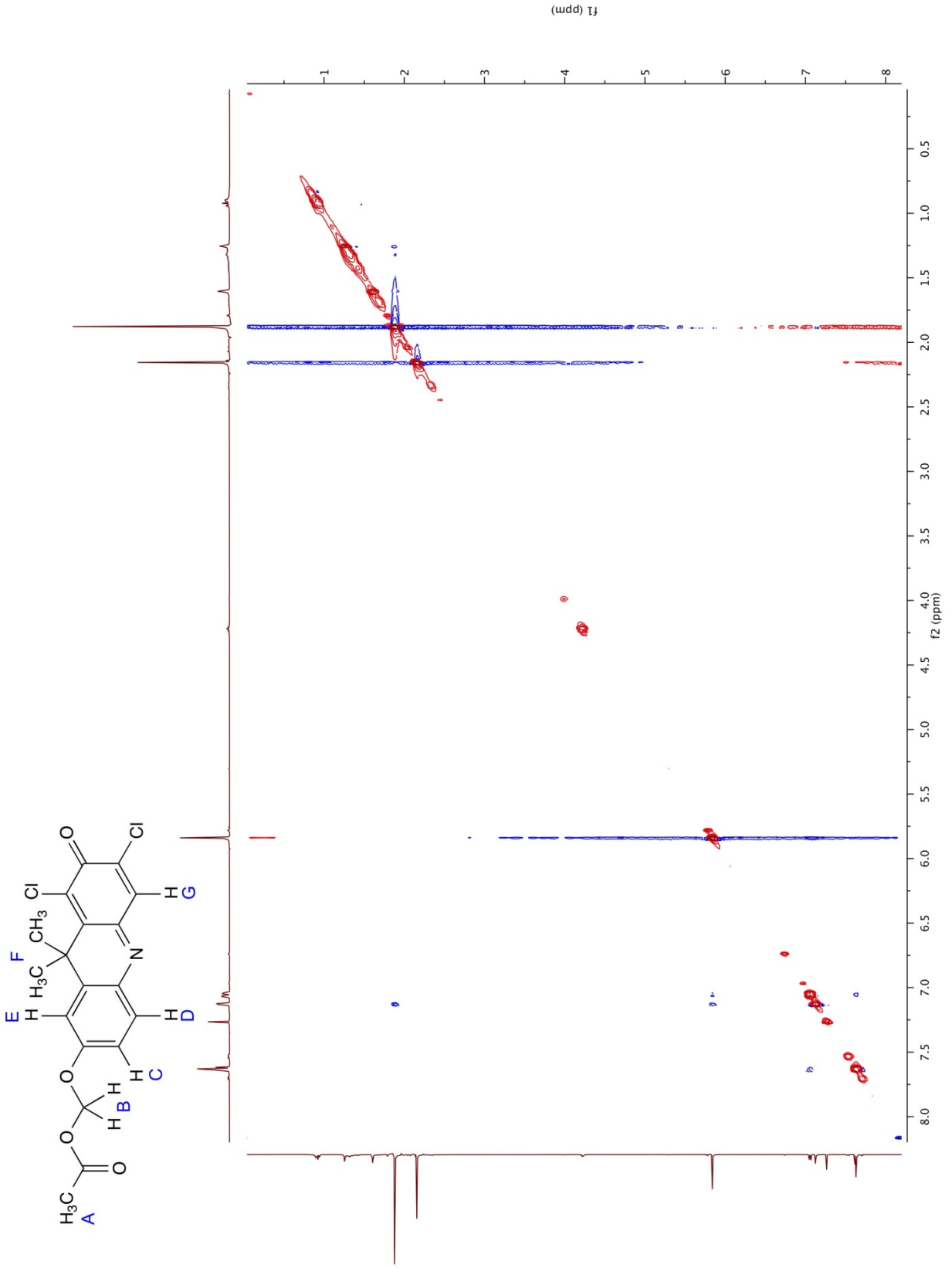

Figure S1. NOESY spectrum of DDAO-AME 1. The methylene protons (H_B) of the AM ether moiety (s, 2H, 5.86 ppm) exhibit Nuclear Overhauser effects (NOEs) with aromatic protons H_E (d, 1H, 7.14 pm) and H_C (dd, 1H, 7.08 ppm), showing that O-alkylation occurred on the side of the molecule opposite the chlorides.

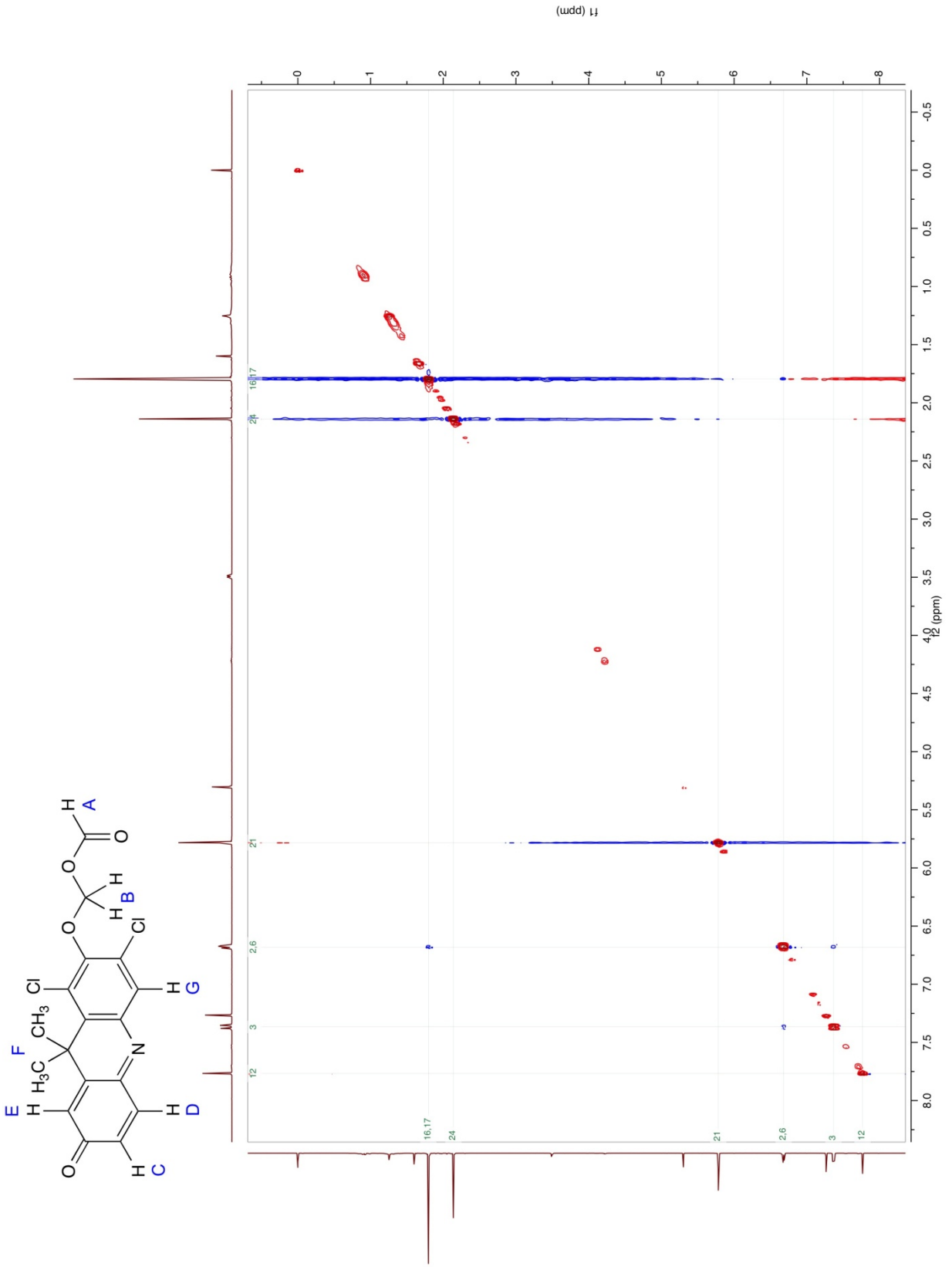

Figure S2. NOESY spectrum of DDAO-AME 2. The methylene protons (H_B) of the AM ether moiety (s, 2H, 5.80 ppm) exhibit no NOEs, indicating that the AM ether group is between the two chlorides.

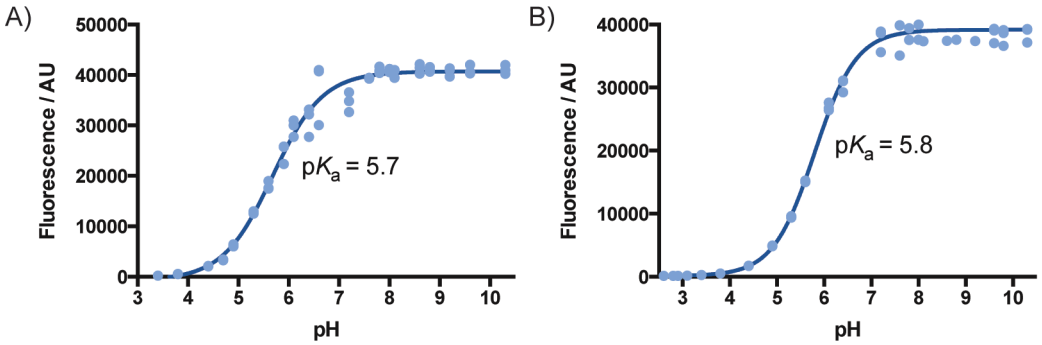

Figure S3. Determination of the pK_a of A) 1 μM DDAO and B) 1 μM resorufin. The fluorescence of DDAO (Ex 635, Em 670) and resorufin (Ex 550, Em 600) was measured in triplicate in buffers at various pH's (ranging from 2.8 to 10.3) and is reported in arbitrary units (AU). Data were fit to a sigmoidal curve and the pK_a of each fluorophore was determined as the inflection point of the curve.

Table S1. Spectral characteristics of fluorogenic probes and parent fluorophores.

Compound	$\lambda_{abs/em}$ (nm)[a]	pK_a	ε ($M^{-1}cm^{-1}$)	ϕ (Solvent)
DDAO	646/660	5.7	33000[b]	0.40 (PBS pH 7.4)[4]
DDAO-AME 1	465/625	—	1400[a]	0.07 (10 mM HEPES pH 7.3) 0.02 (DMSO)
DDAO-AME 2	395/—	—	2300[a]	—
Resorufin	571/588	5.8	57000[c]	0.74 (pH 9.5 buffer)[5]
Res-AME	475/605	—	4100[a]	0.01 (10 mM HEPES pH 7.3) 0.001 (DMSO)

[a] in 10 mM HEPES, pH 7.3 [b] in 0.1 N NaOH [c] in PBS, pH 7.4 [d] in DMSO

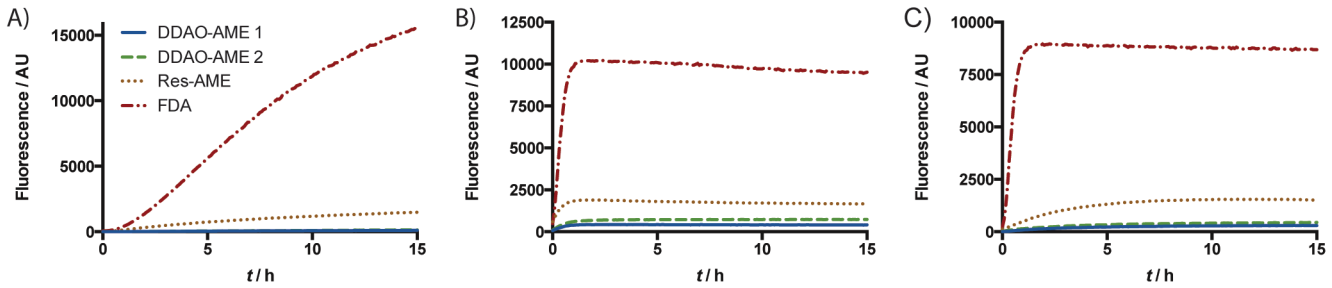

Figure S4. Stability of esterase probes in A) PBS (pH 7.4), B) DMEM-FBS and C) DMEM-FBS (h.i.). Each probe (DDAO-AME 1, DDAO-AME 2, Res-AME, and FDA) was diluted to 25 μM, and parent fluorophore fluorescence (in arbitrary units, AU) was measured every 15 min for 15 h (DDAO: Ex 635, Em 670; resorufin: Ex 550, Em 600; fluorescein: Ex 490, Em 525). Each line represents the average of three replicates. In C, serum was heat-inactivated (h.i.) for 40 min at 56 °C before preparation of the media [DMEM-FBS (h.i.)].

Table S2. Half-life [h] for the hydrolysis of each probe in different buffer conditions.			
Probe	**PBS (pH 7.4)**	**DMEM-FBS**	**DMEM-FBS (heat-inactivated)**
DDAO-AME 1	27	0.3	2.6
DDAO-AME 2	41	0.4	3.1
Res-AME	13	0.3	2.0
FDA	8	0.3	0.3

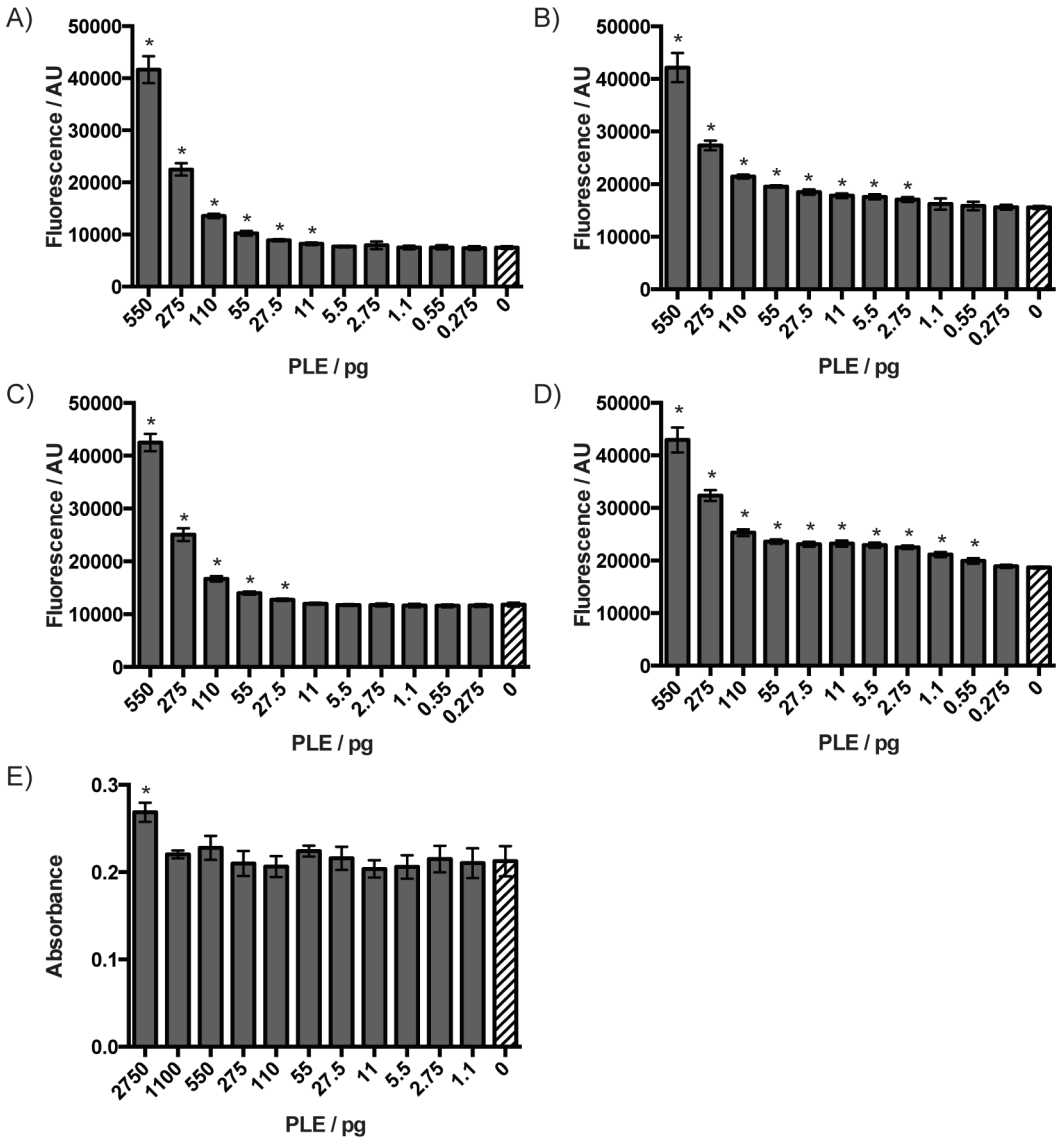

Figure S5. The PLE detection limit after a 10 min incubation with fluorogenic or chromogenic probes. A) DDAO-AME 1 (25 µM). B) DDAO-AME 2 (25 µM). C) Res-AME (25 µM). D) FDA (25 µM). E) *p*-NPA (1700 µM). Enzyme was diluted in 10 mM HEPES (pH 7.3) and incubated with probe for 10 min at 37 °C. The formation of hydrolyzed product was detected by measuring the fluorescence of DDAO (Ex 635 nm, Em 670 nm), resorufin (Ex 550 nm, Em 600 nm), or fluorescein (Ex 490 nm, Em 525 nm). Alternatively, *p*-NPA hydrolysis was detected by measuring the absorbance of *p*-nitrophenol at 348 nm. The detection limit was determined as the lowest statistically significant detectable amount compared to the no enzyme control using an unpaired, two-tailed t test with Welch's correction (*$P < 0.01$). Error bars represent one standard deviation; $n = 6$. Fluorescence is shown in arbitrary units (AU).

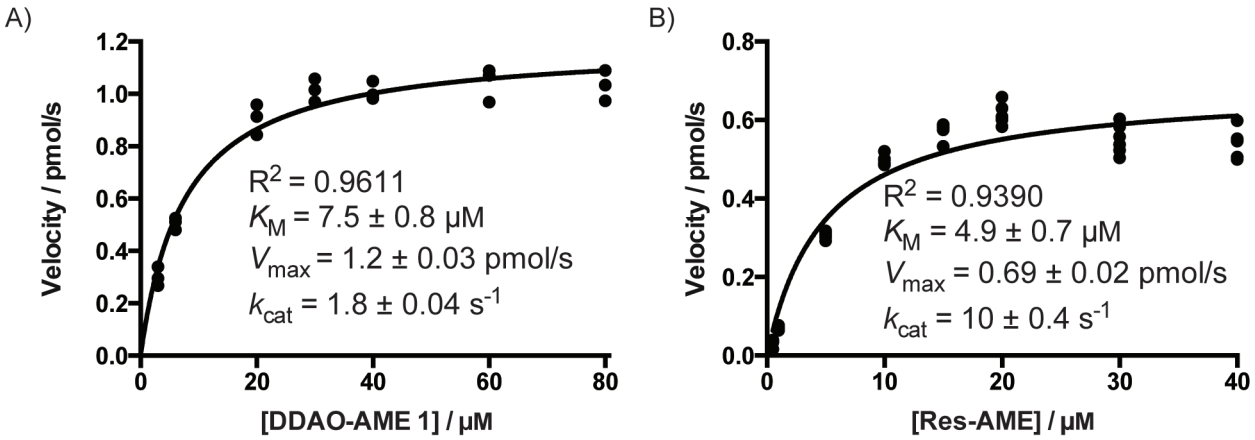

Figure S6. Kinetic evaluation of DDAO-AME 1 and Res-AME with PLE. A) DDAO-AME 1 (ranging from 3-80 µM) was evaluated with 500 ng/mL PLE in triplicate. DDAO: Ex 635, Em 670. B) Res-AME (ranging from 0.5-40 µM) was evaluated with 50 ng/mL PLE in sextuplicate. Resorufin: Ex 550, Em 600. Experiments were performed in 10 mM HEPES buffer (pH 7.3) at 37 °C. Fluorescence generation was measured every 20 or 30 s. All data were fit to a Michaelis-Menten enzyme kinetics curve, and did not fit a substrate inhibition curve.

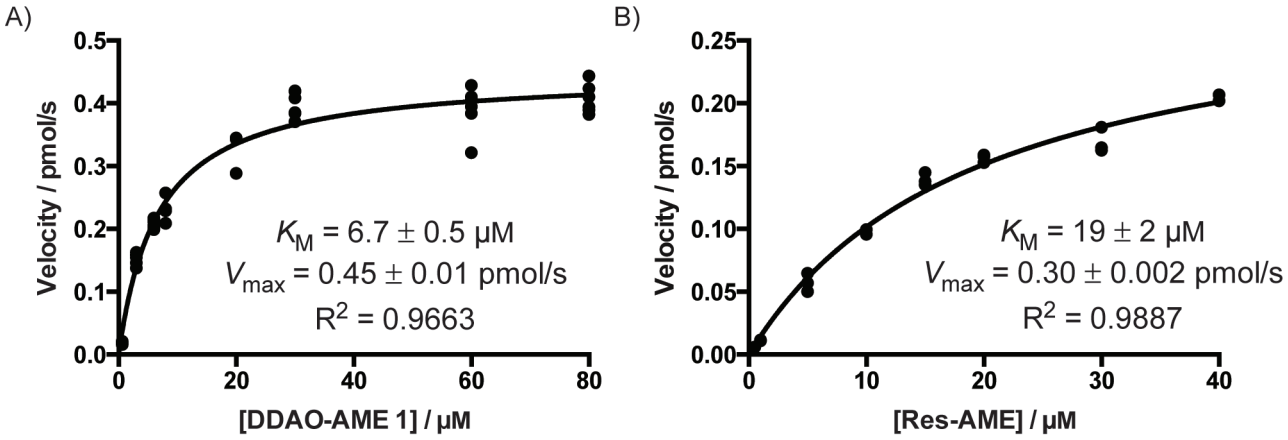

Figure S7. Kinetic evaluation of DDAO-AME 1 and Res-AME with *Bacillus subtilis* esterase. A) DDAO-AME 1 (ranging from 0.6-80 µM) was evaluated with 500 ng/mL *B. subtilis* esterase in sextuplicate. DDAO: Ex 635, Em 670. B) Res-AME (ranging from 0.4-40 µM) was evaluated with 500 ng/mL *B. subtilis* esterase in triplicate. Resorufin: Ex 550, Em 600. Experiments were performed in 10 mM HEPES buffer (pH 7.3) at 37 °C. Fluorescence generation was measured every 20 s. Data were fit to a Michaelis-Menten enzyme kinetics curve.

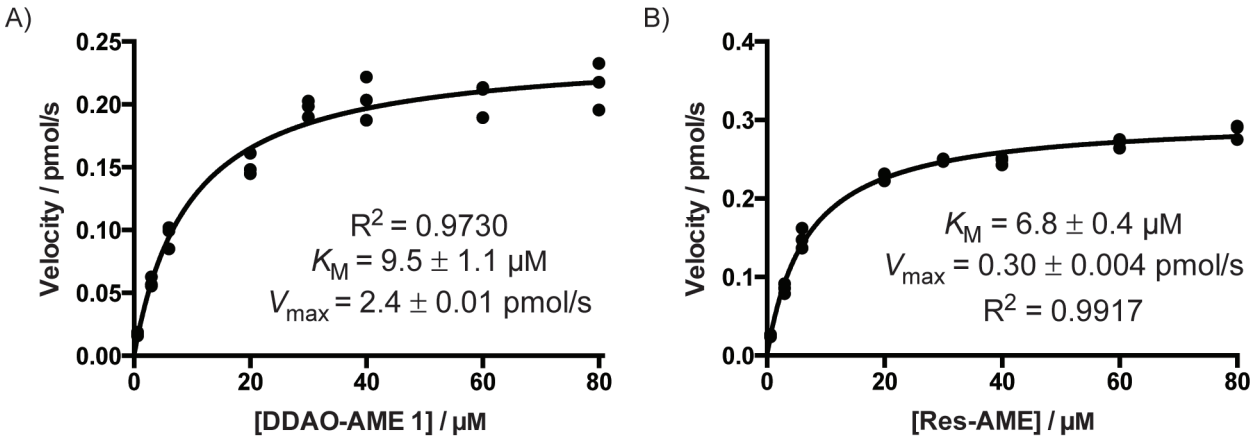

Figure S8. Kinetic evaluation of DDAO-AME 1 and Res-AME with *Candida antarctica* lipase. A) DDAO-AME 1 (ranging from 0.6-80 μM) was evaluated with 10 μg/mL *C. antarctica* lipase. DDAO: Ex 635, Em 670. B) Res-AME (ranging from 0.6-80 μM) was evaluated with 10 μg/mL *C. antarctica* lipase. Resorufin: Ex 550, Em 600. Experiments were performed in triplicate in 10 mM HEPES buffer (pH 7.3) at 37 °C. Fluorescence generation was measured every 20 s. Data were fit to a Michaelis-Menten enzyme kinetics curve.

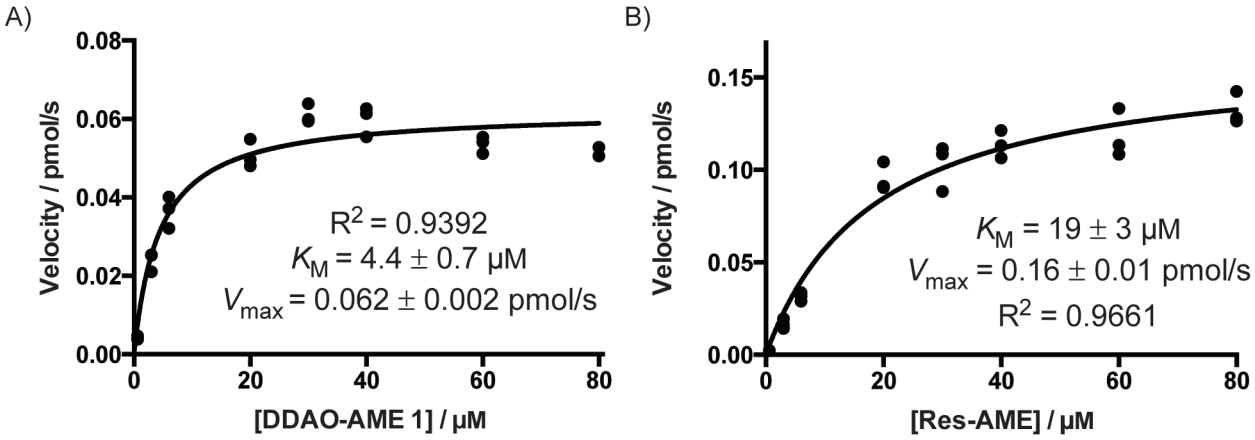

Figure S9. Kinetic evaluation of DDAO-AME 1 and Res-AME with *Mucor miehei* lipase. A) DDAO-AME 1 (ranging from 0.6-80 μM) was evaluated with 10 μg/mL *M. miehei* lipase. DDAO: Ex 635, Em 670. B) Res-AME (ranging from 0.6-80 μM) was evaluated with 20 μg/mL *M. miehei* lipase. Resorufin: Ex 550, Em 600. Experiments were performed in triplicate in 10 mM HEPES buffer (pH 7.3) at 37 °C. Fluorescence generation was measured every 20 s. Data were fit to a Michaelis-Menten enzyme kinetics curve. Data did not fit a substrate inhibition curve.

Table S3. Enzymes used for probe characterization.

Enzyme	**Product #**
Aspergillus sp. lipase	84205
Candida antarctica lipase	65986
Candida rugosa lipase	62316
Mucor miehei lipase	62298
Pseudomonas cepacia lipase	62309
Pseudomonas fluorescens lipase	95608
Rhizopus arrhizus lipase	62305
Rhizopus niveus lipase	62310
porcine pancreas lipase	L3126
porcine liver esterase	E2884
Saccharomyces cerevisiae esterase	46071
Bacillus subtilis esterase	96667

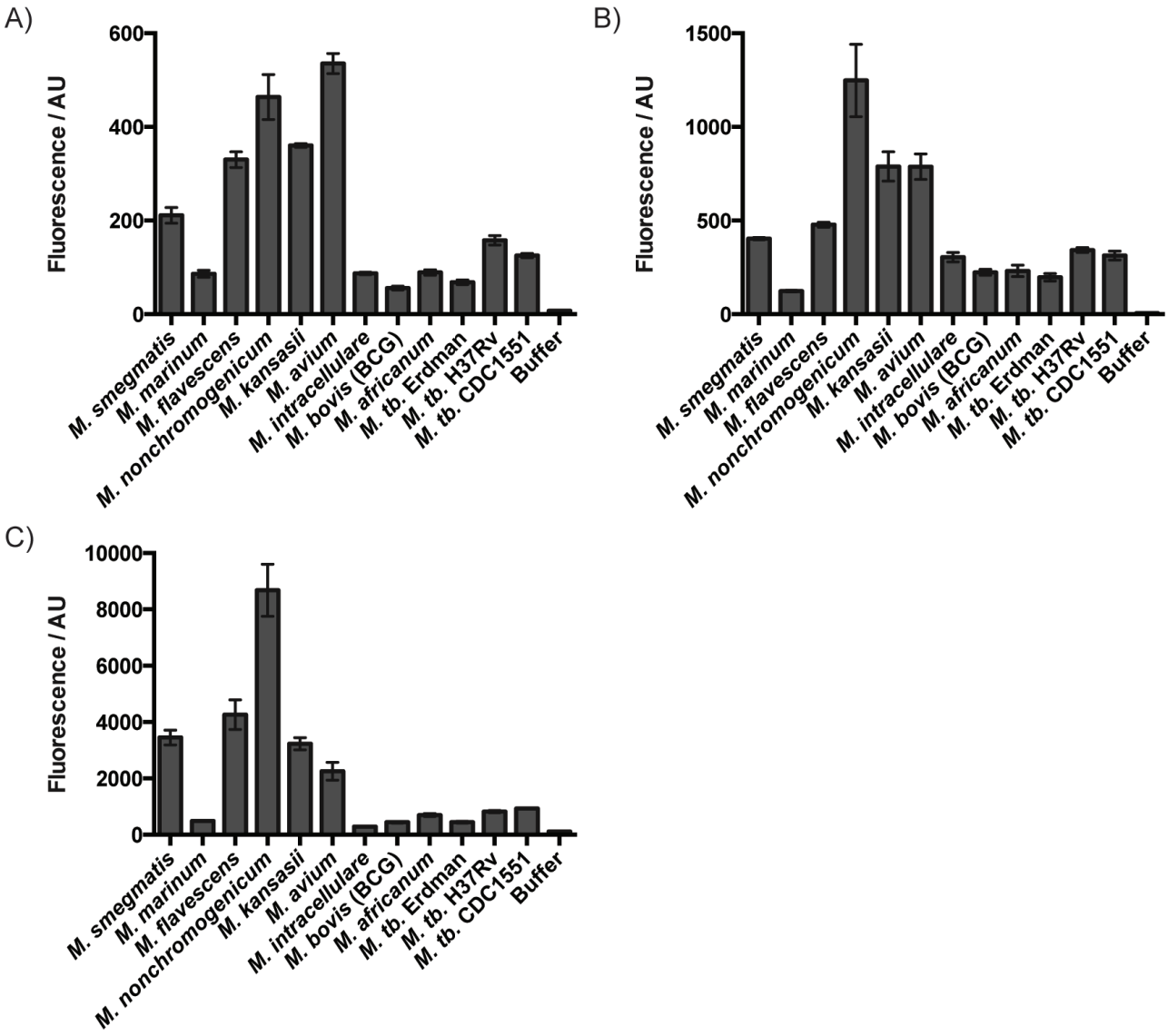

Figure S10. Mycobacterial lysates have esterase activity. Lysates (1 µg) were incubated with 5 µM fluorogenic probe in 10 mM HEPES (pH 7.3) for 10 min at 37 °C. Probe cleavage was detected by an increase in fluorescence (in arbitrary units, AU) compared to the buffer control. A) DDAO-AME 1 (DDAO: Ex 635, Em 670). B) DDAO-AME 2 (DDAO: Ex 635, Em 670). C) Res-AME (resorufin: Ex 550, Em 600). All responses are statistically significant ($P < 0.01$) compared to the no enzyme control as determined by an unpaired, two-tailed t test with Welch's correction. Error bars represent one standard deviation; $n = 3$.

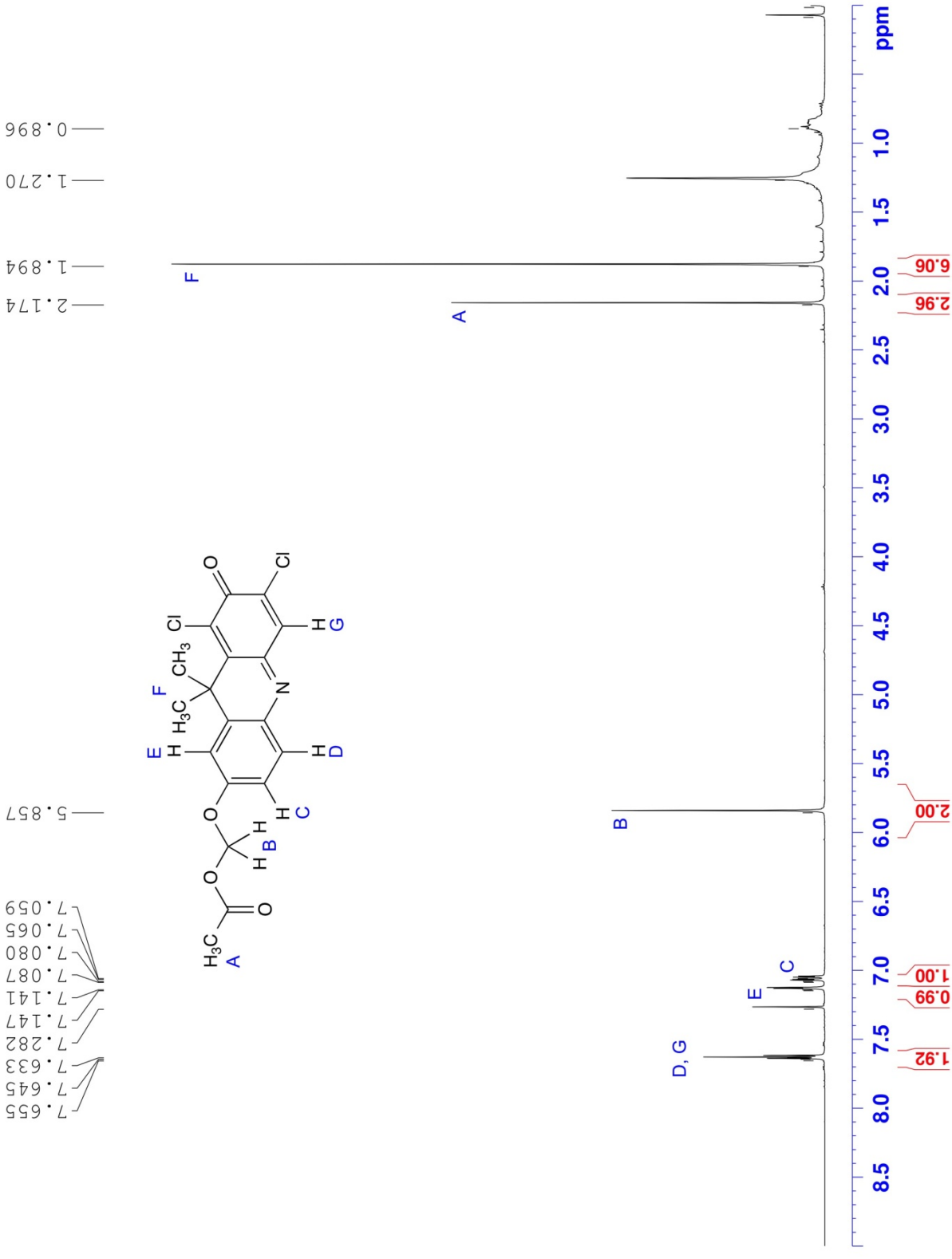

Figure S11. ^{1}H NMR spectrum of DDAO-AME 1; $CDCl_3$, 400 MHz.

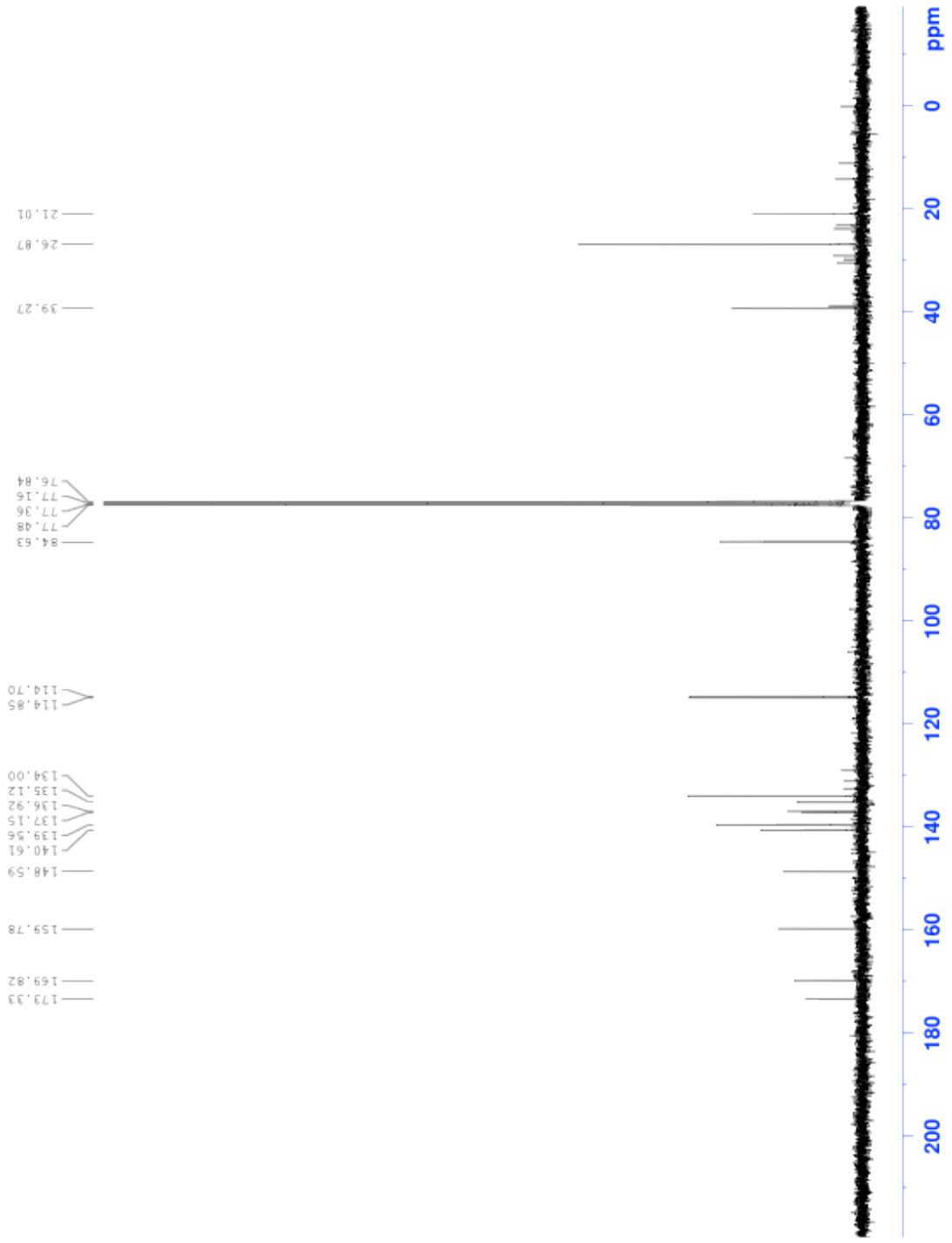

Figure S12. ^{13}C NMR spectrum of DDAO-AME 1; $CDCl_3$, 101 MHz.

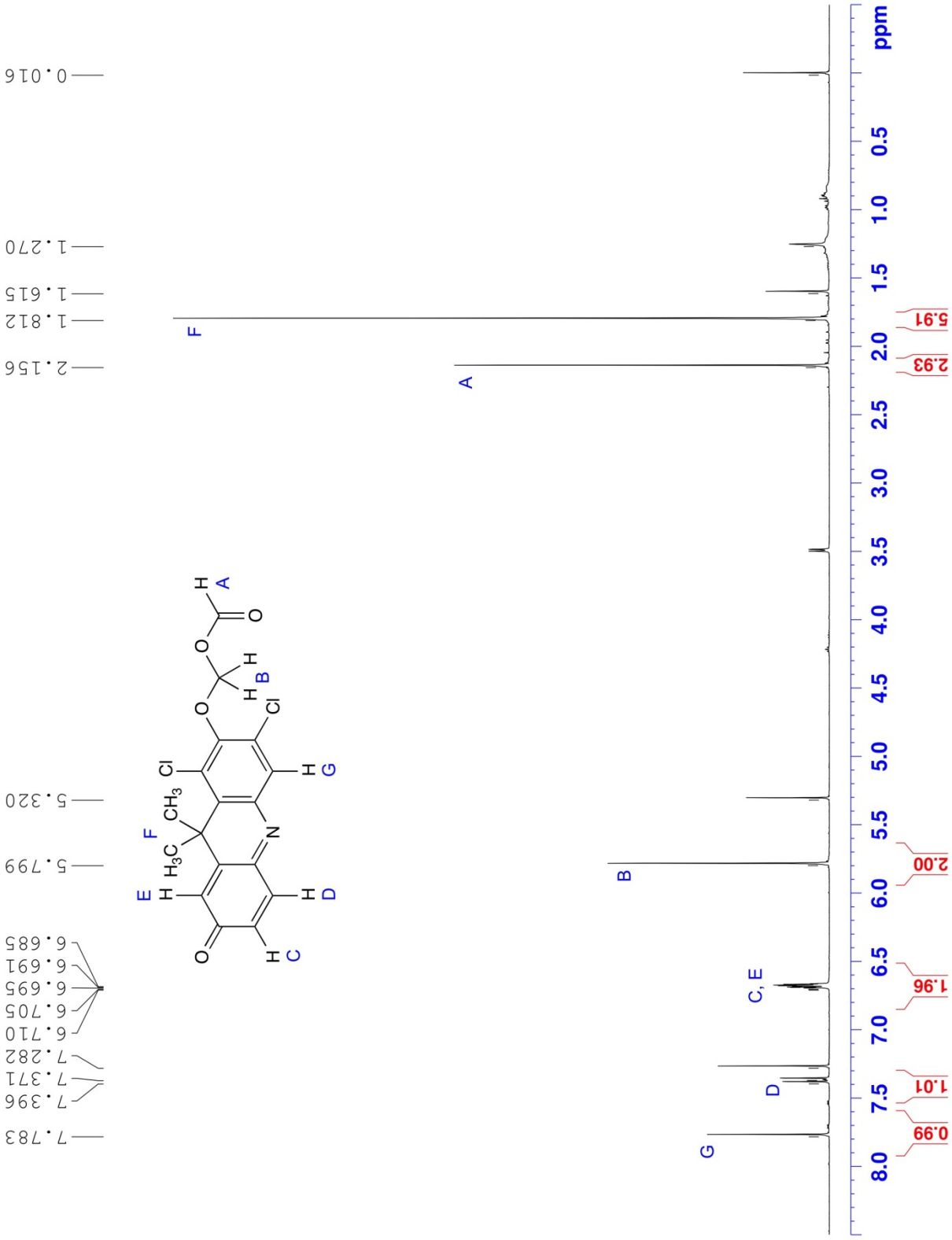

Figure S13. ʻH NMR spectrum of DDAO-AME 2; $CDCl_3$, 400 MHz.

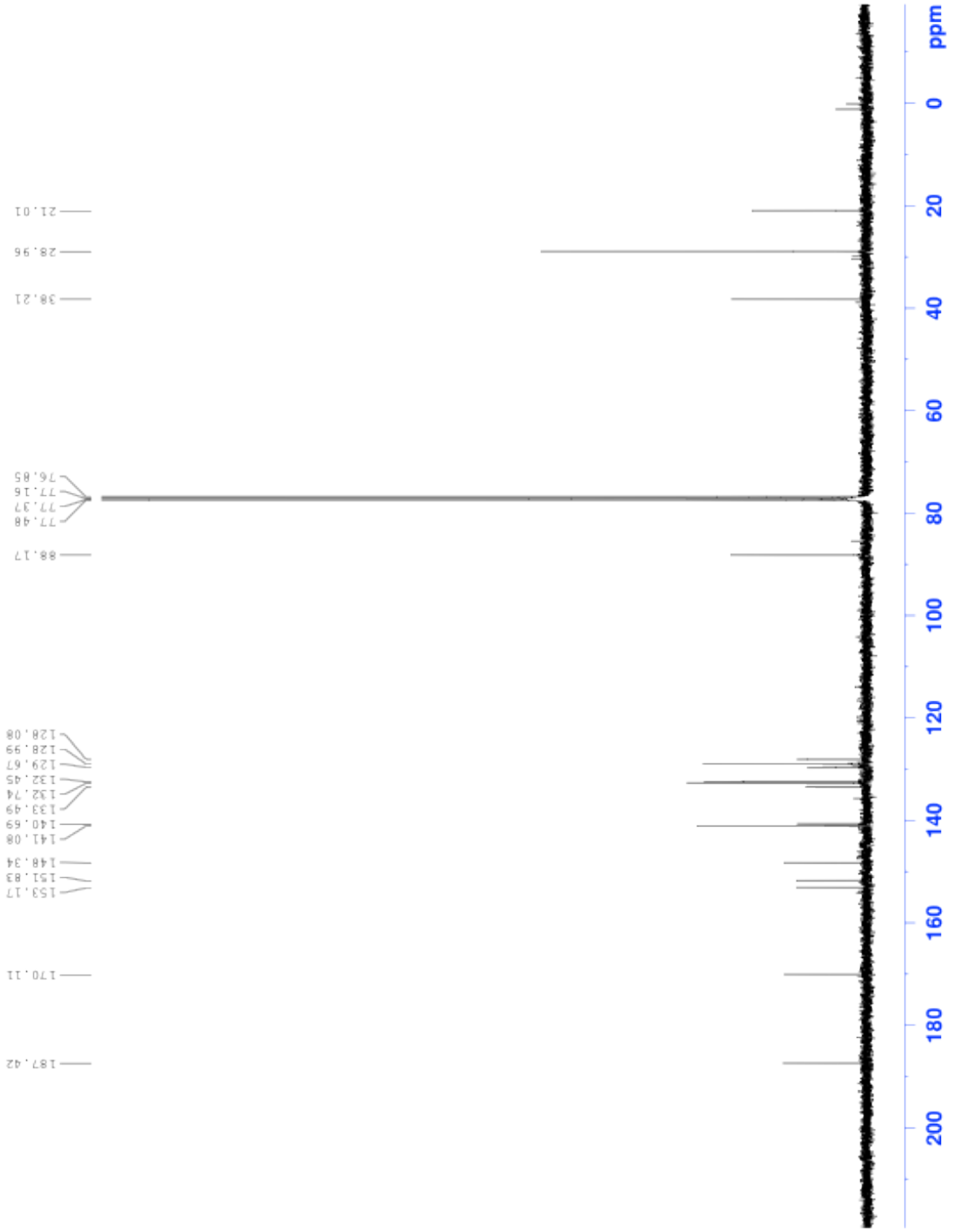

Figure S14. ^{13}C NMR spectrum of DDAO-AME 2; $CDCl_3$, 101 MHz.

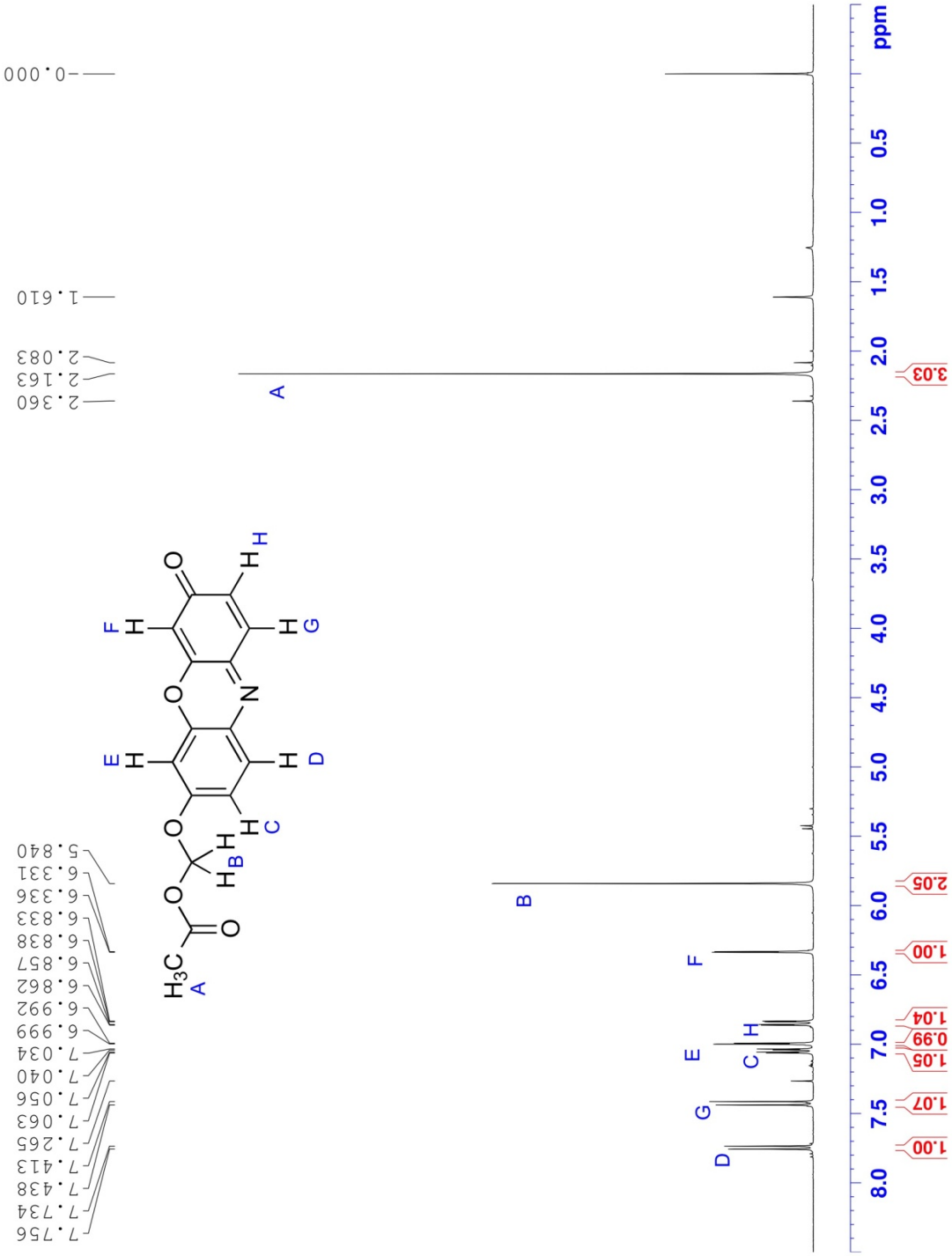

Figure S15. Res-AME ʿH NMR spectrum; $CDCl_3$, 400 MHz.

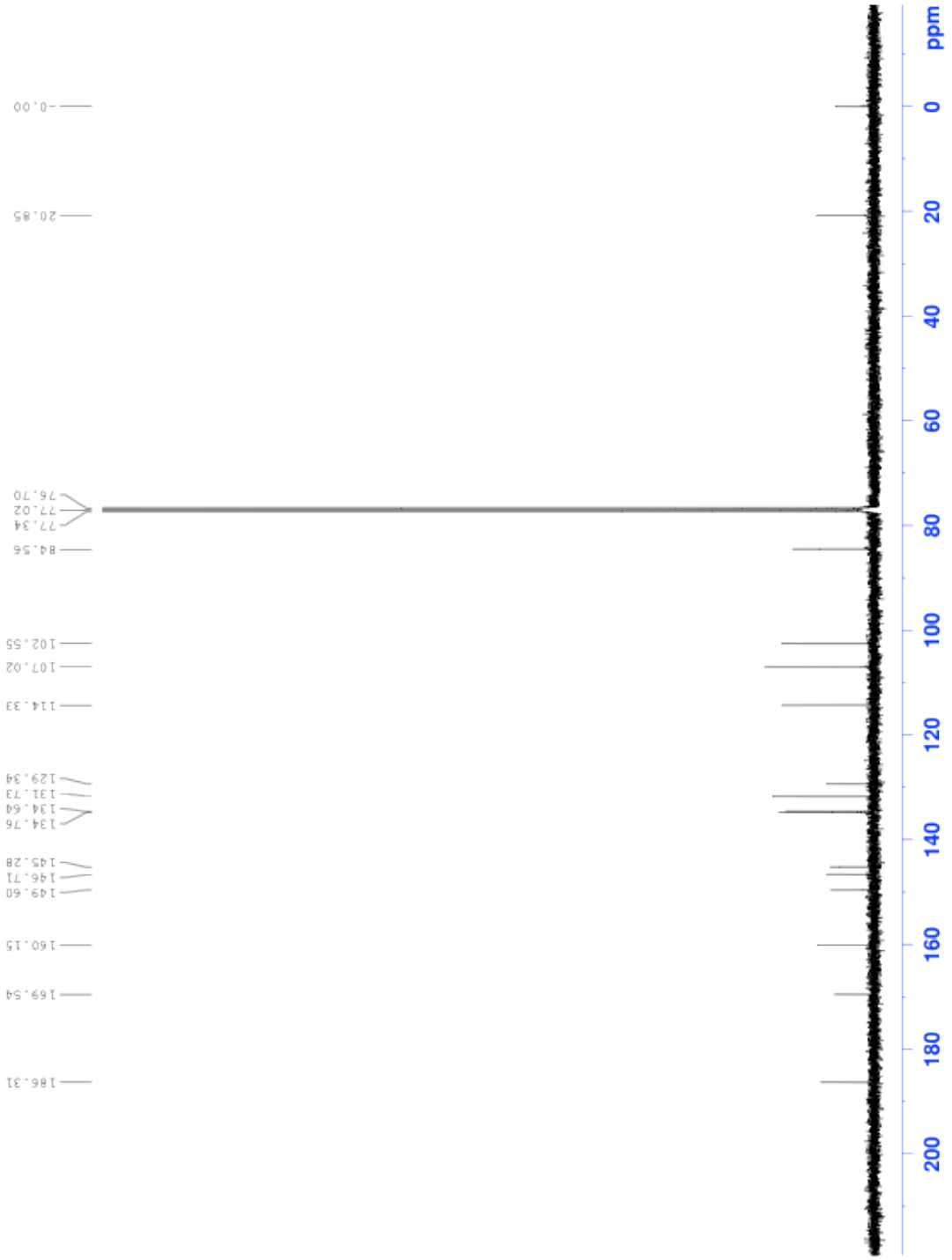

Figure S16. Res-AME ^{13}C NMR spectrum; $CDCl_3$, 101 MHz.

References

[1] L. D. Lavis, T. Y. Chao, R. T. Raines, *Chem. Sci.* **2011**, *2*, 521-530.
[2] S. Fery-Forgues, D. Lavabre, *J. Chem. Educ.* **1999**, *76*, 1260.
[3] R. D. Allison, D. L. Purich, *Methods Enzymol.* **1979**, *63*, 3-22.
[4] K. E. Beatty, M. Williams, B. L. Carlson, B. M. Swarts, R. M. Warren, P. D. van Helden, C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 12911-12916.
[5] C. Bueno, M. L. Villegas, S. G. Bertolotti, C. M. Previtali, M. G. Neumann, M. V. Encinas, *Photochem. Photobiol.* **2002**, *76*, 385-390.
[6] W. S. Rasband, U.S. National Institute of Health, Bethesda, **1997-2007**.