

Terbium(III) Luminescence-Based Assay for Esterase Activity

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Chemical Syntheses

Materials. Silica gel (40 μm , 230–400 mesh) was from SiliCycle. Reagent chemicals were obtained from commercial sources and used without further purification. DCM was dried by passage over a column of alumina. The progress of reactions was monitored by thin-layer chromatography using plates of 250- μm silica 60-F254 from EMD Millipore.

Conditions. All procedures were performed in air at ambient temperature ($\sim 22\text{ }^\circ\text{C}$) and pressure (1.0 atm) unless indicated otherwise.

Solvent Removal. The phrase “concentrated under reduced pressure” refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining a water bath below $40\text{ }^\circ\text{C}$. Residual solvent was removed from samples at high vacuum (<0.1 torr).

NMR Spectroscopy. ^1H and ^{13}C NMR spectra were acquired with Bruker spectrometers operating at 400 and 500 MHz. Chemical shift data are reported in units of δ (ppm) relative to an internal standard (residual solvent or TMS).

Synthesis of Ethyl 2-Thiopheneacetate. Thiophene-2-yl-acetic acid (711.3 mg, 5.003 mmol) was dissolved in ethanol (35 mL), and fuming aqueous hydrochloric acid (3 drops) was added to the resulting solution. The reaction mixture was allowed to stir for 19 h at $70\text{ }^\circ\text{C}$ then cooled to room temperature. The solvent was removed under reduced pressure, and the resulting oil was dissolved in EtOAc. The organic layer was washed 3 times with 5% v/v aqueous NaHCO_3 . The combined aqueous layers were extracted with EtOAc, and the organic extracts were combined and dried with $\text{Na}_2\text{SO}_4(\text{s})$. The organic solvent was removed under reduced pressure to yield the product as a brown oil in quantitative yield. **^1H NMR** (400 MHz, CDCl_3 , δ): 7.24 (d, 1H, $J = 4.9$), 7.02–6.93 (m, 2H), 4.21 (q, 2H, $J = 7.1$ Hz), 3.85 (s, 2H), 1.30 (t, 3H, $J = 7.2$ Hz). **^{13}C NMR** (101 MHz, CDCl_3 , δ): 170.49, 135.22, 126.78, 126.75, 125.00, 61.21, 35.54, 14.17.

Synthesis of Cyclohexyl 2-(Thiophen-2-yl)acetate. Thiophene-2-yl-acetic acid (142 mg, 1.0 mmol), 4-dimethylaminopyridine (3.0 mg, 0.025 mmol) and cyclohexanol (211 μL , 2.0 mmol) were cooled to $0\text{ }^\circ\text{C}$. A solution of dicyclohexylcarbodiimide (227 mg, 1.1 mmol) in DCM (1.1 mL) was added, and the resulting solution was stirred on ice for 15 min and then at room temperature for 3 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude mixture was taken up in EtOAc and washed twice with 1 M HCl, twice with saturated aqueous NaHCO_3 , and once with brine. The organic layer was dried over $\text{MgSO}_4(\text{s})$, concentrated under reduced pressure, and purified by chromatography on silica gel, eluting with 9:1 hexanes/EtOAc (R_f 0.46) to afford the product (0.190 g, 85%) as a brown oil. **^1H NMR** (400 MHz, $\text{DMSO}-d_6$, δ): 7.41 (dd, 1H, $J = 4.4, 2.0$ Hz), 6.98–6.94 (m, 2H), 4.69 (tt, 1H, $J = 8.6, 3.8$ Hz), 3.88 (s, 2H), 1.80–1.73 (m, 2H), 1.67–1.59 (m, 2H), 1.51–1.14 (m, 6H). **^{13}C NMR** (101 MHz, DMSO , δ): 169.51, 135.62, 126.84, 126.66, 125.39, 72.52, 35.07, 30.94, 24.81, 23.01.

Synthesis of 2-Oxo-2-phenylethyl 2-(Thiophen-2-yl)acetate. Thiophene-2-yl-acetic acid (142 mg, 1.0 mmol), 4-dimethylaminopyridine (3 mg, 0.025 mmol) and 2-hydroxyacetophenone (136 mg, mmol) were cooled to $0\text{ }^\circ\text{C}$. A solution of dicyclohexylcarbodiimide (1.1 mmol) in DCM (1.1 mL) was added, and the resulting solution was stirred on ice for 15 min, and then at room temperature for 2 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude mixture was taken up in EtOAc and washed twice with 1 M HCl, twice with saturated aqueous NaHCO_3 , and once with brine. The organic layer was dried over $\text{MgSO}_4(\text{s})$, concentrated under reduced pressure, and purified by chromatography on silica gel, eluting with 2:1 hexanes/EtOAc (R_f 0.50) to afford the product (0.118 g, 45%) as a brown solid.

¹H NMR (400 MHz, DMSO-*d*₆, δ): 7.98–7.94 (m, 2H), 7.69 (t, 1H, $J = 7.4$ Hz), 7.56 (t, 2H, $J = 7.7$ Hz), 7.44 (dd, 1H, $J = 5.1, 1.3$ Hz), 7.06–7.03 (m, 1H), 7.01–6.98 (m, 1H), 5.55 (s, 2H), 4.10 (s, 2H). **¹³C NMR** (101 MHz, DMSO, δ): 192.48, 169.80, 135.01, 133.97, 133.80, 128.91, 127.77, 127.21, 126.73, 125.54, 66.97, 34.17.

Synthesis of 3-(4-Hydroxyphenyl)pentane-2,4-dione. The synthesis of 3-(4-hydroxyphenyl)pentane-2,4-dione was adapted from a literature procedure.¹ Briefly, 4-iodophenol (220 mg, 1 mmol), acetylacetone (0.31 mL, 3.0 mmol), CuI (19 mg, 0.1 mmol), L-proline (23 mg, 0.2 mmol), and CsCO₃ (1.30 g, 4.0 mmol) were dissolved in DMSO (4 mL), and the resulting solution was heated at 60 °C under Ar(g) for 18 h. The reaction mixture was quenched with 1 M HCl (10 mL) and extracted twice with EtOAc, dried over MgSO₄(s), concentrated under reduced pressure, and purified by chromatography on silica gel, eluting with 4:1 hexanes/EtOAc (R_f 0.20) to afford the product (46 mg, 24%) as a white powder. **¹H NMR** (400 MHz, CDCl₃, δ): (100% enol) 16.58 (s, 1H), 7.04 (d, 2H, $J = 8.7$ Hz), 6.87 (d, 2H, $J = 8.7$ Hz), 5.80 (s, 1H), 1.91 (s, 6H). **¹³C NMR** (101 MHz, CDCl₃, δ): (100% enol) 191.71, 155.36, 132.42, 129.01, 115.89, 114.81, 24.29.

Synthesis of 4-(2,4-dioxopentan-3-yl)phenyl 2-(Thiophen-2-yl)acetate. Thiophene-2-yl-acetic acid (20 mg, 0.140 mmol), 4-dimethylaminopyridine (0.5 mg, 0.004 mmol), and 3-(4-hydroxyphenyl)pentane-2,4-dione (35 mg, 0.182 mmol) were cooled to 0 °C. A solution of dicyclohexylcarbodiimide (0.15 mmol) in DCM (0.15 mL) was added, and the resulting solution was stirred on ice for 15 min, and then at room temperature for 5 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude mixture was taken up in EtOAc and washed twice with 1 M HCl, twice with saturated aqueous NaHCO₃, and once with brine. The organic layer was dried over MgSO₄(s), concentrated under reduced pressure, and purified by chromatography on silica gel, eluting with 9:1 hexanes/EtOAc (R_f 0.19) to afford the product (23 mg, 52%) as a yellow solid. **¹H NMR** (500 MHz, CDCl₃, δ): (100% enol) 16.68 (s, 1H), 7.27 (dd, 1H, $J = 5.1, 1.3$ Hz), 7.20–7.12 (m, 4H), 7.06 (dd, 1H, $J = 3.5, 1.3$ Hz), 7.01 (dd, 1H, $J = 5.1, 3.5$ Hz), 4.10 (s, 2H), 1.88 (s, 6H). **¹³C NMR** (126 MHz, CDCl₃, δ): (100% enol) 191.14, 168.93, 150.18, 134.82, 134.33, 132.28, 127.34, 127.12, 125.55, 121.95, 114.41, 35.76, 24.35.

Optimization of 2TA Detection Conditions in Aqueous Media

Methodology for monitoring 2TA in aqueous solution was optimized by varying the components listed in Table S1 while maintaining 2TA at a constant concentration. Unless noted otherwise, the reported emission response is the emission of the indicated assay components with added 2TA divided by the emission of the indicated assay components without 2TA, *i.e.*, RFU_{2TA}/RFU_{blank} . Each assay was conducted in triplicate, and values are the mean \pm SD.

Table S1. Components Varied During Assay Optimization

Component	Variations Tested	Optimum
Buffer Agent	HEPES, Tris, Bis-Tris, <i>N</i> -methyl morpholine, imidazole (with varying levels of Triton X-100)	HEPES
HEPES concentration	5–50 mM (with varying levels of Triton X-100)	5 mM
Surfactant type	Brij 35 or Triton X-100 varying from 50% to >200% of the critical micelle concentration (CMC)	0.1 w/v
Tri- <i>n</i> -octylphosphine oxide (TOPO)	100–500 μ M	350 μ M
pH	6.8–8.2	7.4
Tb ³⁺	0.05–10 mM	4 mM

Buffer type was investigated first. Tris buffer is a common choice in aqueous Tb-sensitization assays, but Tris itself chelates to Tb^{3+} only weakly.^{2,3} This chelation could boost the assay signal by displacing water from the lanthanide ion, thereby limiting the paths of nonradiative decay. Hence, we also tested BIS-TRIS, which might better chelate to Tb^{3+} due to its two additional hydroxy groups. HEPES was chosen as a representative zwitterionic buffer. Given that lanthanides are oxophilic, imidazole and *N*-methyl morpholine (NMM) were chosen as buffers that might chelate only weakly to Tb^{3+} or are too hindered to chelate to Tb^{3+} , respectively, thereby competing less with the sensitizing agent. An excitation scan was conducted in 20 mM buffer, pH 7.6, containing 2TA (500 μ M) and $TbCl_3$ (1 mM) (Figure S1A). Low relative emission was observed with no surfactant present, so Triton X-100 (0–0.11% w/v) was added, and the relative emission was measured. HEPES–NaOH buffer resulted in the strongest emission over a blank control and was chosen for the assay. HEPES–NaOH concentration was then assessed in a similar manner, and a lower concentration of HEPES–NaOH was found to yield a higher relative emission (Figure S1B). Because a HEPES concentration of <5 mM might not offer sufficient buffer capacity, 5 mM HEPES–NaOH was chosen.

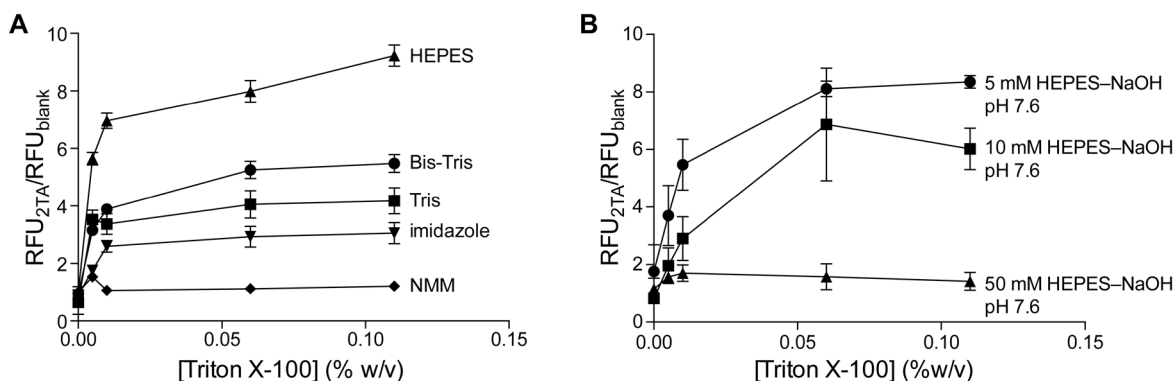


Figure S1. Graph showing data for the optimization of buffer type and concentration. Values are the mean \pm SD from triplicate measurements.

Next, surfactant type and concentration was examined. Studies with buffer type and concentration suggested that an added surfactant led to a desirable increase in the emission intensity, particularly when added at or above the CMC (\sim 0.02% w/v for Triton X-100). We therefore examined Triton X-100 and Brij 35 (which is a nonionic surfactant that does not contain aryl groups and has a CMC of \sim 0.1% w/v) at approximately half the CMC, at the CMC, and well above the CMC in 5 mM HEPES–NaOH buffer at pH 7.6, containing 2TA (100 μ M) and $TbCl_3$ (1 mM). Both surfactants exhibited similar behavior in that the strongest relative emission intensity was observed at and above the CMC (Figure S2). Triton X-100 showed the strongest enhancement of emission intensity and was chosen for use. Additionally, the data from the buffer screen and surfactant screen suggested that at levels of 0.05% w/v and above, the concentration of Triton X-100 has little effect on the emission. Hence, a Triton X-100 concentration of 0.1% w/v was chosen for use.

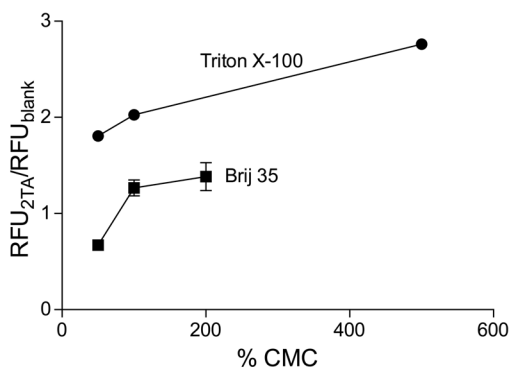


Figure S2. Graph showing data for the optimization of surfactant type and concentration. Values are the mean \pm SD from triplicate measurements.

The synergic agent TOPO was tested at concentrations of 100, 250, 300, 350, 400, and 500 μM in 5 mM HEPES–NaOH buffer, pH 7.6, containing 2TA (100 μM), TbCl_3 (1 mM), and Triton X-100 (0.1 %w/v). An increase in 2TA/blank was observed above 300 μM , and a maximum was observed at 350 μM (Figure S3). Hence, 350 μM was chosen for use.

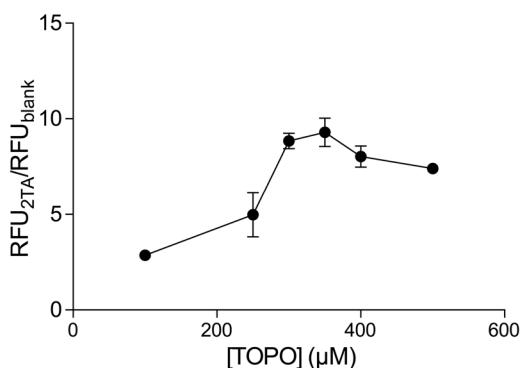


Figure S3. Graph showing data for the optimization of TOPO concentration. Values are the mean \pm SD from triplicate measurements.

Next, the pH of the assay solution was examined. Assays were performed in 5 mM HEPES–NaOH buffer, pH 6.8–8.2, containing 2TA (100 μM), TbCl_3 (1 mM), Triton X-100 (0.1% w/v), and TOPO (350 μM), and the emission intensity monitored over the course of 1 h, during which the intensity was essentially unchanged (Figure S4). A steady response was observed from pH 6.8 to 7.8, above which the intensity decreased. Hence, the pH of 7.4 was deemed to be an acceptable value.

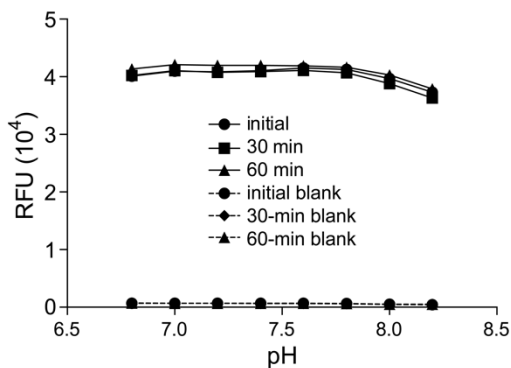


Figure S4. Graph showing data for the optimization of pH.

The final factor explored was the Tb^{3+} concentration. A final TbCl_3 concentration ranging from 0.05 to 10 mM was assessed with 100 μM 2TA, 0.1 %w/v Triton X-100, and 350 μM TOPO. A

plateau was observed above 1 mM (Figure S5), and 4 mM was chosen as the final concentration because of its being well into the plateau region.

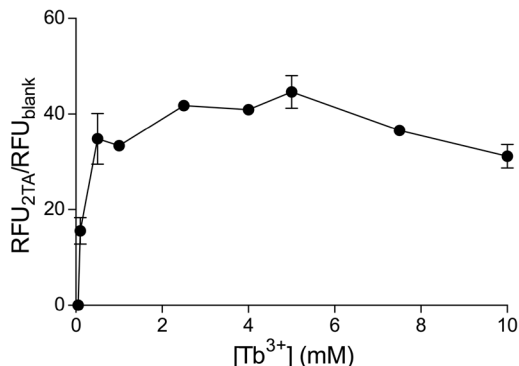


Figure S5. Graph showing data for the optimization of Tb³⁺ concentration. Values are the mean ± SD from triplicate measurements.

The influence of equilibration time on the luminescence emission intensity was accessed by monitoring solutions of 2TA (4–164 μM) at 0, 20, 60, and 120 min after their preparation. The data were fitted by linear regression using Excel (Figure S6).

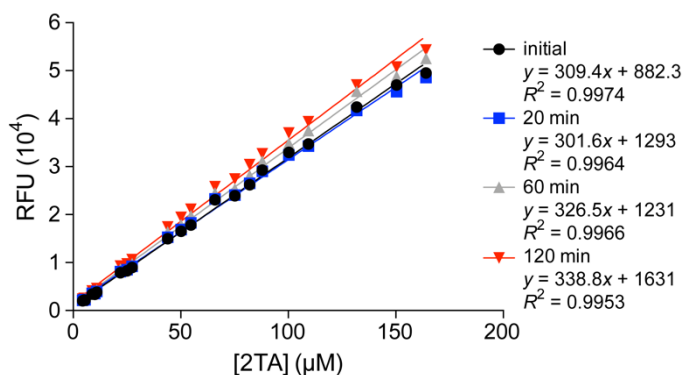


Figure S6. Graph showing the dependence of fluorescence emission on 2TA concentration.

The influence of additives was assessed by taking the 200.5 μM stock solution of 2TA and performing the same dilution sequences as in the linearity studies reported in the main text to yield 100 μL samples in the indicated buffer ranging from 5.0 μM to 150.3 μM. The slope, intercept, and R² were determined for each curve by linear regression using Excel (Figure S7).

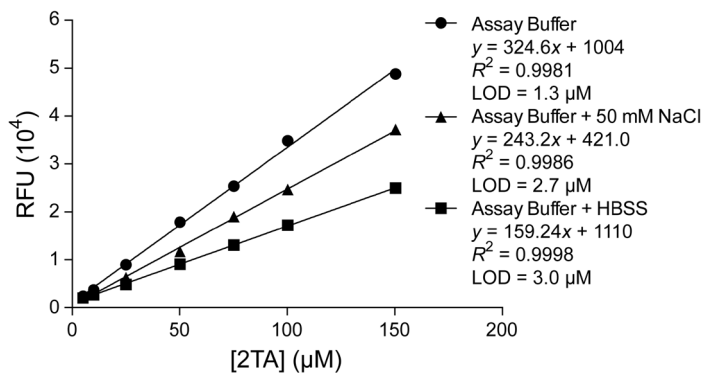


Figure S7. Graph showing the dependence of the fluorescence emission and LOD on additives.

R Script for Nonlinear Least Squares Fit of the Job Plot. In the script, “c” is replaced with “P”, y is intensity, and x is the mole fraction of Tb³⁺.

```

> library(minpack.lm)
> y <-
c(0.03030303,739.2121212,1246.848485,1587.69697,1671.878788,1421.575758,1193.
484848,832.8787879,605.6060606,276.969697,-
69.48484848,0.151515152,743.6666667,1126.121212,1380.69697,1589.242424,1426.5
75758,1232.848485,787.2424242,635.4242424,329.6060606,1.96969697,0.242424242,
860.6666667,1205.757576,1610.242424,1490.606061,1328.484848,1083.939394,920.5
151515,554.8787879,331.6969697,1.515151515)
> x <-
c(0,0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9,1,0,0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0
.9,1,0,0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9,1)

> JobFit <- nlsLM(y~P*(x)^m*(1-x)^n,start=list(P=1000,m=1,n=1))
> summary(JobFit)

Formula: y ~ P * (x)^m * (1 - x)^n

Parameters:
  Estimate Std. Error t value Pr(>|t|)
P 7.389e+03  7.058e+02  10.47 1.55e-11 ***
m 9.034e-01  5.376e-02  16.81 < 2e-16 ***
n 1.473e+00  7.525e-02  19.58 < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 76.48 on 30 degrees of freedom

Number of iterations to convergence: 11
Achieved convergence tolerance: 1.49e-08
> 0.9043/(0.9034+1.473)
[1] 0.3805336

```

R Script for Nonlinear Least Squares Fit to Determine the Apparent K_d Value

```

> library(minpack.lm)
> x <-
c(25,50,100,200,400,600,1000,4000,8000,2000,25,50,100,200,400,600,1000,4000,8
000,2000,25,50,100,200,400,600,1000,4000,8000,2000)
> y <-
c(0,0.032269662,0.048778383,0.11070047,0.402364141,0.564028818,0.670127554,0.
945886481,1,0.842304318,0.012525345,0.030975424,0.044795007,0.106357584,0.363
206258,0.527862063,0.688606394,0.935014884,0.886466587,0.735170192,0.00267475
8,0.03048649,0.041947684,0.114784509,0.363896519,0.499633299,0.6620889,0.9265
01675,0.935791426,0.751621392)
> HillFit <- nlsLM(y ~ x^h/(K^h + x^h),start=list(h=1,K=500))
> summary(HillFit)

Formula: y ~ x^h/(K^h + x^h)

Parameters:
  Estimate Std. Error t value Pr(>|t|)
h  1.39896    0.07416   18.86 <2e-16 ***
K 620.29170   23.87415   25.98 <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Residual standard error: 0.04306 on 28 degrees of freedom

Number of iterations to convergence: 6

Achieved convergence tolerance: 1.49e-08

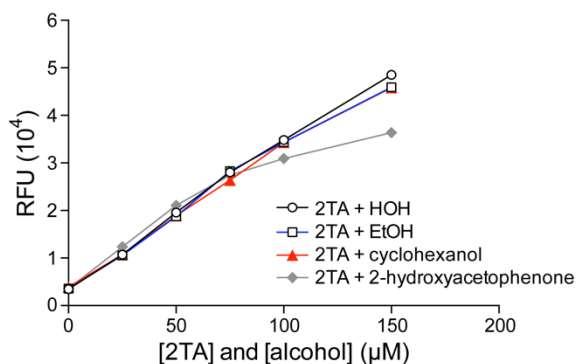


Figure S8. Dependence of fluorescence emission on equimolar 2TA and alcohol concentration.

Table S2. Slope and R^2 Values for Graph in Figure S8

Concentration Range	2TA + H ₂ O		2TA + EtOH		2TA + cyclohexanol		2TA + 2-hydroxyacetophenone	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
25–150 μM	300.1	0.996	282.9	0.987	281.5	0.994	185.3	0.926
25–100 μM	323.3	0.996	323.7	0.993	312.9	1.000	248.0	0.966
25–75 μM	346.5	1.000	354.4	0.998	312.7	0.999	301.1	0.991

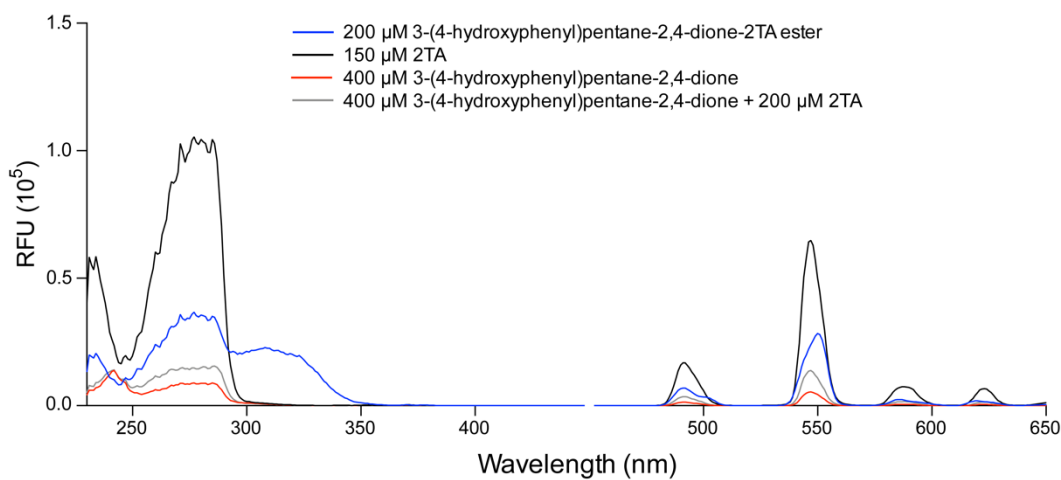


Figure S9. Excitation (<450 nm) and emission (>450 nm) spectra of assay buffer containing combinations of 2TA, 3-(4-hydroxyphenyl)pentane-2,4-dione, and the 2TA ester of 3-phenylpentane-2,4-dione.

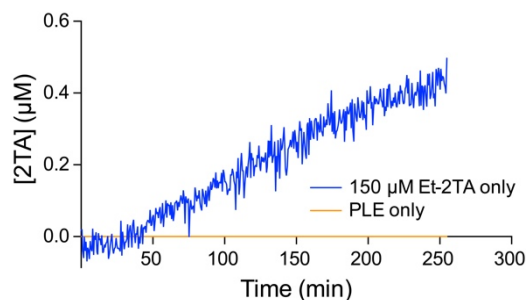


Figure S10. Graph of the nonenzymatic hydrolysis of Et-2TA (150 μM) over a 4-h period in assay buffer.

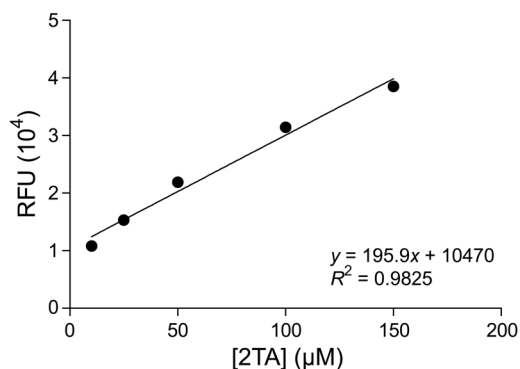


Figure S11. Linearity of the assay in *B. subtilis* OI1085 lysate. Data points are the mean from three samples.

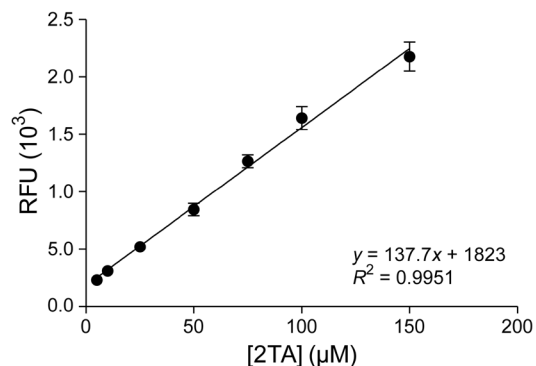


Figure S12. Linearity of the assay in HEK-293T lysate. Samples were prepared in triplicate, and values are the mean \pm SD from triplicate measurements.

Determination of Steady-State Kinetic Parameters Using Initial Rates

Script for performing fit with R software:

```
> library(minpack.lm)
> So <-
c(193.9343411,156.9408101,127.6872406,94.70898427,62.98895834,45.8124176,31.0
2534772,21.91905519,10.99134297,155.38792,121.8746986,99.08168863,70.96045152
,45.0228289,37.87916773,26.84735906,16.25089214,7.839626334,176.5040035,148.5
050984,115.4968944,85.21864322,57.52809515,42.85899156,34.13995541,20.6832675
2,8.433866502)
> Vo <-
c(0.024076855,0.02383575,0.023288318,0.021920329,0.018266907,0.015038088,0.00
4065228,0.009409715,0.008484955,0.028553193,0.021242729,0.021426847,0.0210092
68,0.017360108,0.018652849,0.015028486,0.010633779,0.009138852,0.025050903,0.
026162708,0.024258181,0.024135727,0.021497588,0.020025371,0.018942333,0.01427
3264,0.008044493)
> plot(So,Vo)
> MMfit <- nlsLM(Vo ~ Vmax*So/(Km+So),start=list(Vmax=0.03,Km=25))
> summary(MMfit)
```

Formula: $V_o \sim V_{max} * S_o / (K_m + S_o)$

Parameters:

	Estimate	Std. Error	t value	Pr(> t)	
Vmax	0.029553	0.001954	15.127	4.33e-14	***
Km	30.239777	6.437658	4.697	8.16e-05	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.002961 on 25 degrees of freedom

Number of iterations to convergence: 4

Achieved convergence tolerance: 1.49e-08

Determination of Steady-State Kinetic Parameters Using the Integrated Michaelis–Menten Equation

Script for performing fit with R software of the progress curve for the PLE-catalyzed hydrolysis of ethyl 2-thiopheneacetate:

```
> library(minpack.lm)
> library(lamW)
> t <-
c(0, 24.6, 49.3, 74, 98.6, 123.2, 147.9, 172.5, 197.2, 221.9, 246.5, 271.2, 295.8, 320.5, 3
45.1, 369.8, 394.4, 419.1, 443.7, 468.4, 493, 517.7, 542.3, 567, 591.6, 616.3, 640.9, 665.
6, 690.3, 714.9, 739.6, 764.2, 788.9, 813.5, 838.2, 862.8, 887.5, 912.1, 936.8, 961.4, 986
.1, 1010.7, 1035.4, 1060, 1084.7, 1109.3, 1134, 1158.7, 1183.3, 1207.9, 1232.6, 1257.3, 1
281.9, 1306.6, 1331.2, 1355.9, 1380.5, 1405.2, 1429.8, 1454.5, 1479.1, 1503.8, 1528.4, 1
553.1, 1577.7, 1602.4, 1627, 1651.7, 1676.4, 1701, 1725.7, 1750.3, 1775, 1799.6, 1824.3,
1848.9, 1873.6, 1898.2, 1922.9, 1947.5, 1972.2, 1996.8, 2021.5, 2046.2, 2070.8, 2095.5,
2120.1, 2144.8, 2169.4, 2194.1, 2218.7, 2243.4, 2268, 2292.7, 2317.3, 2342, 2366.6, 2391
.3, 2415.9, 2440.6, 2465.3, 2489.9, 2514.6, 2539.2, 2563.9, 2588.5, 2613.1, 2637.8, 2662
.5, 2687.1, 2711.8, 2736.4, 2761.1, 2785.7, 2810.4, 2835, 2859.7, 2884.3, 2909, 2933.6, 2
958.3, 2982.9, 3007.6, 3032.2, 3056.9, 3081.5, 3106.2, 3130.8, 3155.5, 3180.1, 3204.8, 3
229.4, 3254.1, 3278.7, 3303.4, 3328, 3352.7, 3377.3, 3402, 3426.6, 3451.3, 3476, 3500.6,
3525.3, 3549.9, 3574.6, 3599.2, 3623.9, 3648.5, 3673.2, 3697.8, 3722.5, 3747.1, 3771.8,
3796.4, 3821.1, 3845.7, 3871.1, 3895.9, 3920.6, 3945.2, 3969.9, 3994.5, 4019.2, 4043.8,
4068.5, 4093.1, 4117.8, 4142.4, 4167.1, 4191.7, 4216.4, 4241, 4265.7, 4290.3, 4315, 4339
.7, 4364.3, 4389, 4413.6, 4438.3, 4462.9, 4487.6, 4512.2, 4536.9, 4561.5, 4586.2, 4610.8
, 4635.5, 4660.1, 4684.8, 4709.4, 4734.1, 4758.7, 4783.4, 4808, 4832.7, 4857.3, 4882, 490
6.6, 4931.3, 4955.9, 4980.6, 5005.2, 5029.9, 5054.5, 5079.2, 5103.8, 5128.5, 5153.1, 517
7.8, 5202.4, 5227.1, 5252.7, 5277.4, 5302.1, 5326.7, 5351.4, 5376, 5400.7, 5425.3, 5450,
5474.6, 5499.3, 5523.9, 5548.6)
> P <-
c(0, 0.81, 1.61, 2.28, 2.94, 3.61, 4.27, 4.92, 5.55, 6.18, 6.83, 7.28, 7.92, 8.65, 9.17, 9.7
5, 10.28, 10.72, 11.45, 11.88, 12.42, 12.97, 13.52, 13.97, 14.35, 14.86, 15.48, 15.85, 16.
28, 16.71, 17.07, 17.55, 17.92, 18.62, 18.91, 19.17, 19.86, 20.33, 20.7, 21.04, 21.5, 22.1
9, 22.35, 22.91, 23.37, 23.7, 24.35, 24.97, 25.25, 25.86, 26.32, 26.63, 26.99, 27.81, 27.9
, 28.56, 29.31, 29.55, 30, 30.7, 31.07, 31.53, 31.89, 32.44, 33.03, 33.52, 34, 34.74, 35.12
, 35.57, 35.61, 36.74, 36.78, 37.59, 37.71, 38.58, 38.8, 39.4, 39.35, 40.14, 40.56, 41.39,
41.3, 42.11, 42.31, 43.18, 43.41, 43.95, 44.52, 44.98, 45.28, 45.89, 46.35, 46.74, 47.17,
47.59, 47.95, 48.58, 49.15, 49.07, 49.99, 50.57, 51.13, 51.07, 52.21, 51.9, 52.49, 53, 53.
76, 54.25, 54.1, 54.84, 55.27, 55.77, 56.22, 56.54, 57.03, 57.22, 57.85, 58.36, 58.38, 59.
17, 59.65, 59.78, 60.47, 60.87, 61.39, 61.37, 62.57, 62.54, 62.93, 63.6, 63.56, 64.02, 64.
88, 64.66, 65.57, 65.99, 66.09, 66.71, 66.72, 67.82, 67.61, 68.74, 68.71, 69.49, 70, 69.29
```

```
,70.27,71.32,71.12,71,71.55,72.63,72.97,72.72,73.39,74.01,74.02,74.94,74.75,7
5.3,76.08,76.17,76.54,76.76,77.33,77.51,77.69,78.87,78.8,79.54,79.56,80.28,80
.35,80.68,81.23,81.69,81.73,81.3,82.24,82.87,83.14,83.59,84.41,83.72,84.75,85
.16,84.98,85.27,85.94,86.15,86.9,86.71,87.31,88.24,88.18,87.99,88.6,89.14,89.
34,89.7,90.19,89.93,90.76,90.19,91.11,90.96,91.38,91.92,92.39,92.62,92.76,93.
46,93.14,94.34,94.04,94.2,94.56,95,95.16,95.57,95.68,95.97,96.84,96.62)
> s <- 122
> IntMM <- nlsLM(P~s-K*lambertW0(s/K*exp((s-
V*t)/K)),start=list(K=500,V=0.25)) #guess values for Km and Vmax
> summary(IntMM)
```

Formula: $P \sim s - K * \text{lambertW0}(s/K * \exp((s - V * t)/K))$

Parameters:

	Estimate	Std. Error	t value	Pr(> t)
K	4.486e+01	1.130e+00	39.71	<2e-16 ***
V	2.996e-02	2.742e-04	109.27	<2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.6273 on 224 degrees of freedom

Number of iterations to convergence: 13

Achieved convergence tolerance: 1.49e-08

Script for performing fit with R software of the progress curve for the PLE-catalyzed hydrolysis of cyclohexyl 2-thiopheneacetate:

```
> library(minpack.lm)
> library(lamW)
> t <-
c(0,24.6,49.3,74,98.6,123.2,147.9,172.5,197.2,221.9,246.5,271.2,295.8,320.5,3
45.1,369.8,394.4,419.1,443.7,468.4,493,517.7,542.3,567,591.6,616.3,640.9,665.
6,690.3,714.9,739.6,764.2,788.9,813.5,838.2,862.8,887.5,912.1,936.8,961.4,986
.1,1010.7,1035.4,1060,1084.7,1109.3,1134,1158.7,1183.3,1207.9,1232.6,1257.3,1
281.9,1306.6,1331.2,1355.9,1380.5,1405.2,1429.8,1454.5,1479.1,1503.8,1528.4,1
553.1,1577.7,1602.4,1627,1651.7,1676.4,1701,1725.7,1750.3,1775,1799.6,1824.3,
1848.9,1873.6,1898.2,1922.9,1947.5,1972.2,1996.8,2021.5,2046.2,2070.8,2095.5,
2120.1,2144.8,2169.4,2194.1,2218.7,2243.4,2268,2292.7,2317.3,2342,2366.6,2391
.3,2415.9,2440.6,2465.3,2489.9,2514.6,2539.2,2563.9,2588.5,2613.1,2637.8,2662
.5,2687.1,2711.8,2736.4,2761.1,2785.7,2810.4,2835,2859.7,2884.3,2909,2933.6,2
958.3,2982.9,3007.6,3032.2,3056.9,3081.5,3106.2,3130.8,3155.5,3180.1,3204.8,3
229.4,3254.1,3278.7,3303.4,3328,3352.7,3377.3,3402,3426.6,3451.3,3476,3500.6,
3525.3,3549.9,3574.6,3599.2,3623.9,3648.5,3673.2,3697.8,3722.5,3747.1,3771.8,
3796.4,3821.1,3845.7,3871.1,3895.9,3920.6,3945.2,3969.9,3994.5,4019.2,4043.8,
4068.5,4093.1,4117.8,4142.4,4167.1,4191.7,4216.4,4241,4265.7,4290.3,4315,4339
.7,4364.3,4389,4413.6,4438.3,4462.9,4487.6,4512.2,4536.9,4561.5,4586.2,4610.8
,4635.5,4660.1,4684.8,4709.4,4734.1,4758.7,4783.4,4808,4832.7,4857.3,4882,490
6.6,4931.3,4955.9,4980.6,5005.2,5029.9,5054.5,5079.2,5103.8,5128.5,5153.1,517
7.8,5202.4,5227.1,5252.7,5277.4,5302.1,5326.7,5351.4,5376,5400.7,5425.3,5450,
5474.6,5499.3,5523.9,5548.6,5573.2,5597.9,5622.5,5647.2,5671.8,5696.5,5721.1,
5745.8,5770.5,5795.1,5819.8,5844.4,5869,5893.7,5918.4,5943,5967.7,5992.3,6017
,6041.6,6066.3,6090.9,6115.6,6140.3,6164.9,6189.6,6214.2,6238.9,6263.5,6288.2
,6312.8,6337.5,6362.1,6386.8,6411.4,6436.1,6460.7,6485.4,6510,6534.7,6559.3,6
584,6608.6,6633.3,6657.9,6682.6,6707.2,6731.9,6756.5,6781.2,6805.8,6830.5,685
5.1,6879.8,6904.4,6929.1,6953.7,6978.4,7003,7027.7,7052.4,7077,7101.7,7126.3,
```

```
7151,7175.6,7200.3,7224.9,7249.6,7274.3,7298.9,7323.6,7348.2,7372.9,7397.5,74
22.2,7446.8,7471.5,7496.1,7520.8,7545.4,7570.1,7594.7,7619.4,7644,7668.7,7693
.3,7718,7742.7,7767.3,7792,7816.6,7841.3,7865.9,7890.6,7915.2,7939.9,7964.5,7
989.2,8013.8,8038.5,8063.2,8087.8,8112.5,8137.1,8161.8,8186.4,8211.1,8235.7,8
260.4,8285,8309.7,8334.3,8359,8383.6,8408.3,8433,8457.6,8482.3,8506.9,8531.6,
8556.2,8580.9)
```

```
> P <-
```

```
c(0,0.47,0.88,1.24,1.6,1.94,2.29,2.67,2.98,3.35,3.64,3.93,4.22,4.58,4.84,5.17
,5.42,5.68,6.13,6.3,6.59,6.8,7.13,7.37,7.62,7.9,8.09,8.29,8.55,8.78,9.11,9.25
,9.48,9.68,9.88,10.11,10.33,10.53,10.74,10.86,11.22,11.45,11.59,11.85,12.03,1
2.18,12.48,12.73,12.98,13.21,13.4,13.62,13.83,14.24,14.27,14.61,14.85,15.06,1
5.33,15.65,15.94,16.09,16.27,16.72,17.02,17.1,17.52,17.65,17.97,18.17,18.31,1
8.96,18.89,19.3,19.41,19.81,19.95,20.28,20.41,20.83,21.05,21.45,21.51,21.81,2
1.94,22.35,22.54,22.95,23.07,23.49,23.69,24.02,24.27,24.42,24.69,24.93,25.19,
25.63,25.74,25.89,26.23,26.65,26.96,26.99,27.41,27.37,27.74,28.09,28.44,28.71
,28.87,29.12,29.43,29.8,29.85,30.19,30.55,30.51,30.94,31.09,31.25,31.74,31.85
,31.87,32.41,32.73,33.01,33.18,33.65,33.63,33.72,34.1,34.49,34.53,34.9,34.73,
35.42,35.61,35.61,36.33,36.27,36.65,36.76,37.48,37.15,37.63,38.23,37.75,38.21
,38.76,38.63,38.71,39.17,39.74,39.99,39.79,40.24,40.51,40.43,40.89,40.79,41.2
3,41.64,41.97,42.03,42.43,42.57,42.74,42.72,43.46,43.41,43.86,43.69,44.3,44.5
,44.68,44.81,45.11,45.32,45.35,45.71,46.12,46.3,46.39,47.03,46.82,47.34,47.3,
47.39,47.79,48,48.17,48.71,48.49,49.04,49.61,49.27,49.54,49.84,49.77,50.17,50
.52,50.88,50.66,51,51.15,51.55,51.34,51.55,52.03,52.41,52.56,52.57,52.93,53.0
8,53.55,53.4,53.67,54.09,54.4,54.58,54.49,54.55,54.9,55.55,55.33,56.22,55.6,5
5.85,56.04,56.41,56.55,56.61,56.9,56.91,56.87,57.88,57.22,58.2,57.86,58.12,58
.45,58.74,58.86,59.32,59.19,59.45,59.77,59.83,59.81,60.37,60.25,60.68,60.52,6
0.74,61.3,61.38,61.67,61.37,61.65,61.94,61.9,62.22,62.25,62.12,62.82,62.77,62
.67,63.39,62.95,63.82,63.29,64.73,64.23,63.67,64.33,64.24,64.79,64.78,65.18,6
4.94,65.27,65.1,65.75,66.16,65.46,65.89,66.38,66.22,66.31,66.92,66.03,66.86,6
7.17,66.75,67.18,67.75,67.86,67.86,67.67,67.85,67.99,68.2,68.87,68.49,69.44,6
8.63,69.36,69.09,69.15,69.23,70.11,69.39,70.37,70.12,70.35,70.52,70.54,70.05,
71.02,70.95,71.4,70.58,71.89,71.67,71.82,71.72,71.67,72.16,72.35,72.16,72.39,
72.61,72.73,72.7,73.02,73.2,73,73.42,73.58,73.47,73.78,73.81,73.55,74.17,74.5
3,73.98,74.13,74.77)
```

```
> s <- 93
```

```
> IntMM <- nlsLM(P~s-K*lambertW0(s/K*exp((s-
V*t)/K)),start=list(K=200,V=0.25)) #guess values for Km and Vmax, note So is
assumed equivalent to Pinfinity
```

```
> summary(IntMM)
```

```
Formula: P ~ s - K * lambertW0(s/K * exp((s - V * t)/K))
```

```
Parameters:
```

	Estimate	Std. Error	t value	Pr(> t)
K	3.892e+01	6.758e-01	57.59	<2e-16 ***
V	1.618e-02	1.116e-04	145.06	<2e-16 ***

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.4439 on 347 degrees of freedom
```

```
Number of iterations to convergence: 16
```

```
Achieved convergence tolerance: 1.49e-08
```

References

- (1) Jiang, Y.; Wu, N.; Wu, H.; He, M. An efficient and mild CuI/L-proline-catalyzed arylation of acetylacetone or ethyl cyanoacetate. *Synlett* **2005**, 2731-2734.
- (2) Arnaud, N.; Georges, J. Improved detection of salicylic acids using terbium-sensitized luminescence in aqueous micellar solutions of cetyltrimethylammonium chloride. *Analyst* **1999**, *124*, 1075-1078.
- (3) Bebawy, L. I.; El Kelani, K.; Abdel Fattah, L. Fluorimetric determination of some antibiotics in raw material and dosage forms through ternary complex formation with terbium (Tb^{3+}). *J. Pharm. Biomed. Anal.* **2003**, *32*, 1219-1225.