

SUPPLEMENTARY MATERIALS

Characterization of *P. falciparum* dipeptidyl aminopeptidase 3 specificity reveals structural factors responsible for differences in amino acid preferences between peptide-based substrates and covalent inhibitors.

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Supplementary Results

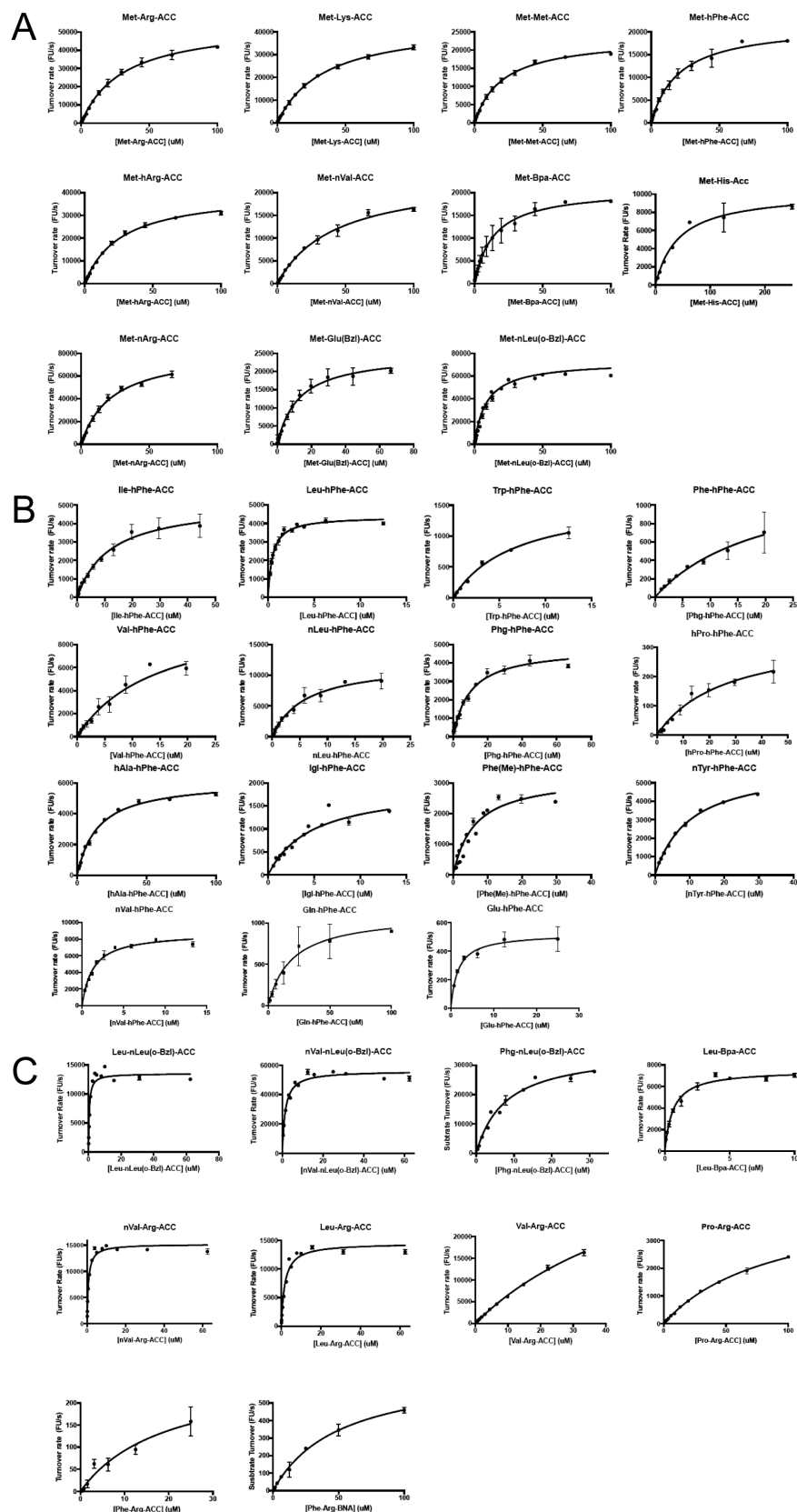


Figure S1. Michaelis-Menten fits for rDPAP3.

The turnover rate of the indicated substrates was measured at different concentrations and 1 nM rDPAP3 in assay buffer. Data was fitted in Prism to a Michaelis-Menten model. K_m , k_{cat} , and k_{cat}/K_m values are reported in Table 1. (A) Substrates with P2 Met. (B) Substrates with P1 hPhe. (C) Substrates with selected P1 and P2 residues.

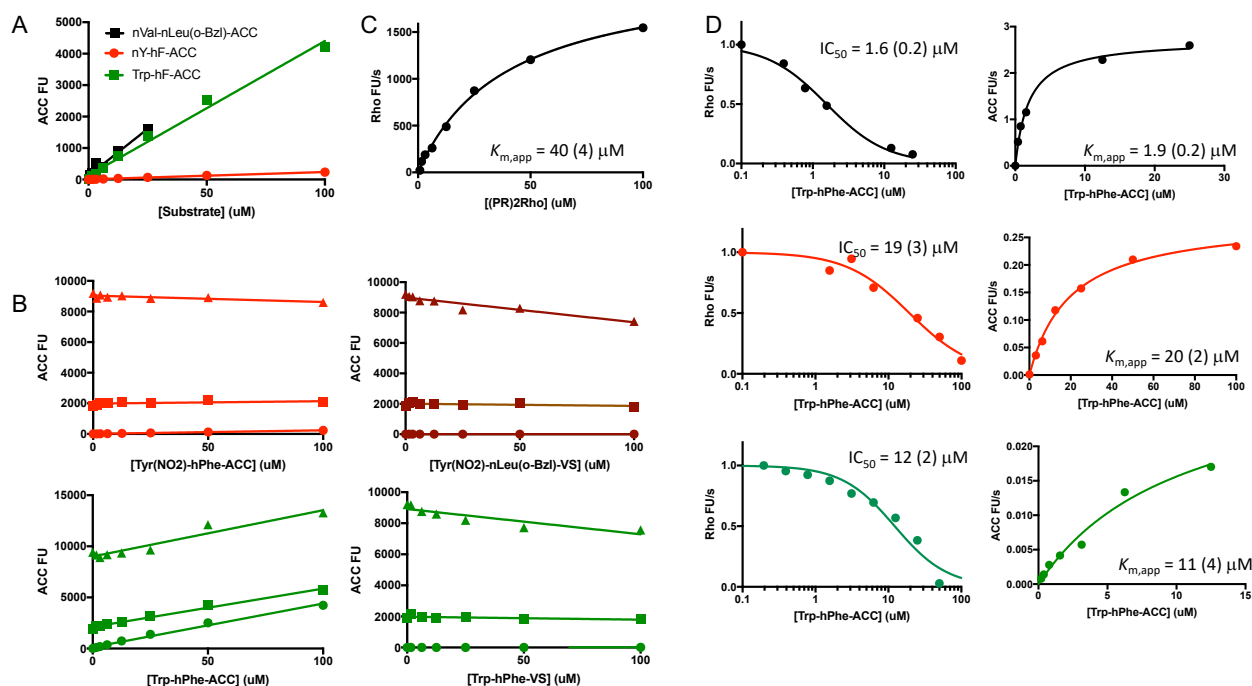


Figure S2. Substrate quenching and substrate competition studies. (A) Background fluorescence signal of selected substrates measured in assay buffer. Note that Tyr(NO₂)-hPhe-ACC shows a very low level of background fluorescence compared to Trp-hPhe-ACC or nVal-nLeu(o-Bzl)-ACC (Fig. S2A) likely indicating intramolecular quenching between ACC and Tyr(NO₂). (B) ACC fluorescence signal measured at increasing concentrations of the indicated substrates and inhibitors in the presence at 0 (circles), 1 (squares) or 5 (triangles) μM of free ACC. (C) (PR)₂Rho turnover by rDPAP3. (D) Turnover rates of (PR)₂Rho (left graphs) and ACC substrates (right graphs) measured at 40 μM of (PR)₂Rho and increasing concentrations of ACC substrates. $K_{m,app}$ and IC_{50} values are indicated in each graph.

Figure S3. Irreversible inhibition fits. Attached as a separate file. Substrate turnover by rDPAP3 (left row), DPAP1 (middle row) or CatC (right row) was measured for at least 40 min at different inhibitor concentrations. The reaction progress curves (FU vs time) at each inhibitor concentration were fitted to Eq. 5 to obtain k_{obs} values (Top graphs). These values were then fitted to Eqs. 6 and 7 as a function of inhibitor concentration to obtain k_{inact} , K_i , and k_{inact}/K_i . The fits for the vinyl sulfone library (Table 2) are shown the first 36 pages, and those for the compounds listed in Table 3 the pages 37-51.

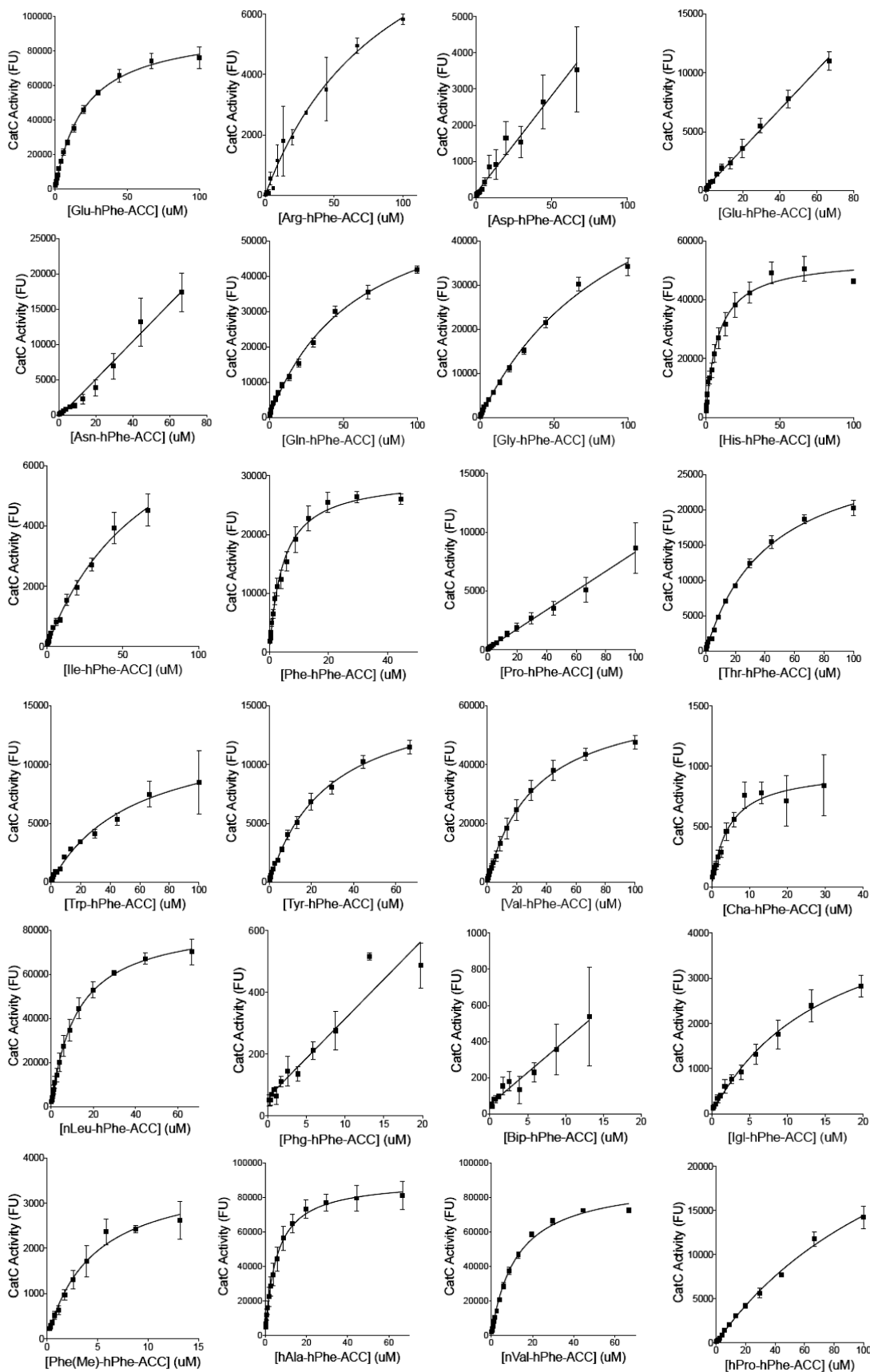


Figure S4. Michaelis Menten fits for CatC. The turnover rate of the indicate substrates was measured at different concentrations and 1 nM CatC in assay buffer. Data was fitted in Prism to a Michaelis Menten model. K_m , k_{cat} , and k_{cat}/K_m values are reported in Table S1.

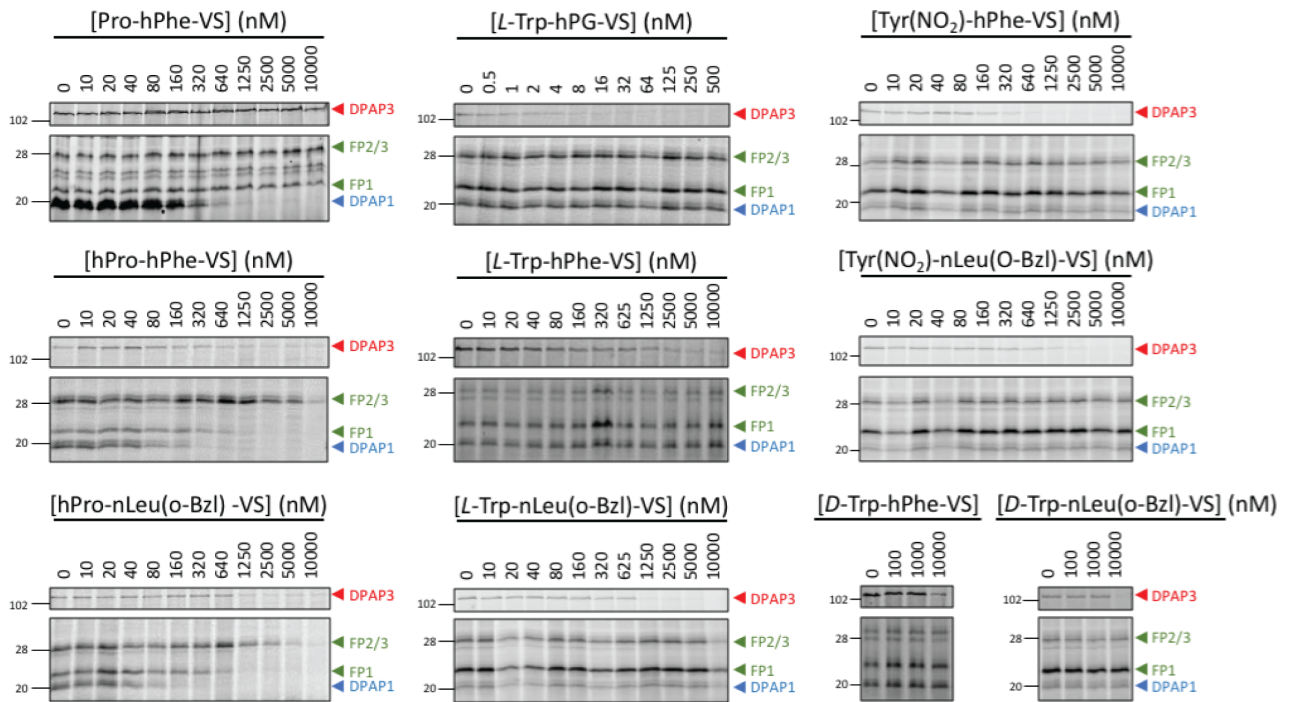


Figure S5. Selective inhibition of DPAP1 or DPAP3 in live parasites. Intact mature schizonts were pre-treated for 30 min with a dose response of inhibitor followed by 1 h treatment with FY01 to label the residual level of DPAPs and FPs activities. Fluorescent bands corresponding to the different cysteine proteases labelled by the probe are indicated by arrows. Dose response curves are shown in Fig. 4 and the IC₅₀ values for each protease and inhibitor reported in Table 4.

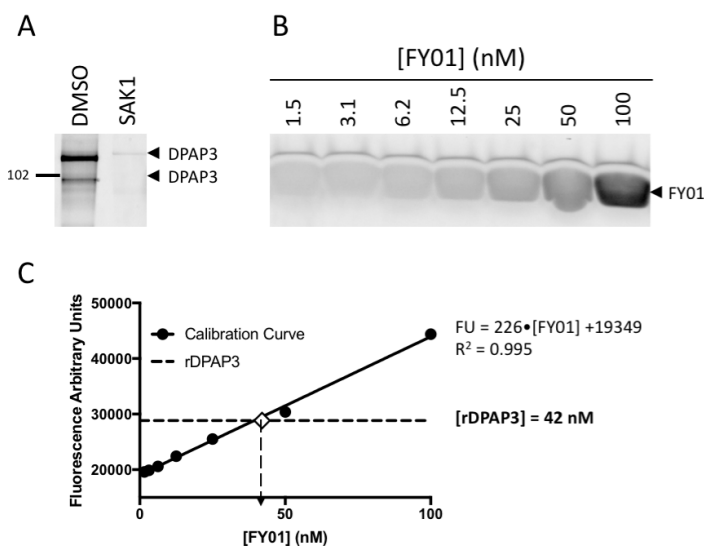


Figure S6. Active site titration of rDPAP3 using activity-based probes.

(A) Labelling of purified rDPAP3 by FY01 in the presence or absence of SAK1. Our stock of rDPAP3 was diluted 20-fold in assay buffer, treated with DMSO or 1 μ M SAK1 for 30 min, and labelled with FY01 for 1 h. Samples were run on an SDS-PAGE gel and DPAP3 labelling measured using a flatbed fluorescence scanner. (B) Calibration curve of free probe measured on the same SDS-PAGE gel as the one shown in A. (C) The fluorescence signal for rDPAP3 labelling and free probe was quantified using ImageJ, and the concentration of labelled rDPAP3 calculated based on the calibration curve.

Supplementary Materials and Methods

Synthesis of H₂N-P2-P1-ACC fluorescent substrates

To 1g (0.74mmol) of Fmoc-protected Rink Amide resin (Iris Biotech GmbH, Germany) was added to a glass solid-phase reaction vessel. Next, 5 mL of dichloromethane (DCM) was added and the resin was gently stirred once per 10 minutes for 1 h, then filtered and washed three times with *N,N*-dimethylformamide (DMF). Fmoc protecting group was removed using 20% piperidine in DMF (in three cycles: 5 min, 5 min, and 25 min), filtered each time and rinsed with DMF (six times). A ninhydrin test was performed to confirm resin Fmoc de-protection. Next, 2.5 eq of Fmoc-ACC-OH (1.85 mmol, 816 mg) was pre-activated with 2.5 eq of HOBt (1.85 mmol, 278 mg) and 2.5 eq of DICl (1.85 mmol, 242 μ l) in DMF for 3 min and the mixture was added to the resin. Reaction was gently agitated for 24 h at room temperature. Next, resin was washed five times with DMF and reaction was repeated using 1.5 eq of above reagents to improve the yield of ACC coupling. After 24h of gentle stirring, resin was washed with DMF and Fmoc protecting group was removed from ACC with the use of 20% piperidine in DMF (5 min, 5 min, and 25 min), filtered and washed with DMF (six times). Resin was subsequently washed with DCM (3 times) and MeOH (3 times), dried over P₂O₅ and divided into eight equal portions (0.09 mmol per portion). Each portion of the H₂N-ACC-resin was placed in to the wells of semiautomatic FlexChem solid phase synthesizer cartridge (SciGene, USA). Then, to each well 2.5 eq of Fmoc-P1-OH (0.225 mmol) with 2.5 eq of HATU (0.225 mmol, 86 g), and 2.5 eq of 2,4,6-collidine (0.225 mmol, 30 μ l) in DMF were added. Reactions were carried out for 24 h with gentle agitation of reaction cartridge, followed by washing the resin five times with DMF. P1 coupling reactions were repeated using 1.5 eq of above reagents. P1 Fmoc protecting group was removed from each substrate using 20% piperidine in DMF (5 min, 5 min, and 25 min), and the resin was washed six times with DMF. A ninhydrin test was performed to confirm P1 Fmoc de-protection. Next, 2.5 eq Fmoc-P2-OH (0.225 mmol) was pre-activated with 2.5 eq of HOBt (0.225 mmol, 34 mg) and 2.5 eq of DICl (0.225 mmol, 30 μ l) in DMF, added to the cartridge wells containing 1 eq of H₂N-P1-ACC-resin and gentle agitated for 3 hours. A ninhydrin test confirmed the complete P2 coupling. Next, resin was filtered and washed with DMF (six times). Fmoc-protecting group was removed using 20% piperidine in DMF (5 min, 5 min, and 25 min), followed by washing the resin six time with DMF and performing a ninhydrin test. Next, the HN2-P2-P1-ACC-resin product was washed with DMF (six times), DCM (three times) and MeOH (three times), dried over P₂O₅ and cleaved from the resin with a mixture of TFA : TIPS : H₂O (v/v/v 95/2.5/2.5). The crude product was purified by HPLC (Waters system), lyophilized and dissolved in DMSO to a final concentration of 20mM. Each substrate was analyzed by analytical HPLC and High Resolution Mass Spectrometry.

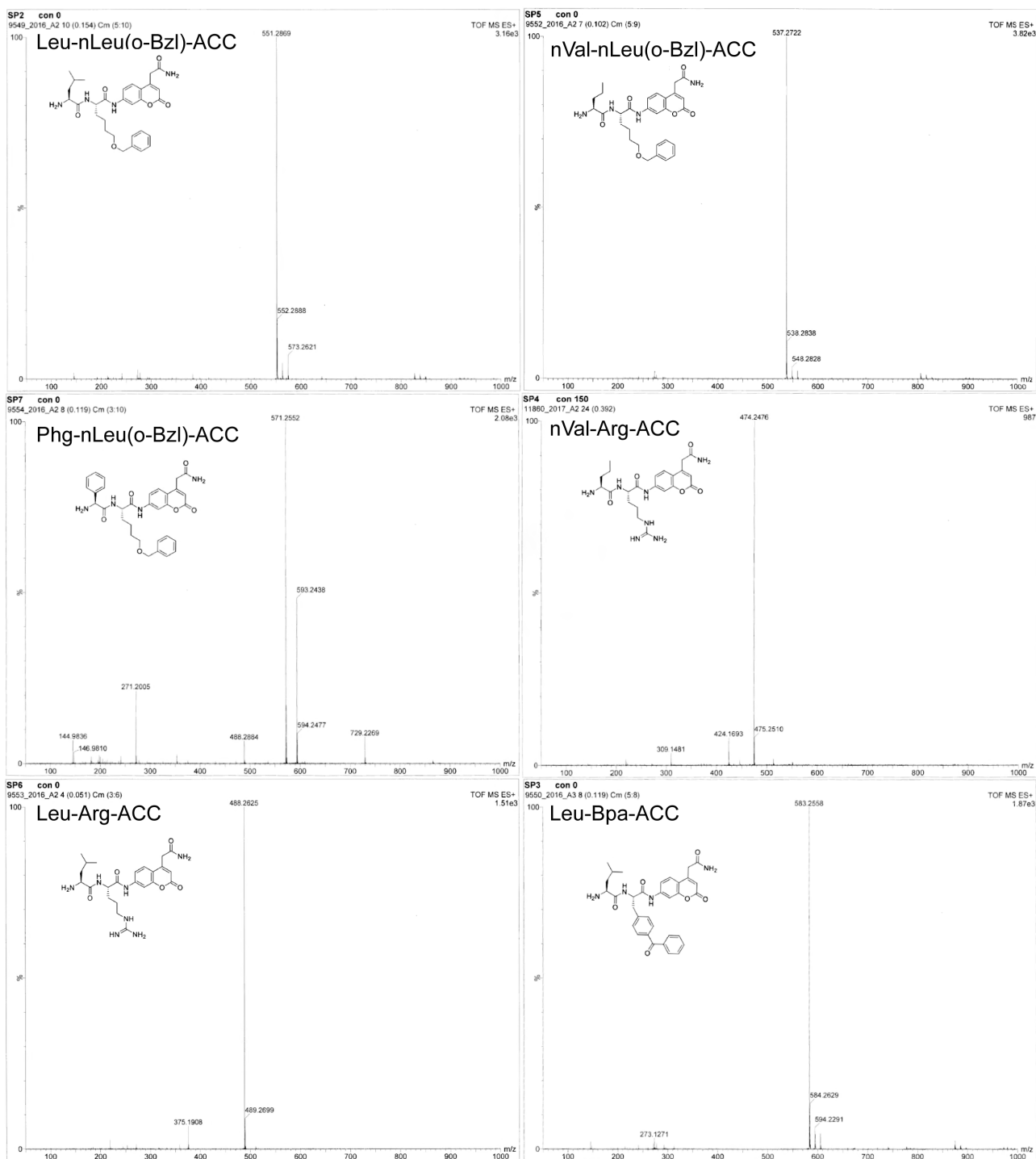


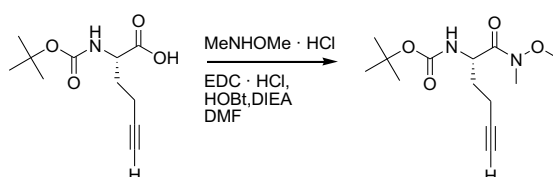
Figure S7. High resolution mass spectra of DPAP fluorogenic substrates.

Synthesis of vinyl sulfone inhibitors.

The synthesis of vinyl sulfone inhibitors with natural and unnatural amino acids in the position P1 and P2, is based on a peptide coupling between the activated carboxylic acid of amino acid in the P2, and the unprotected amine of the amino acid P1 bearing the vinyl sulfone. The coupling reaction is followed by the removal of the Boc protecting group of P2 to afford the desired inhibitor. The

synthesis of the vinyl sulfone was done following previously reported methods^{19,24}. Briefly, the carboxylic acid of the amino acid in P1 is coupled with N,O-Dimethylhydroxylamine to form a Weinreb-Nahm amide. The amide is reduced to the aldehyde with LiAlH₄. With the carbonyl at hand, in Horner-Wadsworth-Emmons (HWE) type reaction with diethyl phenylsulfonylmethylphosphonate, the ε-alkene is formed, which is the nucleophilic trap for the active Cys residue of the protease. The tert-butyloxycarbonyl protecting group is removed in mild acid conditions, and coupled with the activated carboxylic acid as indicated above.

tert-Butyl (S)-1-(N-methoxy-N-methylcarbamoyl)pent-4-ynylcarbamate Lm1msed13



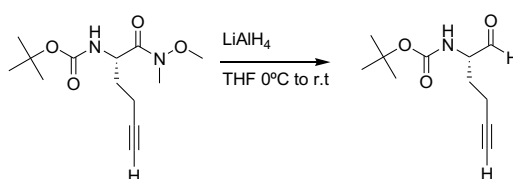
To a solution of (S)-2-(Boc-amino)-5-hexynoic acid (0.6 g, 2.64 mmol) in dry DMF (14 mL) at 0 °C was added EDC (607 mg, 3.17 mmol), HOBT (427 mg, 3.17 mmol), N,O-dimethylhydroxylamine hydrochloride (308 mg, 3.17 mmol) and DIPEA (0.7 mL, 7.92 mmol). The reaction was stirred at rt for 12 h. The resulting solution was evaporated in vacuo to give a light yellow oil, which was diluted with aqueous NH₄Cl (10 wt%) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (silica gel; using 20% EtOAc in hexanes) provided the protected product *tert*-butyl (S)-1-(N-methoxy-N-methylcarbamoyl)pent-4-ynylcarbamate as a white foam (0.64 g, 2.37 mmol, 90%).

¹H NMR (DCCl₃, δ): 1.41 (s, 9H), 1.72 (td, J = 20.9, 6.9 Hz, 2H), 1.96 (t, J = 2.6 Hz, 1H), 2.27 (dt, J = 7.2, 7.0, 2.5 Hz, 2H), 3.19 (s, 3H), 3.76 (s, 3H), 4.73 (dt, J = 8.7, 4.4 Hz, 1H), 5.21 (d, J = 8.1 Hz, 1H).

¹³C NMR (DCCl₃, δ): 15.0 (CH₂), 28.3 (CH₃), 32.0 (CH₂), 32.2 (CH₃), 49.8 (CH), 61.6 (CH₃), 68.7 (CH), 79.7 (C), 83.2 (C), 155.4 (C), 172.4 (C).

ESI-MS: [M+H]⁺ calcd. for C₁₃H₂₂N₂O₄Na = 293.1772 found 293.1478. (M.W. 270.3248)

tert-Butyl (S)-1-formylpent-4-ynylcarbamate Lm1msed14



To a solution of *tert*-Butyl (S)-1-(N-methoxy-N-methylcarbamoyl)pent-4-ynylcarbamate (0.62 g, 2.3 mmol) in dry THF (23 mL) at 0 °C was added LiAlH₄ (0.1 g, 2.76 mmol) over 10 min, with vigorous

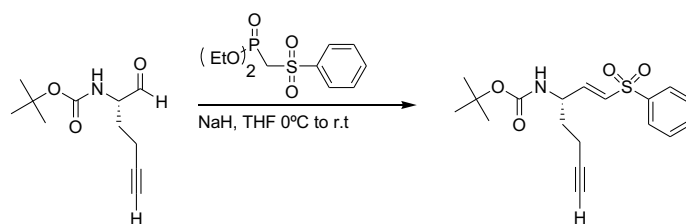
stirring. The mixture was stirred for an additional 20 min at 0 °C, whereupon cold water was carefully added until effervescence ceased. A cold HCl solution (1 M) was added to break up the gelatinous emulsion until pH 6~7. Upon dilution with H₂O (150 mL) and extraction with EtOAc (3 x 75 mL), the combined organic extracts dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (silica gel; using 30% EtOAc in hexanes) provided the product *tert*-Butyl (S)-1-formylpent-4-ynylcarbamate as a white solid (0.44 g, 2.08, 90%).

¹H NMR (*DCCl*₃ δ): 1.44 (s, 9H), 1.85 (dt, *J* = 14.2, 14.0, 7.1 Hz, 2H), 2.01 (t, *J* = 2.6 Hz, 1H), 2.32 (dt, *J* = 6.7, 2.5 Hz, 2H), 4.29 (bs, 1H), 5.21 (bs, 1H), 9.64 (s, 1H).

¹³C NMR (*DCCl*₃ δ): 14.5 (CH₂), 27.9 (CH₂), 28.1 (CH₃), 59.0 (CH), 70.0 (CH), 80.2 (C), 82.5 (C), 155.3 (C), 199.0 (CH)

ESI-MS: [M+H]⁺ calcd. for C₁₁H₁₇NO₃Na = 234.1101 found 234.1105. (M.W. 211.2576)

tert-Butyl (S,E)-1-(phenylsulfonyl)hept-1-en-6-yn-3-ylcarbamate



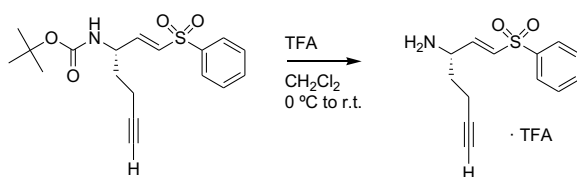
To a cooled (0°C) suspension of hexane-washed NaH (60% in mineral oil; 0.1 g, 2.38 mmol) in dry THF (10 mL) was added drop-wise diethyl[benzenesulfonyl]methylphosphonate (0.64 g, 2.18 mmol) in dry THF (10 mL) via syringe. The mixture was stirred for an additional 30 min at 0 °C and *tert*-butyl (S)-1-formylpent-4-ynylcarbamate (0.42 g, 2.0 mmol) in dry THF (10 mL) was added drop-wise. The stirring was continued for 1 h, before a cold 10 wt% NH₄Cl solution was added to break up the gelatinous emulsion until pH 6~7. The solution was concentrated in vacuo, diluted with water (100 mL) and extracted with EtOAc (3 x 75 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated under vacuum. Purification by flash column chromatography (silica gel; using 40% EtOAc in hexanes) provided the product *tert*-butyl (S,E)-1-(phenylsulfonyl)hept-1-en-6-yn-3-ylcarbamate as a white foam (0.46 g, 1.30 mmol, 65%).

¹H NMR (*DCCl*₃ δ): 1.37 (s, 9H), 1.80 (ddd, *J* = 21.5, 13.8, 6.9 Hz, 2H), 2.00 (t, *J* = 2.6 Hz, 1H), 2.27 (dt, *J* = 6.9, 2.5 Hz, 2H), 4.47 (bs, 1H), 4.69 (d, *J* = 8.6 Hz, 1H), 6.46 (dd, *J* = 15.0, 1.5 Hz, 1H), 6.89 (dd, *J* = 15.0, 5.0 Hz, 1H), 7.50-7.61 (m, 3H) 7.87 (d, *J* = 7.0 Hz, 2H).

¹³C NMR (*DCCl*₃ δ): 15.1 (CH₂), 28.2 (CH₃), 32.7 (CH₂), 50.4 (CH), 69.9 (CH), 80.2 (C), 82.4 (C), 127.6 (CH), 129.2 (CH), 130.9 (CH), 133.4 (CH), 140.1 (C), 145.5 (CH), 154.8 (C).

ESI-MS: [M+H]⁺ calcd. for C₁₈H₂₃NO₄Na = 372.1240 found 372.1254. (M.W. 349.4445)

(S,E)-1-(phenylsulfonyl)hept-1-en-6-yn-3-amine

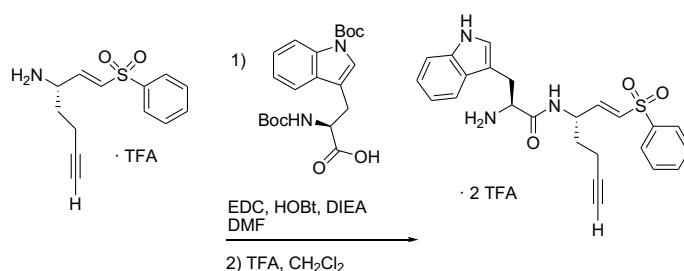


$^1\text{H NMR}$ ($\text{MeOD}-d_4$ δ): 1.90-2.07 (m, 2H), 2.20-2.31 (m, 2H), 2.40 (t, $J = 2.6$ Hz, 1H), 4.14 (dt, $J = 7.9, 6.0$ Hz, 1H), 6.88 (dd, $J = 15.2, 7.4$ Hz, 1H), 7.02 (d, $J = 15.2$ Hz, 1H), 7.61-7.74 (m, 3H), 7.92-7.95 (m, 2H).

$^{13}\text{C NMR}$ ($\text{MeOD}-d_4$ δ): 15.3 (CH_2), 32.1 (CH_2), 51.6 (CH), 72.0 (CH), 82.1 (C), 129.1 (CH), 130.8 (CH), 135.3 (CH), 137.1 (CH), 139.9 (CH), 140.8 (C), 162 (q, C, TFA).

ESI-MS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{13}\text{H}_{16}\text{NO}_2\text{S} = 250.0896$ found 250.0890. (M.W. 363.3520)

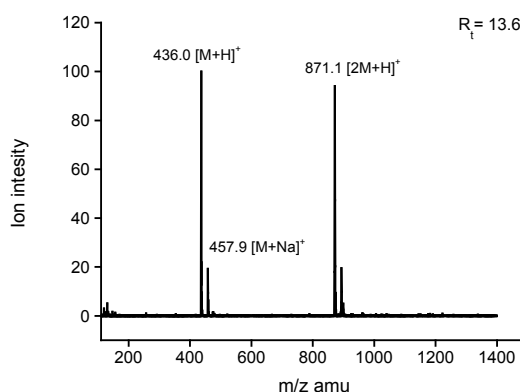
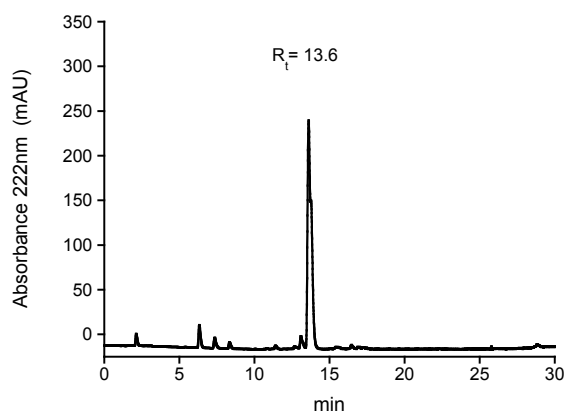
(2S)-2-Amino-3-(1H-indol-3-yl)-N-((S,E)-1-(phenylsulfonyl)hept-1-en-6-yn-3-yl)propanamide (Trp-hPG-VS)



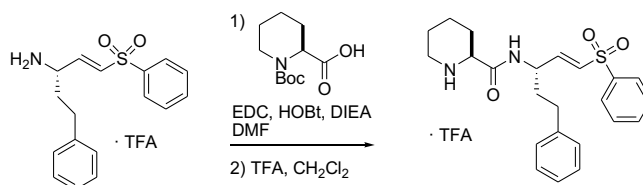
$^1\text{H NMR}$ (MeOD_4 δ): 1.73-1.86 (m, 2H), 2.21 (dt, $J = 7.2, 2.6$ Hz, 2H), 2.28 (bs, 1H), 3.20-3.35 (m, 2H, partial overlapping with MeOD signal), 4.12 (dd, $J = 7.80, 7.15$ Hz, 1H), 4.70 (dd, $J = 13.3, 4.8$ Hz, 1H), 6.15 (dd, $J = 15.1, 1.5$ Hz, 1H), 6.78 (dd, $J = 15.1, 5.4$ Hz, 1H), 7.05-7.08 (m, 1H), 7.14 (bs, 2H), 7.43 (d, $J = 8.2$ Hz, 1H), 7.57-7.64 (m, 3H), 7.67-7.70 (m, 1H), 7.84 (dd, $J = 8.4, 1.2$ Hz, 2H).

$^{13}\text{C NMR}$ (MeOD_4 δ): 15.2 (CH_2), 28.7 (CH_2), 32.6 (CH_2), 50.2 (CH), 55.1 (CH), 70.7 (CH), 83.3 (C), 108.0 (C), 122.4 (CH), 118.8 (CH), 120.1 (CH), 122.7 (CH), 125.2 (CH), 128.3 (C), 128.5 (CH), 130.3 (CH), 132.2 (CH), 134.6 (CH), 138.2 (C), 141.6 (C), 145.9 (CH), 169.9 (C).

ESI-MS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{24}\text{H}_{26}\text{N}_3\text{O}_3\text{S} = 436.1689$ found 436.1690. (M.W. 663.5853).



**(2S)-N-((S,E)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)piperidine-2-carboxamide
Lm1msed42 (hPro-hPhe-VS)**



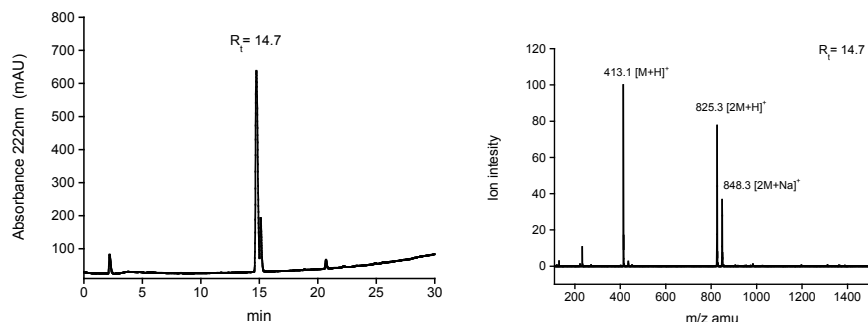
To a solution of (S)-N-Boc-Piperidine-2-carboxylic acid (211 mg, 0.92 mmol) in DMF (3 mL) was added EDC/HCl (177 mg, 0.92 mmol), HOBT (125 mg, 0.92 mmol) and DIEA (0.6 mL, 3.1 mmol). After 10 min, (S)-3-benzenesulfonyl-1-phenethylallylamine trifluoroacetate (TFA·HphVSPH, X) (320 mg, 0.77 mmol) in DMF (3 mL) was added drop-wise. The reaction was stirred at rt for 12 h. The resulting solution was evaporated in vacuo to give a light yellow oil, which was diluted with aqueous NH₄Cl (10 wt%) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (silica gel; using 40% EtOAc in hexanes) provided the protected product as a white foam.

To a solution of this intermediate in CH₂Cl₂ (5 mL) at 0 °C was added trifluoroacetic acid (5 mL). The solution was stirred at room temperature overnight, then evaporated to dryness in vacuo and finally freeze-dried yielding a white solid that was identified as the trifluoroacetic salt of the desired product (216 mg, 0.41 mmol, 52%).

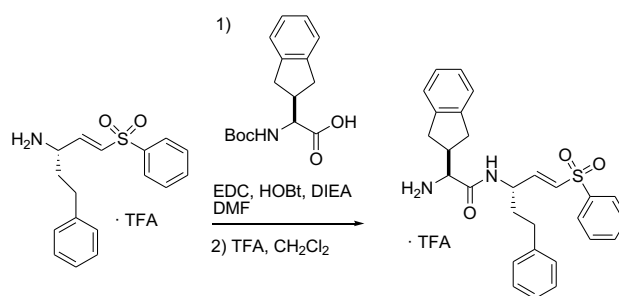
¹H NMR (*MeOD-d₄* δ): 1.59-1.69 (m, 3H), 1.84-1.99 (m, 4H), 2.19 (d, *J* = 9.5 Hz, 1H), 2.57-2.67 (m, 2H), 3.01 (t, *J* = 10.9 Hz, 1H), 3.38 (d, *J* = 12.6 Hz, 1H), 3.82 (dd, *J* = 11.1, 2.8 Hz, 1H), 4.57 (dd, *J* = 13.9, 5.7 Hz, 1H), 6.56 (d, *J* = 15.2 Hz, 1H), 6.91 (dd, *J* = 15.1, 5.9 Hz, 1H), 7.14-7.18 (m, 3H), 7.22-7.27 (m, 2H), 7.58-7.62 (m, 2H), 7.67-7.71 (m, 1H), 7.88 (d, *J* = 7.6 Hz, 2H).

¹³C NMR (*MeOD-d₄* δ): 22.8 (CH₂), 23.1 (CH₂), 28.7 (CH₂), 33.0 (CH₂), 36.2 (CH₂), 44.9 (CH₂), 50.8 (CH), 59.0 (CH), 127.3 (CH), 128.7 (CH), 129.5 (CH), 129.6 (CH), 130.7 (CH), 132.4 (CH), 134.9 (CH), 141.7 (C), 141.9 (C), 146.8 (CH), 169.8 (C).

ESI-MS: [M+H]⁺ calcd. for C₂₃H₂₉N₂O₃S = 413.1893 found 413.1896. (M.W. 526.5684)



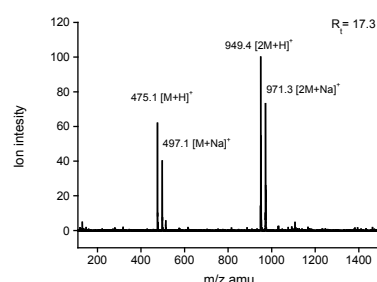
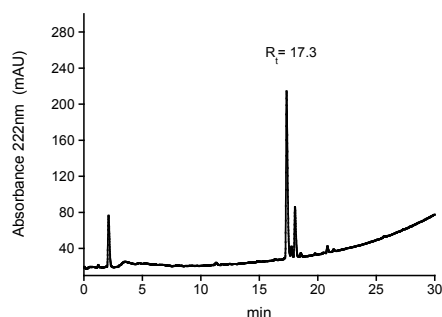
(2S)-2-amino-2-(2,3-dihydro-1H-inden-2-yl)-N-((S,E)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)acetamide Lm1msed44 (Igl-hPhe-VS)



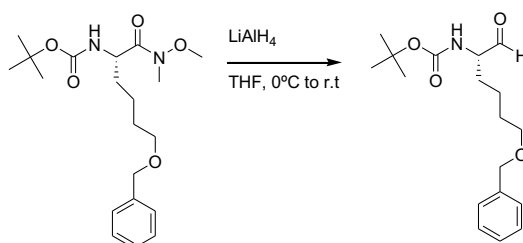
$^1\text{H NMR}$ ($\text{MeOD-}d_4$ δ): 1.96 (td, $J = 15.3, 7.5$ Hz, 2H), 2.66 (dd, $J = 15.6, 8.3$ Hz, 2H), 2.88 (t, $J = 5.60$ Hz, 4H), 3.00-3.06 (m, 1H), 3.95 (dd, $J = 5.4, 1.2$ Hz, 1H), 4.60 (q, $J = 6.8$ Hz, 1H), 6.64 (d, $J = 15.2$ Hz, 1H), 6.89 (dd, $J = 15.1, 6.6$ Hz, 1H), 7.16 (bs, 7H), 7.24 (d, $J = 7.1$ Hz, 2H), 7.54-7.58 (m, 2H), 7.62-7.68 (m, 1H), 7.85 (d, $J = 7.68$ Hz, 2H).

$^{13}\text{C NMR}$ ($\text{MeOD-}d_4$ δ): 32.9 (CH_2), 36.2 (CH_2), 36.3 (CH_2), 36.4 (CH_2), 43.1 (CH), 51.2 (CH), 57.8 (CH), 125.5 (CH), 127.3 (CH), 128.0 (CH), 128.7 (CH), 129.5 (CH), 129.6 (CH), 130.7 (CH), 133.2 (CH), 134.9 (CH), 141.6 (C), 142.0 (C), 142.1 (C), 142.2 (C), 146.0 (CH), 169.2 (C).

ESI-MS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{28}\text{H}_{31}\text{N}_2\text{O}_3\text{S} = 475.2050$ found 475.2049. (M.W. 588.6377)



***tert*-Butyl (S)-5-(benzyloxy)-1-formylpentylcarbamate Lm1msed46**



To a solution of *tert*-butyl (S)-1-(N-methoxy-N-methylcarbamoyl)-5-(benzyloxy) pentylcarbamate (2.2 g, 5.8 mmol) in dry THF (50 mL) at 0°C was added LiAlH_4 (0.26 g, 7 mmol) over 10 min, with vigorous stirring. The mixture was stirred for an additional 20 min at 0°C , whereupon cold water

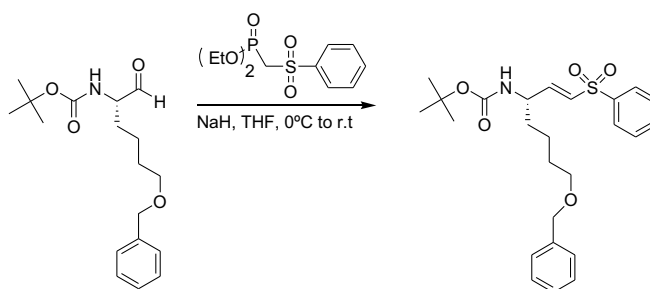
was carefully added until effervescence ceased. A cold HCl solution (1 M) was added to break up the gelatinous emulsion until pH 6~7. Upon dilution with H₂O (150 mL) and extraction with EtOAc (3 x 75 mL), the combined organic extracts dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (silica gel; using 40% EtOAc in hexanes) provided the product *tert*-Butyl (S)-5-(benzyloxy)-1-formylpentylcarbamate as a yellow oil (1.36 g, 4.23 mmol, 73%).

¹H NMR (*DCCl*₃, δ): 1.44 (s, 9H), 1.49-1.72 (m, 6H), 3.47 (t, *J* = 6.2 Hz, 2H), 4.21 (dd, *J* = 12.4, 6.3 Hz, 1H), 4.84 (s, 2H), 5.08 (d, *J* = 6.4 Hz, 1H), 7.32 (s, 5H), 9.56 (s, 1H).

¹³C NMR (*DCCl*₃): 22.0 (CH₂), 28.2 (CH₃), 28.9 (CH₂), 29.3 (CH₂), 59.7 (CH), 69.7 (CH₂), 72.9 (CH₂), 80.0 (C), 127.5 (CH), 127.6 (CH), 128.3 (CH), 138.4 (C), 155.5 (C), 199.9 (CH).

ESI-MS: [M+H]⁺ calcd. for C₁₈H₂₇N₁O₄Na = 344.1832 found 344.1832. (M.W. 321.4113)

tert-Butyl (S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-ylcarbamate Lm1msed47



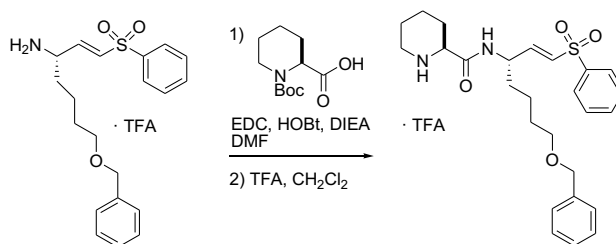
To a cooled (0°C) suspension of hexane-washed NaH (60% in mineral oil; 0.36 g, 9 mmol) in dry THF (50 mL) was added drop-wise diethyl[(benzenesulfonyl)methyl]phosphonate (2.4 g, 8.2 mmol) in dry THF (10 mL) via syringe. The mixture was stirred for an additional 30 min at 0 °C and *tert*-butyl (S)-5-(benzyloxy)-1-formylpentylcarbamate (2.30 g, 7.2 mmol) in dry THF (10 mL) was added drop-wise. The stirring was continued for 1 h, before a cold 10 wt% NH₄Cl solution was added to break up the gelatinous emulsion until pH 6~7. The solution was concentrated in vacuo, diluted with water (100 mL) and extracted with EtOAc (3 x 75 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated under vacuum. Purification by flash column chromatography (silica gel; using 60% EtOAc in hexanes) provided the product *tert*-Butyl (S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-ylcarbamate (8) as a light yellow oil (2.74 g, 6.0 mmol, 83%).

¹H NMR (*DCCl*₃, δ): 1.37 (s, 9H), 1.42-1.46 (m, 4H), 1.58-1.61 (m, 2H), 3.44 (t, *J* = 6.1 Hz, 2H), 4.34 (bs, 1H), 4.47 (s, 2H), 4.57 (d, *J* = 8.4 Hz, 1H), 6.40 (dd, *J* = 15.0, 1.2 Hz, 1H), 6.87 (dd, *J* = 15.0, 4.7 Hz, 1H), 7.32 (bs, 5H), 7.48-7.63 (m, 3H), 7.86 (d, *J* = 7.2 Hz, 1H).

¹³C NMR (*DCCl*₃): 22.4 (CH₂), 28.2 (CH₃), 29.2 (CH₂), 33.9 (CH₂), 50.9 (CH), 69.6 (CH₂), 72.9 (CH₂), 79.9 (C), 127.5 (CH), 127.6 (CH), 128.3 (CH), 129.2 (CH), 130.1 (CH), 133.4 (CH), 138.3 (C), 140.2 (C), 146.6 (CH), 154.8 (C).(*) Bidimensional experiments show that two CH share the same magnetic shift.

ESI-MS: [M+H]⁺ calcd. for C₂₀H₃₂N₂O₅Na = 482.1972 found 482.1972. (M.W. 459.5982)

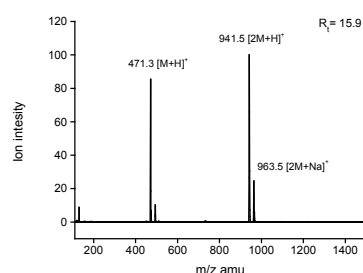
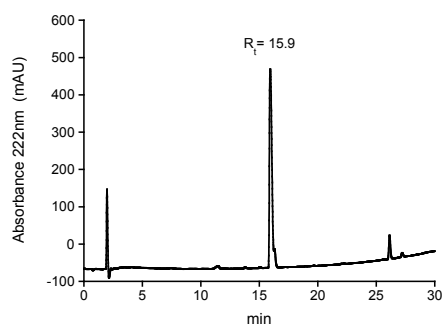
(2S)-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)piperidine-2-carboxamide
Lm1msed55 (hPro-nLeu(O-Bzl)-VS)



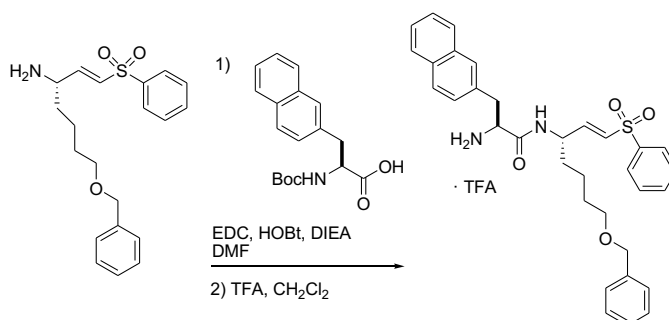
$^1\text{H NMR}$ (*MeOD-d₄* δ): 1.35-1.45 (m, 2H), 1.56-1.69 (m, 7H), 1.82-1.88 (m, 2H), 2.14 (d, $J = 9.5$ Hz, 1H), 2.97 (t, $J = 12.4$ Hz, 1H), 3.36 (d, $J = 12.9$ Hz, 1H), 3.45 (t, $J = 6.3$ Hz, 2H), 3.78 (dd, $J = 11.2, 2.9$ Hz, 1H), 4.46 (s, 2H), 4.56 (dd, $J = 13.3, 5.9$ Hz, 1H), 6.56 (d, $J = 15.2$ Hz, 1H), 6.89 (dd, $J = 15.2, 5.7$ Hz, 1H), 7.30-7.33 (bs, 5H), 7.59 (t, $J = 7.7$ Hz, 2H), 7.68 (t, $J = 7.4$ Hz, 1H), 7.87 (d, $J = 7.6$ Hz, 2H).

$^{13}\text{C NMR}$ (*MeOD-d₄* δ): 22.7 (CH_2), 22.9 (CH_2), 23.6 (CH_2), 28.6 (CH_2), 30.0 (CH_2), 33.9 (CH_2), 44.9 (CH_2), 51.2 (CH), 58.9 (CH), 70.9 (CH_2), 73.8 (CH_2), 128.6 (CH), 128.7 (CH), 128.9 (CH), 129.4 (CH), 130.6 (CH), 132.0 (CH), 134.9 (CH), 139.7 (C), 141.5 (C), 147.2 (CH), 169.8 (C).

ESI-MS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{26}\text{H}_{35}\text{N}_2\text{O}_4\text{S} = 471.2312$ found 471.2314. (M.W. 584.6481)



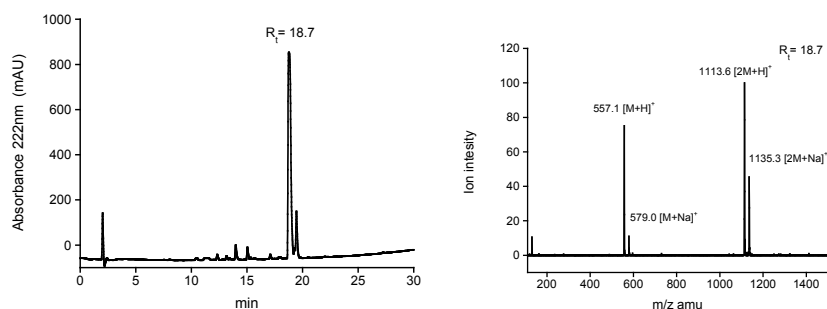
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(naphthalen-2-yl)propanamide
Lm1msed56 (2Nal-nLeu(O-Bzl)-VS)



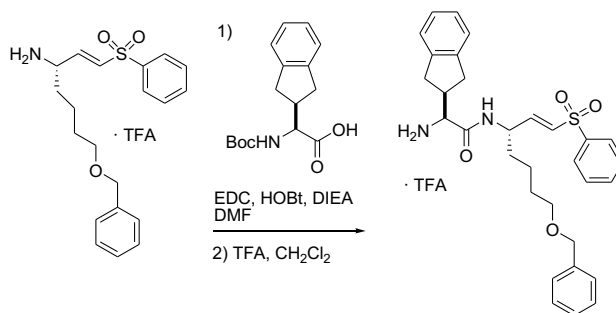
¹H NMR (MeOD-*d*₄ δ): 1.29-1.37 (m, 2H), 1.50-1.61 (m, 4H), 3.18-3.28 (m, 3H), 3.41 (t, J = 6.3 Hz, 1H), 4.15 (t, J = 7.3 Hz, 1H), 4.53 (q, J = 6.4 Hz, 1H), 4.40 (s, 2H), 6.27 (d, J = 15.2 Hz, 1H), 6.74 (dd, J = 15.2, 5.8 Hz, 1H), 7.30 (bs, 6H), 7.51 (bs, 4H), 7.63 (t, J = 7.4 Hz, 1H), 7.69-7.74 (m 3H), 7.83-7.87 (m, 3H).

¹³C NMR (MeOD-*d*₄ δ): 23.5 (CH₂), 30.2 (CH₂), 34.1 (CH₂), 38.8 (CH₂), 51.4 (CH), 55.7 (CH), 70.9 (CH₂), 73.9 (CH₂), 127.3 (CH), 127.6 (CH), 128.1 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 128.9 (CH), 129.0 (CH), 129.4 (CH), 129.6 (CH), 130.1 (CH), 130.6 (CH), 132.2 (CH), 132.9 (C), 134.3 (C), 134.8 (CH), 135.0 (C), 139.7 (C), 141.6 (C), 146.8 (CH), 169.3 (C).

ESI-MS: [M+H]⁺ calcd. for C₃₃H₃₇N₂O₄S = 557.2469 found 557.2464. (M.W. 670.7383)



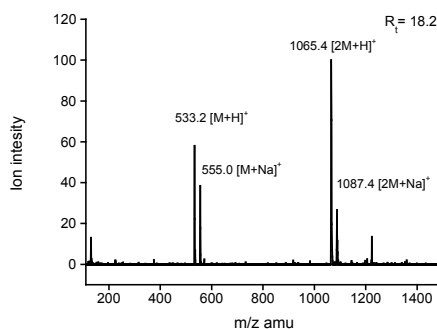
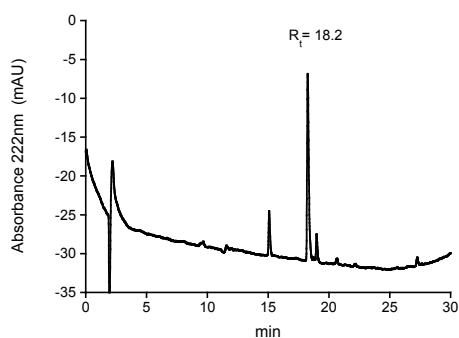
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-2-(2,3-dihydro-1H-inden-2-yl)acetamide Lm1msed57 (Igl-nLeu(O-Bzl)-VS)



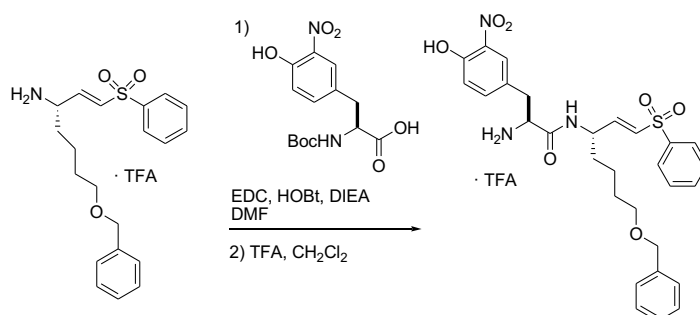
¹H NMR (MeOD-*d*₄ δ): 1.37-1.47 (m, 2H), 1.58-1.70 (m, 4H), 2.84 (dd, J = 18.7, 5.2 Hz, 4H), 2.99-3.04 (m, 1H), 3.47 (t, J = 6.2 Hz, 2H), 3.89 (d, J = 6.8 Hz, 1H), 4.47 (s, 2H), 4.58 (dd, J = 13.9, 6.8 Hz, 1H), 6.63 (d, J = 15.1 Hz, 1H), 6.86 (dd, J = 15.1, 6.5 Hz, 1H), 7.16 (bs, 4H), 7.32 (bs, 5H), 7.54 (t, J = 7.7 Hz, 2H), 7.60-7.67 (m, 1H), 7.84 (d, J = 7.8 Hz, 2H).

¹³C NMR (MeOD-*d*₄ δ): 23.7 (CH₂), 30.2 (CH₂), 34.0 (CH₂), 36.3 (CH₂), 43.1 (CH), 51.5 (CH), 57.8 (CH), 71.0 (CH₂), 73.9 (CH₂), 125.5 (CH), 128.0 (CH), 128.6 (CH), 128.7 (CH), 128.9 (CH), 129.4 (CH), 130.7 (CH), 132.9 (CH), 134.9 (CH), 139.6 (C), 142.1 (C), 142.2 (C), 146.4 (CH), 169.2 (C).

ESI-MS: [M+H]⁺ calcd. for C₃₁H₃₇N₂O₄S = 533.2469 found 533.2463. (M.W. 646.7169)



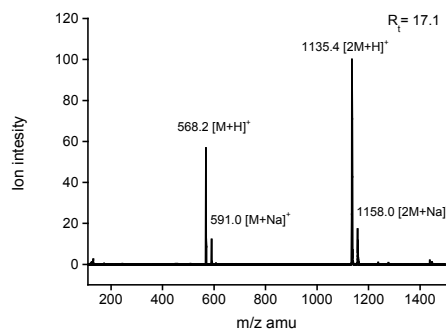
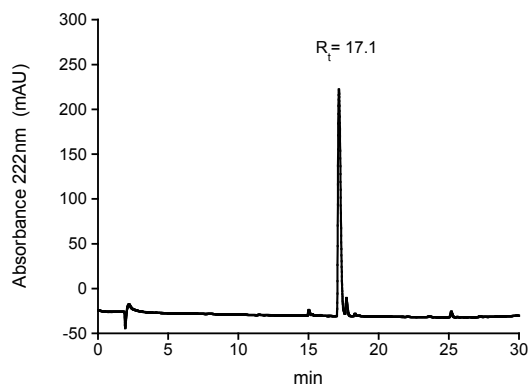
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(4-hydroxy-3-nitrophenyl)propanamide Lm1msed58 (Tyr(NO2)-nLeu(O-Bzl)-VS)



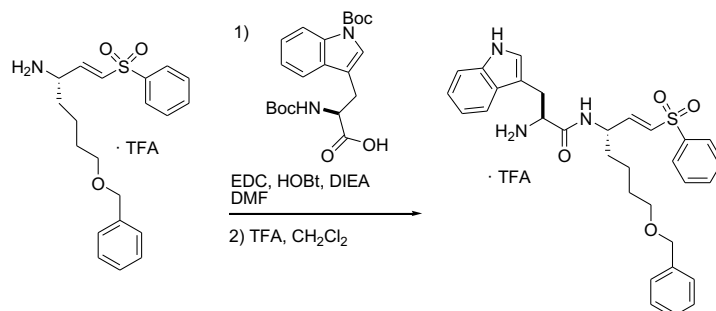
¹H NMR (*MeOD-d₄*, δ): 1.35-1.41 (m, 2H), 1.54-1.63 (m, 4H), 3.10 (dq, $J = 14.1, 7.4$ Hz, 2H), 3.45 (t, $J = 6.2$ Hz, 2H), 4.05 (t, $J = 7.3$ Hz, 1H), 4.45 (s, 2H), 4.55 (dd, $J = 12.9, 6.3$ Hz, 1H), 6.25 (d, $J = 15.2$ Hz, 1H), 6.72 (dd, $J = 15.2, 5.8$ Hz, 1H), 7.15 (d, $J = 8.6$ Hz, 1H), 7.30 (bs, 5H), 7.44 (dd, $J = 8.6, 1.9$ Hz, 1H), 7.58 (t, $J = 7.6$ Hz, 2H), 7.67 (t, $J = 7.4$ Hz, 1H), 7.85 (d, $J = 7.6$ Hz, 2H), 7.99 (d, $J = 1.8$ Hz, 1H).

¹³C NMR (*MeOD-d₄*, δ): 23.5 (CH₂), 30.2 (CH₂), 34.2 (CH₂), 37.3 (CH₂), 51.3 (CH), 55.3 (CH), 70.9 (CH₂), 73.9 (CH₂), 121.8 (CH), 127.0 (CH), 127.4 (CH), 128.7 (CH), 128.8 (CH), 128.9 (CH), 129.4 (CH), 130.6 (C), 132.3 (CH), 134.9 (CH), 135.9 (C), 138.9 (CH), 139.7 (C), 141.6 (C), 146.7 (CH), 155.1 (C), 168.8 (C).

ESI-MS: [M+H]⁺ calcd. for C₂₉H₃₄N₃O₇S = 568.2112 found 568.2102. (M.W. 681.6766).



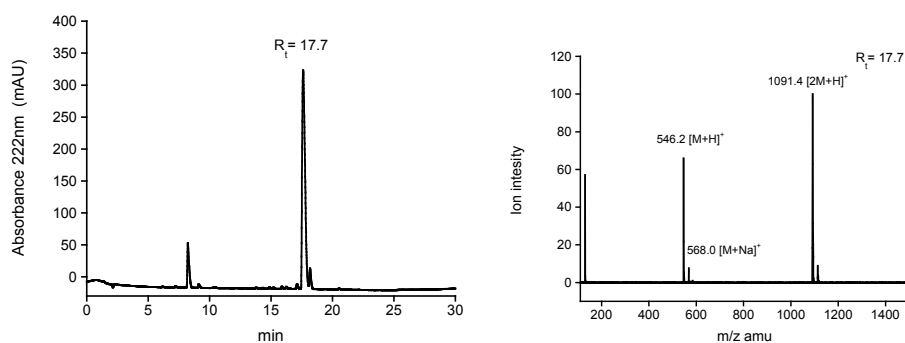
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(1H-indol-3-yl)propanamide Lm1msed68 (Trp-nLeu(O-Bzl)-VS)



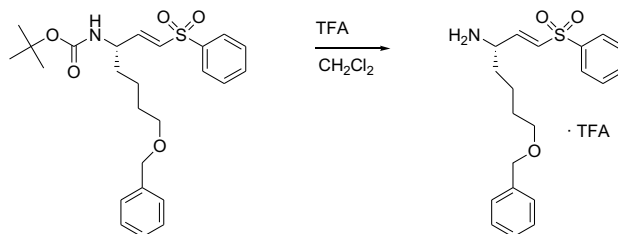
$^1\text{H NMR}$ ($\text{MeOD-}d_4$ δ): 1.31-1.37 (m, 2H), 1.49-1.59 (m, 4H), 3.20 (dd, $J = 14.5, 7.0$ Hz, 1H), 3.34 (d, $J = 7.8$ Hz, 1H), 3.43 (t, $J = 6.2$ Hz, 2H), 4.09 (t, $J = 7.4$ Hz, 1H), 4.44 (s, 2H), 4.52 (q, $J = 6.2$ Hz, 1H), 6.12 (d, $J = 15.2$ Hz, 1H), 6.73 (dd, $J = 15.2, 5.5$ Hz, 1H), 7.05 (t, $J = 7.4$ Hz, 1H), 7.21 (bs, 2H), 7.30 (bs, 5H), 7.40 (d, $J = 8.1$ Hz, 1H), 7.54 (t, $J = 7.6$ Hz, 2H), 7.59-7.66 (m, 2H), 7.80 (d, $J = 7.8$ Hz, 2H).

$^{13}\text{C NMR}$ ($\text{MeOD-}d_4$ δ): 23.5 (CH_2), 29.0 (CH_2), 30.2 (CH_2), 34.2 (CH_2), 51.2 (CH), 54.9 (CH), 71.0 (CH_2), 73.9 (CH_2), 108.0 (C), 112.9 (CH), 119.1 (CH), 120.4 (CH), 123.0 (CH), 125.5 (CH), 128.1 (C), 128.7 (CH), 128.8 (C), 128.9 (CH), 129.4 (CH), 130.6 (CH), 132.0 (CH), 134.8 (CH), 138.2 (C), 139.7 (C), 141.6 (C), 146.8 (CH), 169.8 (C).

ESI-MS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{31}\text{H}_{36}\text{N}_3\text{O}_4\text{S} = 546.2421$ found 546.2438. (M.W. 773.7390)



(S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-amine trifluoroacetate Lm1msed69



To a cooled (0°C) solution of *tert*-butyl (S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-ylcarbamate (0.2 g, 0.43 mmol) in CH_2Cl_2 (5 mL) was added drop-wise TFA (1 mL) via syringe and stirred for 30 min. After checking by HPLC-MS that all the starting material was consumed, the solvent was removed under reduced pressure, and the residual TFA was removed by co-distillation

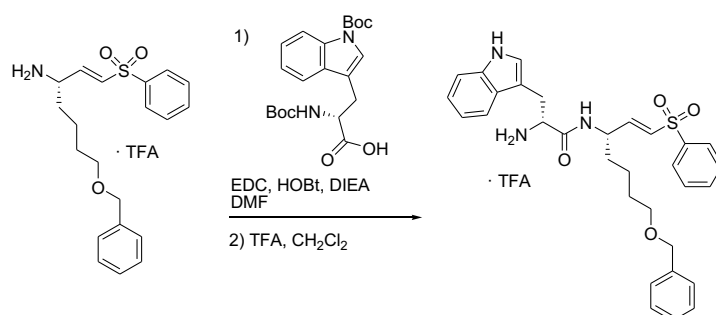
with CH_2Cl_2 and finally dried in vacuo to give (S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-amine as a trifluoroacetic salt (0.2 g; 0.43 mmol, 99%). This material was pure enough to be used in the next step without further purification.

$^1\text{H NMR}$ (MeOD_4 δ): 1.35-1.44 (m, 2H), 1.55-1.66 (m, 2H), 1.71-1.83 (m, 2H), 3.45 (t, $J = 6.0$ Hz, 2H), 3.98 (dd, $J = 13.9, 6.9$ Hz, 1H), 4.46 (s, 2H), 6.86 (dd, $J = 15.3, 6.9$ Hz, 1H), 6.97 (d, $J = 15.3$ Hz, 1H), 7.32 (bs, 5H), 7.56-7.62 (m, 2H), 7.69 (d, $J = 7.1$ Hz, 1H), 7.91 (d, $J = 7.1$ Hz, 2H).

$^{13}\text{C NMR}$ (MeOD_4): 22.8 (CH_2), 29.7 (CH_2), 32.9 (CH_2), 52.2 (CH), 70.4 (CH_2), 73.6 (CH_2), 128.5 (CH), 128.6 (CH), 128.7 (CH), 129.2 (CH), 130.5 (CH), 134.9 (CH), 136.2 (CH), 139.4 (C), 140.6 (C), 140.8 (CH), 161 (q, C, TFA).

ESI-MS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{20}\text{H}_{25}\text{NO}_3\text{SNa} = 382.1447$ found 382.1458. (M.W. 473.5057)

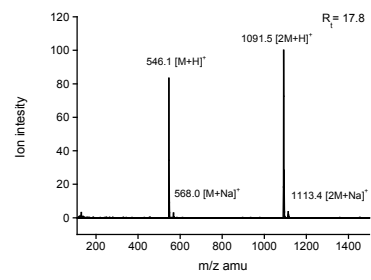
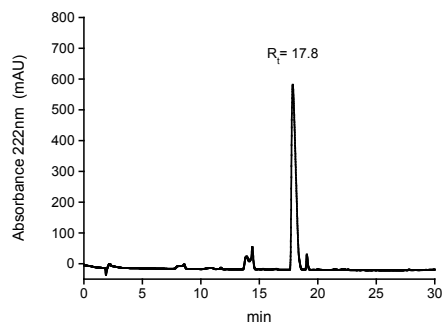
(2R)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(1H-indol-3-yl)propanamide Lm1msed71 (L-Trp-nLeu(O-Bzl)-VS)



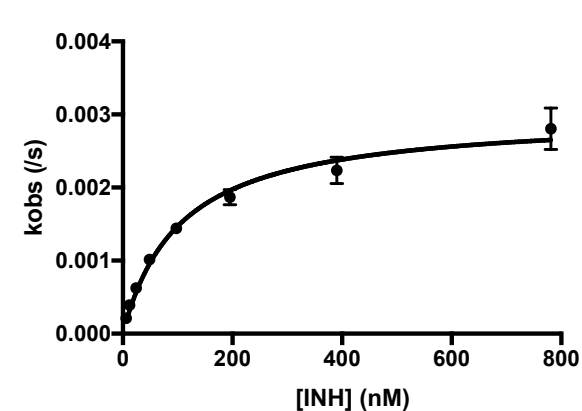
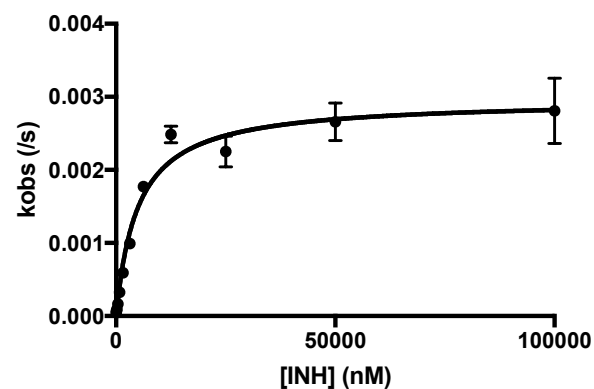
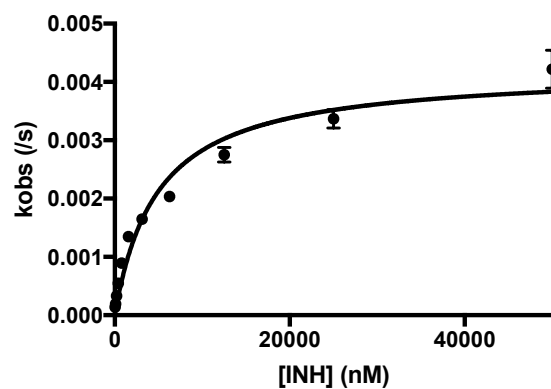
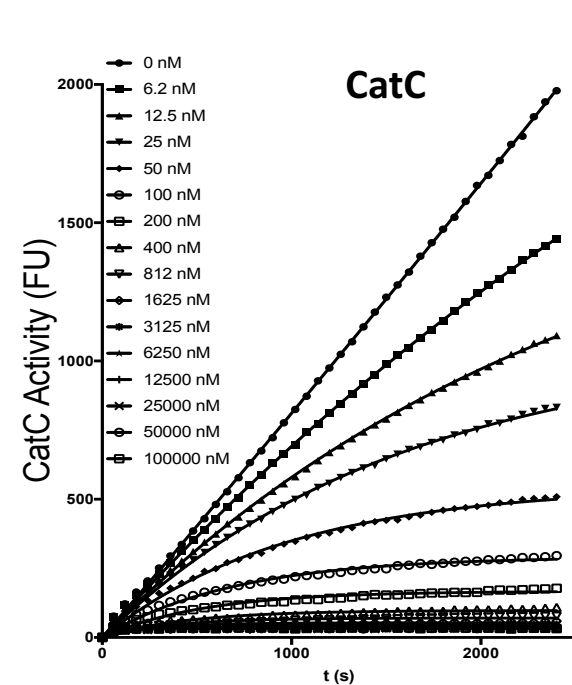
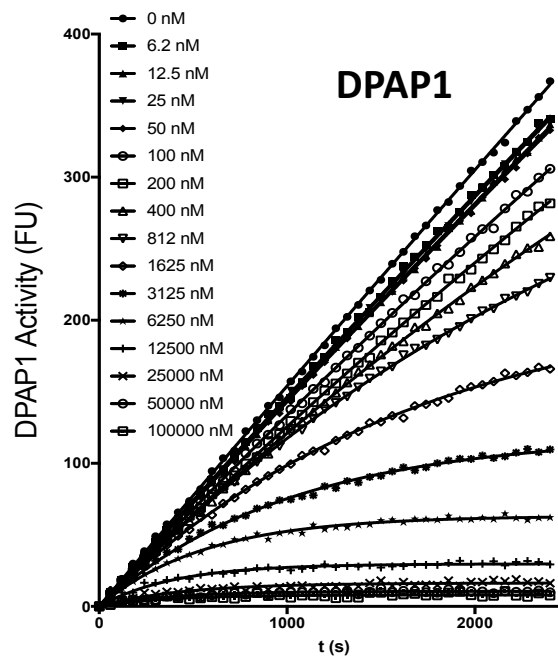
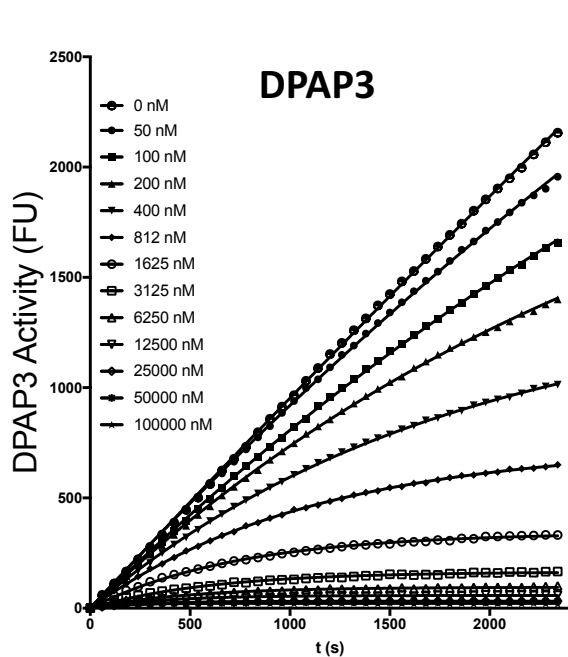
$^1\text{H NMR}$ ($\text{MeOD}-d_4$ δ): 0.95-1.02 (m, 2H), 1.09-1.18 (m, 1H), 1.28-1.35 (m, 1H), 1.37-1.45 (m, 2H), 3.21 (dd, $J = 14.4, 7.2$ Hz, 1H), 3.33-3.38 (m, 3H), 4.05 (t, $J = 7.6$ Hz, 1H), 4.43 (bs, 3H), 6.61 (d, $J = 15.2$ Hz, 1H), 6.81 (dd, $J = 15.1, 5.2$ Hz, 1H), 7.04 (t, $J = 7.4$ Hz, 1H), 7.11 (t, $J = 7.5$ Hz, 1H), 7.16 (s, 1H), 7.28 (s, 2H), 7.29 (s, 2H), 7.35 (d, $J = 8.1$ Hz, 1H), 7.54-7.59 (m, 4H), 7.66 (t, $J = 7.4$ Hz, 1H), 7.84 (d, $J = 7.5$ Hz, 2H).

$^{13}\text{C NMR}$ ($\text{MeOD}-d_4$ δ): 23.2 (CH_2), 29.0 (CH_2), 30.1 (CH_2), 33.9 (CH_2), 51.3 (CH), 55.3 (CH), 70.9 (CH_2), 73.9 (CH_2), 108.2 (C), 112.7 (CH), 119.1 (CH), 120.3 (CH), 122.9 (CH), 125.5 (CH), 128.4 (C), 128.7 (CH), 128.8 (CH), 129.4 (CH), 130.6 (CH), 132.0 (CH), 134.8 (CH), 138.2 (C), 139.8 (C), 141.7 (C), 147.0 (CH), 169.8 (C).

ESI-MS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{31}\text{H}_{36}\text{N}_3\text{O}_4\text{S} = 546.2421$ found 546.2411. (M.W. 773.7390)



P2: Gly



$$k_{inact} = 0.0042 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 3,300 \pm 750 \text{ nM}$$

$$k_{inact}/K_i = 1,300 \pm 250 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0030 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 3,300 \pm 500 \text{ nM}$$

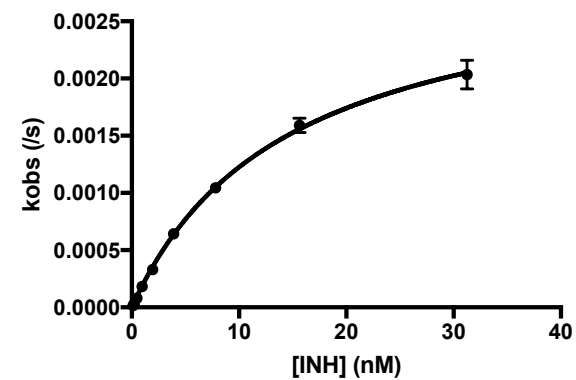
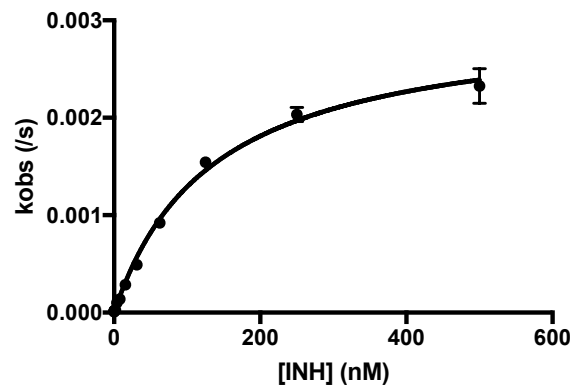
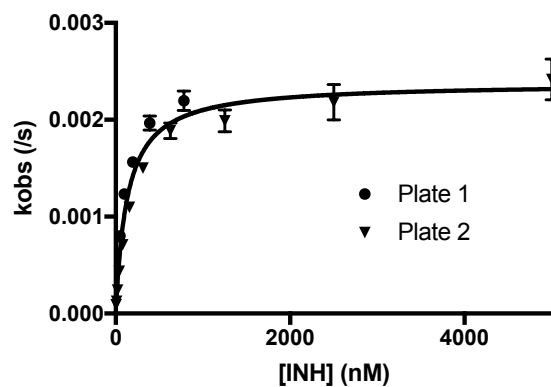
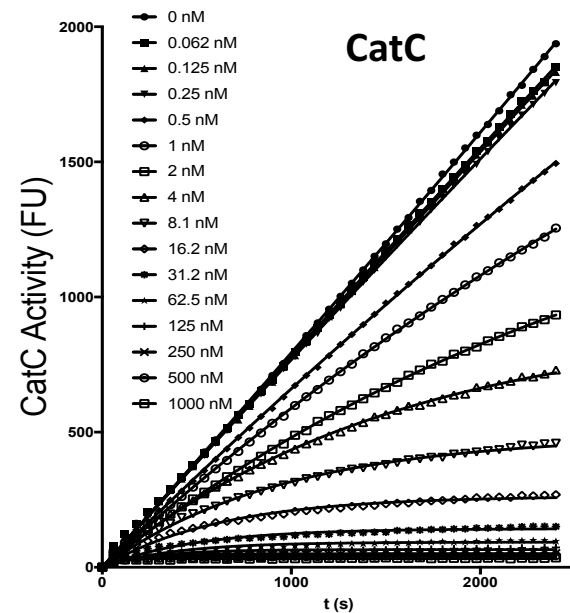
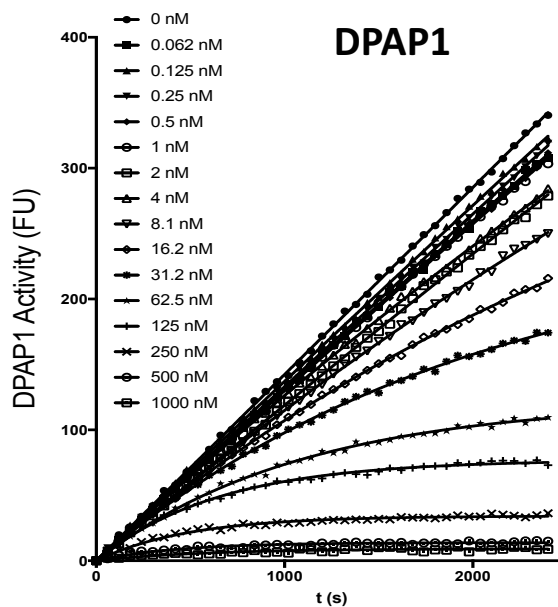
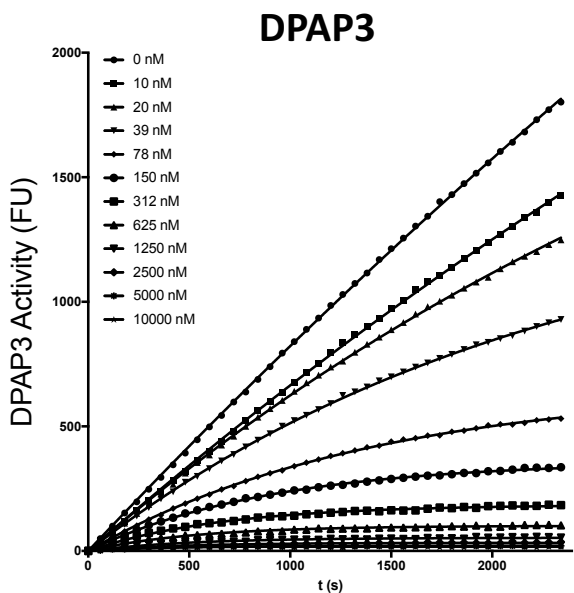
$$k_{inact}/K_i = 900 \pm 100 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0030 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 70 \pm 9 \text{ nM}$$

$$k_{inact}/K_i = 43,000 \pm 4,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Ala



$$k_{inact} = 0.0024 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 88 \pm 13 \text{ nM}$$

$$k_{inact}/K_i = 27,000 \pm 3,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00303 \pm 0.00009 \text{ s}^{-1}$$

$$K_i = 90 \pm 7 \text{ nM}$$

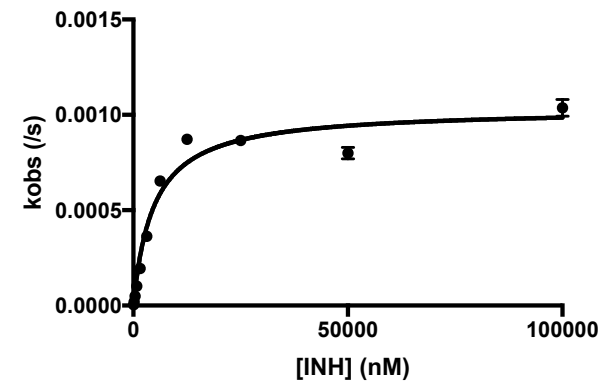
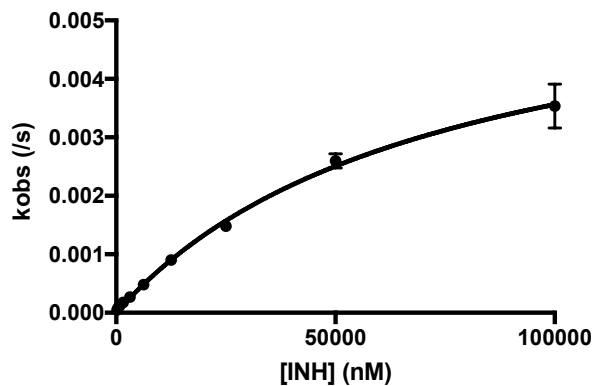
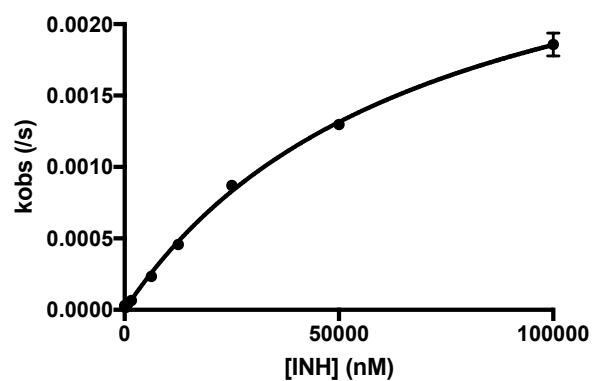
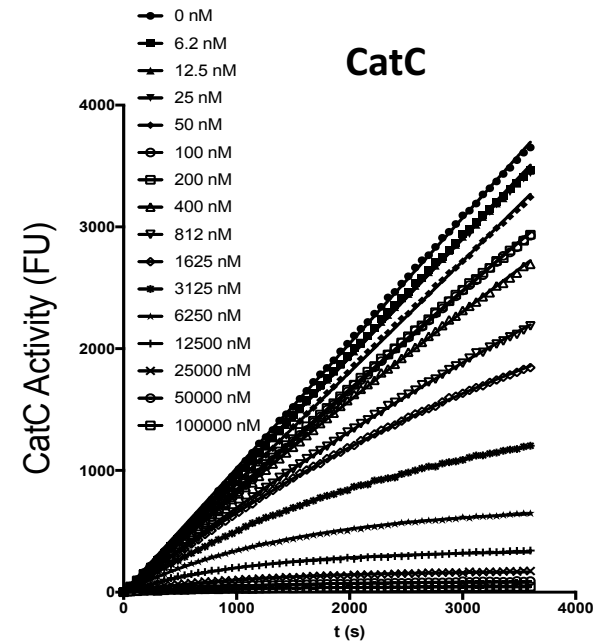
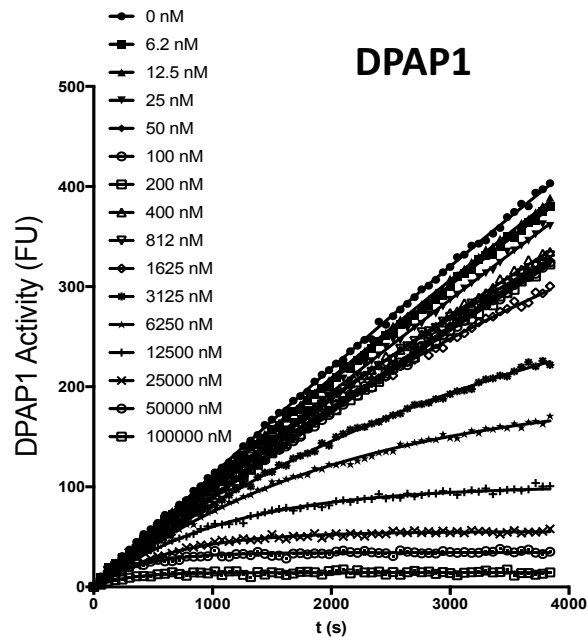
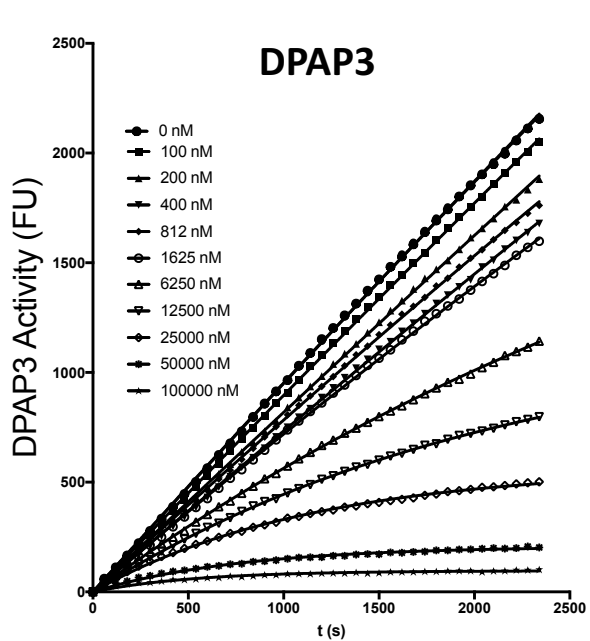
$$k_{inact}/K_i = 34,000 \pm 2,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00300 \pm 0.00007 \text{ s}^{-1}$$

$$K_i = 9.7 \pm 0.4 \text{ nM}$$

$$k_{inact}/K_i = 310,000 \pm 8,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Acpc



$$k_{inact} = 0.0032 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 47,000 \pm 3,000 \text{ nM}$$

$$k_{inact}/K_i = 67.7 \pm 0.1 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0062 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 49,000 \pm 4,000 \text{ nM}$$

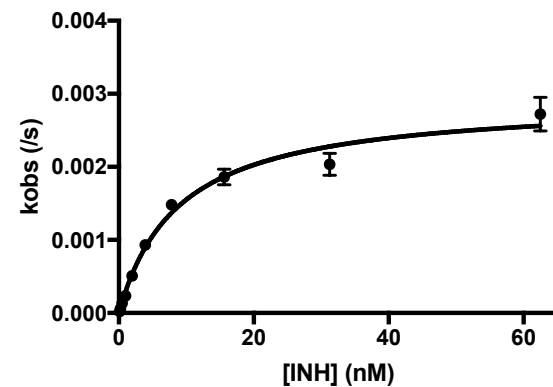
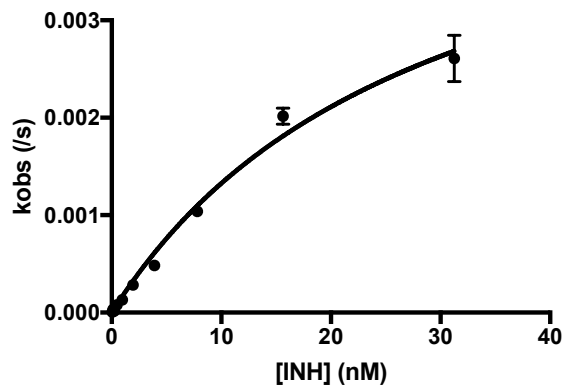
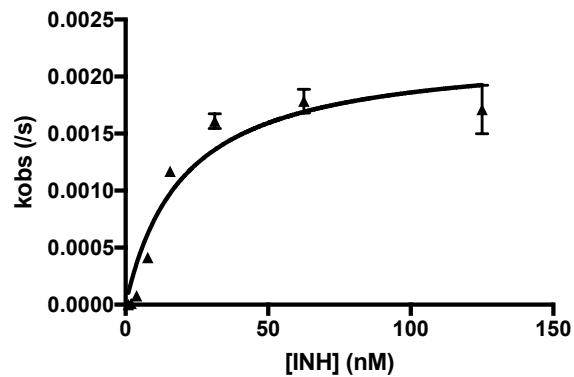
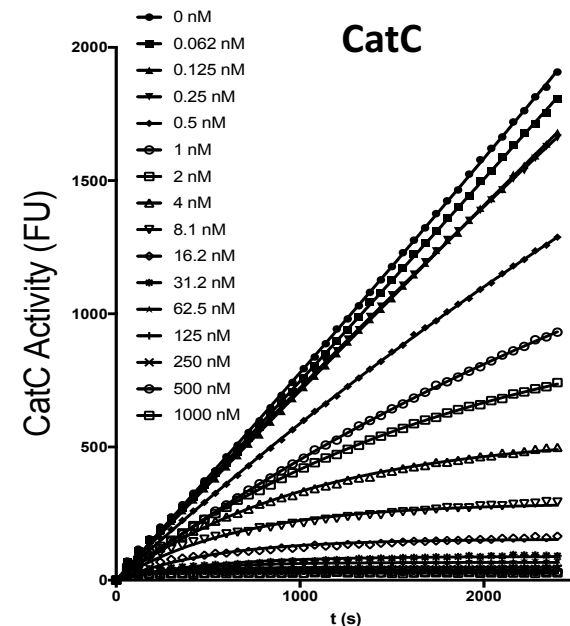
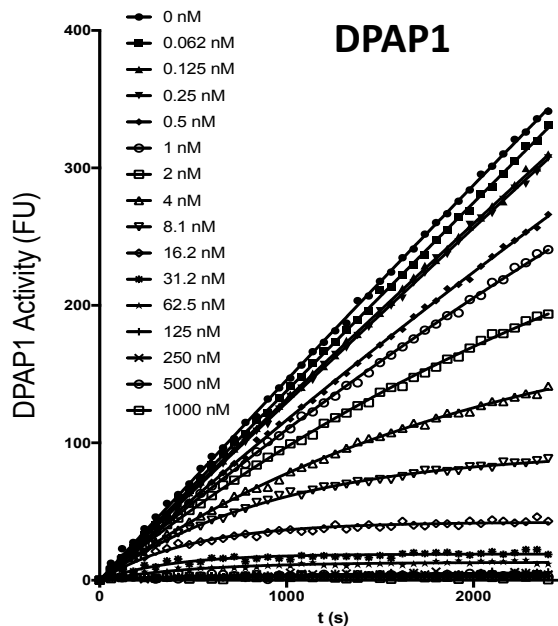
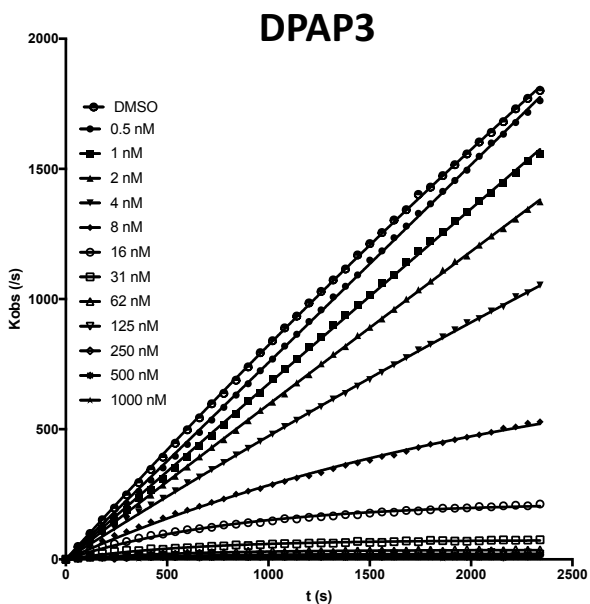
$$k_{inact}/K_i = 127 \pm 6 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00103 \pm 0.00006 \text{ s}^{-1}$$

$$K_i = 3,200 \pm 700 \text{ nM}$$

$$k_{inact}/K_i = 330 \pm 60 \text{ M}^{-1}\text{s}^{-1}$$

P2: hAla



$$k_{\text{inact}} = 0.0022 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 13.5 \pm 6 \text{ nM}$$

$$k_{\text{inact}}/K_i = 166,000 \pm 50,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0052 \pm 0.0007 \text{ s}^{-1}$$

$$K_i = 19 \pm 4 \text{ nM}$$

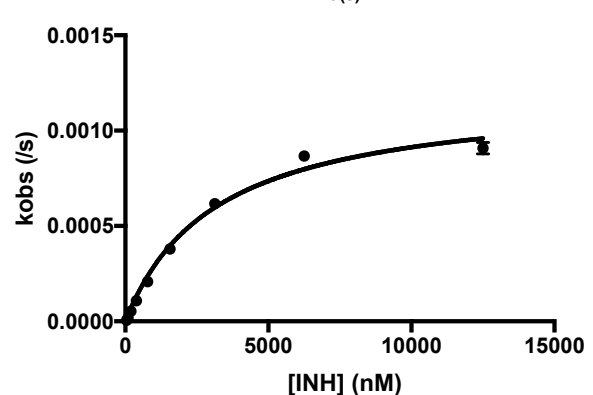
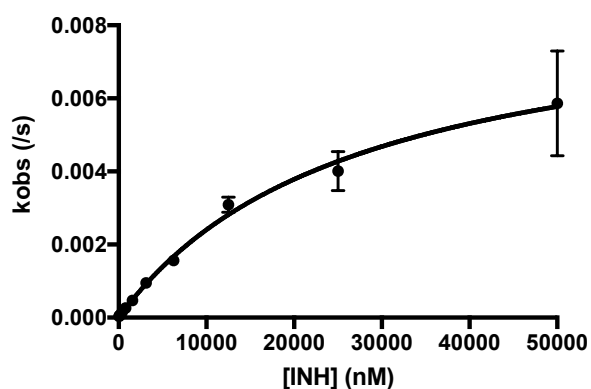
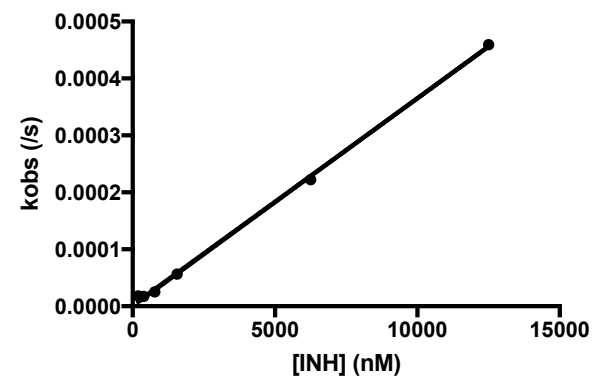
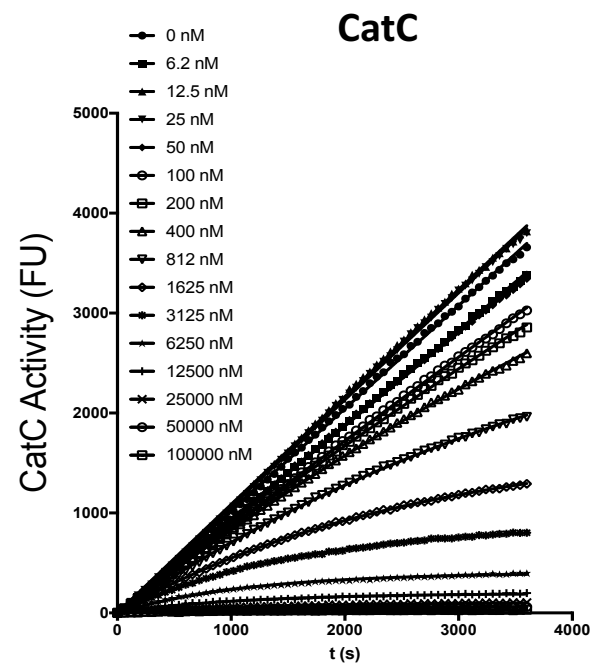
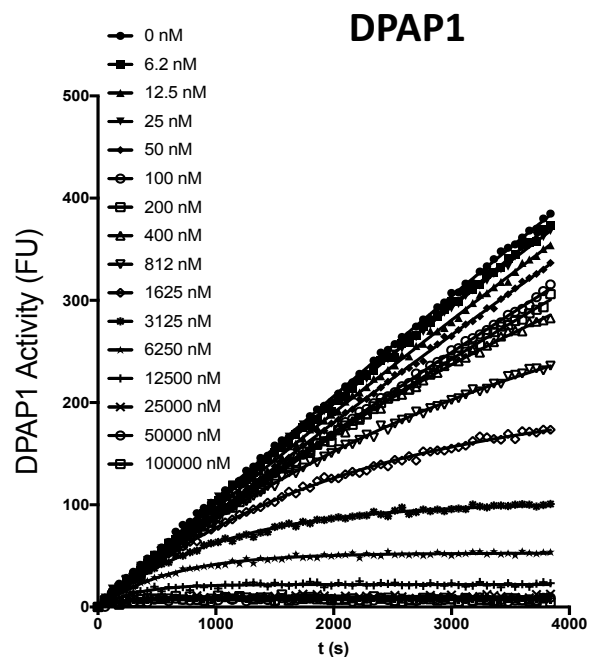
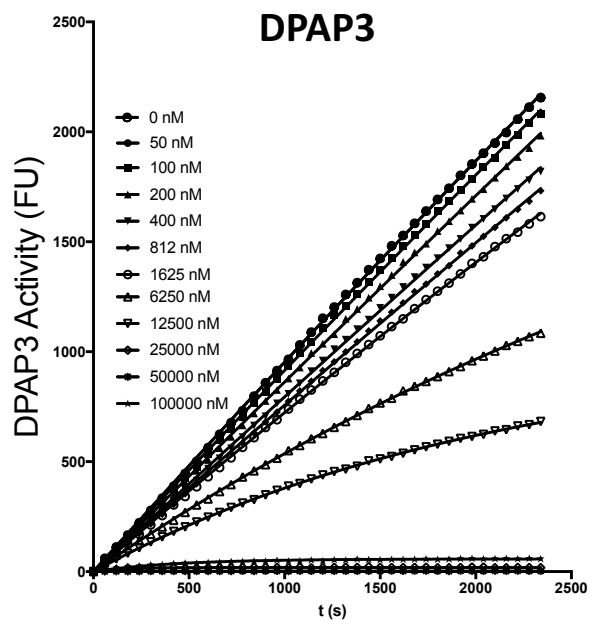
$$k_{\text{inact}}/K_i = 270,000 \pm 30,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0029 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 5.9 \pm 0.9 \text{ nM}$$

$$k_{\text{inact}}/K_i = 500,000 \pm 50,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Aib

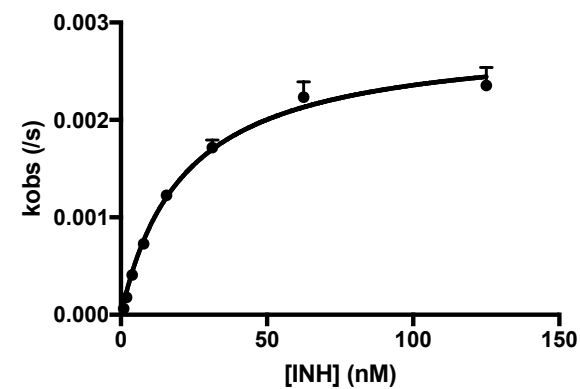
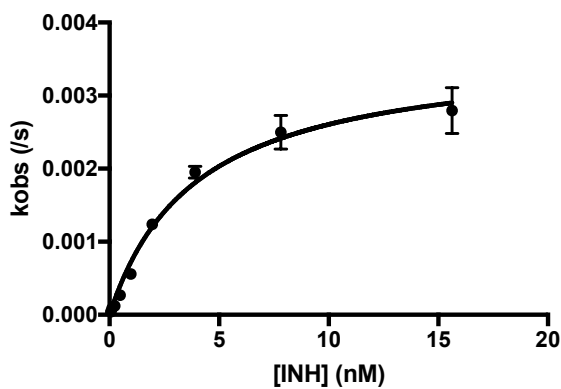
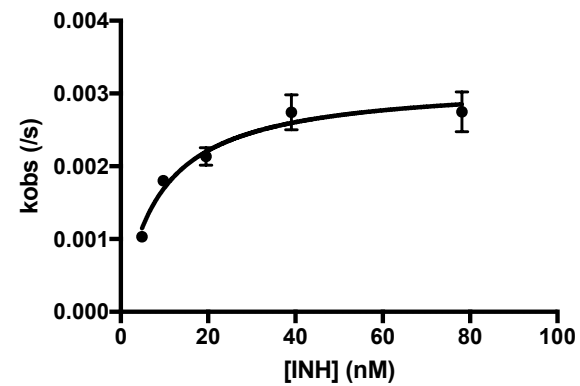
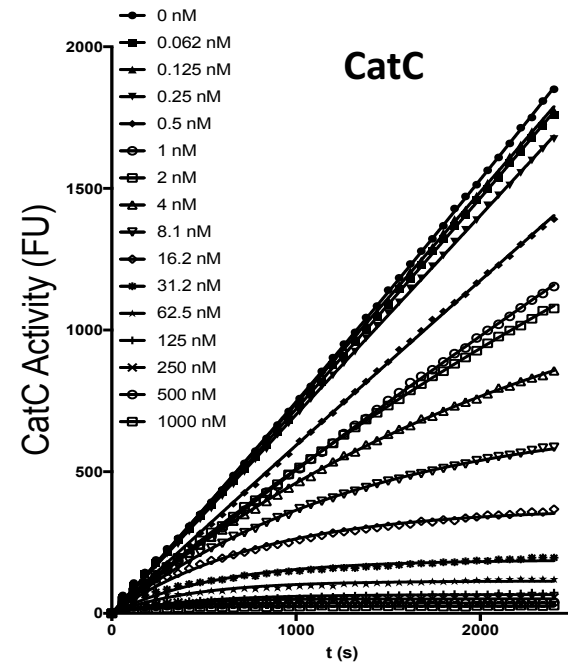
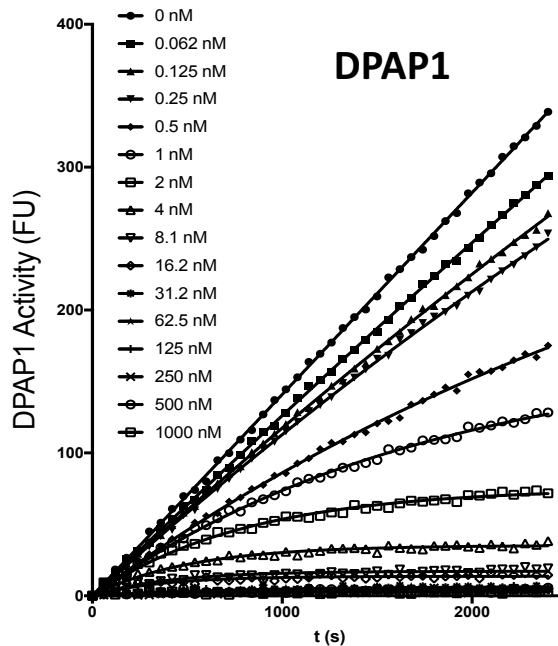
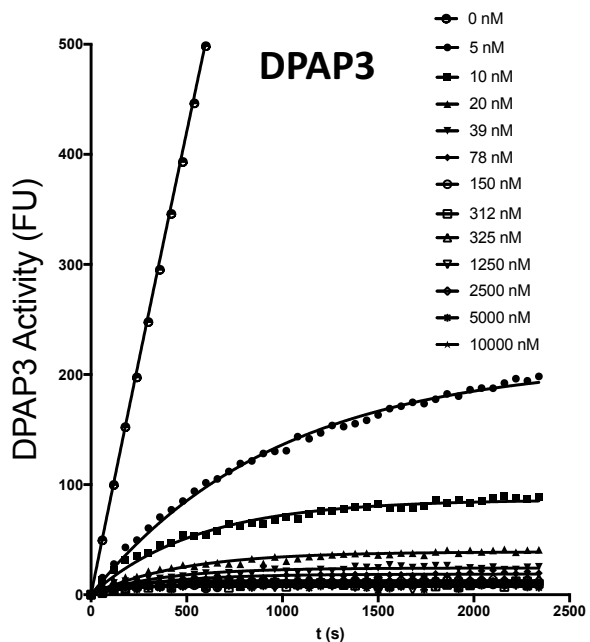


k_{inact} = N.S.
 K_i = N.S.
 $k_{inact}/k_i = 36.3 \pm 0.6 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.0089 \pm 0.0005 \text{ s}^{-1}$
 $K_i = 18,000 \pm 2,000 \text{ nM}$
 $k_{inact}/K_i = 490 \pm 30 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.00120 \pm 0.00007 \text{ s}^{-1}$
 $K_i = 2,100 \pm 300 \text{ nM}$
 $k_{inact}/K_i = 570 \pm 60 \text{ M}^{-1}\text{s}^{-1}$

P2: Val



$$k_{inact} = 0.0032 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 5.7 \pm 1.1 \text{ nM}$$

$$k_{inact}/K_i = 554,000 \pm 84,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0036 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 2.6 \pm 0.4 \text{ nM}$$

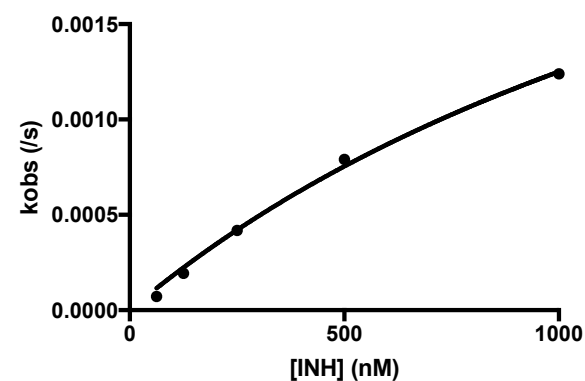
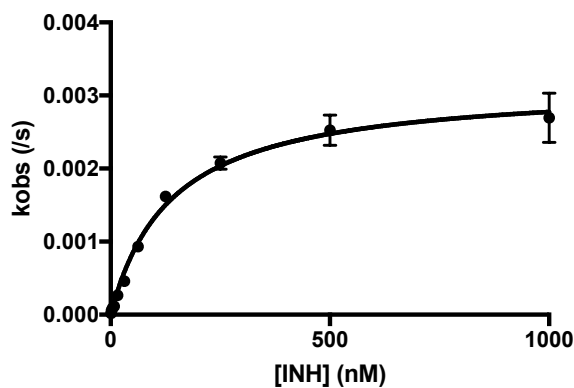
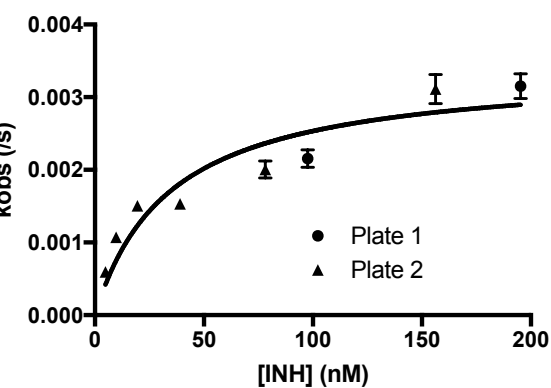
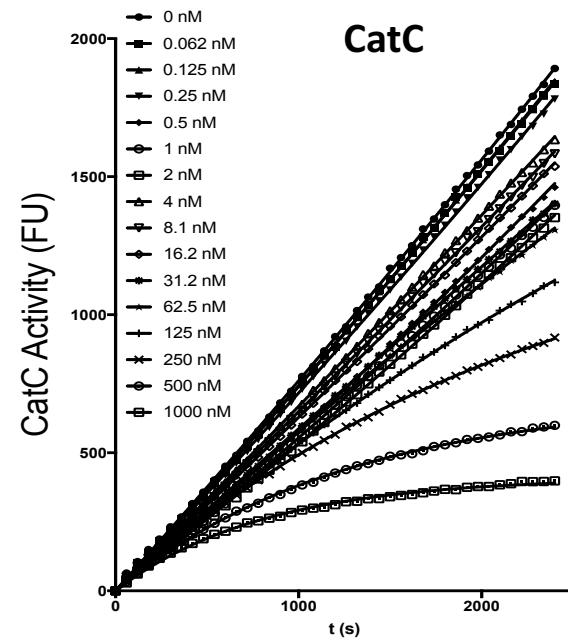
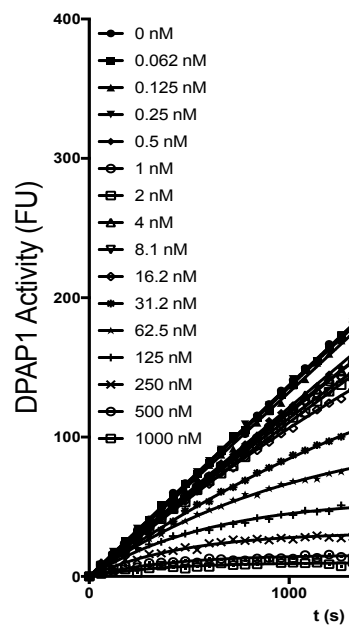
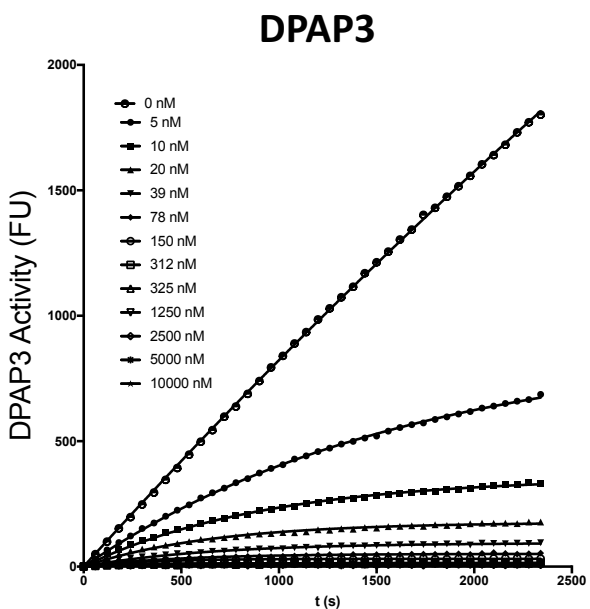
$$k_{inact}/K_i = 1,380,000 \pm 140,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0029 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 14 \pm 2 \text{ nM}$$

$$k_{inact}/K_i = 200,000 \pm 15,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Ile



$$k_{inact} = 0.0034 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 23 \pm 9 \text{ nM}$$

$$k_{inact}/K_i = 148,000 \pm 45,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0032 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 92 \pm 8 \text{ nM}$$

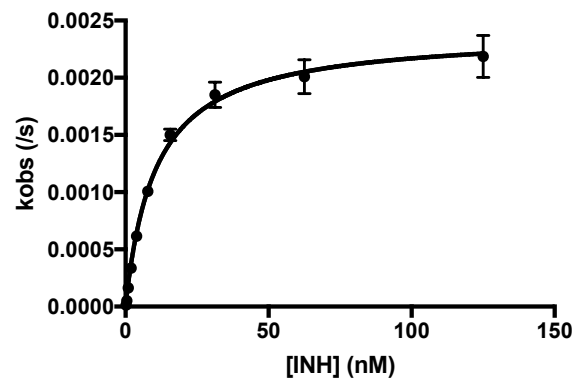
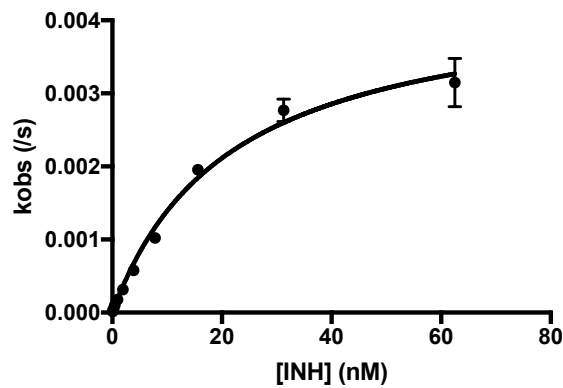
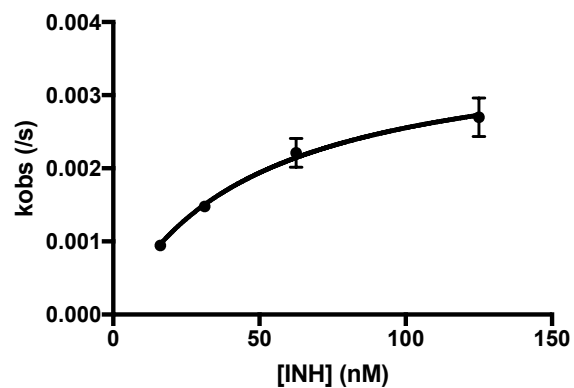
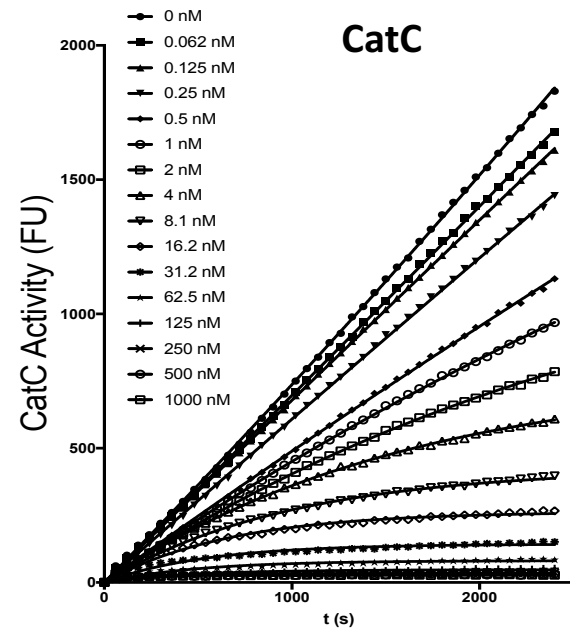
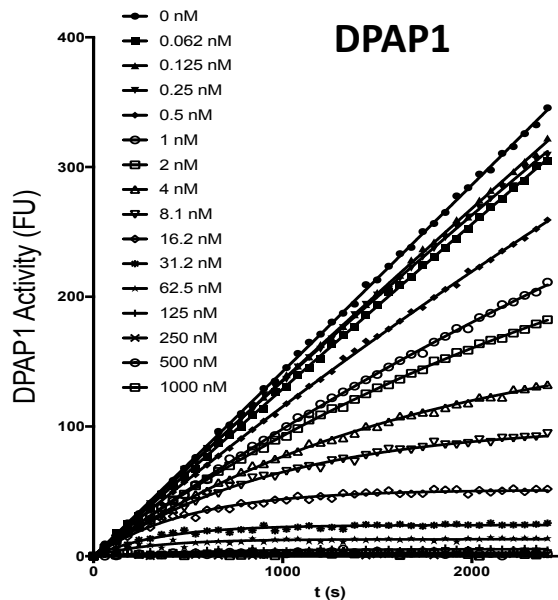
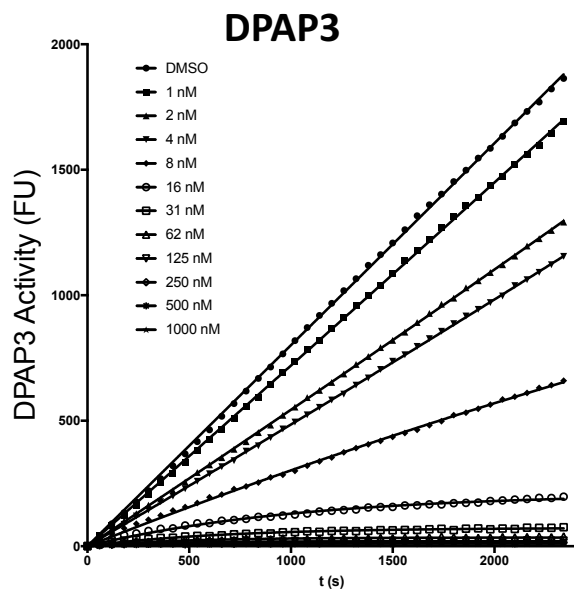
$$k_{inact}/K_i = 34,000 \pm 3,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0037 \pm 0.0008 \text{ s}^{-1}$$

$$K_i = 1,300 \pm 400 \text{ nM}$$

$$k_{inact}/K_i = 2,900 \pm 300 \text{ M}^{-1}\text{s}^{-1}$$

P2: nLeu



$$k_{inact} = 0.0037 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 31 \pm 3 \text{ nM}$$

$$k_{inact}/K_i = 120,000 \pm 7,500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0044 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 14 \pm 2 \text{ nM}$$

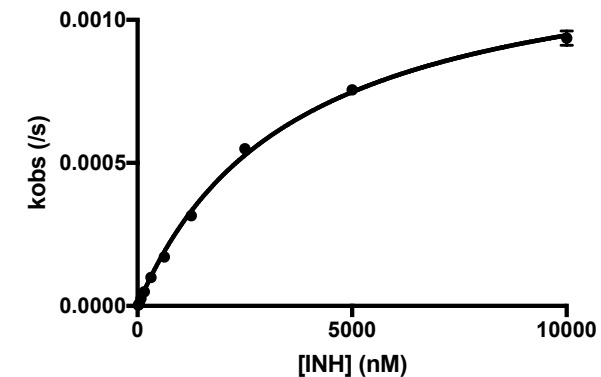
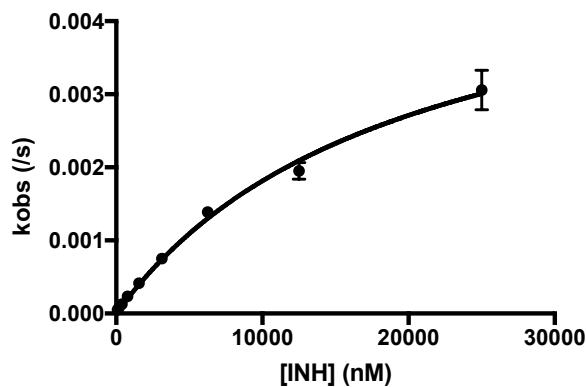
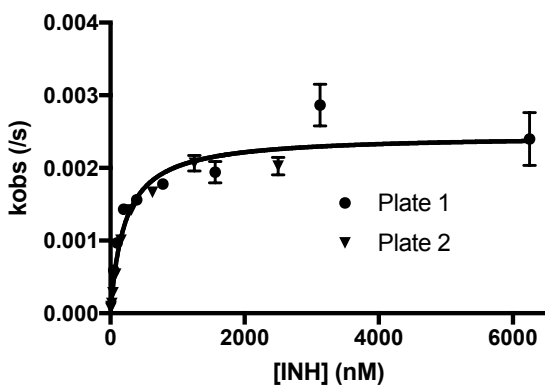
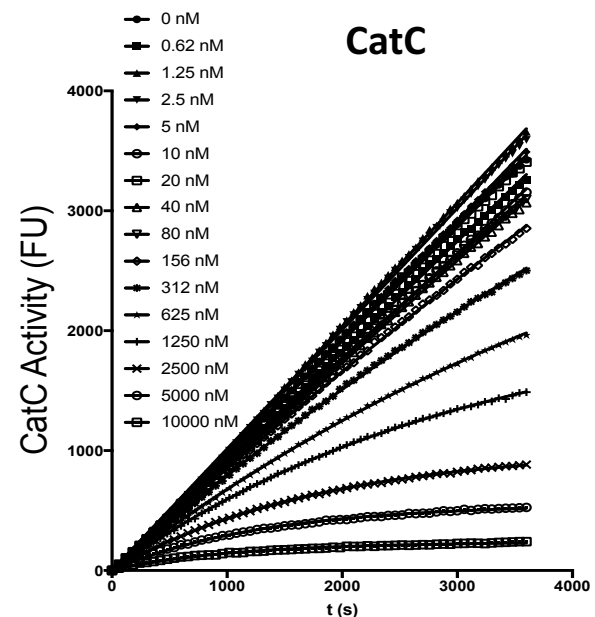
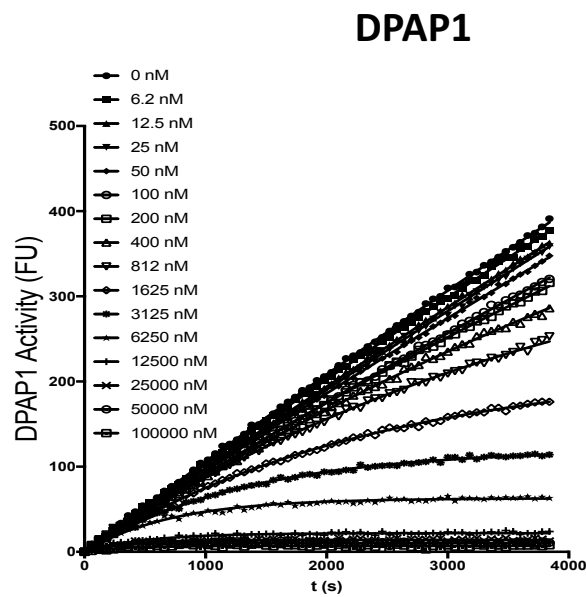
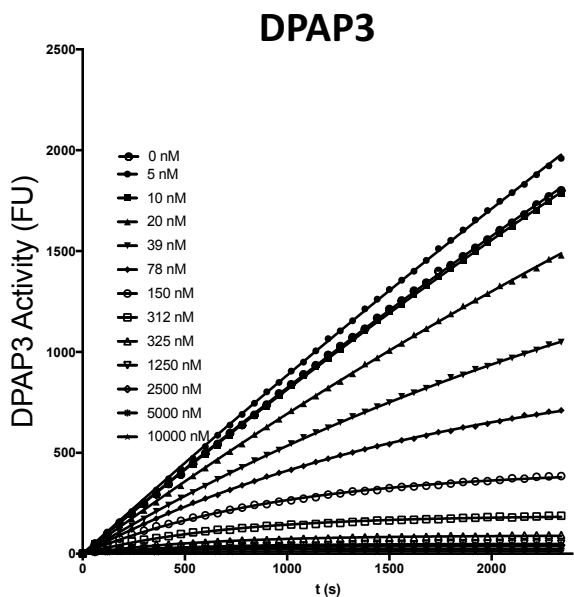
$$k_{inact}/K_i = 305,000 \pm 24,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0024 \pm 0.00005 \text{ s}^{-1}$$

$$K_i = 7.0 \pm 0.5 \text{ nM}$$

$$k_{inact}/K_i = 340,000 \pm 20,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Cba



$$k_{inact} = 0.0025 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 140 \pm 25 \text{ nM}$$

$$k_{inact}/K_i = 17,000 \pm 2,500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0054 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 13,000 \pm 2,000 \text{ nM}$$

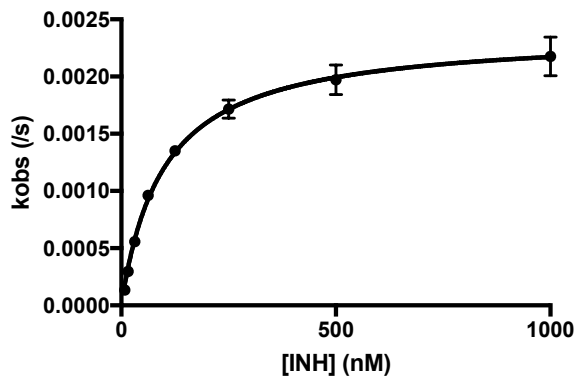
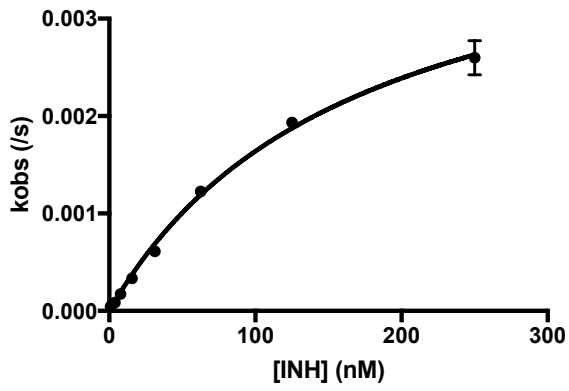
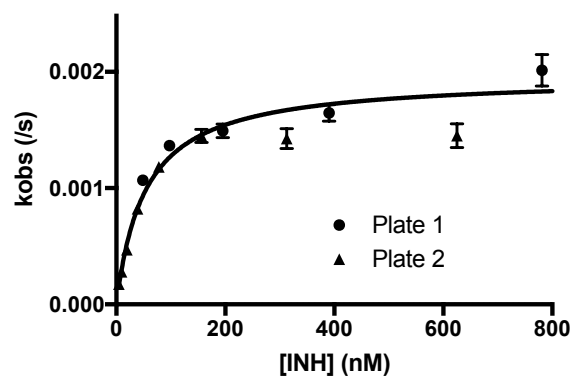
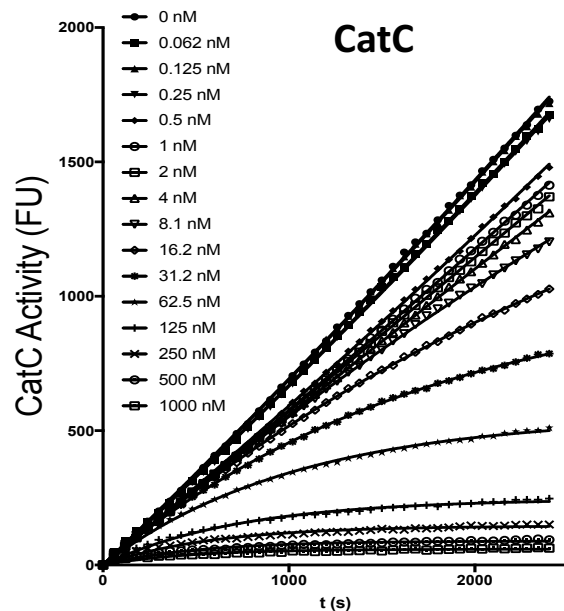
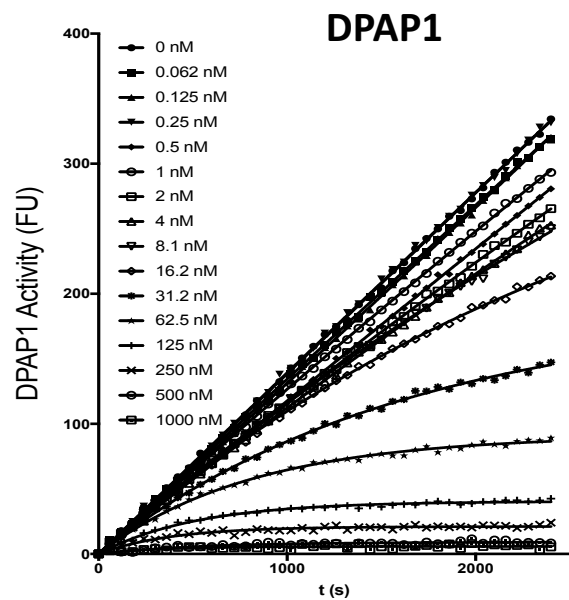
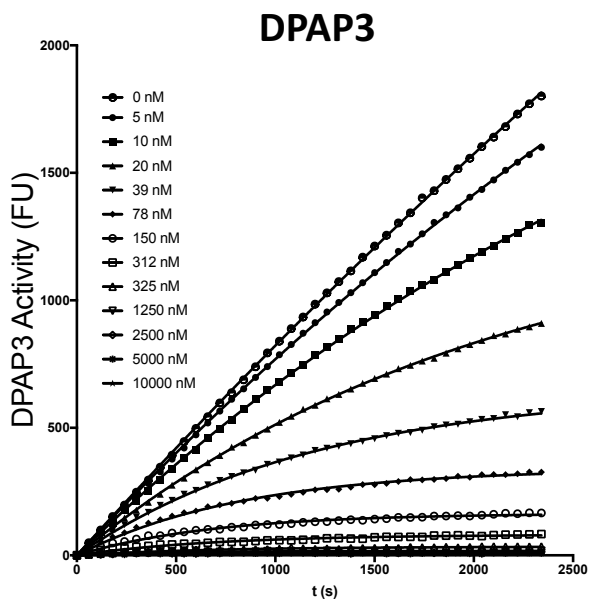
$$k_{inact}/K_i = 410 \pm 30 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00129 \pm 0.00003 \text{ s}^{-1}$$

$$K_i = 2,400 \pm 140 \text{ nM}$$

$$k_{inact}/K_i = 530 \pm 20 \text{ M}^{-1}\text{s}^{-1}$$

P2: Thr



$$k_{inact} = 0.0020 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 36 \pm 8 \text{ nM}$$

$$k_{inact}/K_i = 55,000 \pm 10,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0044 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 112 \pm 10 \text{ nM}$$

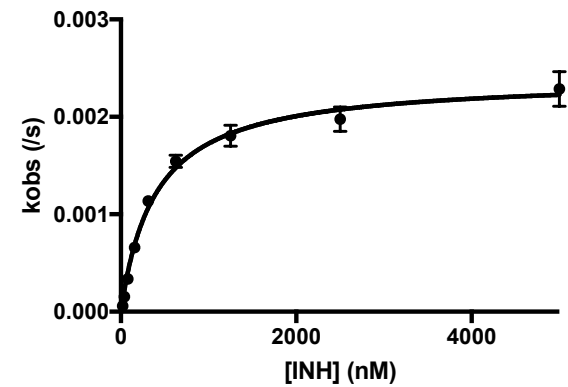
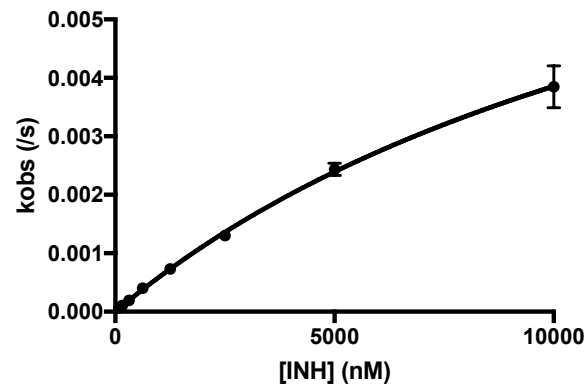
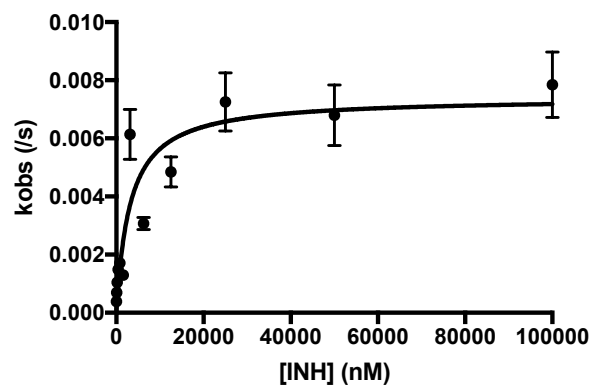
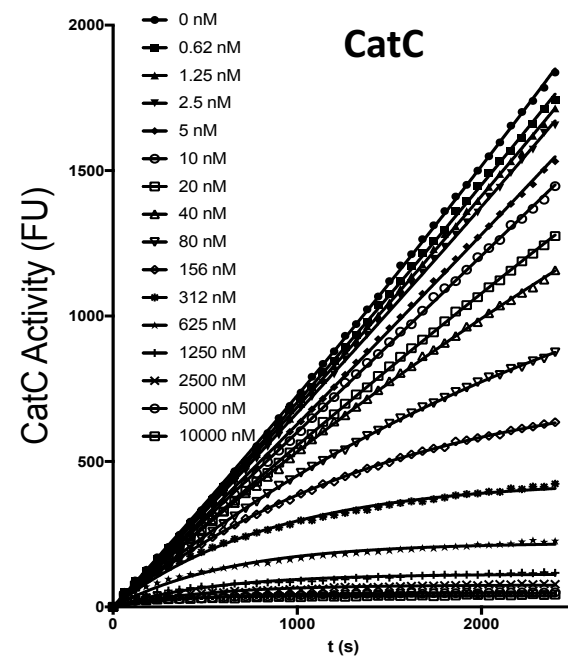
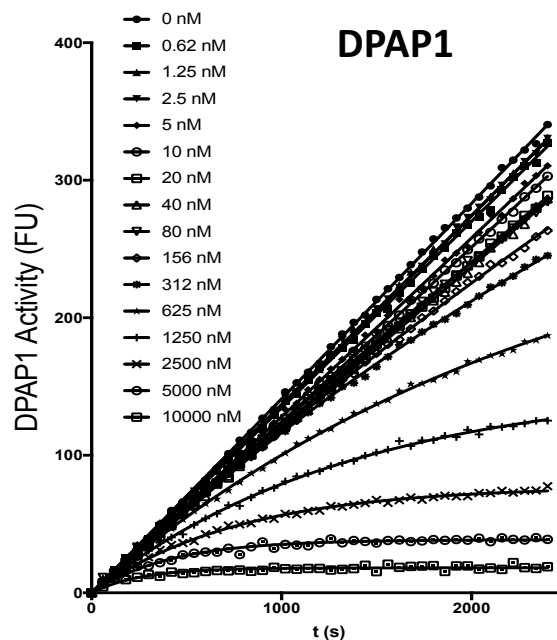
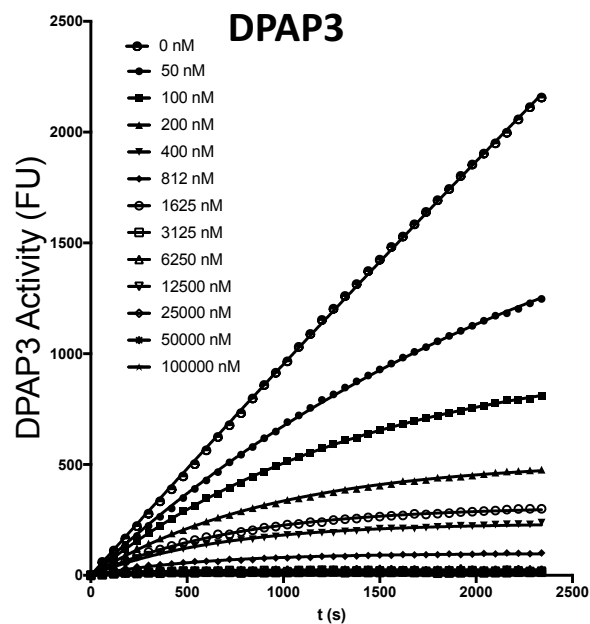
$$k_{inact}/K_i = 39,000 \pm 2,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00238 \pm 0.00003 \text{ s}^{-1}$$

$$K_i = 65 \pm 3 \text{ nM}$$

$$k_{inact}/K_i = 36,000 \pm 1,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Asn



$$k_{inact} = 0.0074 \pm 0.0008 \text{ s}^{-1}$$

$$K_i = 2.1 \pm 1 \text{ } \mu\text{M}$$

$$k_{inact}/K_i = 3,500 \pm 1,400 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0099 \pm 0.0005 \text{ s}^{-1}$$

$$K_i = 10,500 \pm 750 \text{ nM}$$

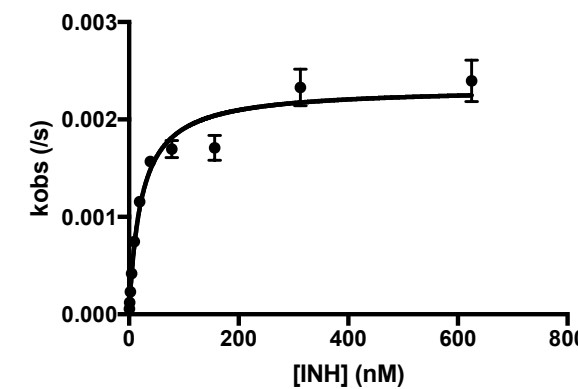
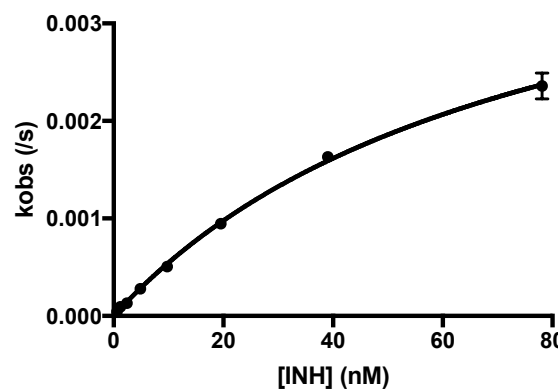
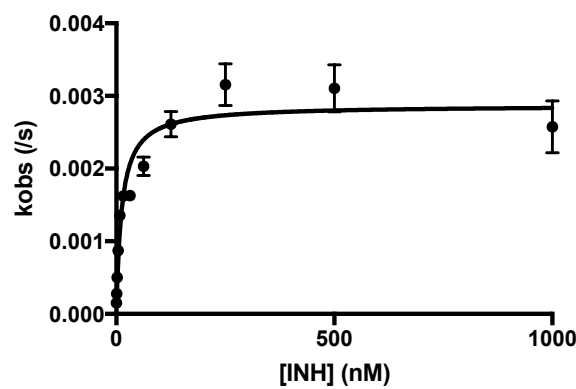
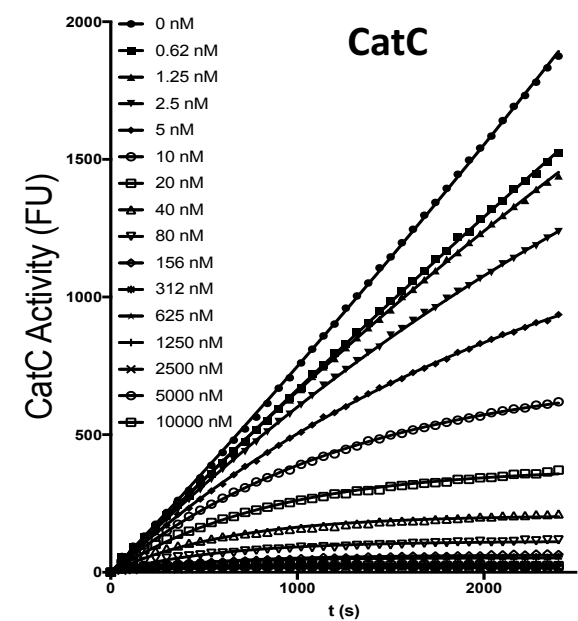
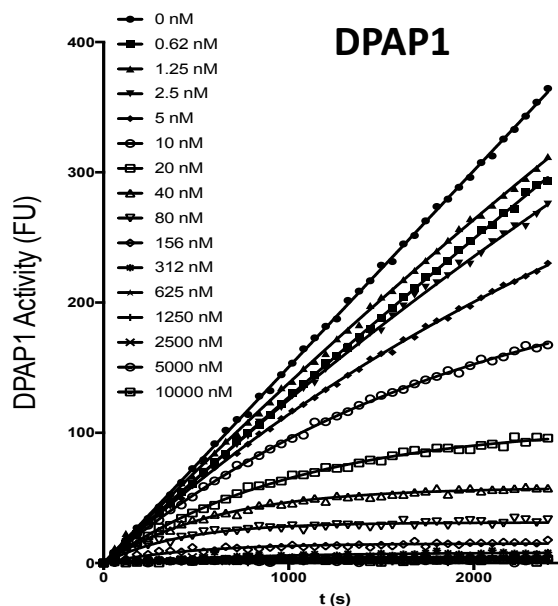
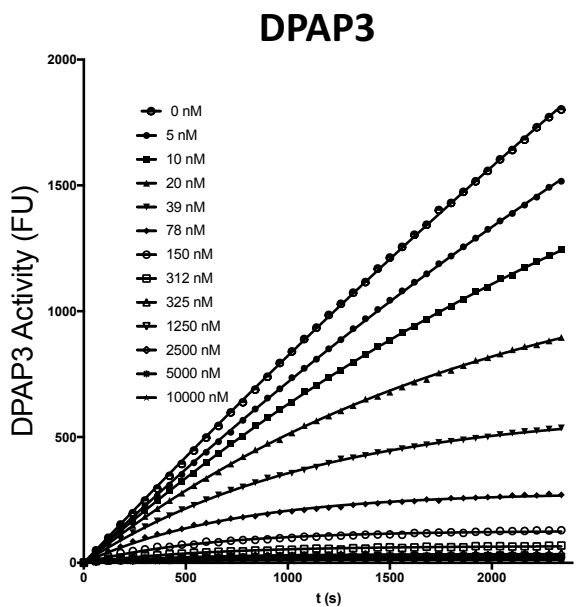
$$k_{inact}/K_i = 950 \pm 20 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00240 \pm 0.00007 \text{ s}^{-1}$$

$$K_i = 260 \pm 30 \text{ nM}$$

$$k_{inact}/K_i = 9,200 \pm 700 \text{ M}^{-1}\text{s}^{-1}$$

P2: Gln

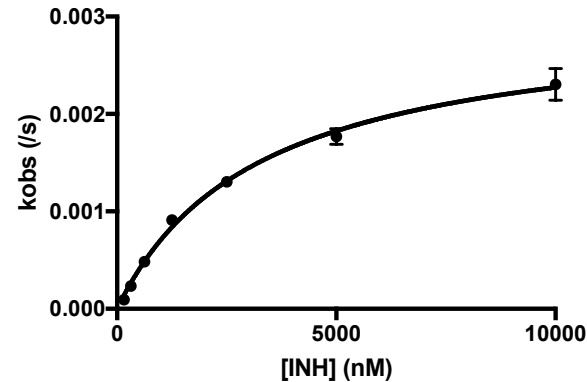
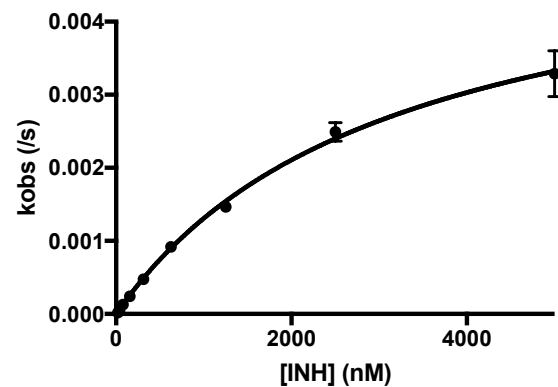
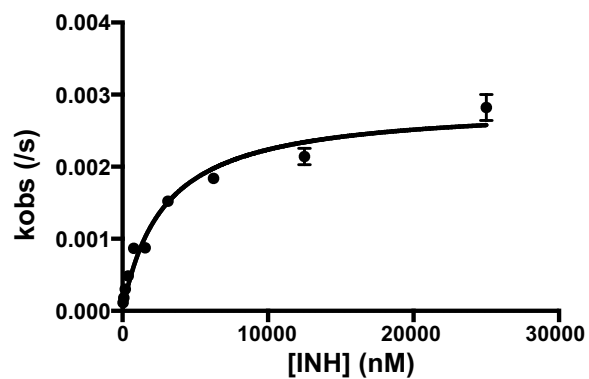
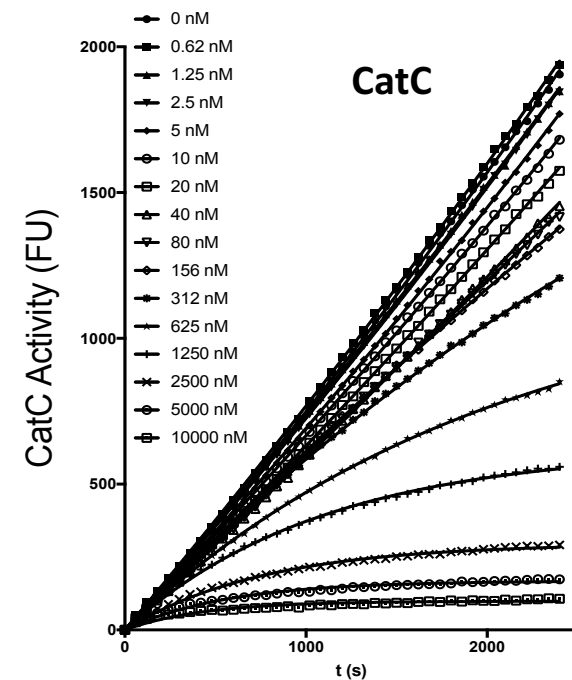
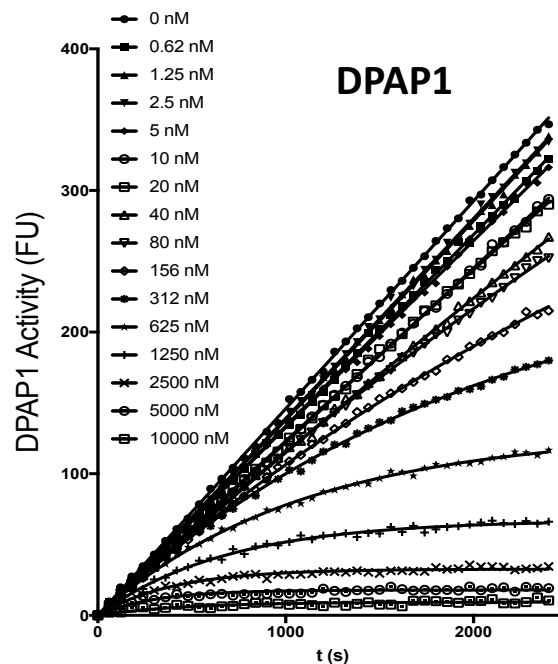
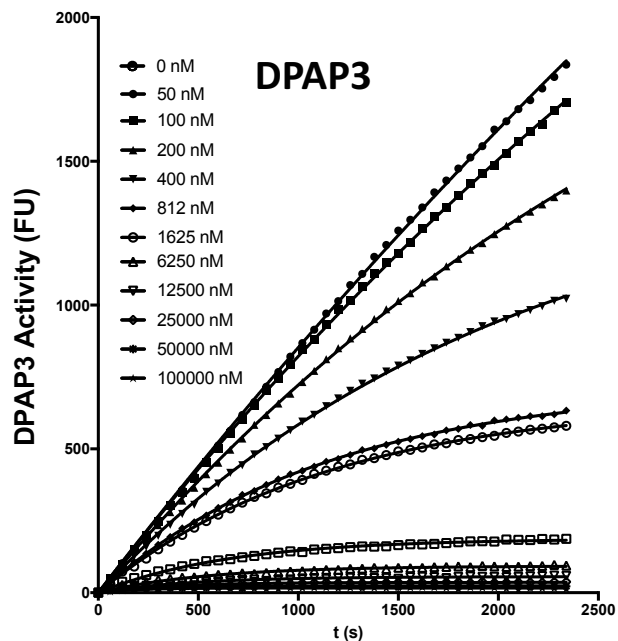


$k_{inact} = 0.0029 \pm 0.0002 \text{ s}^{-1}$
 $K_i = 80 \pm 20 \text{ nM}$
 $k_{inact}/K_i = 35,000 \pm 8,000 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.0047 \pm 0.0002 \text{ s}^{-1}$
 $K_i = 50 \pm 3 \text{ nM}$
 $k_{inact}/K_i = 93,000 \pm 3,000 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.0023 \pm 0.0001 \text{ s}^{-1}$
 $K_i = 15 \pm 3 \text{ nM}$
 $k_{inact}/K_i = 160,000 \pm 25,000 \text{ M}^{-1}\text{s}^{-1}$

P2: Asp



$$i_{inact} = 0.0029 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 1,900 \pm 400 \text{ nM}$$

$$k_{inact}/K_i = 1,500 \pm 200 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0054 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 2,100 \pm 200 \text{ nM}$$

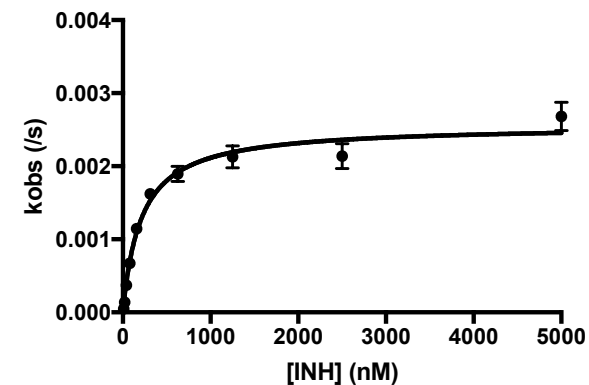
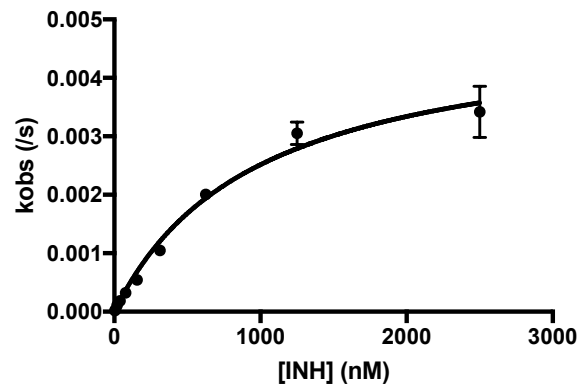
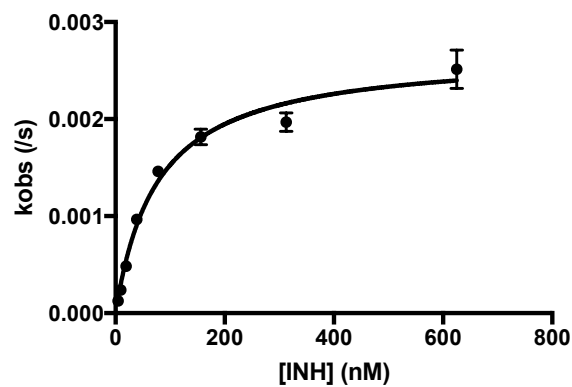
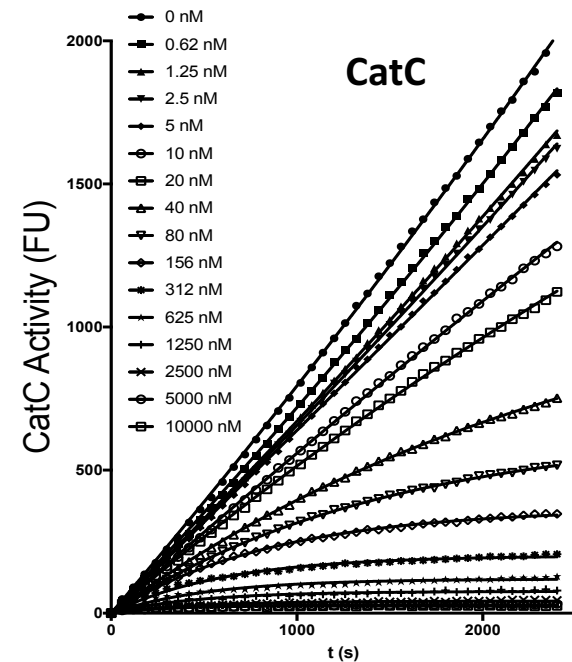
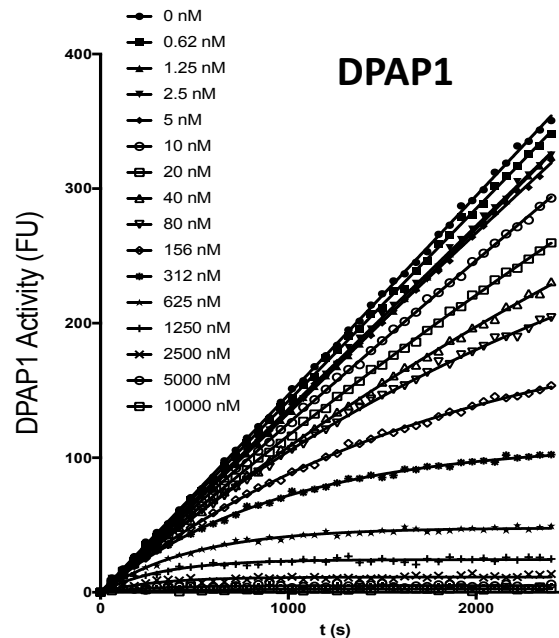
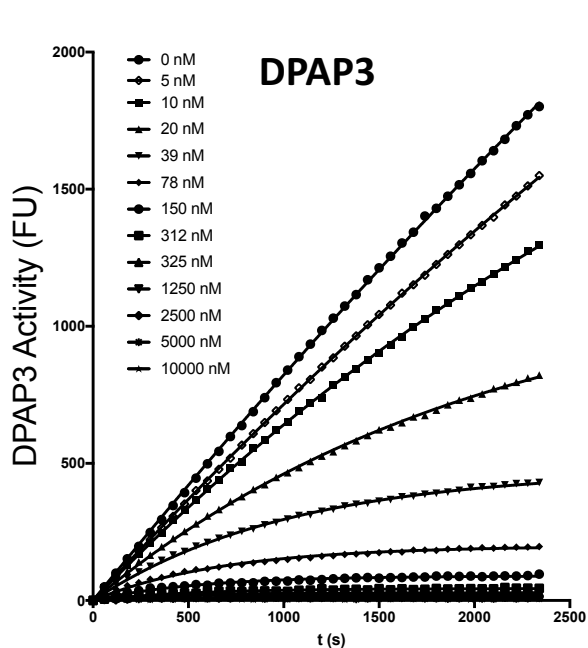
$$k_{inact}/K_i = 2,600 \pm 100 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0030 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 2,200 \pm 200 \text{ nM}$$

$$k_{inact}/K_i = 1,400 \pm 200 \text{ M}^{-1}\text{s}^{-1}$$

P2: Glu



$$k_{inact} = 0.0027 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 51 \pm 8 \text{ nM}$$

$$k_{inact}/K_i = 53,000 \pm 6,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0050 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 650 \pm 100 \text{ nM}$$

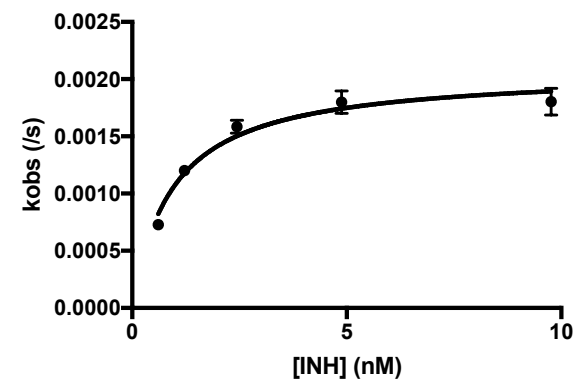
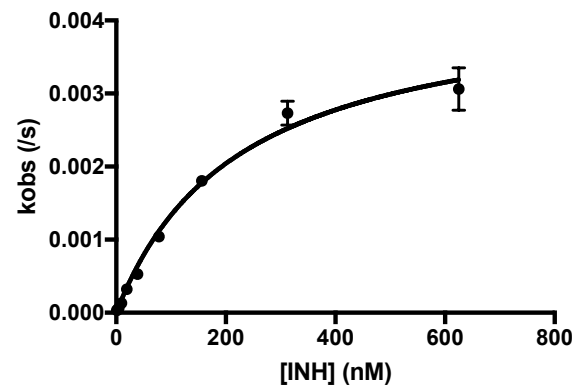
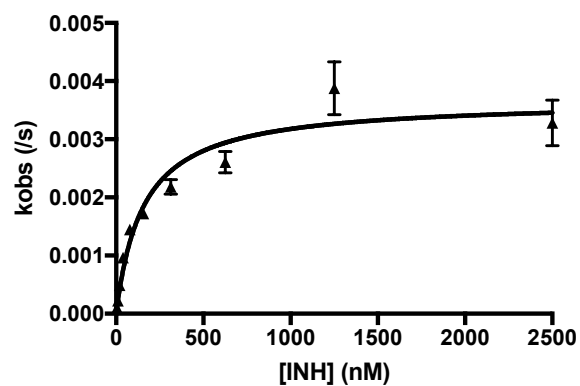
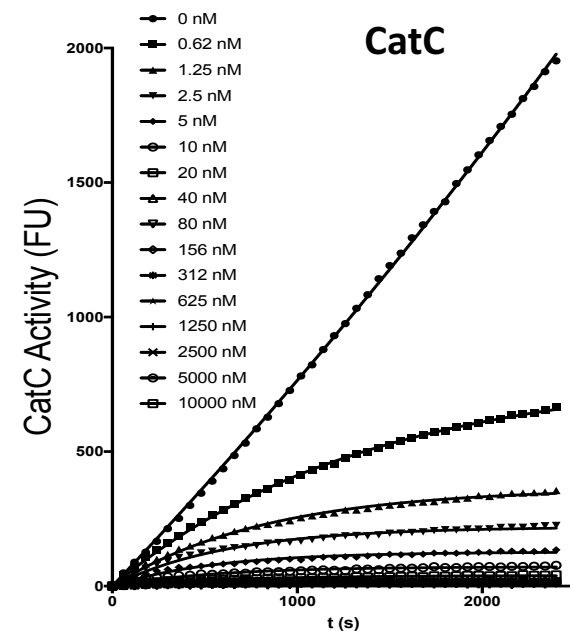
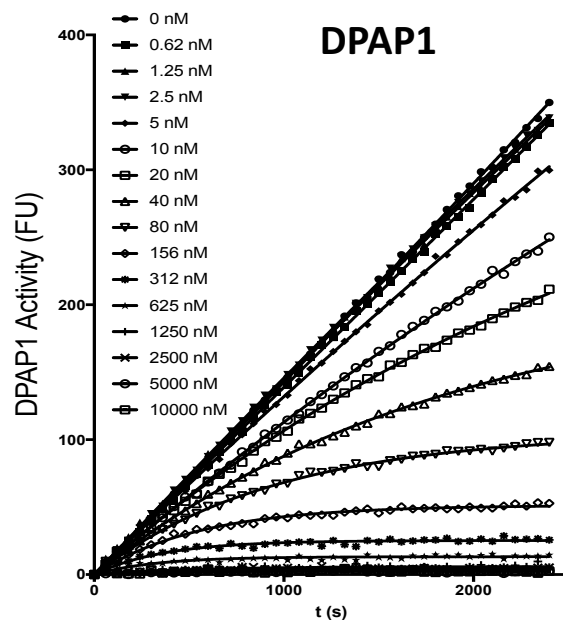
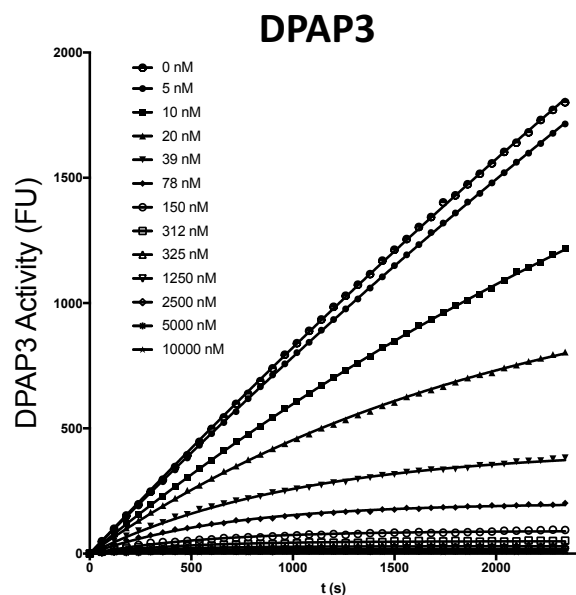
$$k_{inact}/K_i = 7,600 \pm 750 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0026 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 140 \pm 20 \text{ nM}$$

$$k_{inact}/K_i = 18,000 \pm 2,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: His



$$k_{inact} = 0.0037 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 100 \pm 25 \text{ nM}$$

$$k_{inact}/K_i = 35,500 \pm 7,500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0043 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 150 \pm 20 \text{ nM}$$

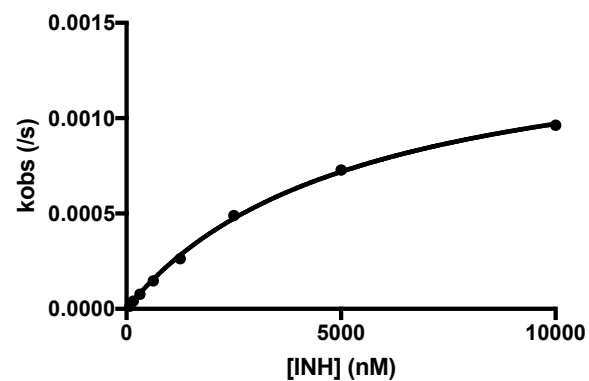
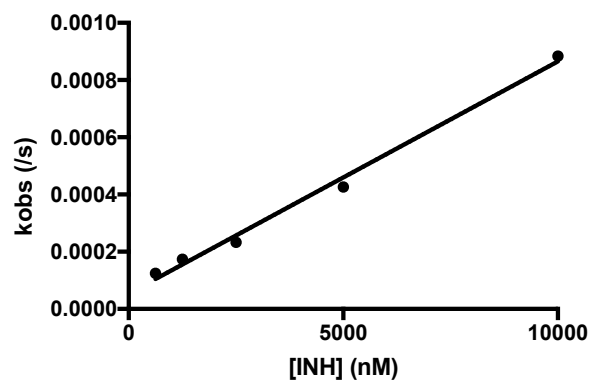
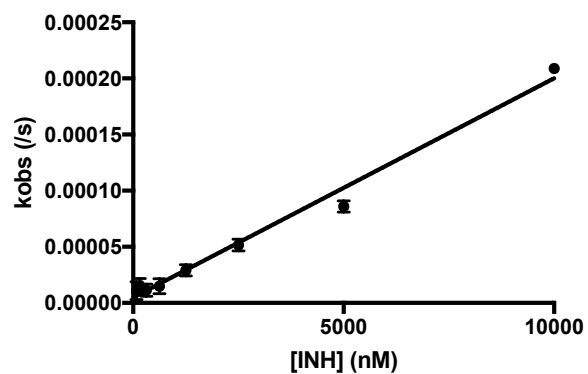
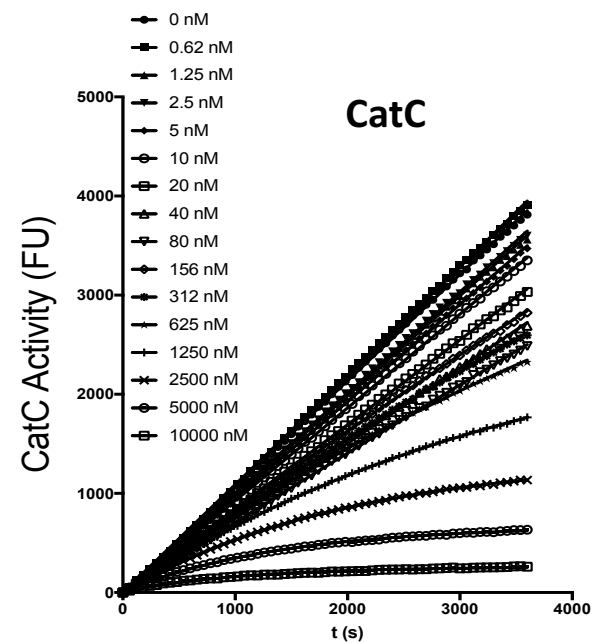
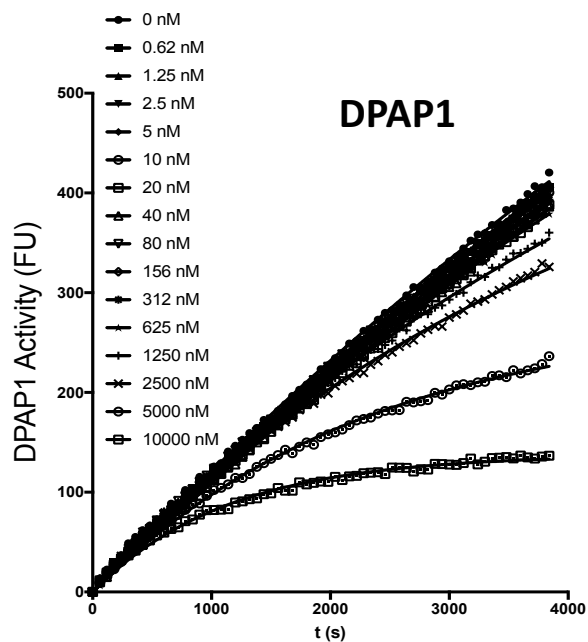
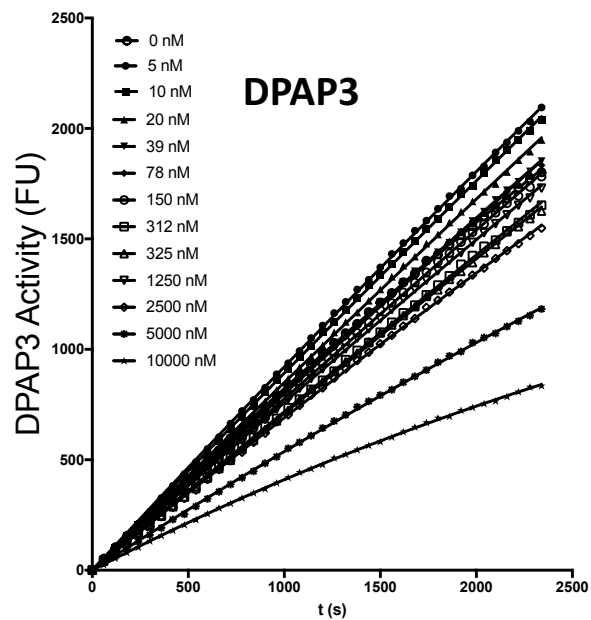
$$k_{inact}/K_i = 29,000 \pm 3,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0021 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 0.6 \pm 0.1 \text{ nM}$$

$$k_{inact}/K_i = 3,350,000 \pm 500,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Lys

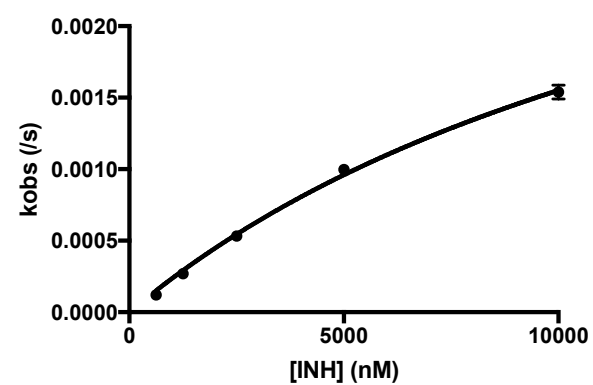
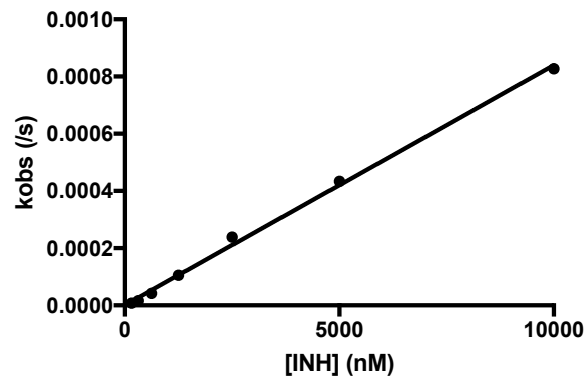
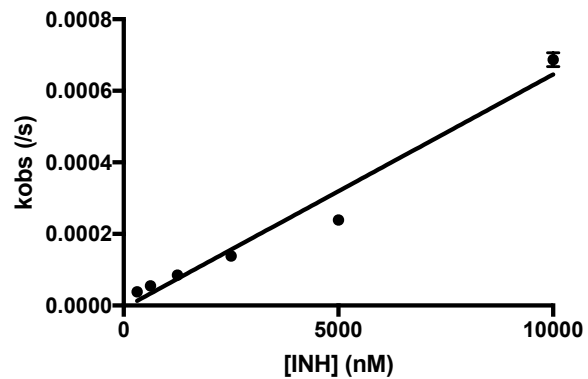
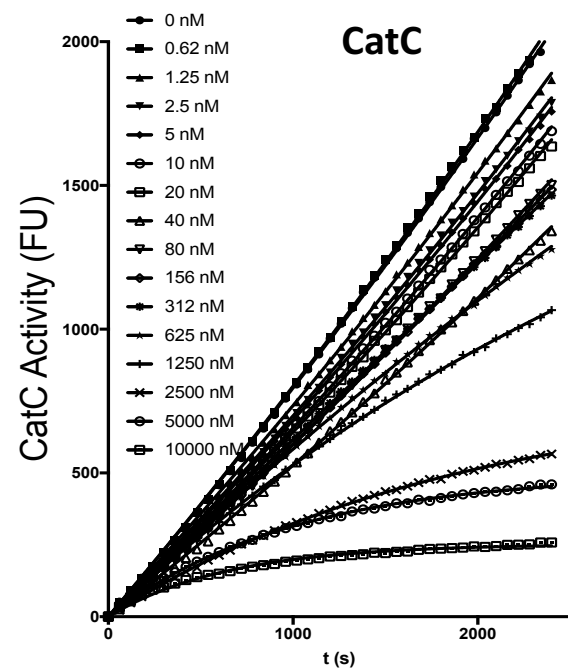
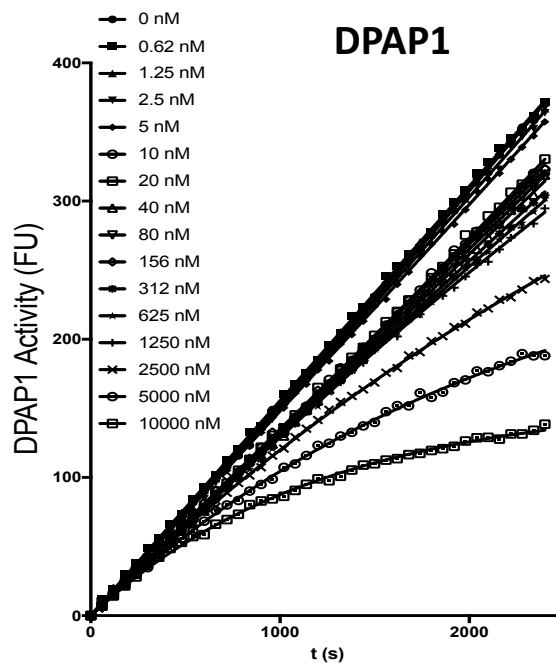
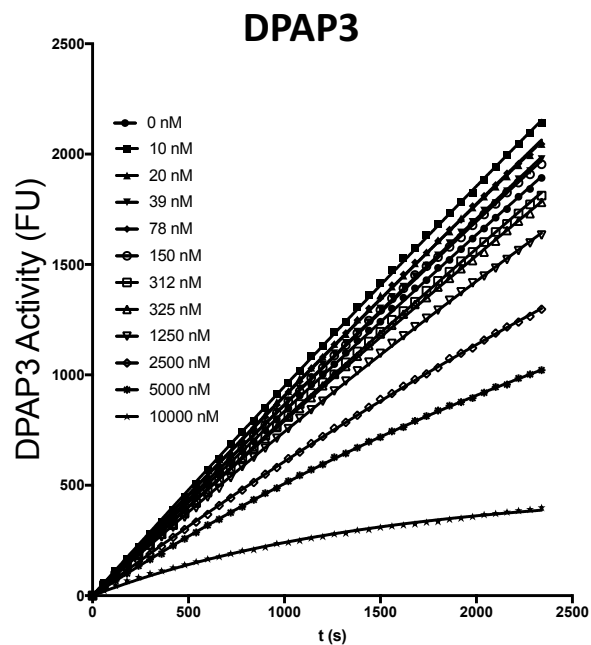


k_{inact} = N.S.
 K_i = N.S.
 $k_{inact}/k_i = 20 \pm 1 \text{ M}^{-1}\text{s}^{-1}$

k_{inact} = N.S.
 K_i = N.S.
 $k_{inact}/K_i = 81 \pm 4 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.00149 \pm 0.00005 \text{ s}^{-1}$
 $K_i = 3,600 \pm 200 \text{ nM}$
 $k_{inact}/K_i = 420 \pm 20 \text{ M}^{-1}\text{s}^{-1}$

P2: Arg



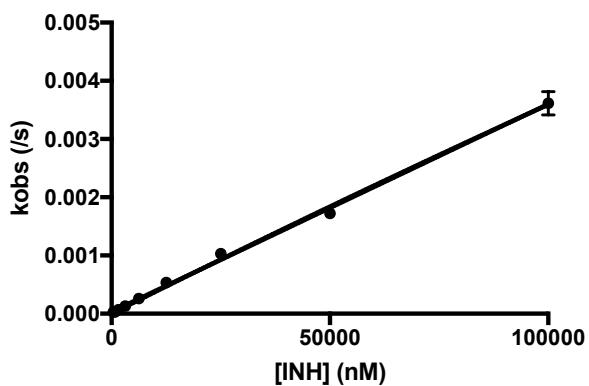
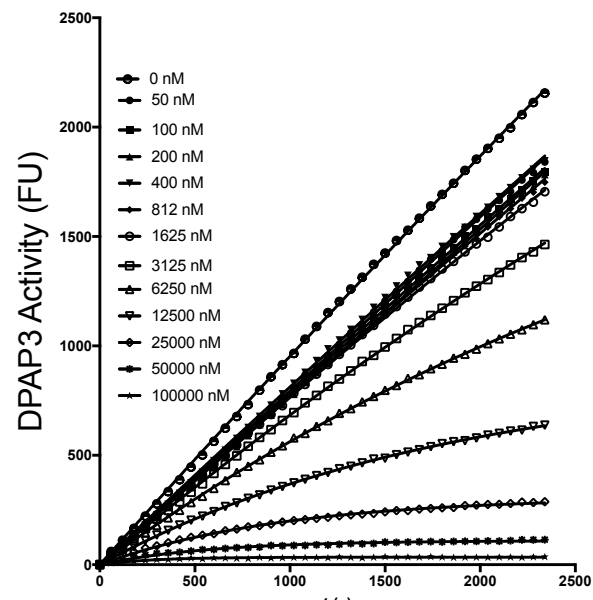
k_{inact} = N.S.
 K_i = N.S.
 $k_{inact}/k_i = 65 \pm 6 \text{ M}^{-1}\text{s}^{-1}$

k_{inact} = N.S.
 K_i = N.S.
 $k_{inact}/K_i = 84 \pm 2 \text{ M}^{-1}\text{s}^{-1}$

k_{inact} = $0.0040 \pm 0.0005 \text{ s}^{-1}$
 K_i = $11,000 \pm 2,000 \text{ nM}$
 $k_{inact}/K_i = 380 \pm 20 \text{ M}^{-1}\text{s}^{-1}$

P2: Phg

DPAP3

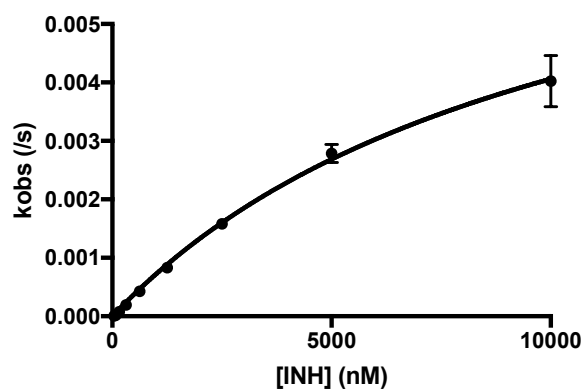
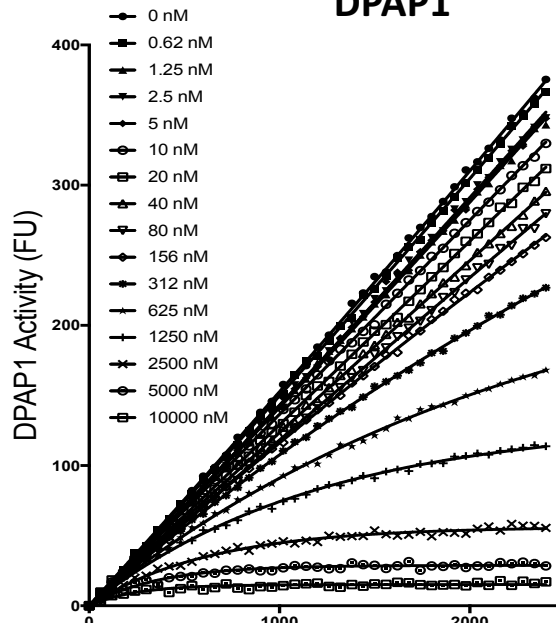


$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

$$K_{inact}/k_i = 35.7 \pm 0.7 \text{ M}^{-1}\text{s}^{-1}$$

DPAP1

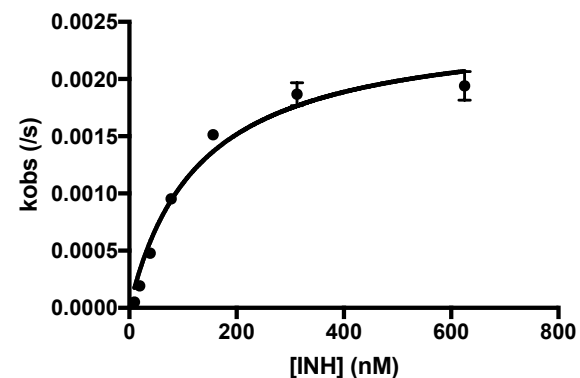
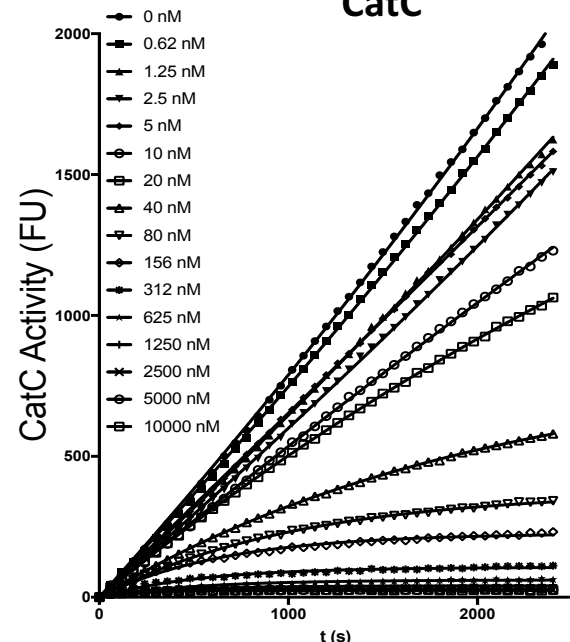


$$k_{inact} = 0.0083 \pm 0.0005 \text{ s}^{-1}$$

$$K_i = 7,000 \pm 650 \text{ nM}$$

$$K_{inact}/k_i = 1,190 \pm 50 \text{ M}^{-1}\text{s}^{-1}$$

CatC

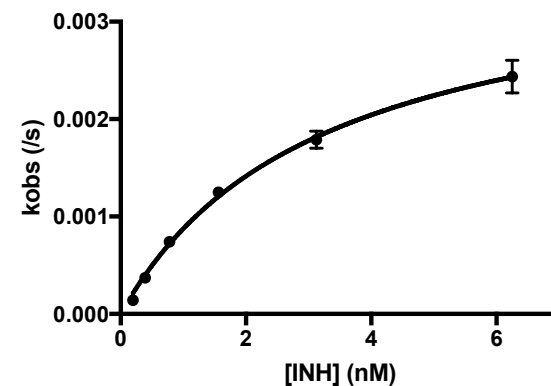
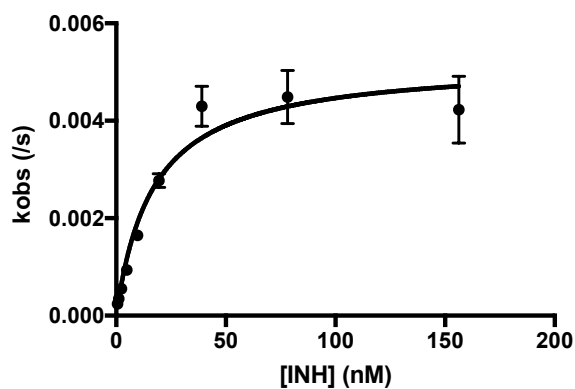
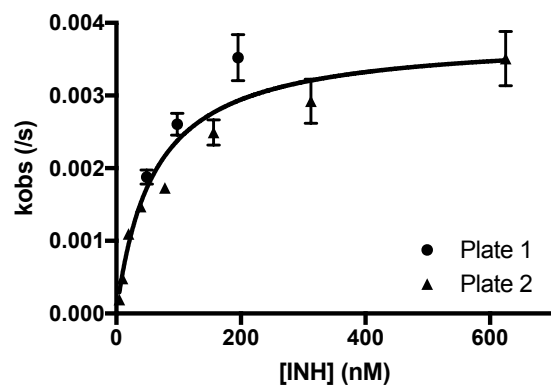
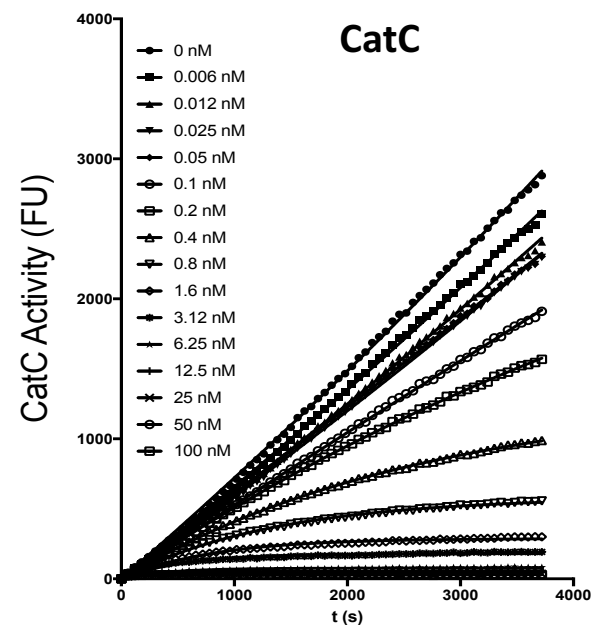
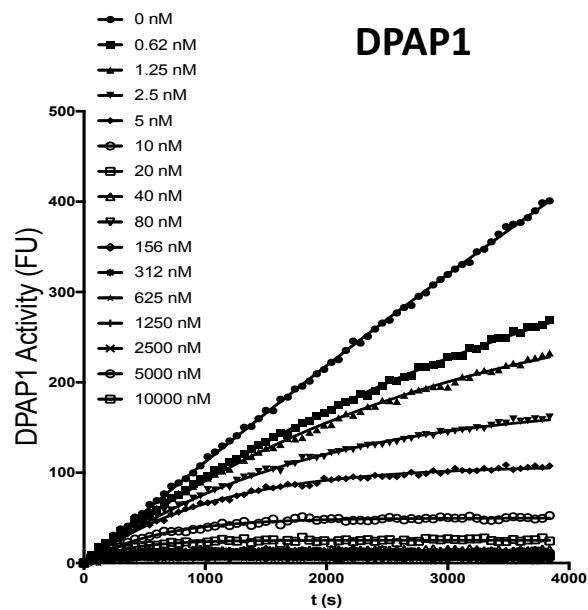
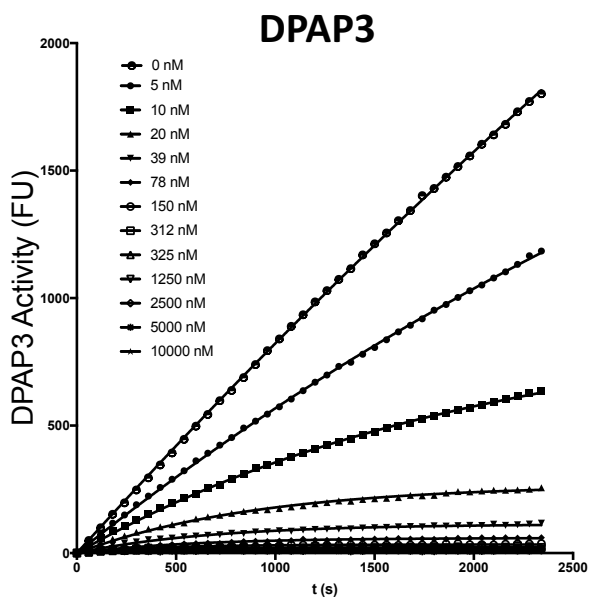


$$k_{inact} = 0.0025 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 86 \pm 20 \text{ nM}$$

$$K_{inact}/k_i = 29,000 \pm 5,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: 2fa



$$k_{inact} = 0.0038 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 39 \pm 10 \text{ nM}$$

$$k_{inact}/K_i = 97,000 \pm 18,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0052 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 11 \pm 3 \text{ nM}$$

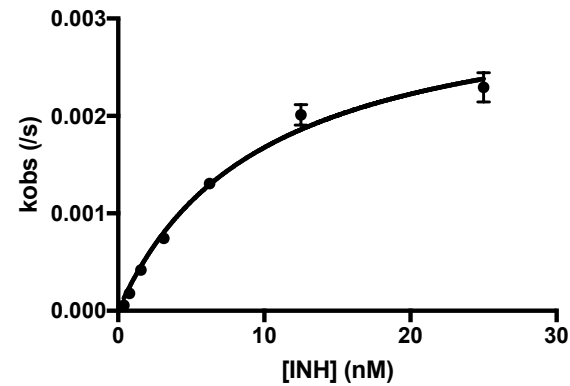
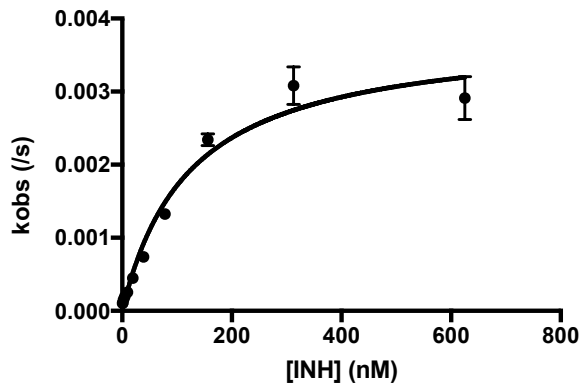
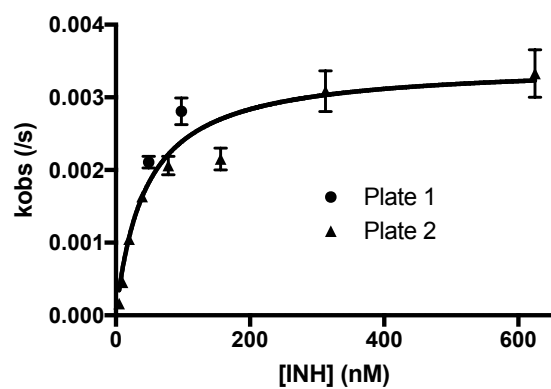
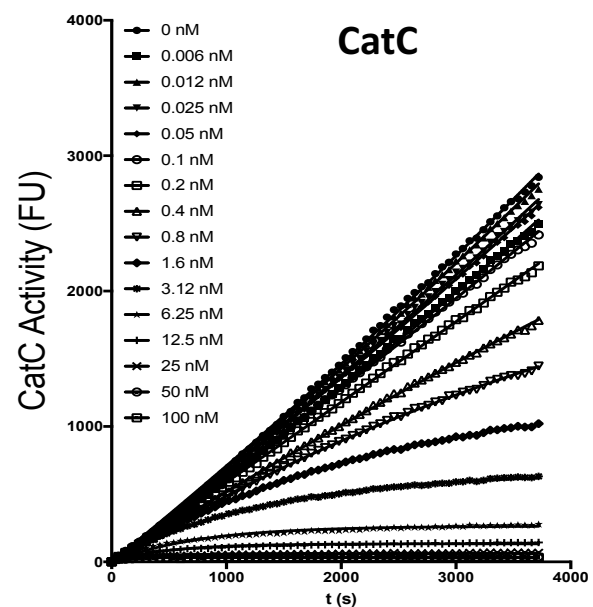
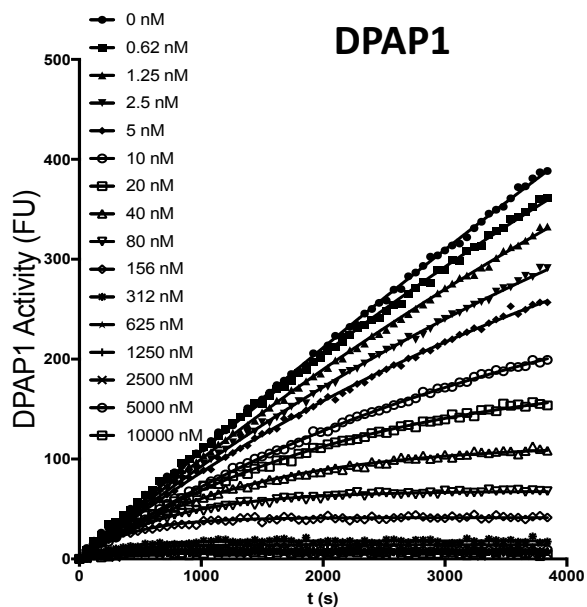
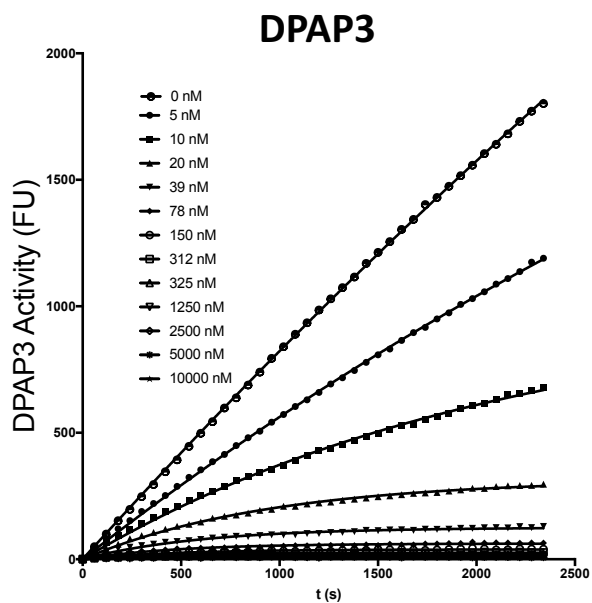
$$k_{inact}/K_i = 470,000 \pm 90,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0037 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 2.1 \pm 0.2 \text{ nM}$$

$$k_{inact}/K_i = 1,720,000 \pm 90,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: 2ta



$$k_{inact} = 0.0034 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 29 \pm 7 \text{ nM}$$

$$k_{inact}/K_i = 118,000 \pm 23,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0038 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 82 \pm 20 \text{ nM}$$

$$k_{inact}/K_i = 47,000 \pm 7,000 \text{ M}^{-1}\text{s}^{-1}$$

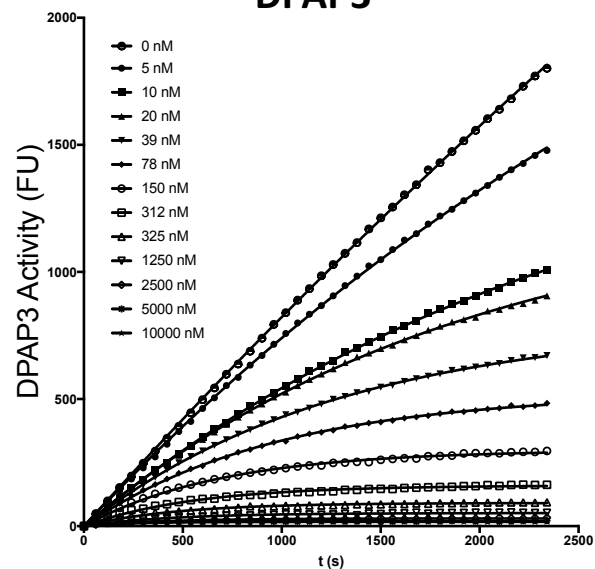
$$k_{inact} = 0.0033 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 6.5 \pm 1 \text{ nM}$$

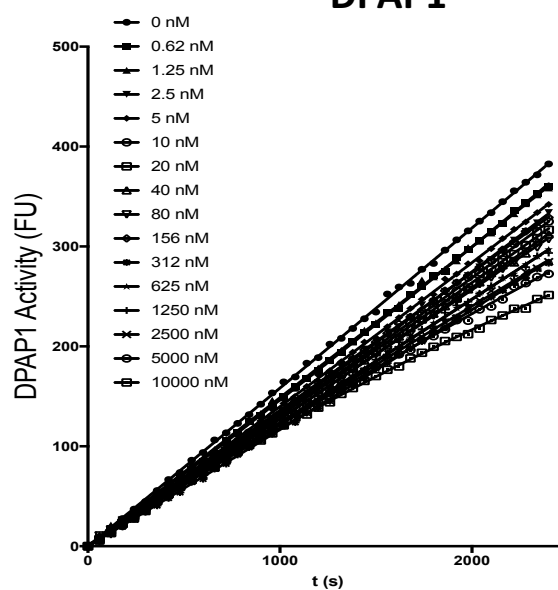
$$k_{inact}/K_i = 510,000 \pm 55,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Cha

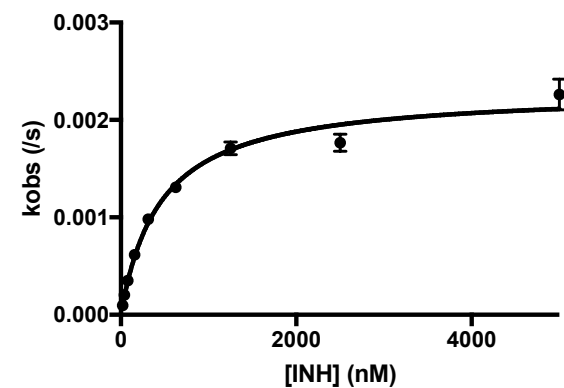
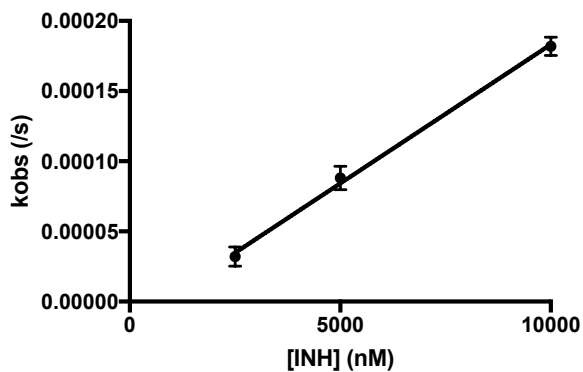
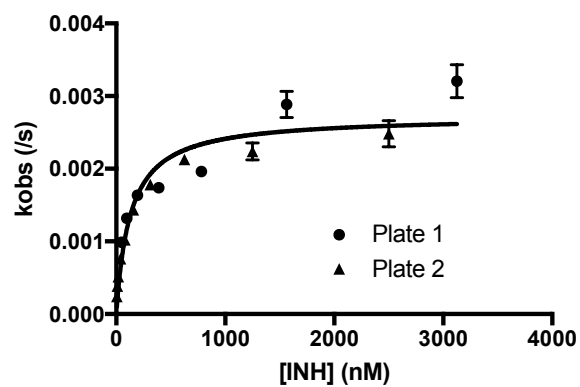
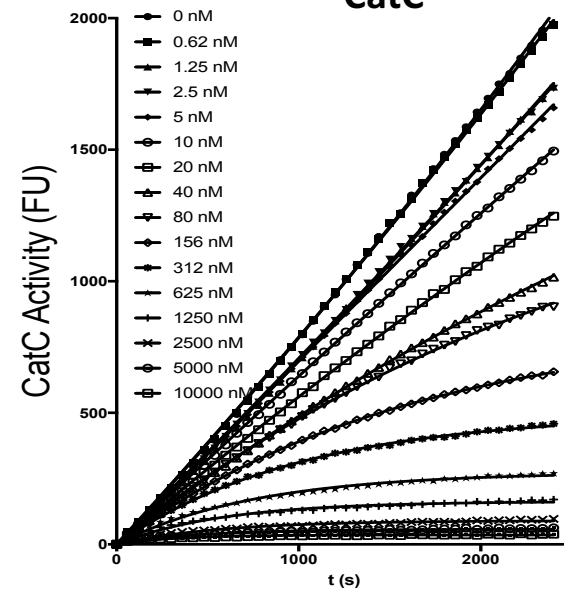
DPAP3



DPAP1



CatC



$$k_{inact} = 0.0027 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 90 \pm 20 \text{ nM}$$

$$k_{inact}/K_i = 31,600 \pm 5,500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

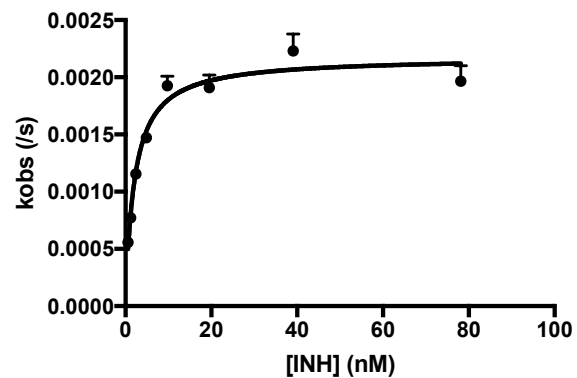
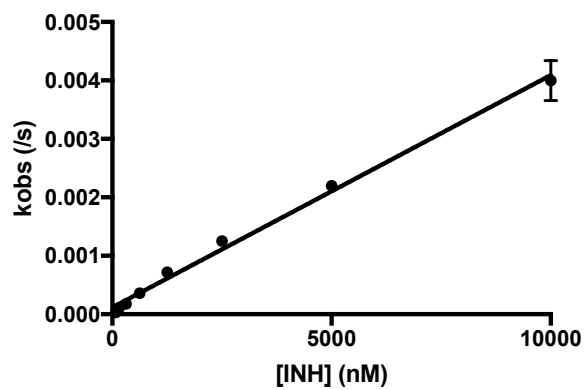
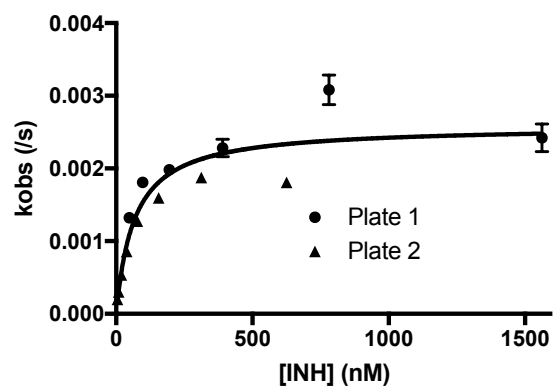
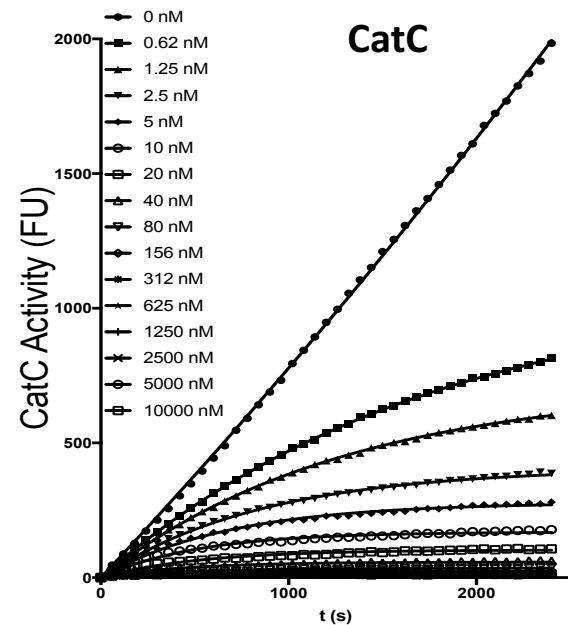
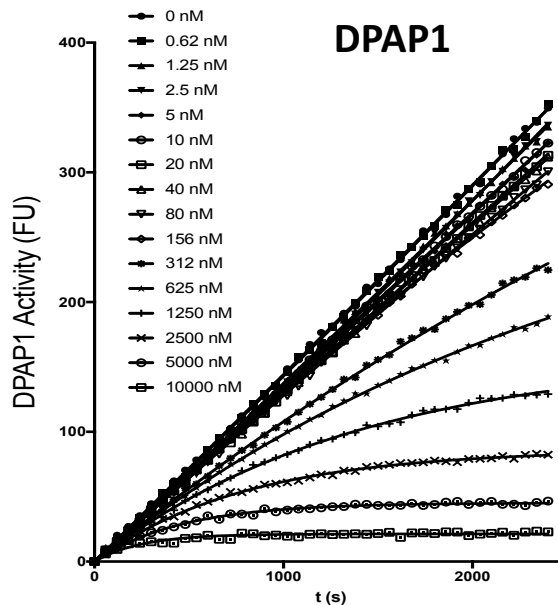
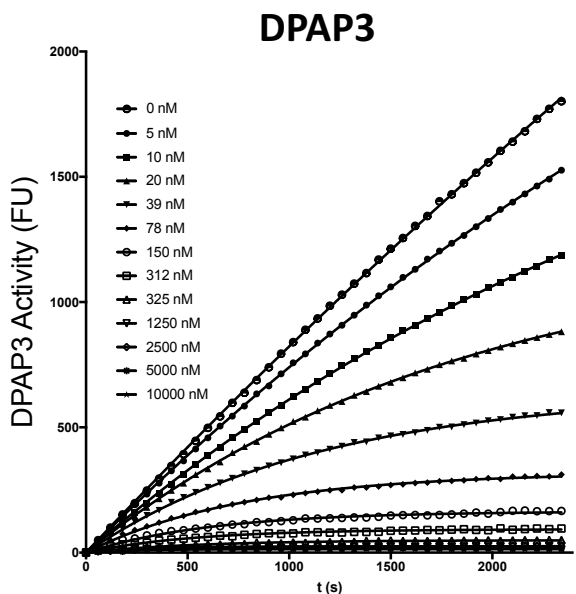
$$k_{inact}/K_i = 20 \pm 1 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0023 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 300 \pm 40 \text{ nM}$$

$$k_{inact}/K_i = 7,700 \pm 800 \text{ M}^{-1}\text{s}^{-1}$$

P2: Phe



$$k_{inact} = 0.0026 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 45 \pm 13 \text{ nM}$$

$$k_{inact}/K_i = 58,000 \pm 13,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

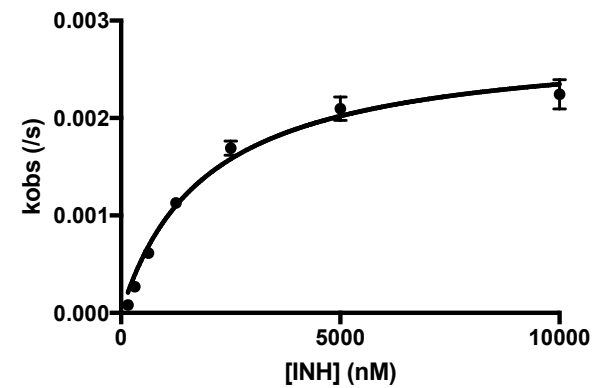
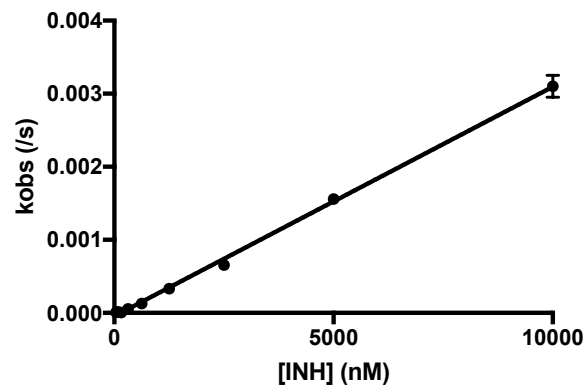
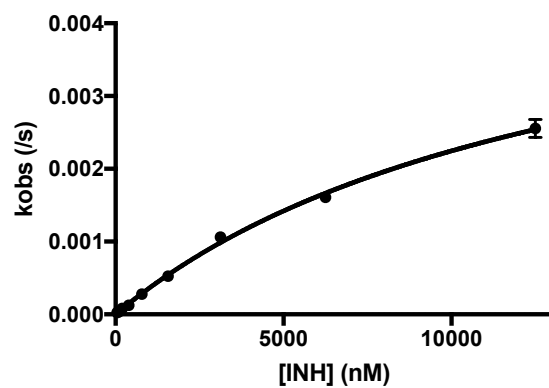
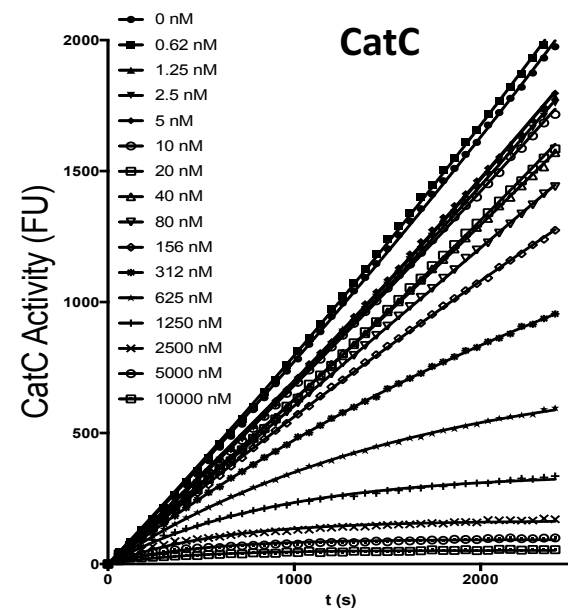
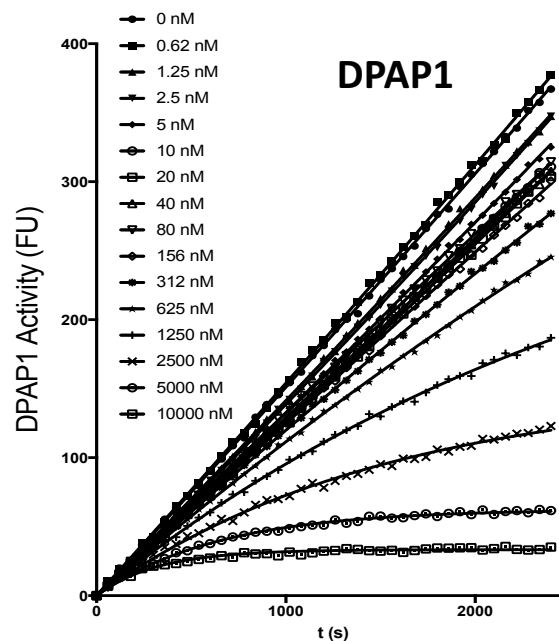
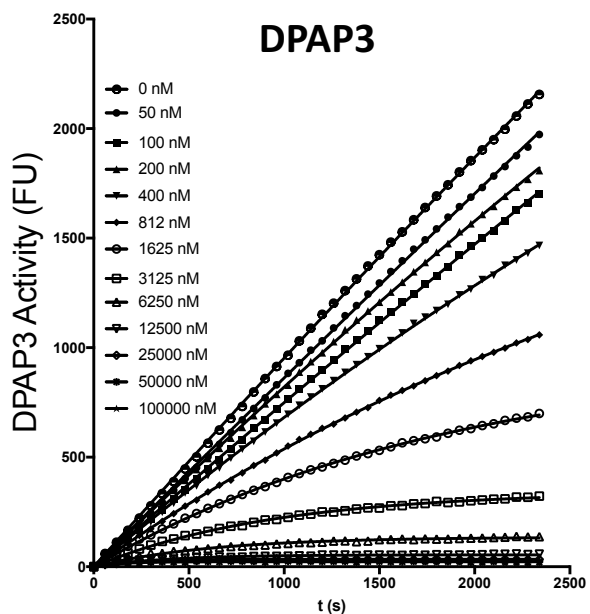
$$k_{inact}/K_i = 400 \pm 10 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00218 \pm 0.00008 \text{ s}^{-1}$$

$$K_i = 1.4 \pm 0.2 \text{ nM}$$

$$k_{inact}/K_i = 1,600,000 \pm 200,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Phe(Me)



$$k_{inact} = 0.054 \pm 0.004 \text{ s}^{-1}$$

$$K_i = 9,200 \pm 300 \text{ nM}$$

$$k_{inact}/K_i = 580 \pm 25 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

$$k_{inact}/K_i = 314 \pm 5 \text{ M}^{-1}\text{s}^{-1}$$

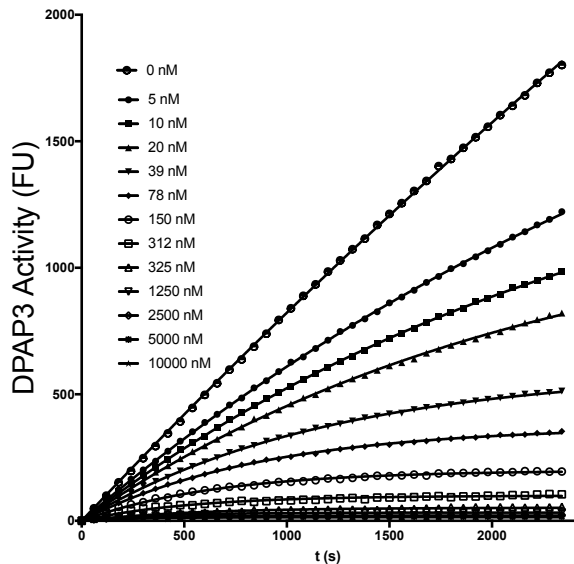
$$k_{inact} = 0.0028 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 1,300 \pm 200 \text{ nM}$$

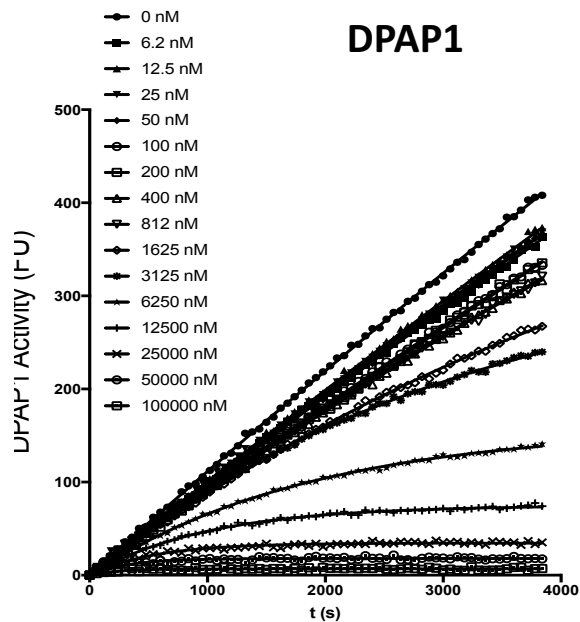
$$k_{inact}/K_i = 2,200 \pm 300 \text{ M}^{-1}\text{s}^{-1}$$

P2: Phe(I)

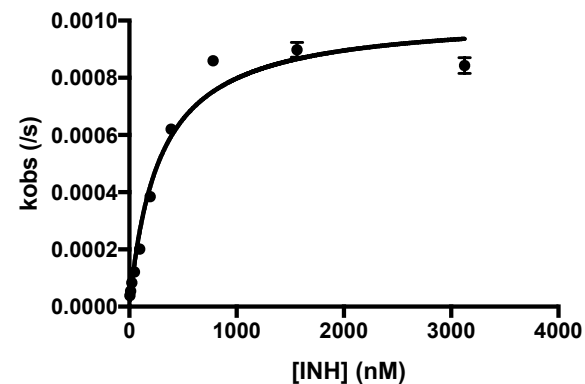
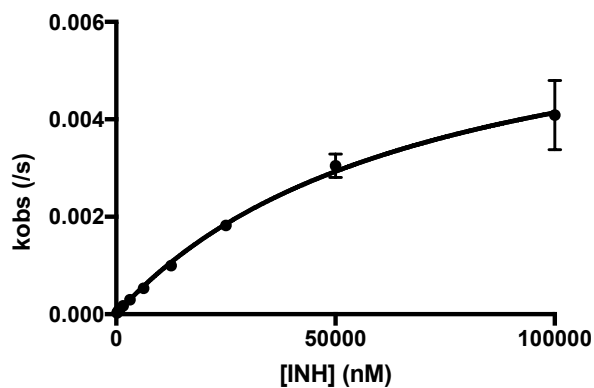
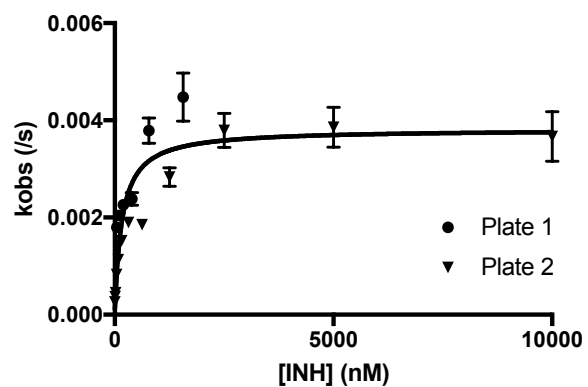
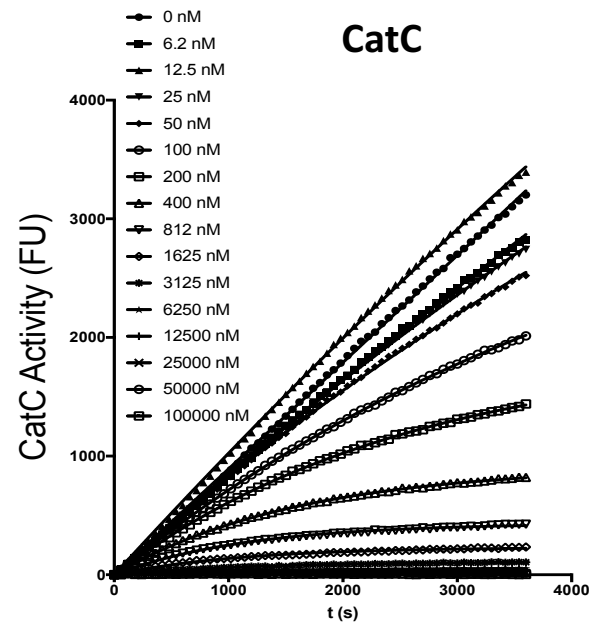
DPAP3



DPAP1



CatC



$$k_{inact} = 0.0038 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 110 \pm 30 \text{ nM}$$

$$k_{inact}/K_i = 36,000 \pm 10,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0070 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 47,000 \pm 3,500 \text{ nM}$$

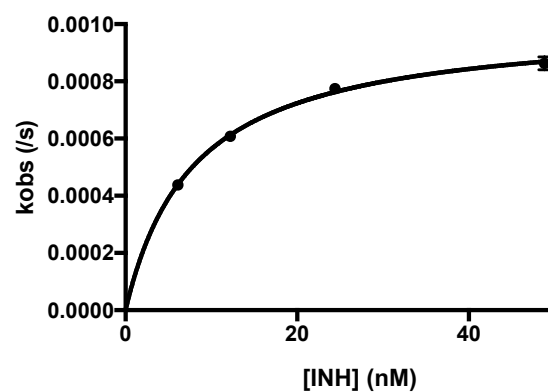
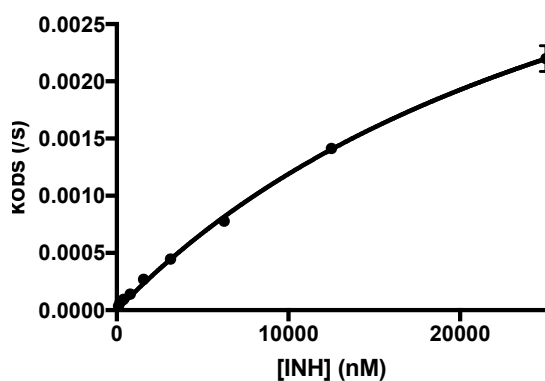
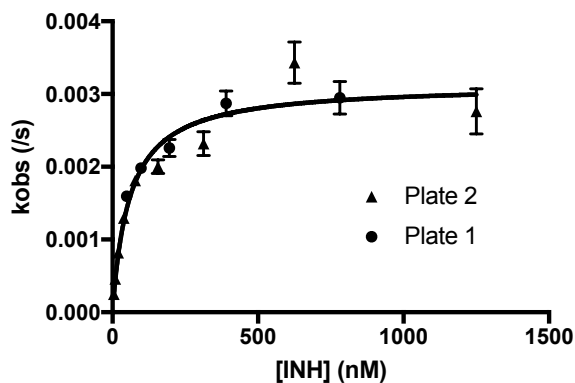
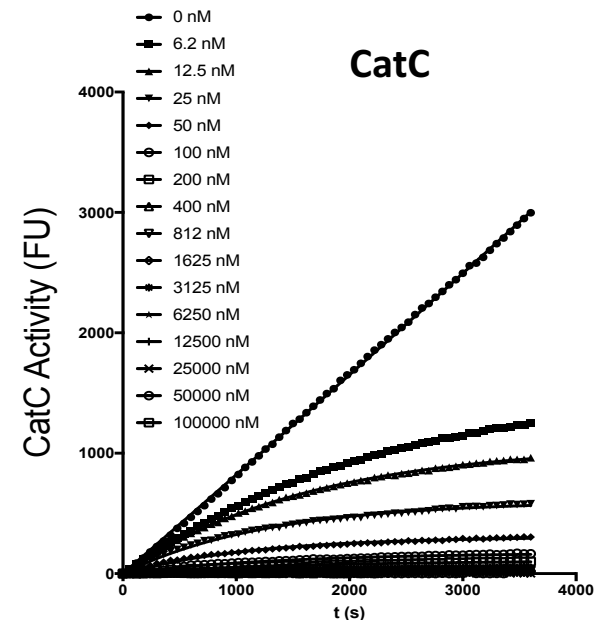
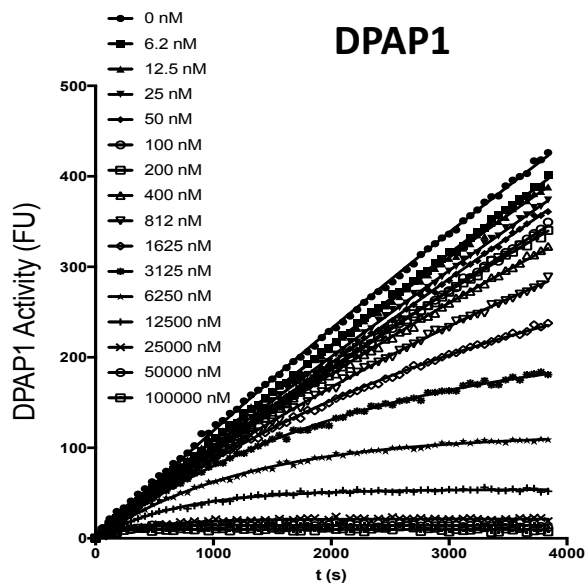
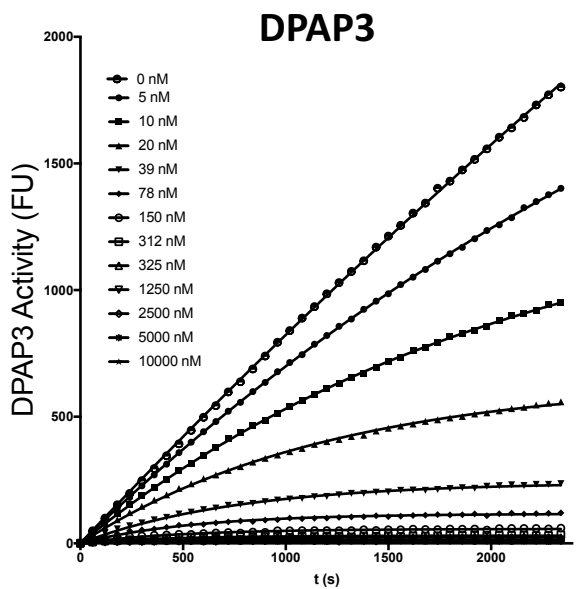
$$k_{inact}/K_i = 151 \pm 6 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00102 \pm 0.00006 \text{ s}^{-1}$$

$$K_i = 185 \pm 40 \text{ nM}$$

$$k_{inact}/K_i = 5,500 \pm 900 \text{ M}^{-1}\text{s}^{-1}$$

P2: Phe(NH₂)



$$k_{\text{inact}} = 0.0031 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 39 \pm 7 \text{ nM}$$

$$k_{\text{inact}}/K_i = 80,000 \pm 12,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0050 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 22,000 \pm 2,500 \text{ nM}$$

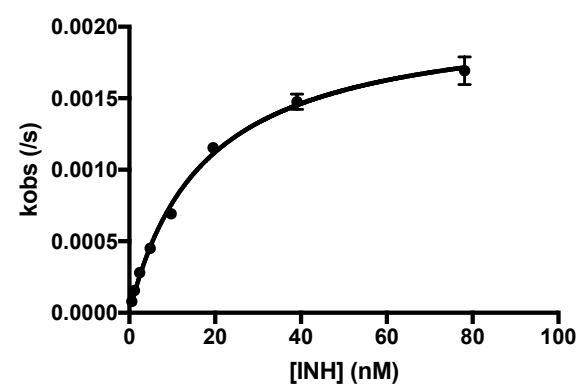
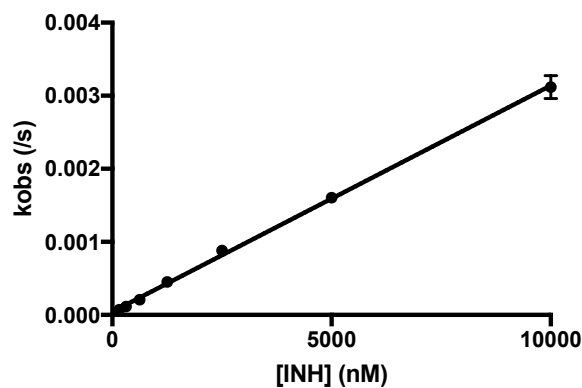
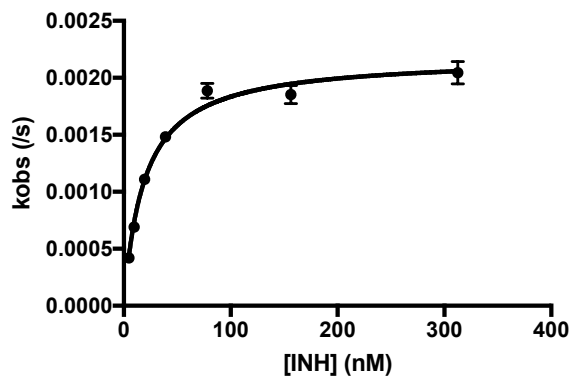
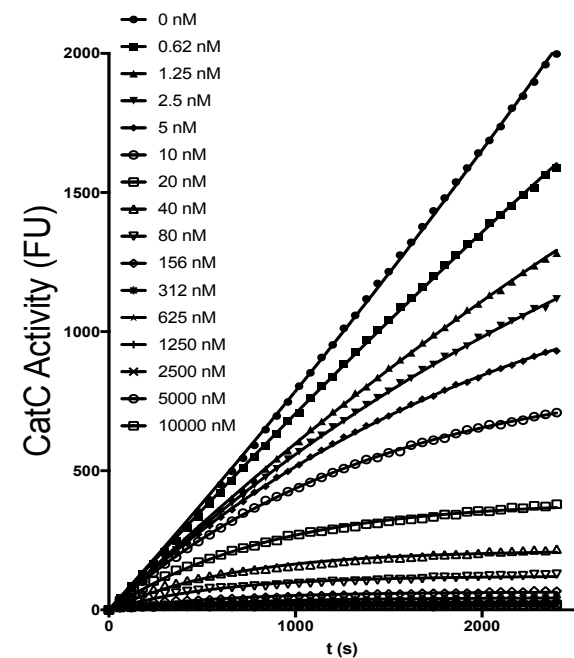
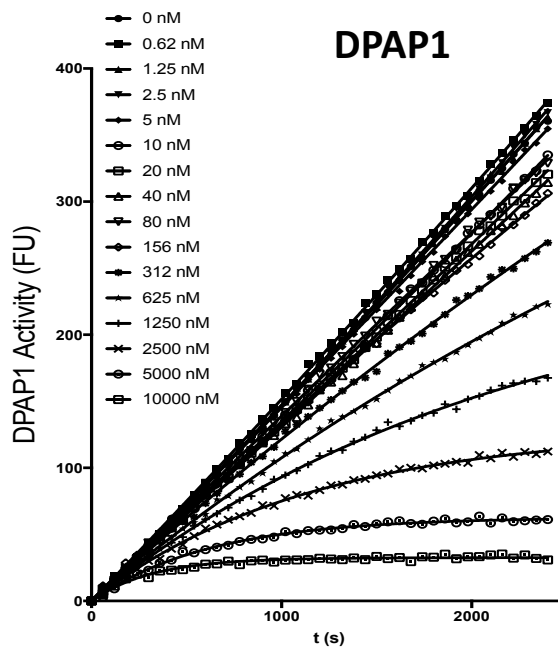
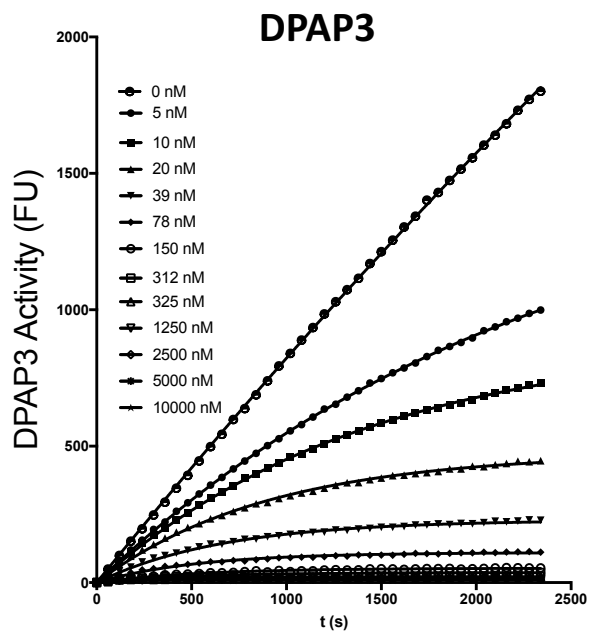
$$k_{\text{inact}}/K_i = 230 \pm 10 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.00101 \pm 0.00002 \text{ s}^{-1}$$

$$K_i = 5.3 \pm 0.3 \text{ nM}$$

$$k_{\text{inact}}/K_i = 190,000 \pm 9,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Tyr



$$k_{inact} = 0.00218 \pm 0.00007 \text{ s}^{-1}$$

$$K_i = 12.7 \pm 1.5 \text{ nM}$$

$$k_{inact}/K_i = 172,000 \pm 16,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

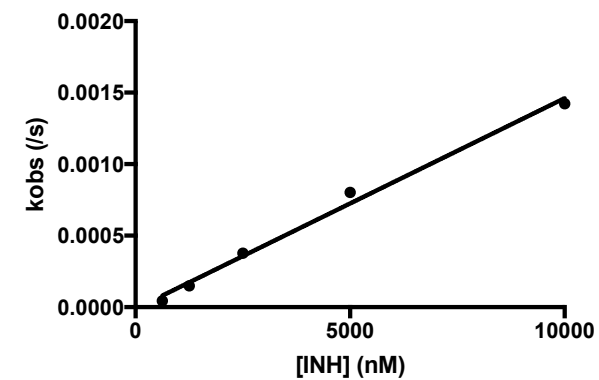
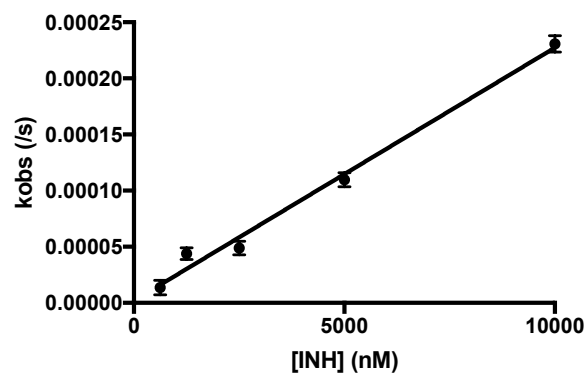
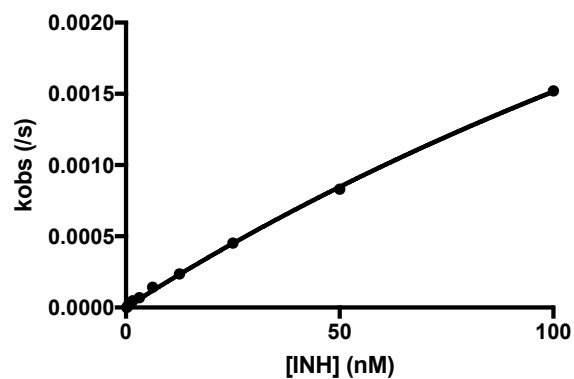
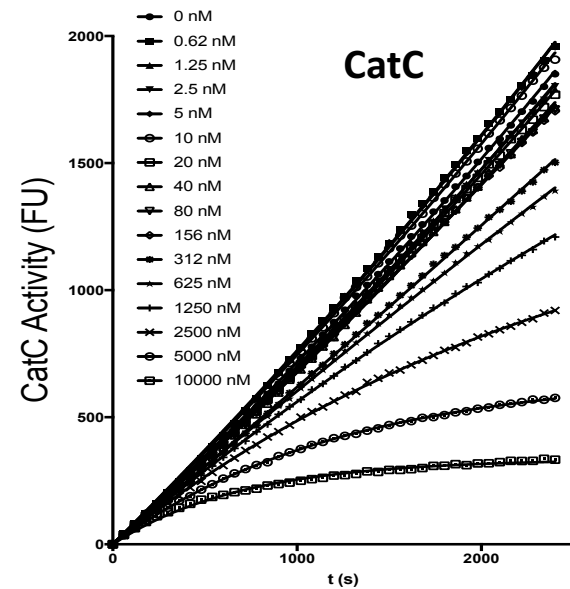
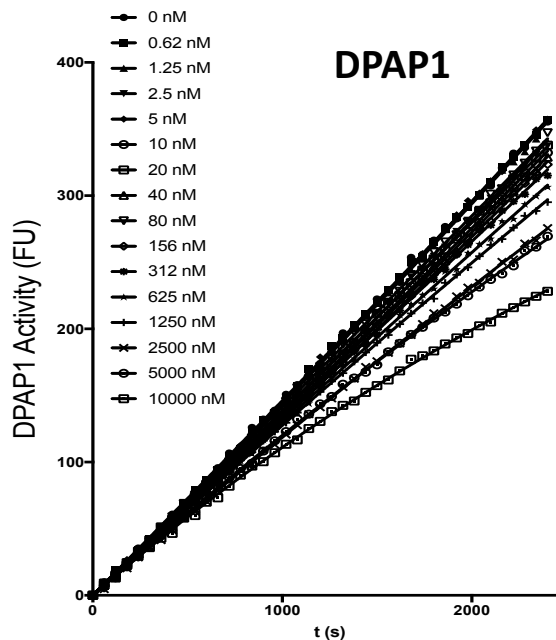
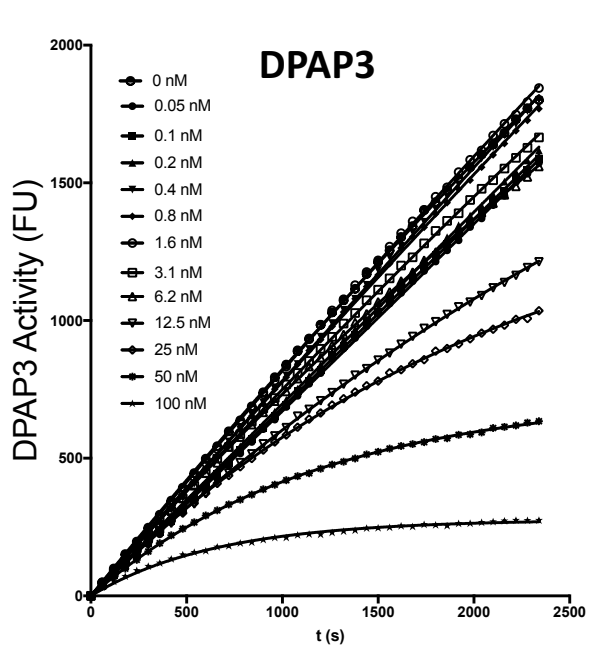
$$k_{inact}/K_i = 310 \pm 5 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00210 \pm 0.00007 \text{ s}^{-1}$$

$$K_i = 11.5 \pm 0.9 \text{ nM}$$

$$k_{inact}/K_i = 182,000 \pm 10,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Tyr(NO₂)



$$k_{inact} = 0.0072 \pm 0.0009 \text{ s}^{-1}$$

$$K_i = 250 \pm 40 \text{ nM}$$

$$k_{inact}/K_i = 28,800 \pm 800 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

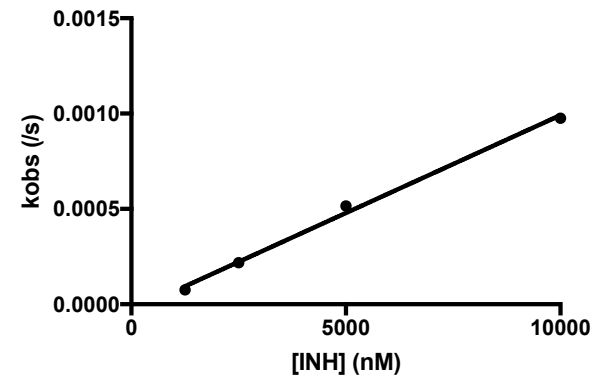
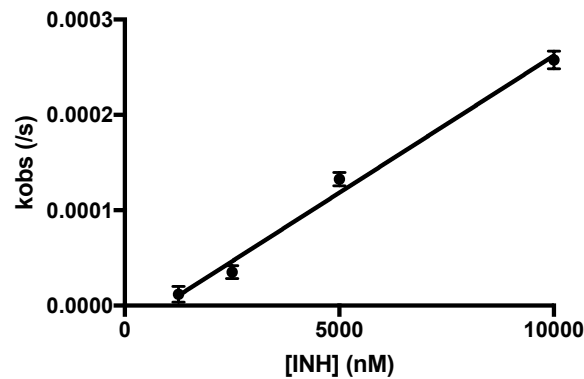
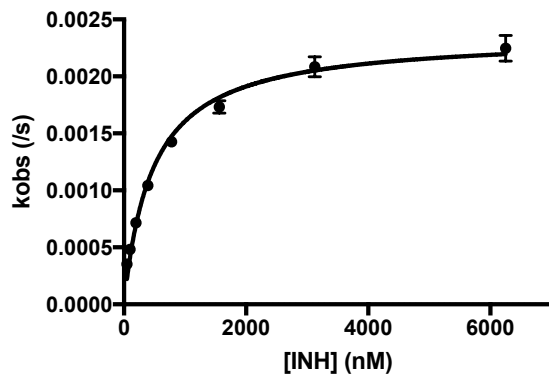
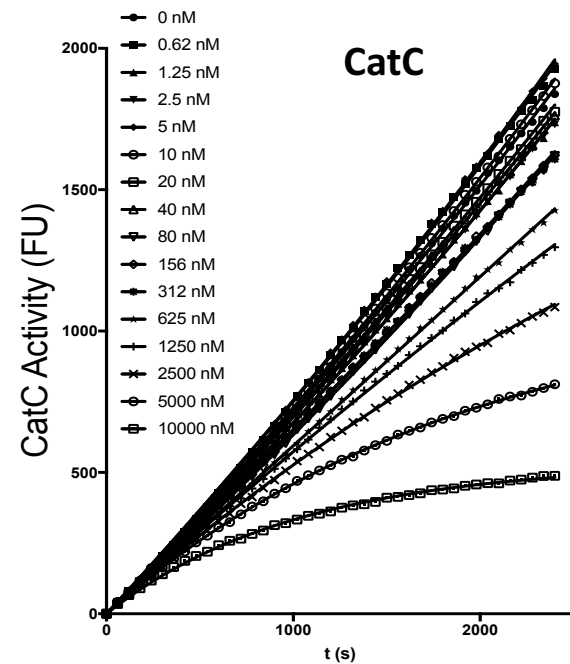
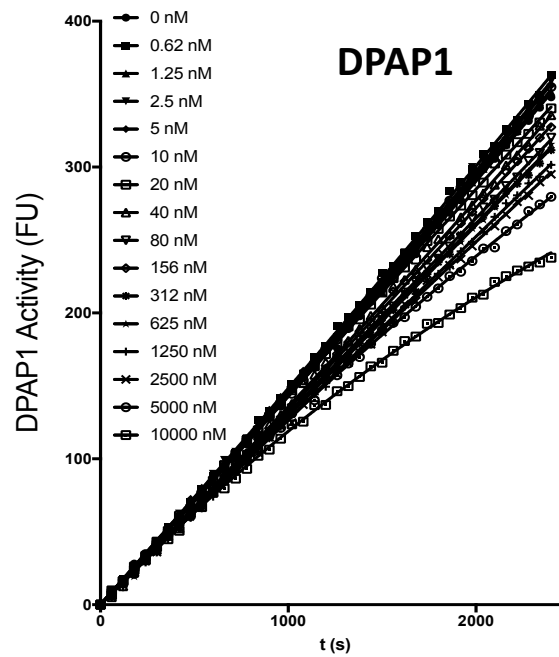
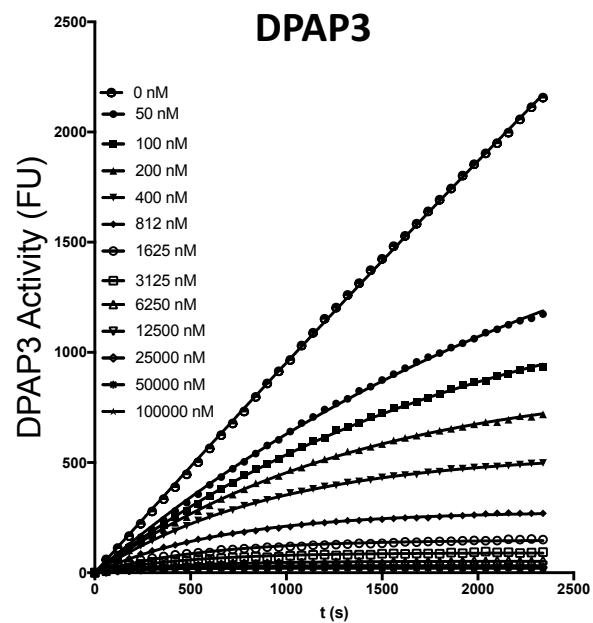
$$k_{inact}/K_i = 23 \pm 2 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

$$k_{inact}/K_i = 147 \pm 8 \text{ M}^{-1}\text{s}^{-1}$$

P2: hPhe



$$k_{inact} = 0.00236 \pm 0.00008 \text{ s}^{-1}$$

$$K_i = 310 \pm 30 \text{ nM}$$

$$k_{inact}/K_i = 7,600 \pm 700 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

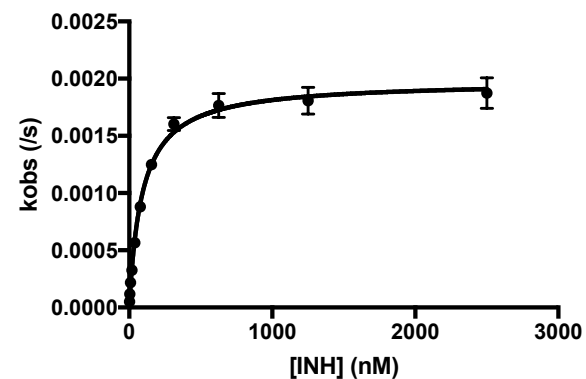
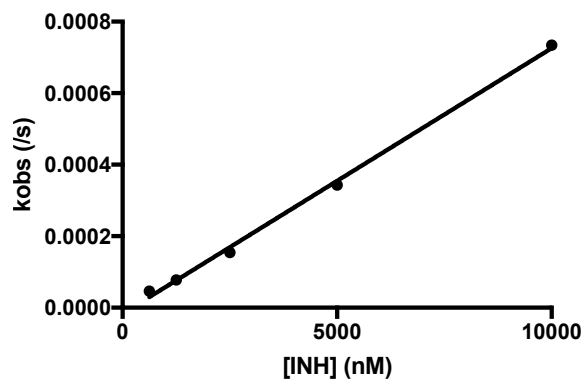
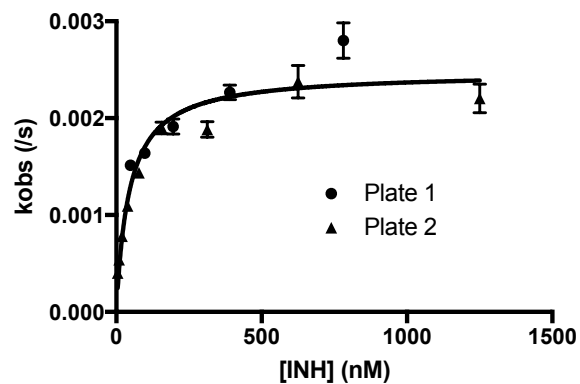
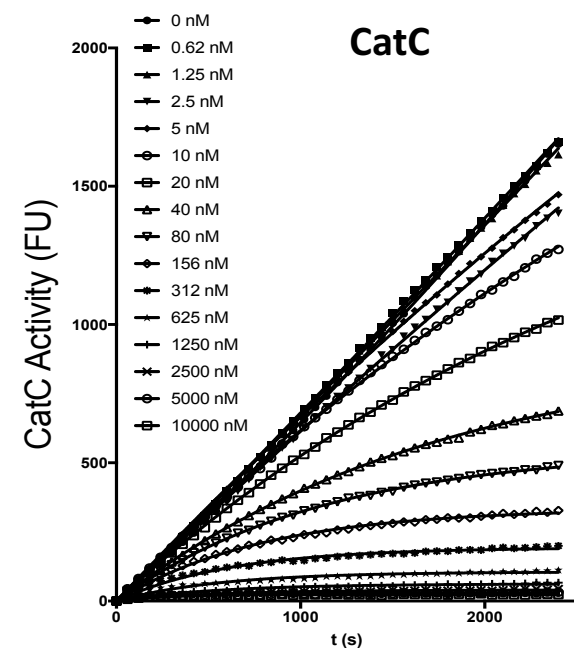
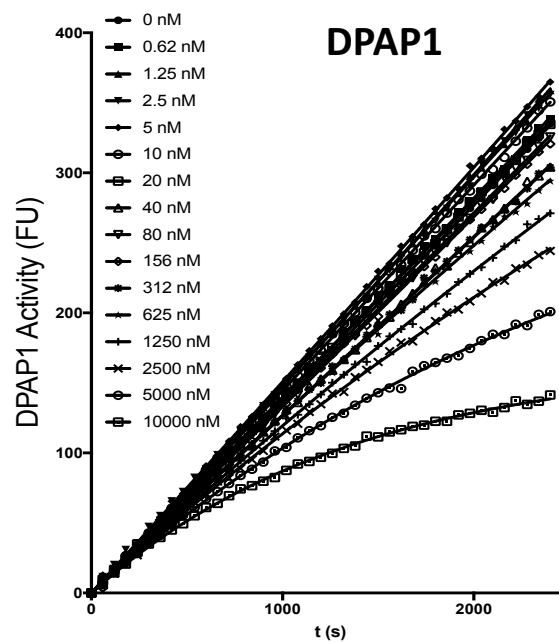
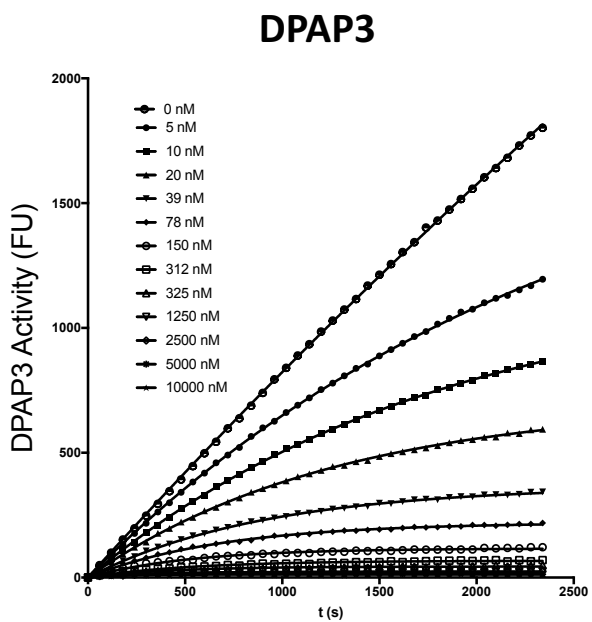
$$k_{inact}/K_i = 29 \pm 2 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

$$k_{inact}/K_i = 103 \pm 5 \text{ M}^{-1}\text{s}^{-1}$$

P2: Trp



$$k_{inact} = 0.0025 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 30 \pm 6 \text{ nM}$$

$$k_{inact}/K_i = 84,000 \pm 14,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

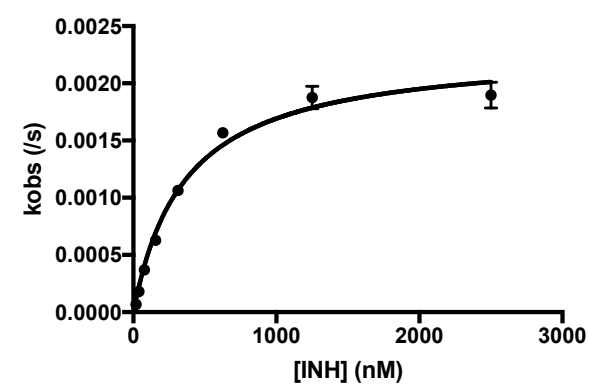
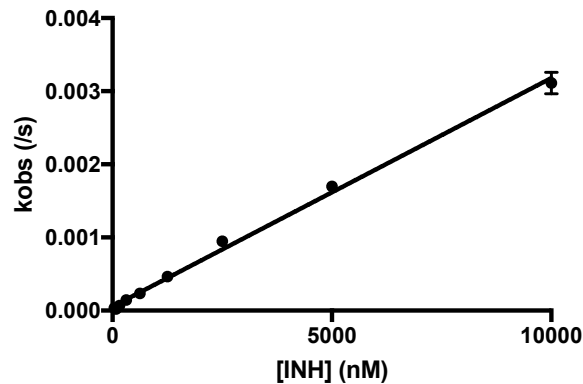
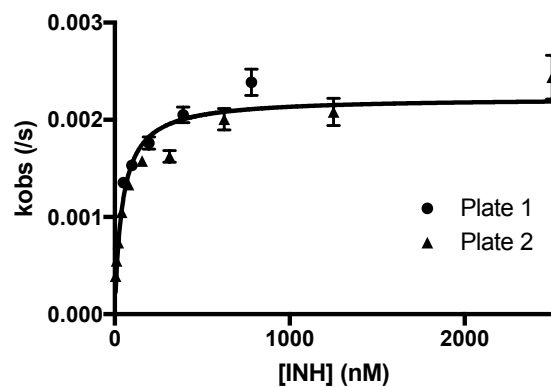
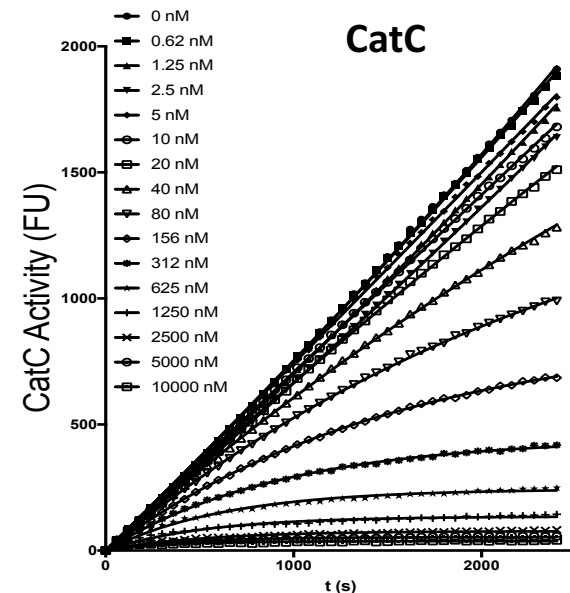
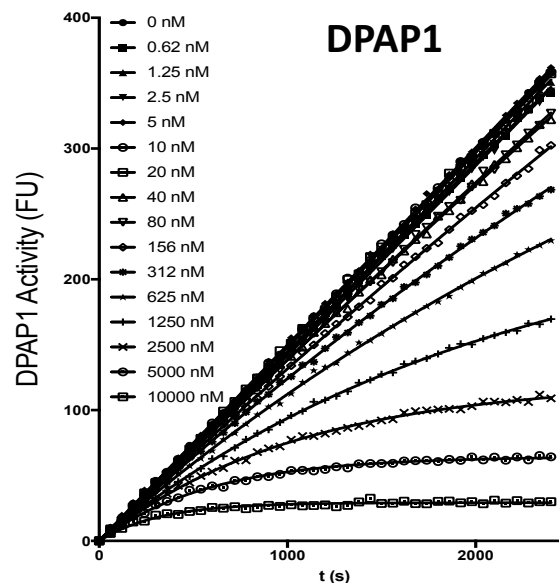
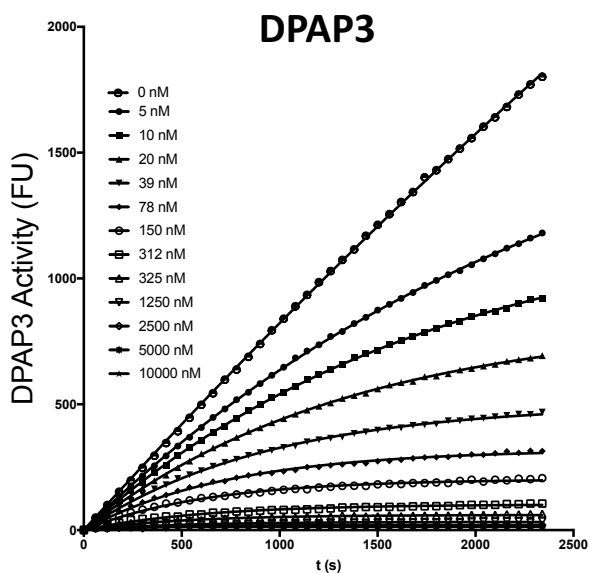
$$k_{inact}/K_i = 74 \pm 2 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00198 \pm 0.00003 \text{ s}^{-1}$$

$$K_i = 61 \pm 4 \text{ nM}$$

$$k_{inact}/K_i = 32,000 \pm 2,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Igl



$$k_{inact} = 0.0022 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 29 \pm 5 \text{ nM}$$

$$k_{inact}/K_i = 78,000 \pm 12,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

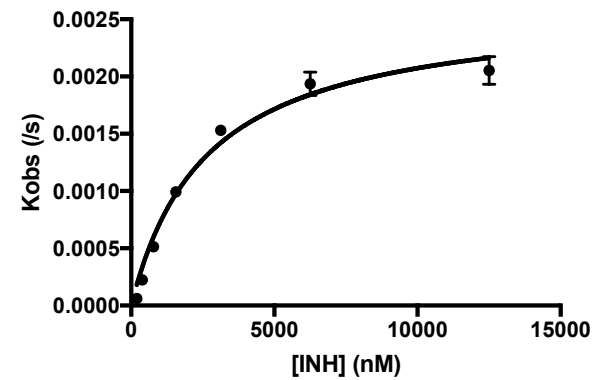
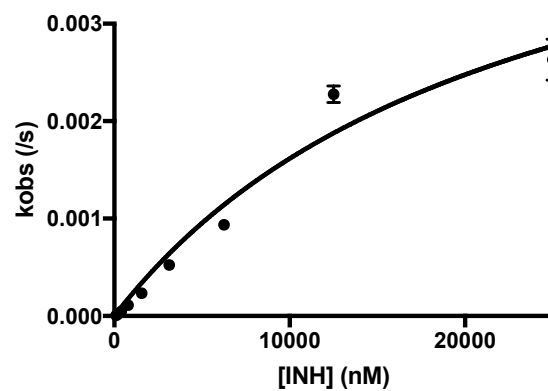
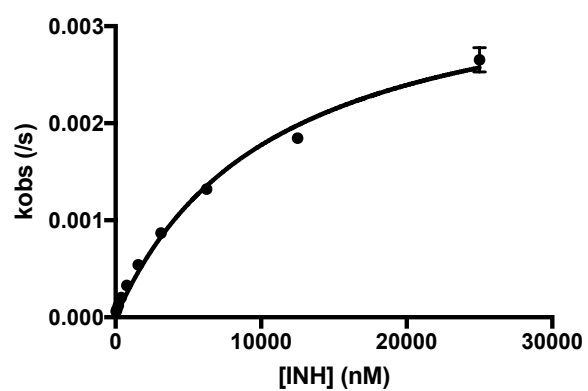
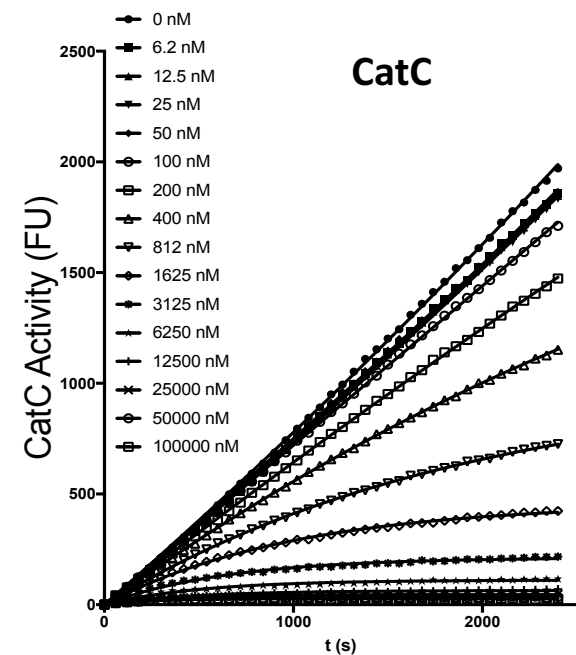
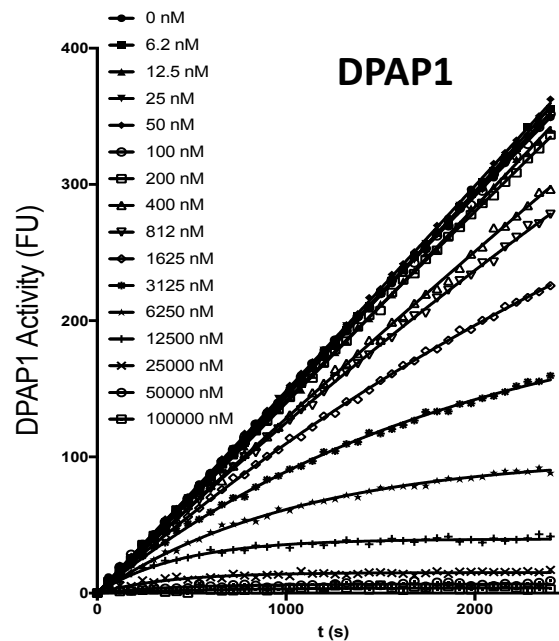
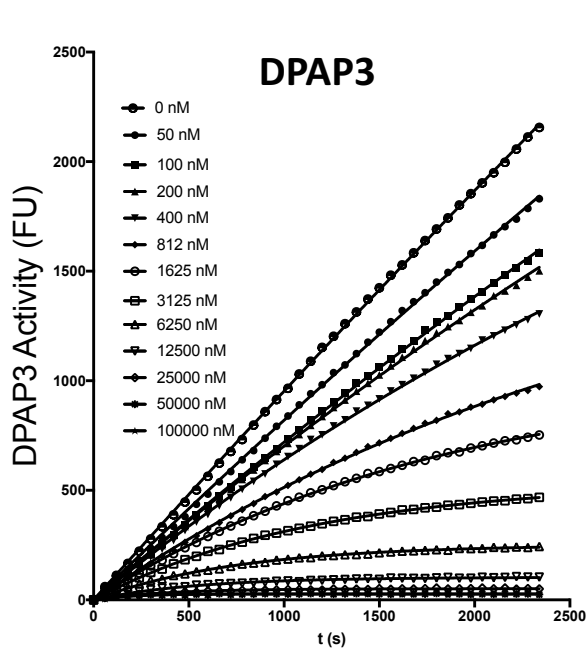
$$k_{inact}/K_i = 313 \pm 7 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0023 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 240 \pm 40 \text{ nM}$$

$$k_{inact}/K_i = 10,000 \pm 1,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: 2NaI



$$k_{inact} = 0.0037 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 7,100 \pm 1,000 \text{ nM}$$

$$k_{inact}/K_i = 510 \pm 40 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0053 \pm 0.0013 \text{ s}^{-1}$$

$$K_i = 15,200 \pm 6,500 \text{ nM}$$

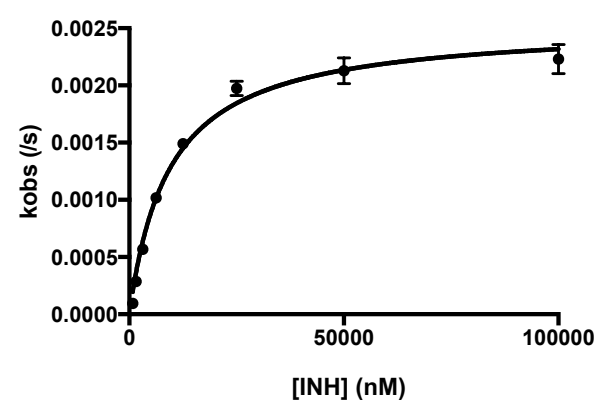
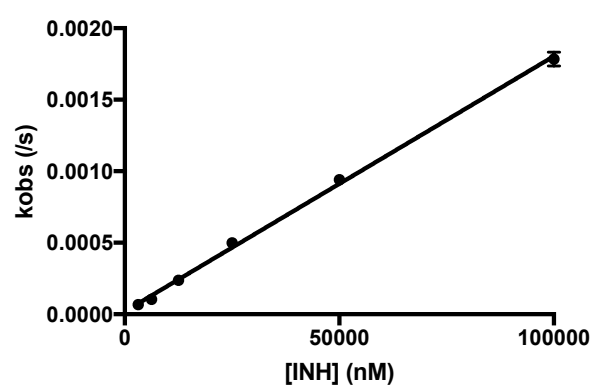
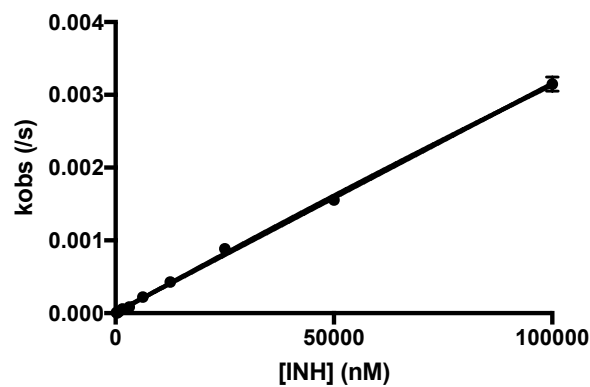
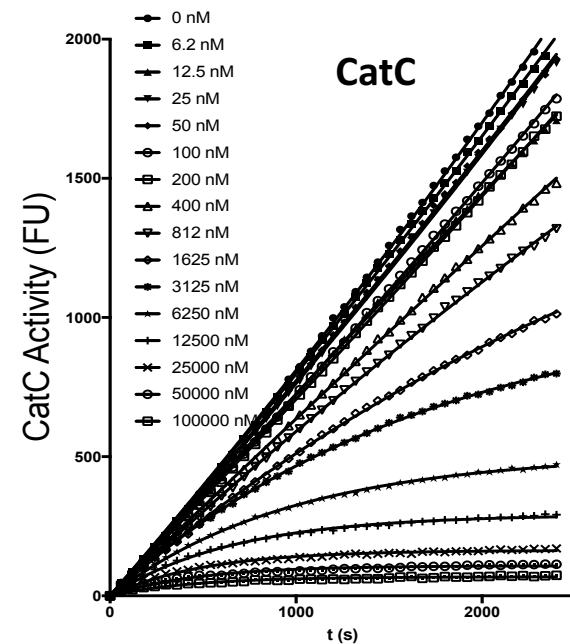
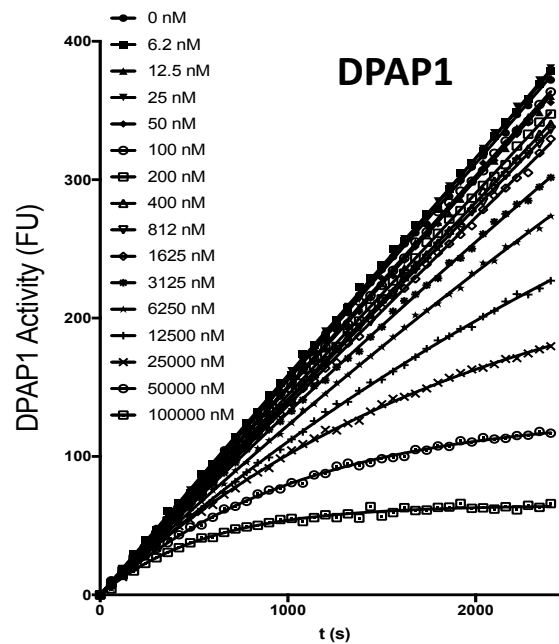
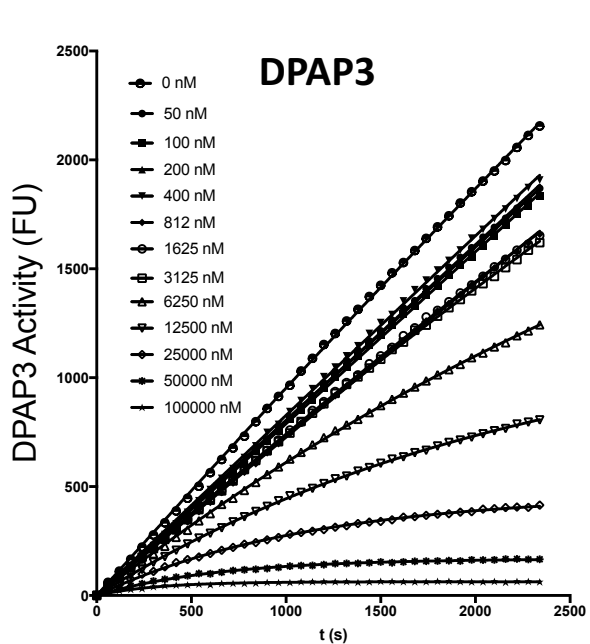
$$k_{inact}/K_i = 350 \pm 70 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0026 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 1,800 \pm 400 \text{ nM}$$

$$k_{inact}/K_i = 1,500 \pm 400 \text{ M}^{-1}\text{s}^{-1}$$

P2: Bip

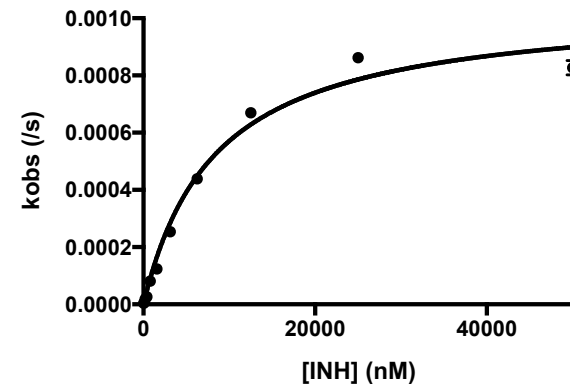
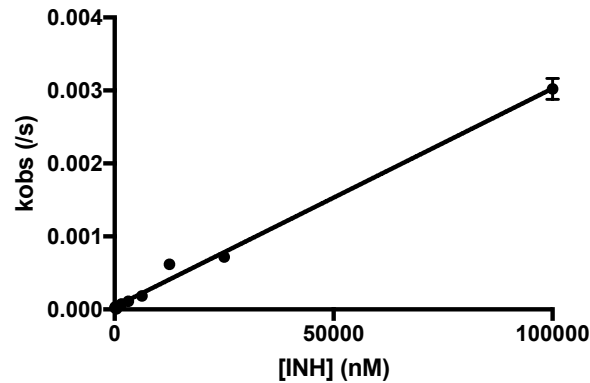
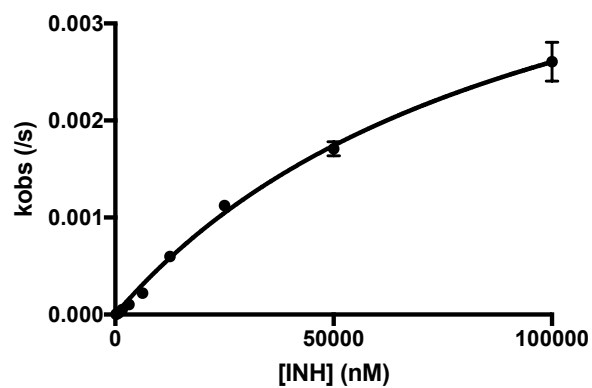
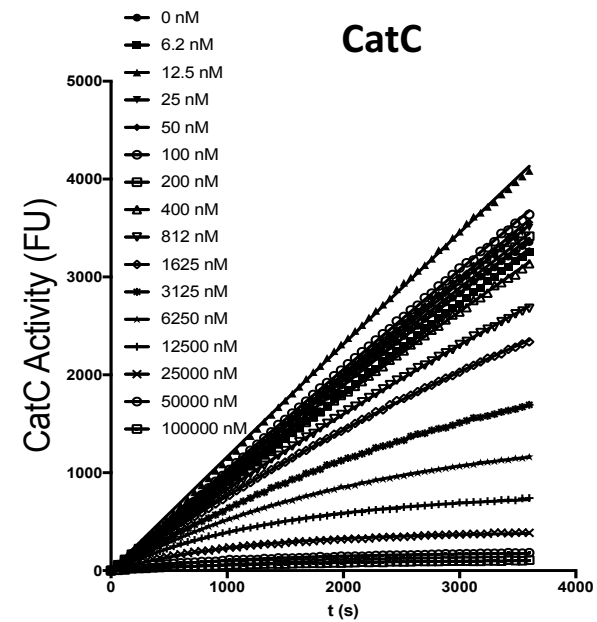
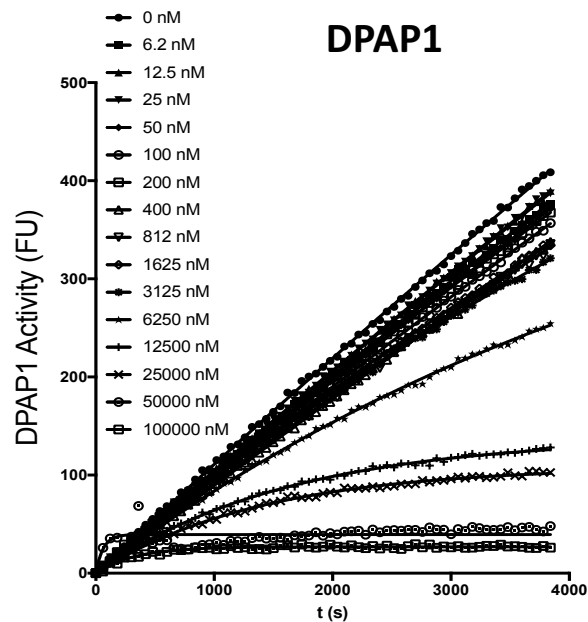
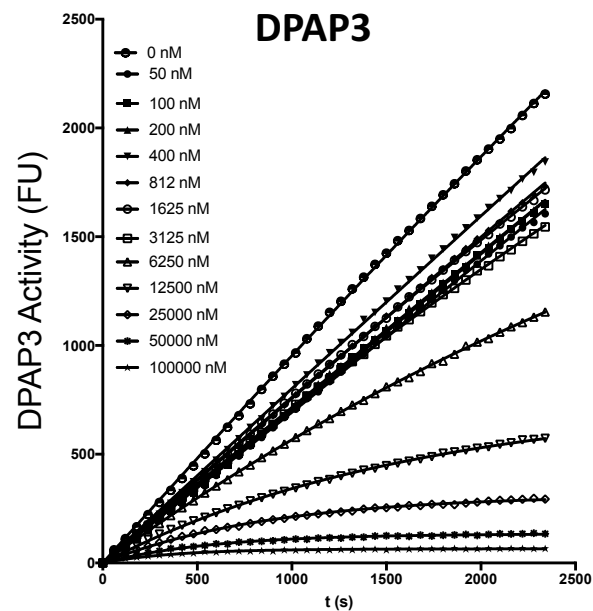


k_{inact} = N.S.
 K_i = N.S.
 $k_{inact}/K_i = 31.4 \pm 0.4 \text{ M}^{-1}\text{s}^{-1}$

k_{inact} = N.S.
 K_i = N.S.
 $k_{inact}/K_i = 17.8 \pm 0.4 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.00253 \pm 0.00009 \text{ s}^{-1}$
 $K_i = 6,200 \pm 800 \text{ nM}$
 $k_{inact}/K_i = 410 \pm 40 \text{ M}^{-1}\text{s}^{-1}$

P2: Inp



$$k_{inact} = 0.0052 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 65,000 \pm 8,000 \text{ nM}$$

$$k_{inact}/K_i = 79 \pm 4 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = \text{N.S.}$$

$$K_i = \text{N.S.}$$

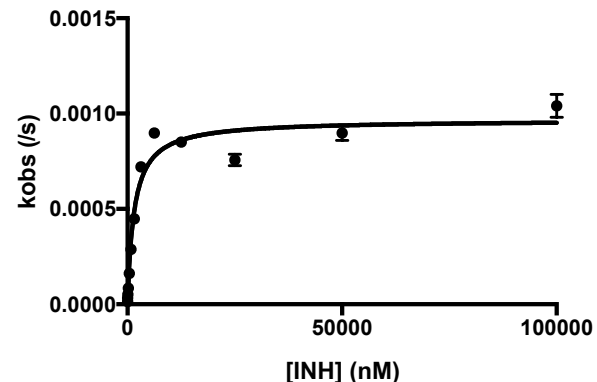
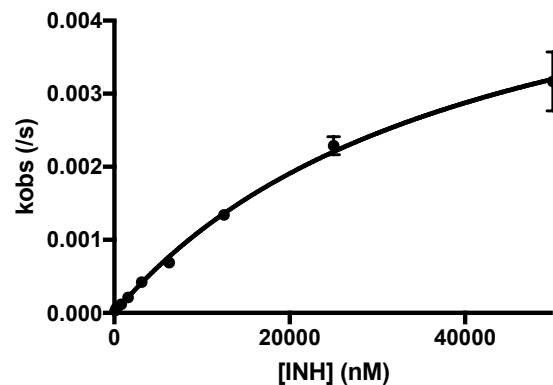
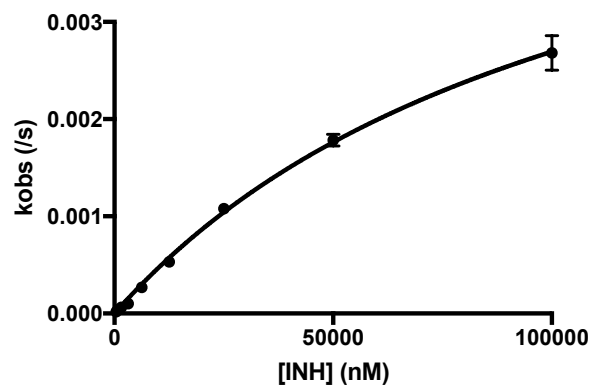
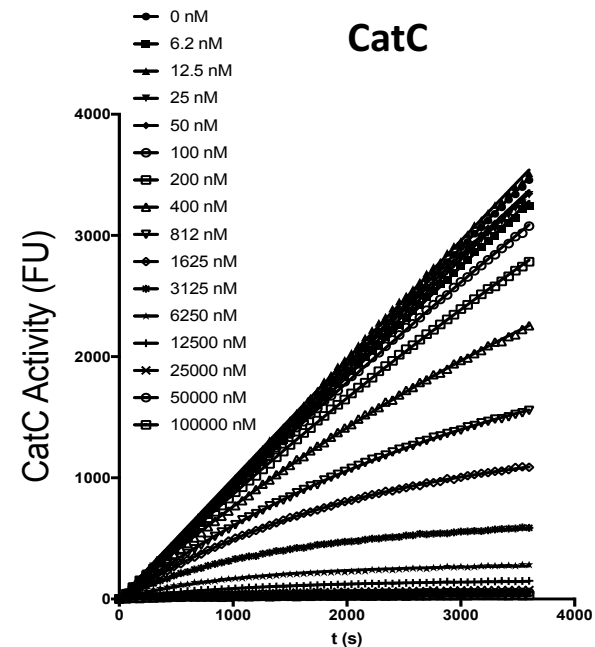
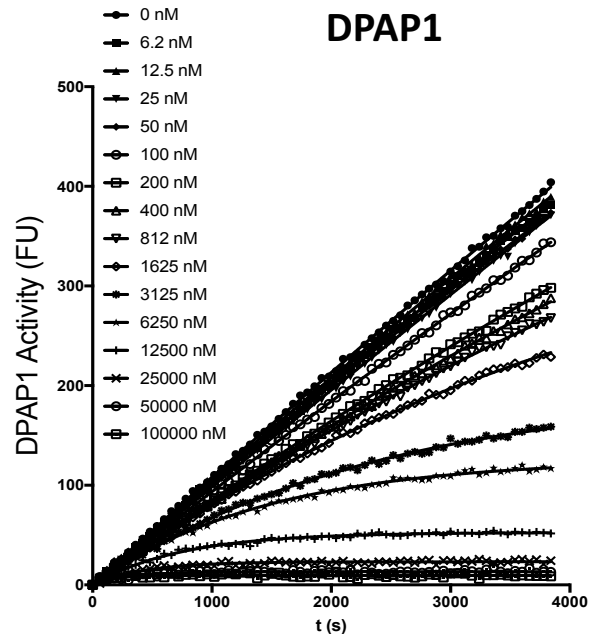
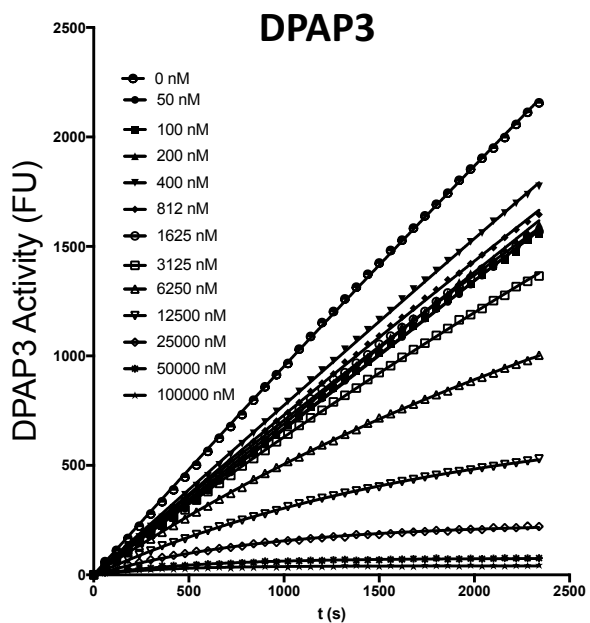
$$k_{inact}/K_i = 30 \pm 1 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00105 \pm 0.00006 \text{ s}^{-1}$$

$$K_i = 5,600 \pm 900 \text{ nM}$$

$$k_{inact}/K_i = 190 \pm 20 \text{ M}^{-1}\text{s}^{-1}$$

P2: Amc



$$k_{inact} = 0.0057 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 75,000 \pm 7,000 \text{ nM}$$

$$k_{inact}/K_i = 76 \pm 3 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0058 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 27,000 \pm 2,500 \text{ nM}$$

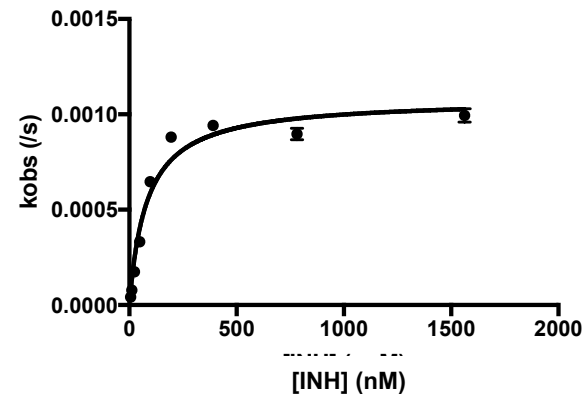
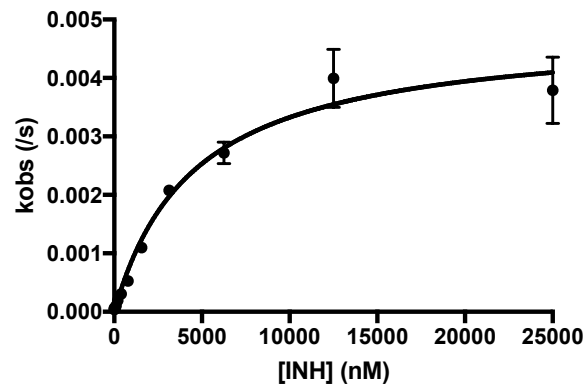
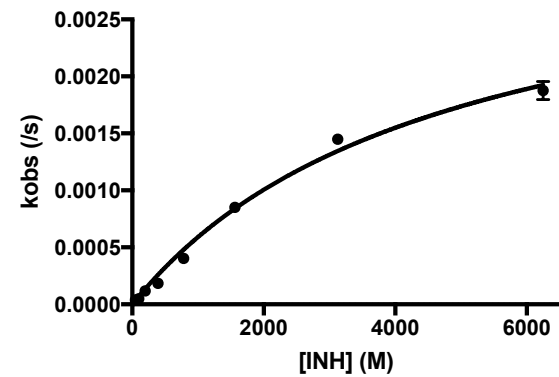
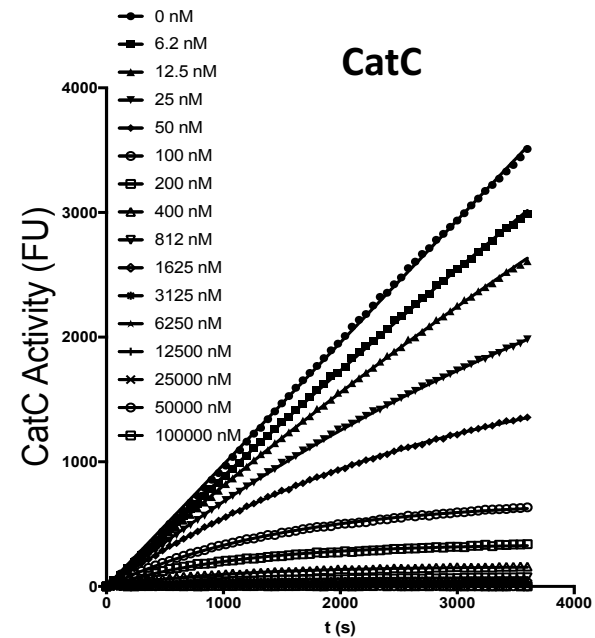
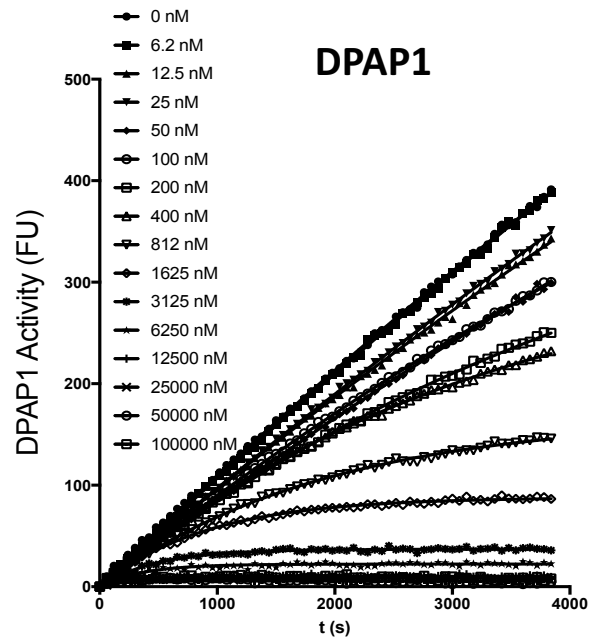
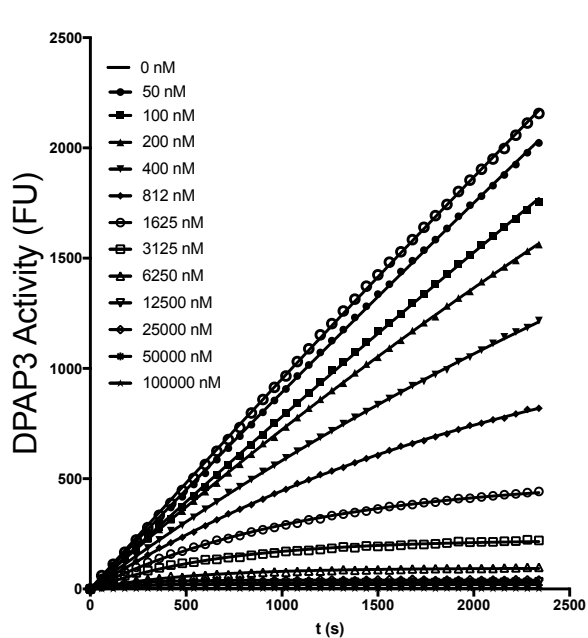
$$k_{inact}/K_i = 213 \pm 9 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00097 \pm 0.00005 \text{ s}^{-1}$$

$$K_i = 1,000 \pm 200 \text{ nM}$$

$$k_{inact}/K_i = 940 \pm 170 \text{ M}^{-1}\text{s}^{-1}$$

P2: 3Abz



$$k_{inact} = 0.0034 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 3,100 \pm 600 \text{ nM}$$

$$k_{inact}/K_i = 1,100 \pm 100 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0048 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 3,000 \pm 500 \text{ nM}$$

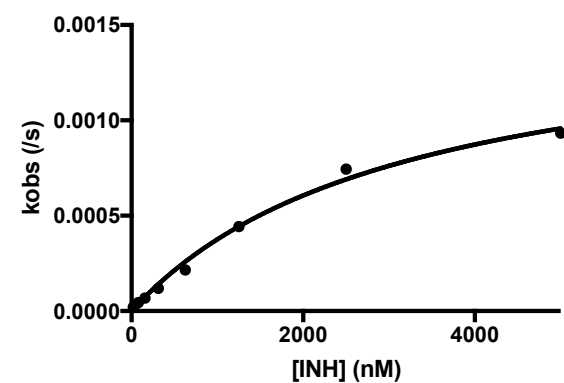
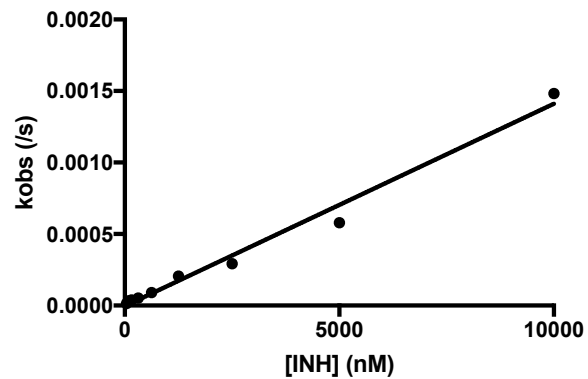
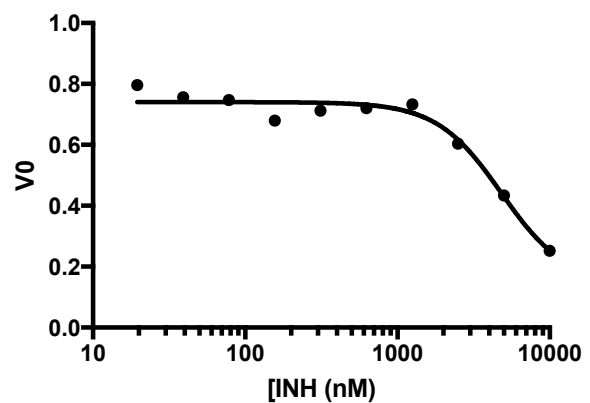
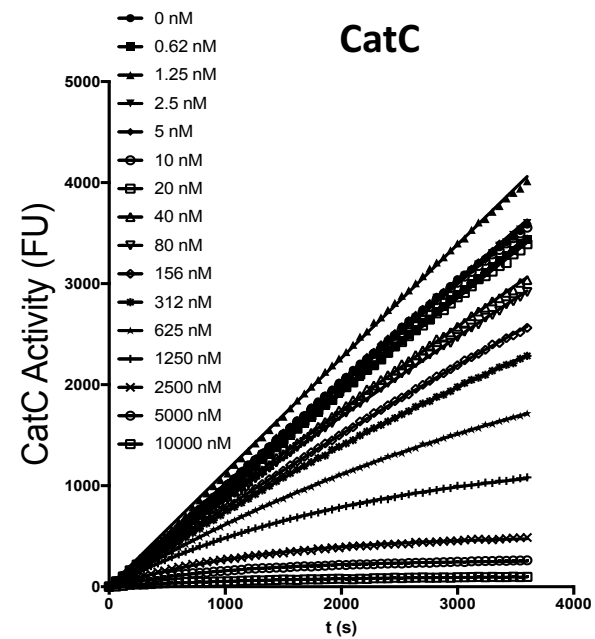
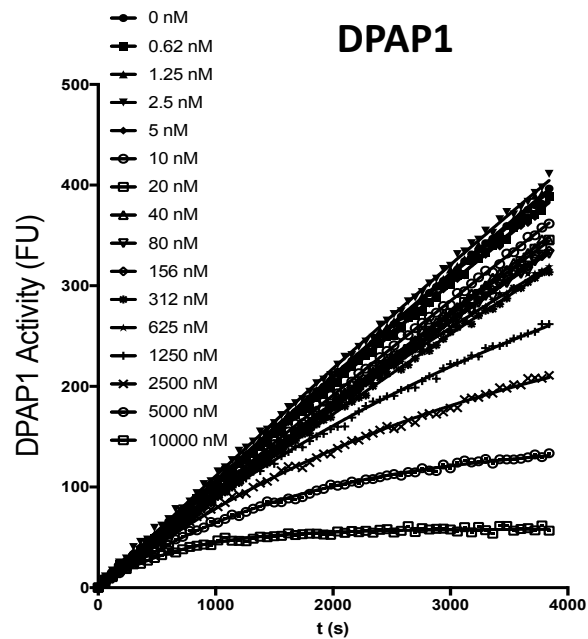
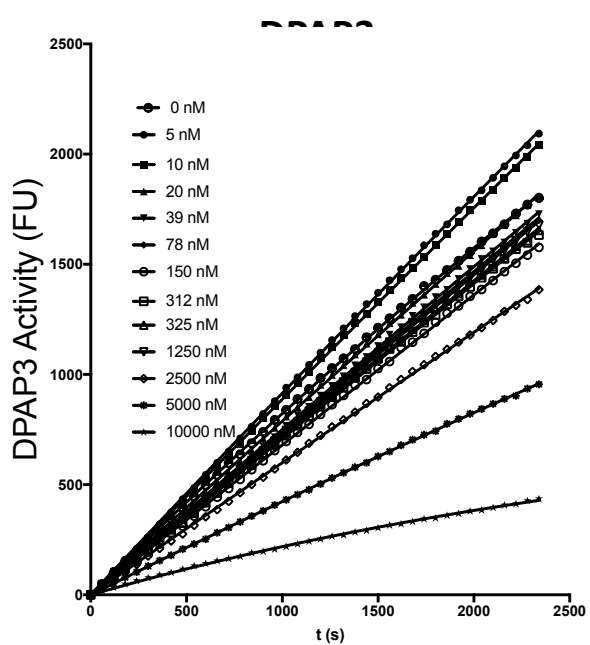
$$k_{inact}/K_i = 1,600 \pm 200 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00108 \pm 0.00007 \text{ s}^{-1}$$

$$K_i = 55 \pm 12 \text{ nM}$$

$$k_{inact}/K_i = 20,000 \pm 4,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Amb

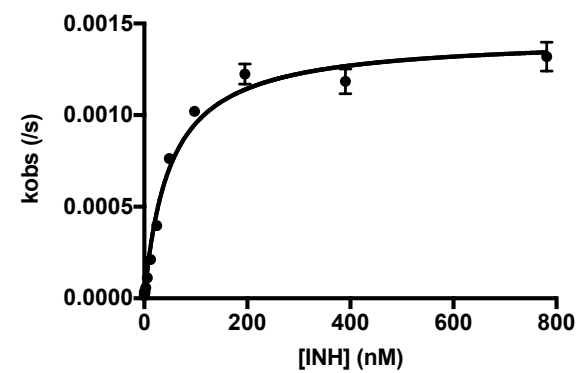
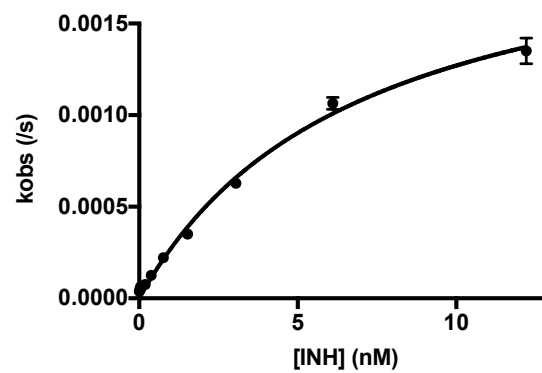
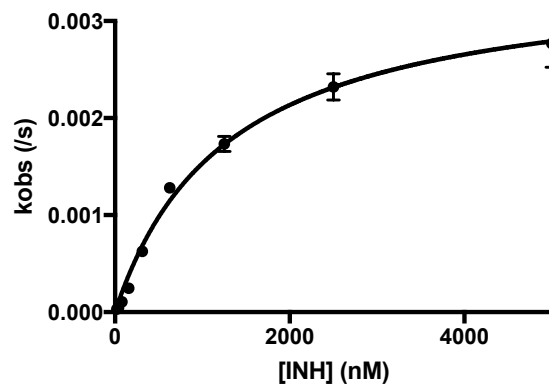
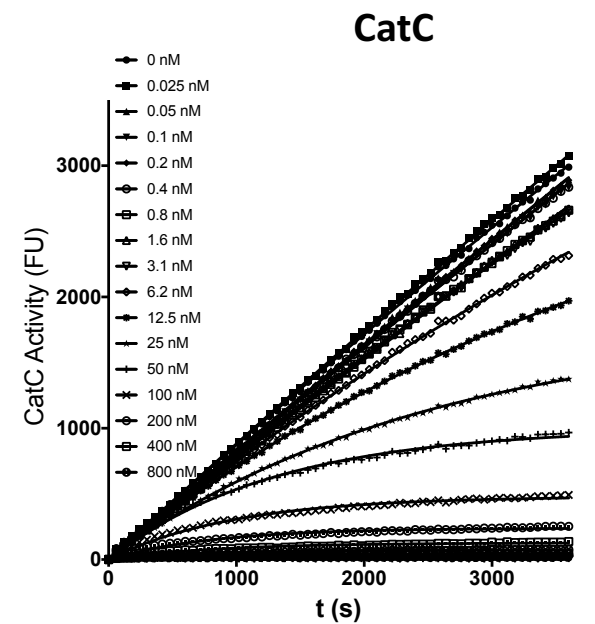
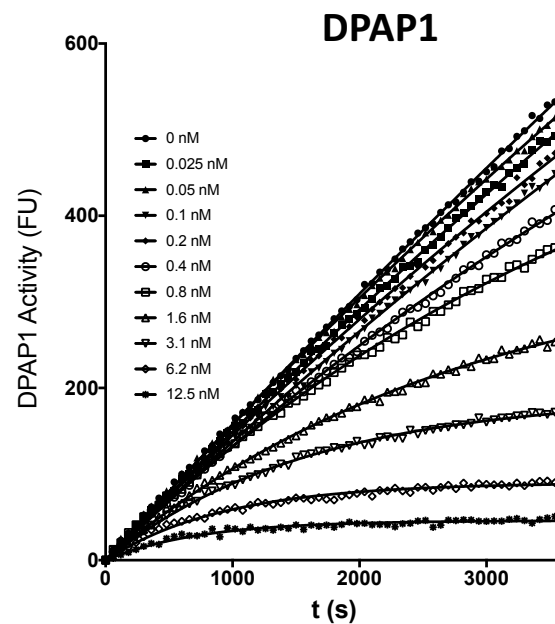
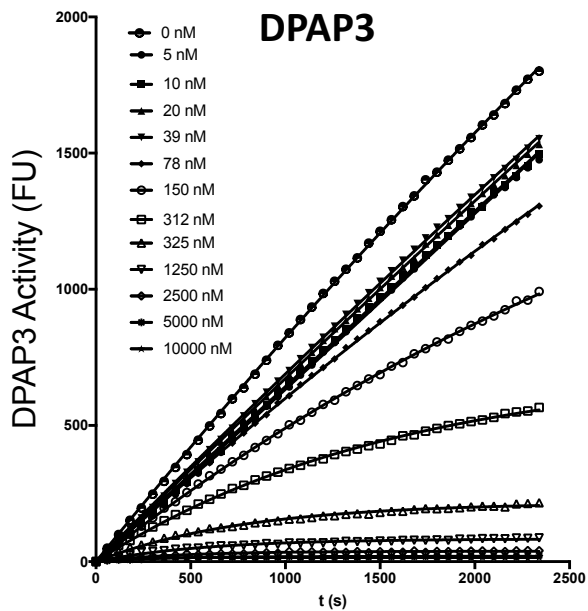
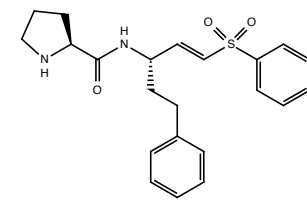


$k_{inact} = \text{N/A}$
 $K_i = 3,200 \pm 100 \text{ nM}$
 $k_{inact}/K_i = \text{N/A}$

$k_{inact} = \text{N.S.}$
 $K_i = \text{N.S.}$
 $k_{inact}/K_i = 140 \pm 7 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.0016 \pm 0.0002 \text{ s}^{-1}$
 $K_i = 2,100 \pm 300 \text{ nM}$
 $k_{inact}/K_i = 740 \pm 60 \text{ M}^{-1}\text{s}^{-1}$

P2: Pro; P1: hPhe



$$k_{inact} = 0.0035 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 860 \pm 100 \text{ nM}$$

$$k_{inact}/K_i = 4,100 \pm 300 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0021 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 4.6 \pm 0.7 \text{ nM}$$

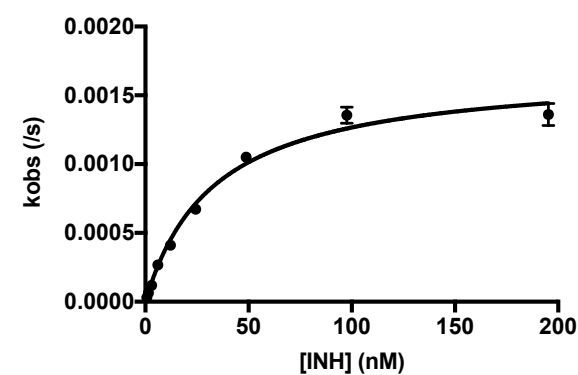
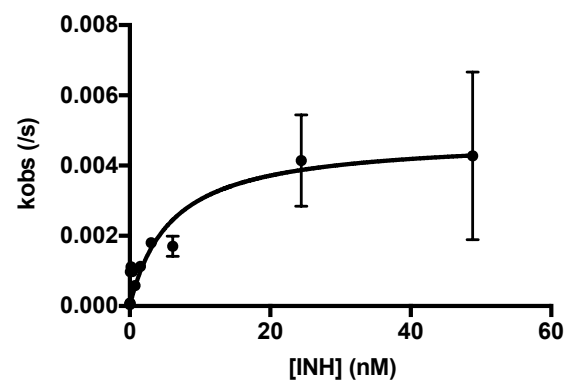
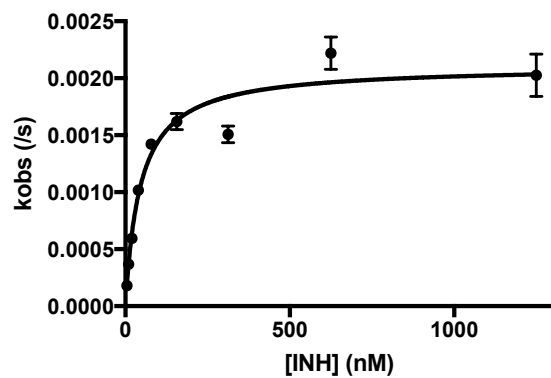
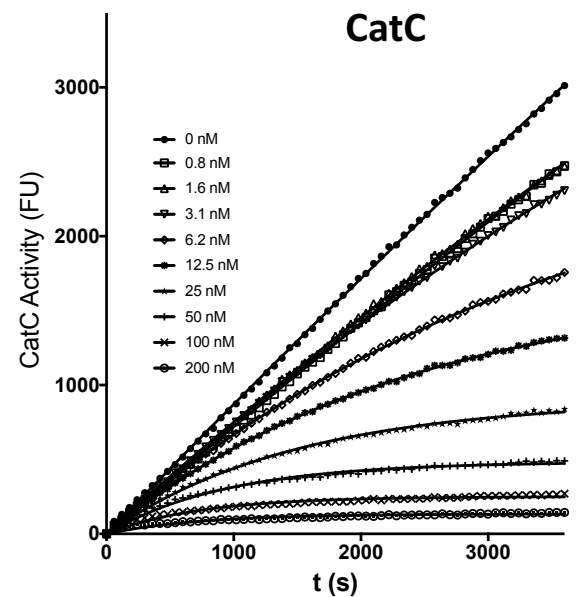
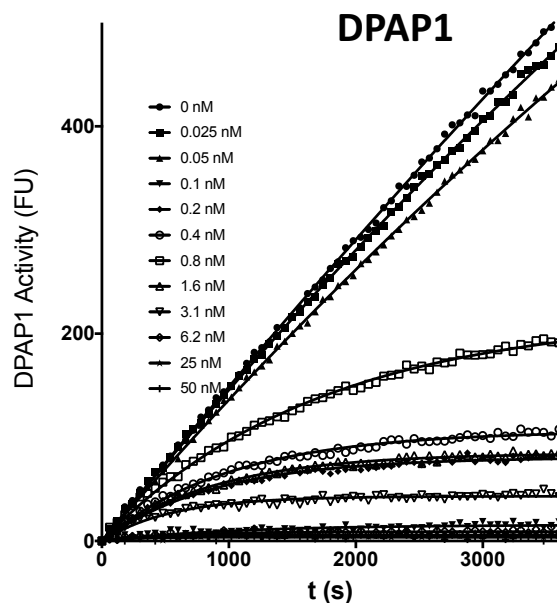
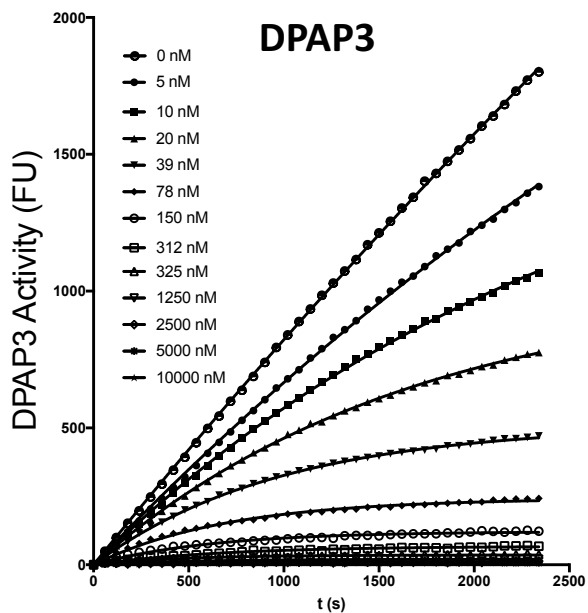
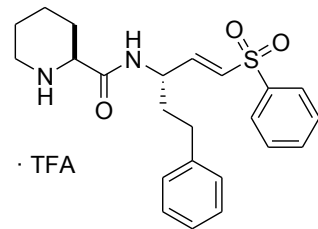
$$k_{inact}/K_i = 470,000 \pm 35,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00145 \pm 0.00007 \text{ s}^{-1}$$

$$K_i = 34 \pm 5 \text{ nM}$$

$$k_{inact}/K_i = 42,000 \pm 5,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: hPro; P1: hPhe

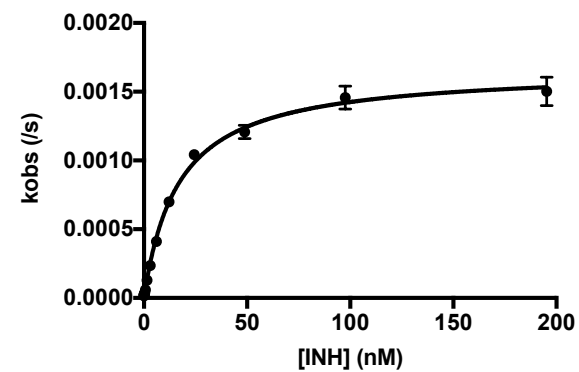
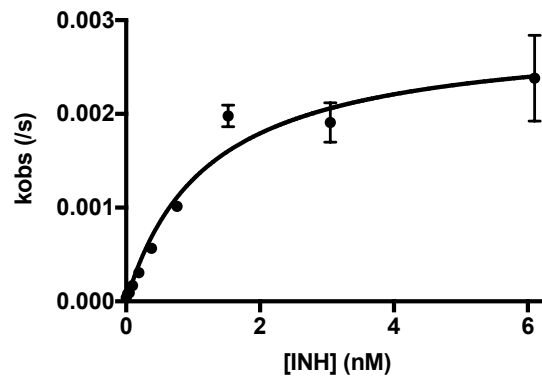
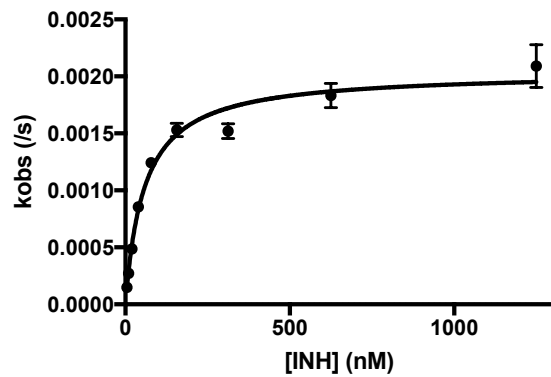
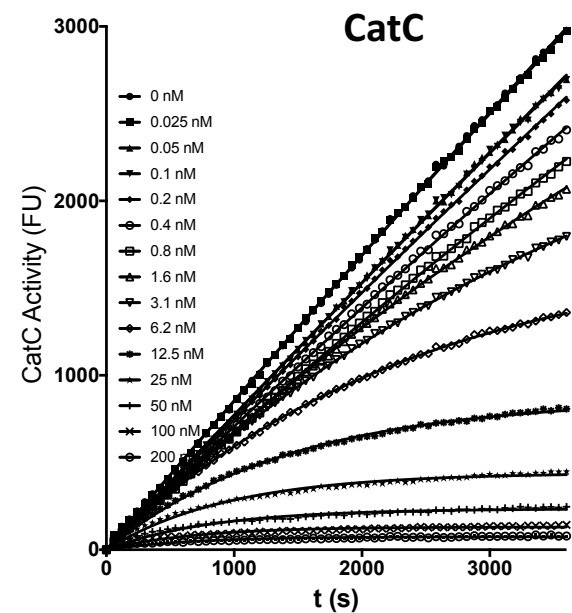
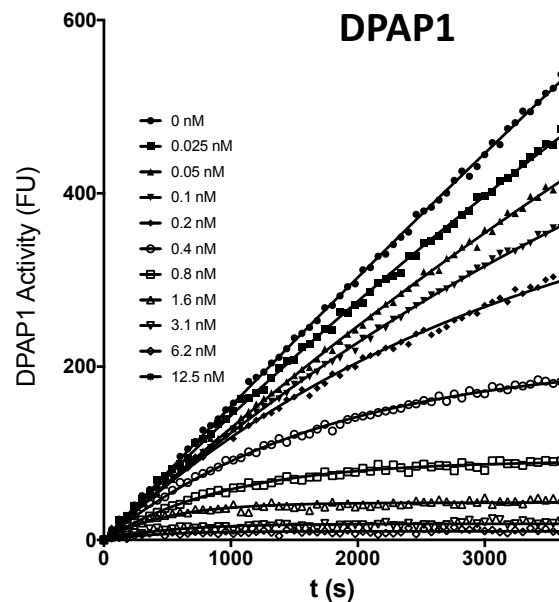
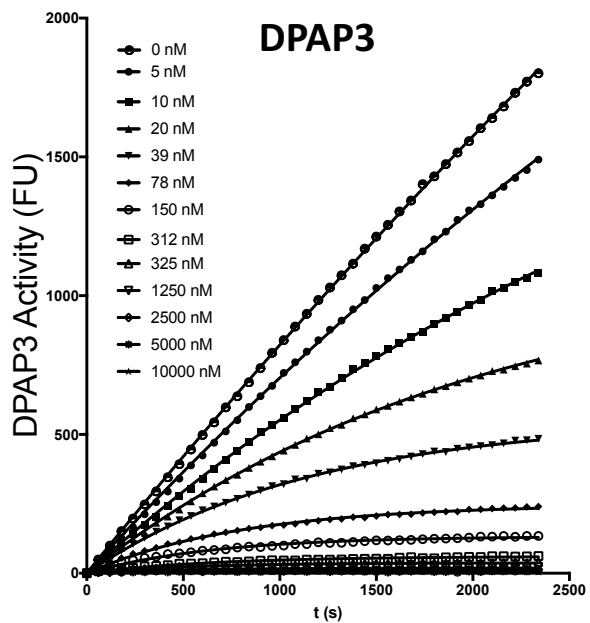
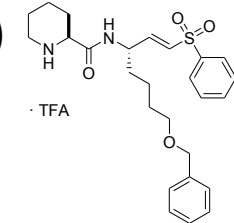


$$k_{\text{inact}} = 0.0021 \pm 0.0001 \text{ s}^{-1}$$
$$K_i = 30 \pm 7 \text{ nM}$$
$$k_{\text{inact}}/K_i = 69,000 \pm 13,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0048 \pm 0.0006 \text{ s}^{-1}$$
$$K_i = 3.9 \pm 1.5 \text{ nM}$$
$$k_{\text{inact}}/K_i = 1,230,000 \pm 400,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.00167 \pm 0.00008 \text{ s}^{-1}$$
$$K_i = 22 \pm 3 \text{ nM}$$
$$k_{\text{inact}}/K_i = 76,000 \pm 800 \text{ M}^{-1}\text{s}^{-1}$$

P2: hPro; P1: nLeu(oBzl)



$$k_{inact} = 0.0020 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 39 \pm 6 \text{ nM}$$

$$k_{inact}/K_i = 53,000 \pm 6,500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0029 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 0.8 \pm 0.2 \text{ nM}$$

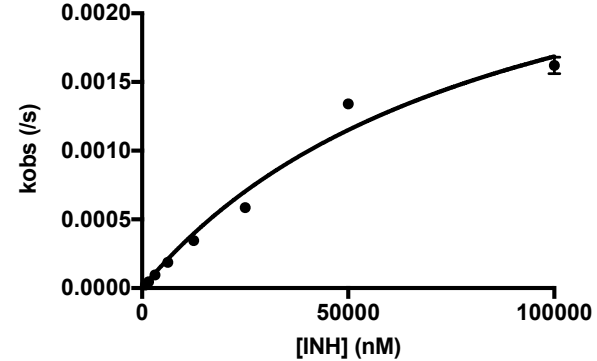
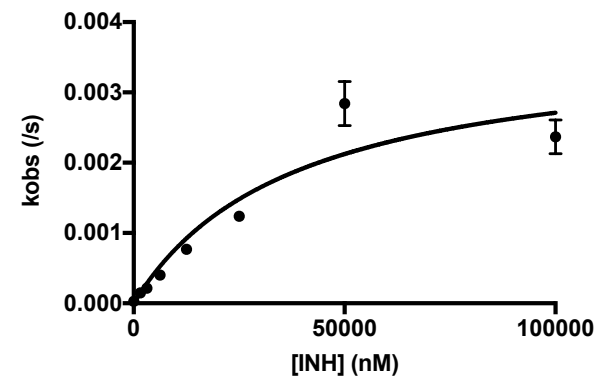
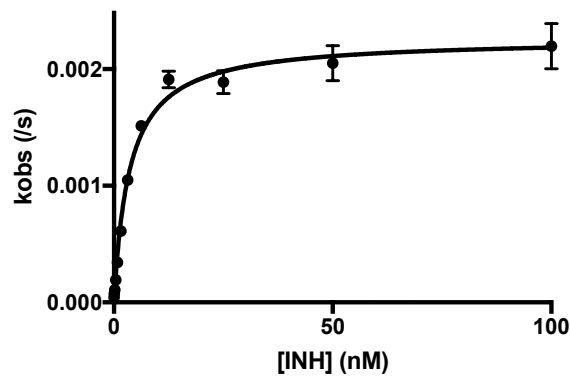
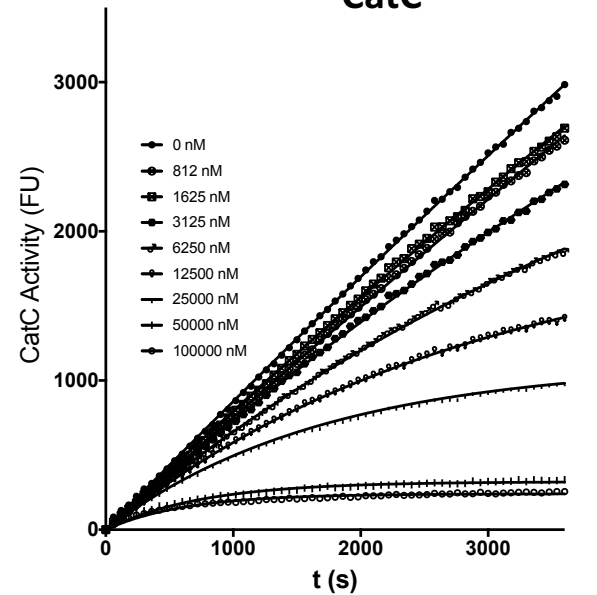
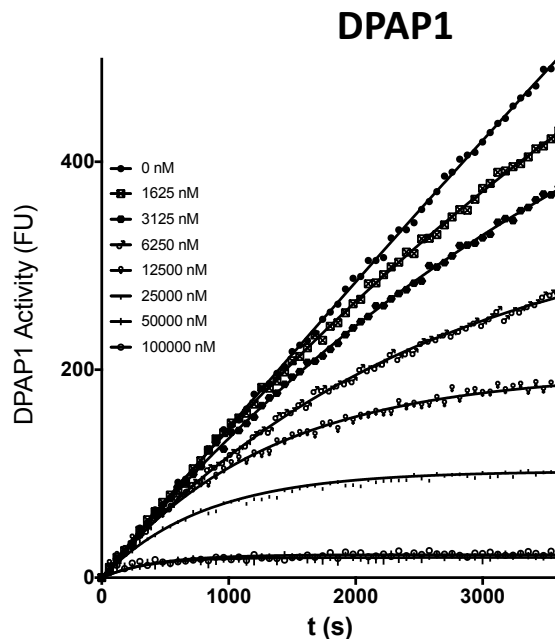
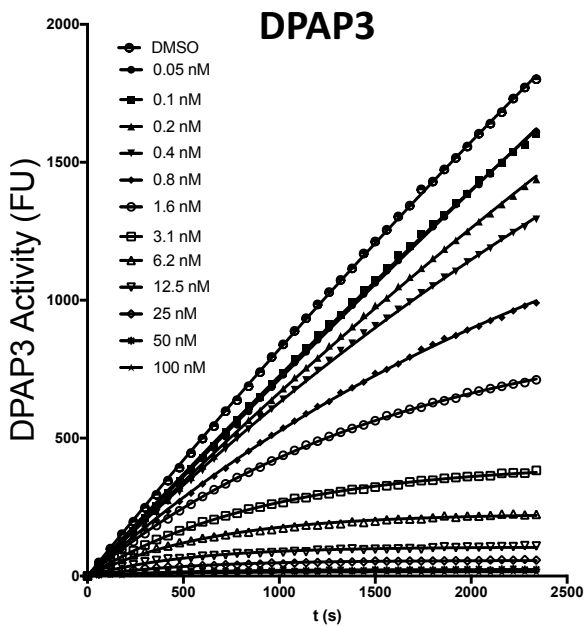
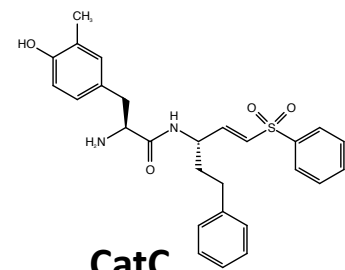
$$k_{inact}/K_i = 3,560,000 \pm 150,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00167 \pm 0.00003 \text{ s}^{-1}$$

$$K_i = 11.2 \pm 0.6 \text{ nM}$$

$$k_{inact}/K_i = 150,000 \pm 6,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Tyr(NO₂); P1: hPhe



$$k_{inact} = 0.00226 \pm 0.00005 \text{ s}^{-1}$$

$$K_i = 2.4 \pm 0.2 \text{ nM}$$

$$k_{inact}/K_i = 950,000 \pm 74,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0038 \pm 0.001 \text{ s}^{-1}$$

$$K_i = 25,000 \pm 1,400 \text{ nM}$$

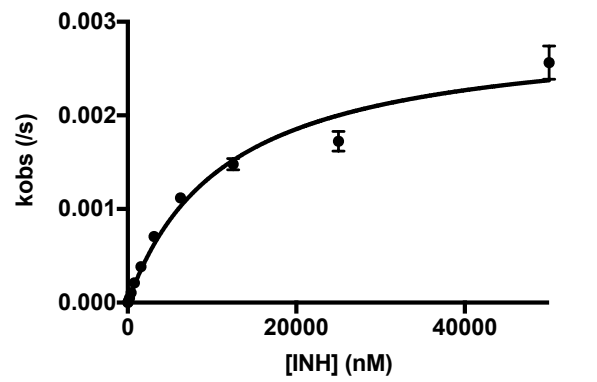
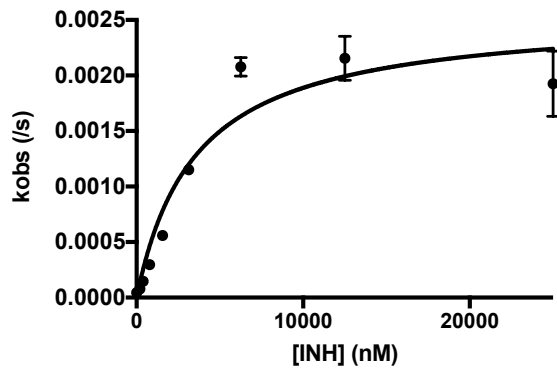
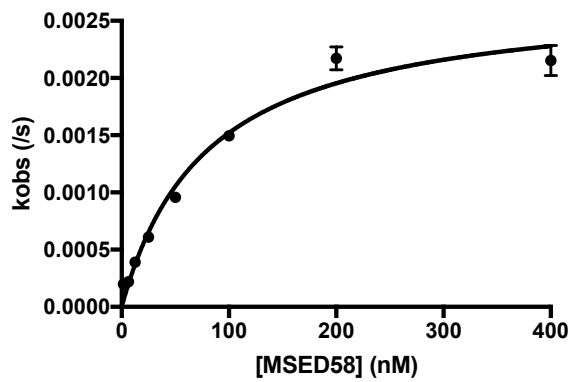
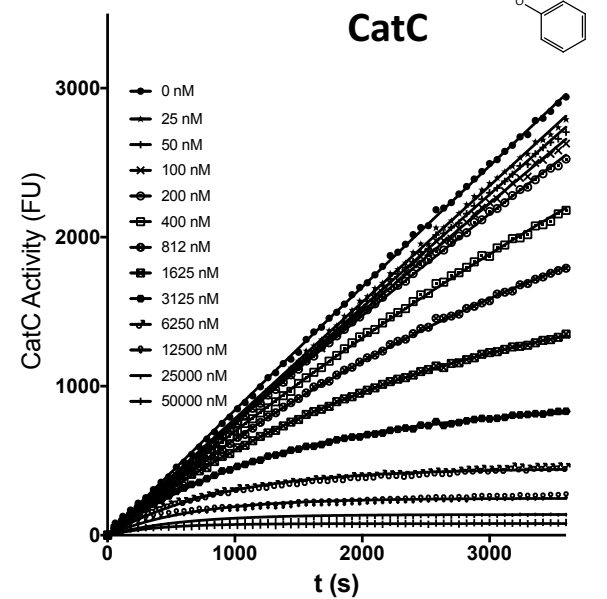
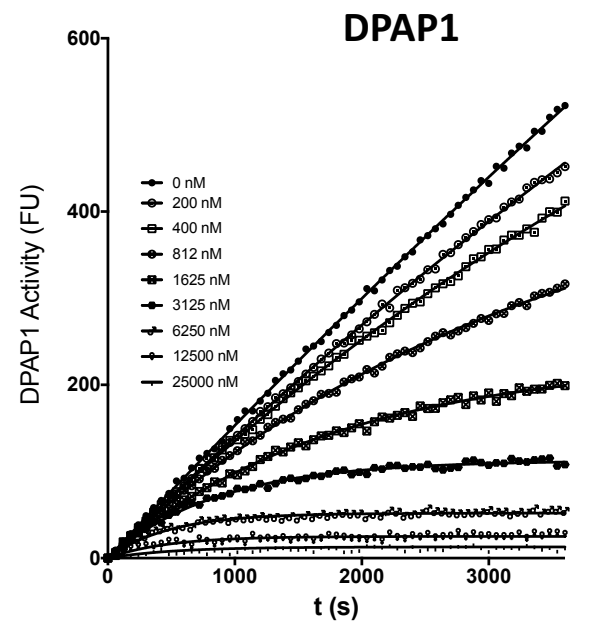
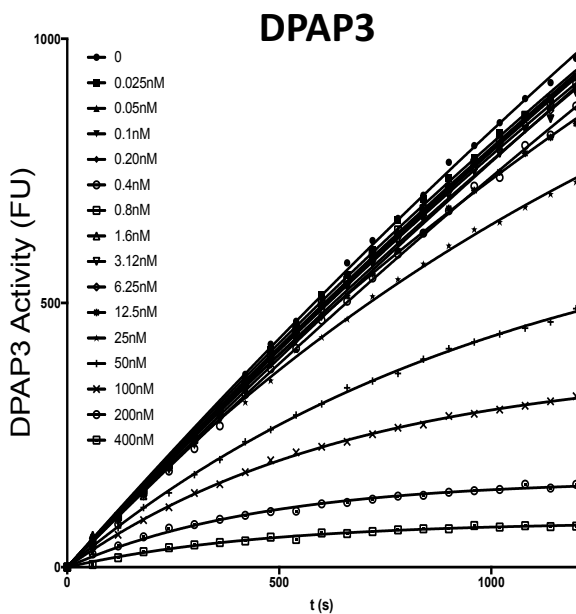
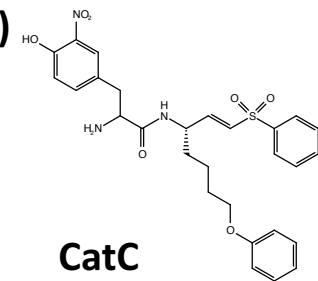
$$k_{inact}/K_i = 150 \pm 50 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0032 \pm 0.0006 \text{ s}^{-1}$$

$$K_i = 58,000 \pm 20,000 \text{ nM}$$

$$k_{inact}/K_i = 54 \pm 8 \text{ M}^{-1}\text{s}^{-1}$$

P2: Tyr(NO₂); P1: nLeu(o-Bzl)

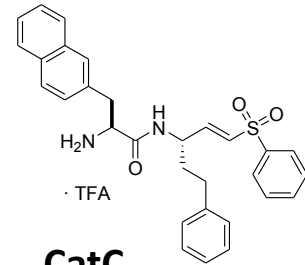


$k_{inact} = 0.0027 \pm 0.0002 \text{ s}^{-1}$
 $K_i = 53 \pm 10 \text{ nM}$
 $k_{inact}/K_i = 51,000 \pm 7,000 \text{ M}^{-1}\text{s}^{-1}$

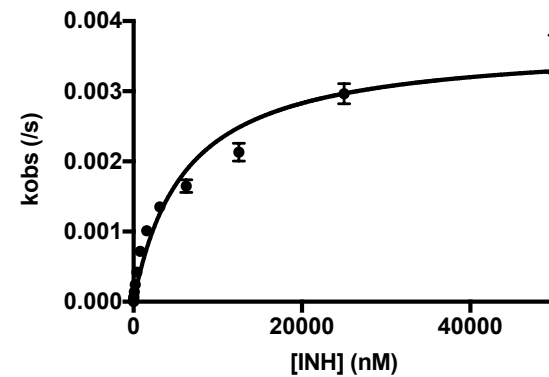
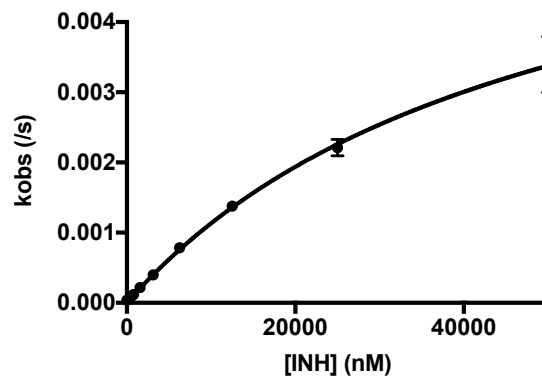
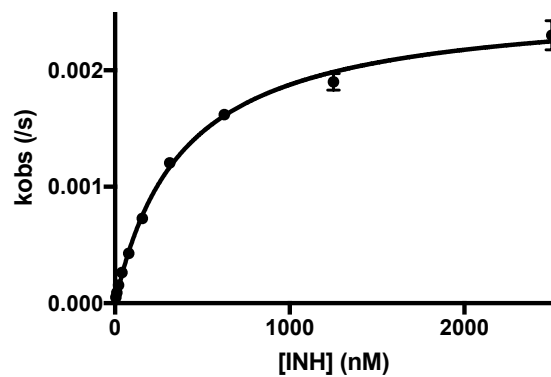
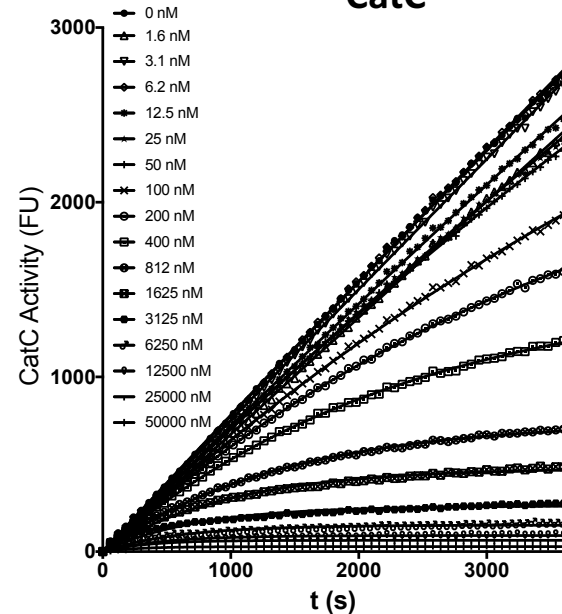
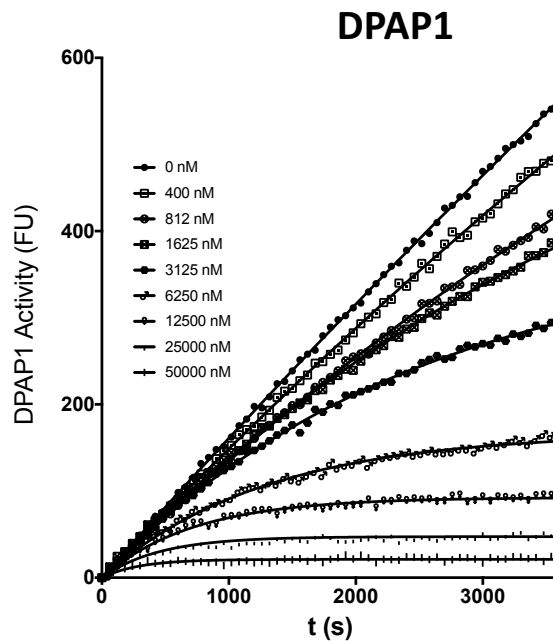
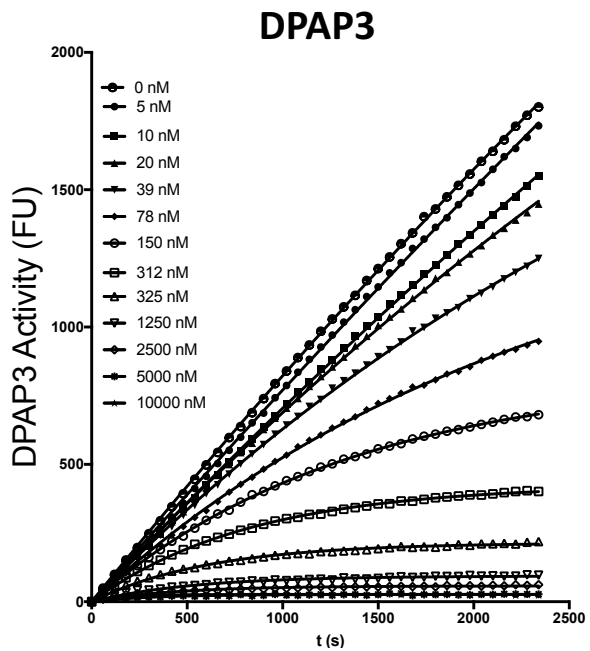
$k_{inact} = 0.0026 \pm 0.0003 \text{ s}^{-1}$
 $K_i = 2,400 \pm 900 \text{ nM}$
 $k_{inact}/K_i = 1,100 \pm 300 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.0029 \pm 0.0002 \text{ s}^{-1}$
 $K_i = 7,700 \pm 1,400 \text{ nM}$
 $k_{inact}/K_i = 380 \pm 50 \text{ M}^{-1}\text{s}^{-1}$

P2: 2NaI; P1:hPhe



CatC



$$k_{\text{inact}} = 0.00259 \pm 0.00007 \text{ s}^{-1}$$

$$K_i = 254 \pm 16 \text{ nM}$$

$$k_{\text{inact}}/K_i = 10,200 \pm 500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0067 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 33,000 \pm 2,000 \text{ nM}$$

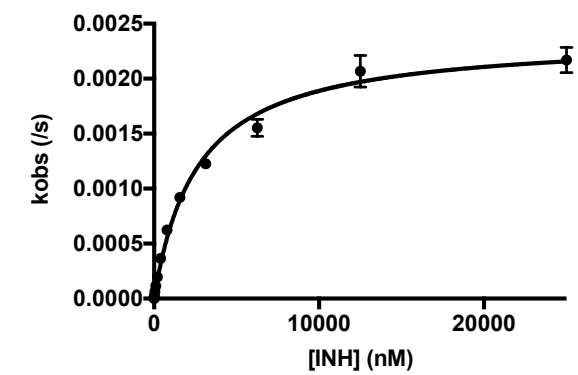
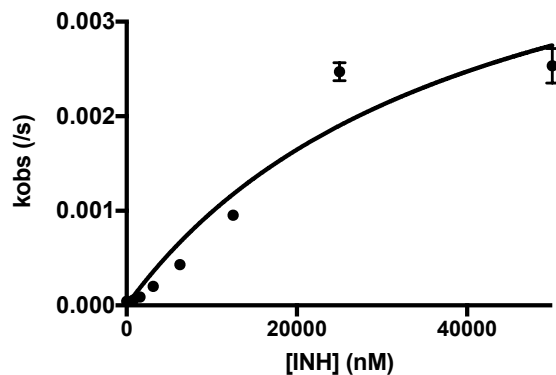
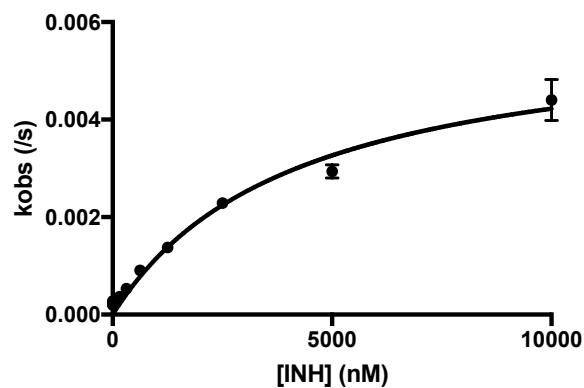
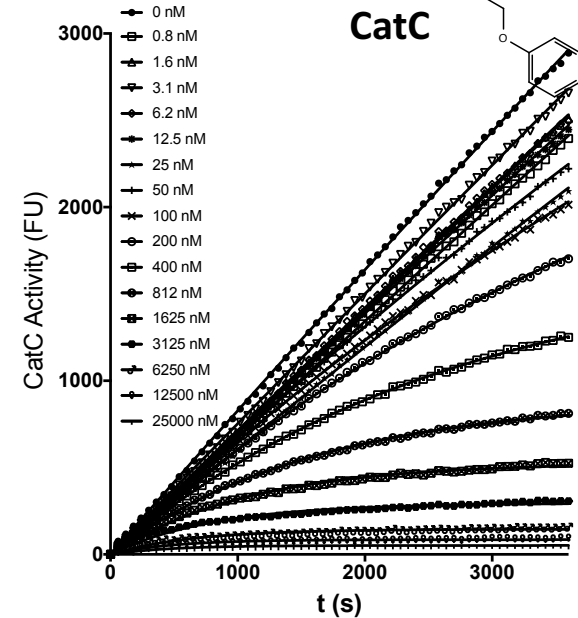
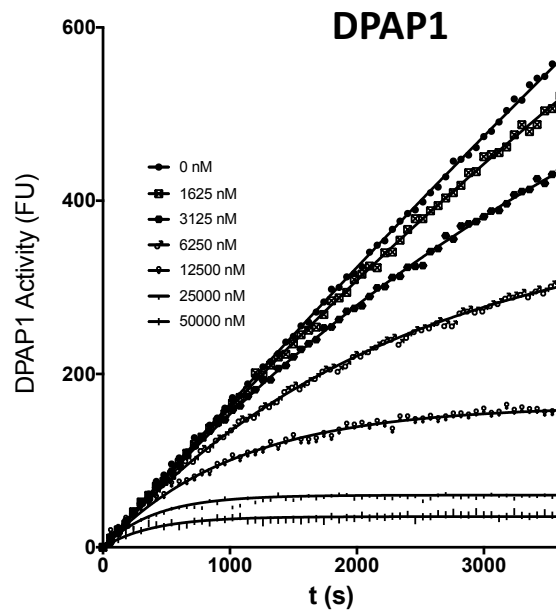
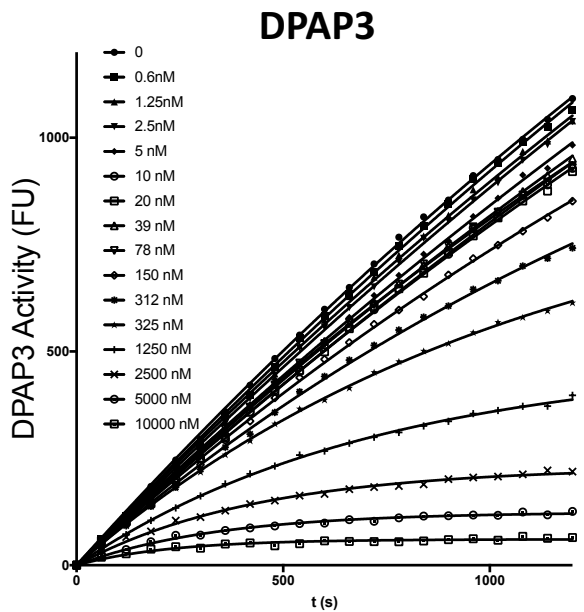
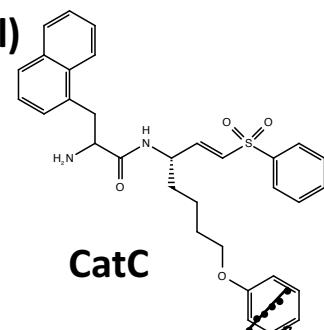
$$k_{\text{inact}}/K_i = 200 \pm 5 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0037 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 4,000 \pm 700 \text{ nM}$$

$$k_{\text{inact}}/K_i = 900 \pm 100 \text{ M}^{-1}\text{s}^{-1}$$

P2: 2NaI; P1: nLeu(o-Bzl)



$$k_{\text{inact}} = 0.0060 \pm 0.0007 \text{ s}^{-1}$$

$$K_i = 2,800 \pm 600 \text{ nM}$$

$$k_{\text{inact}}/K_i = 2,200 \pm 300 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.005 \pm 0.002 \text{ s}^{-1}$$

$$K_i = 27,000 \pm 16,000 \text{ nM}$$

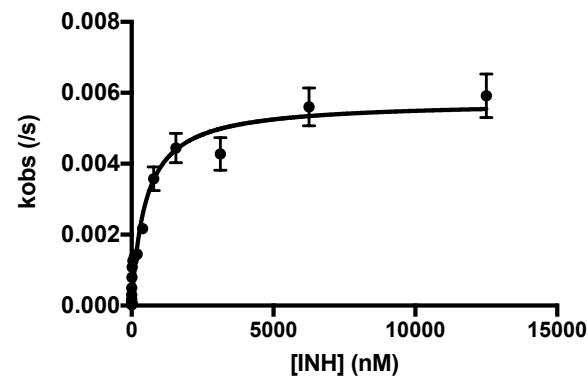
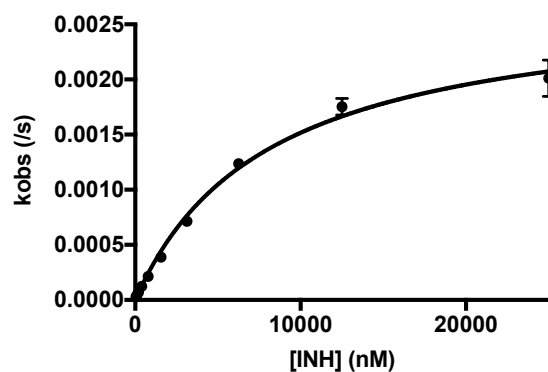
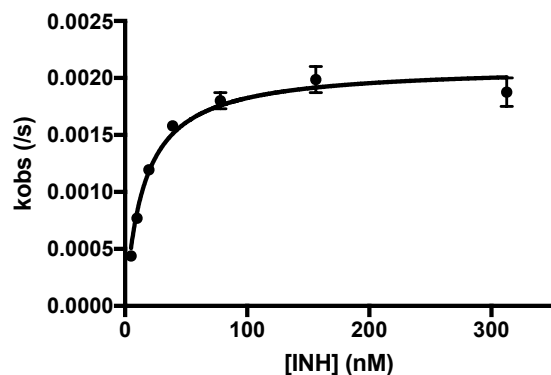
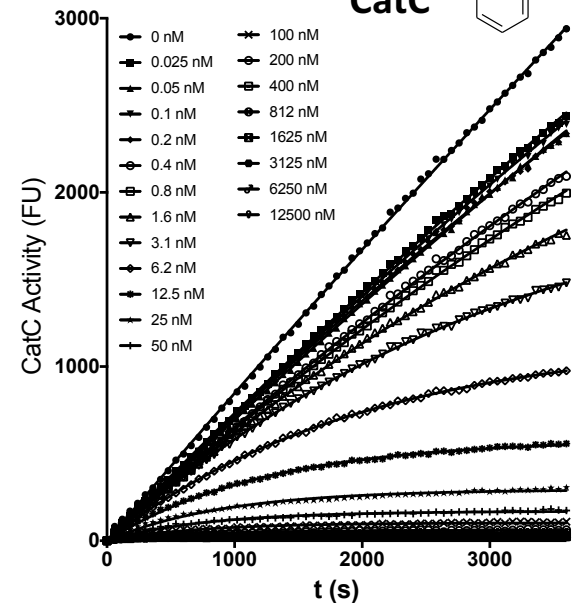
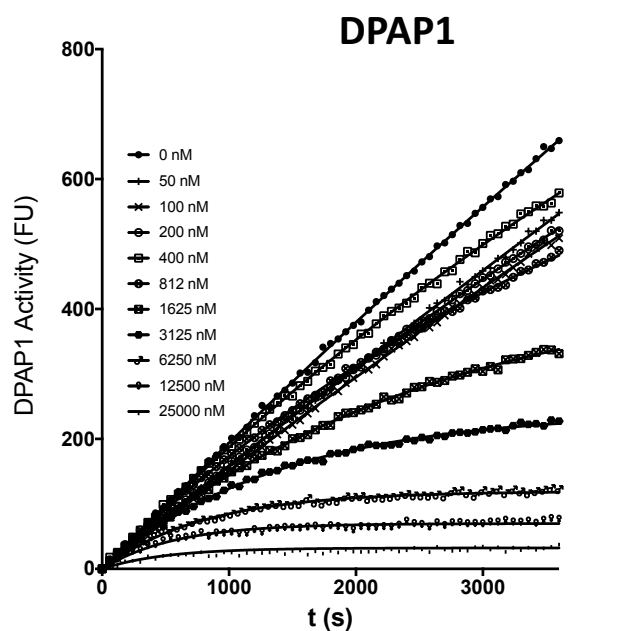
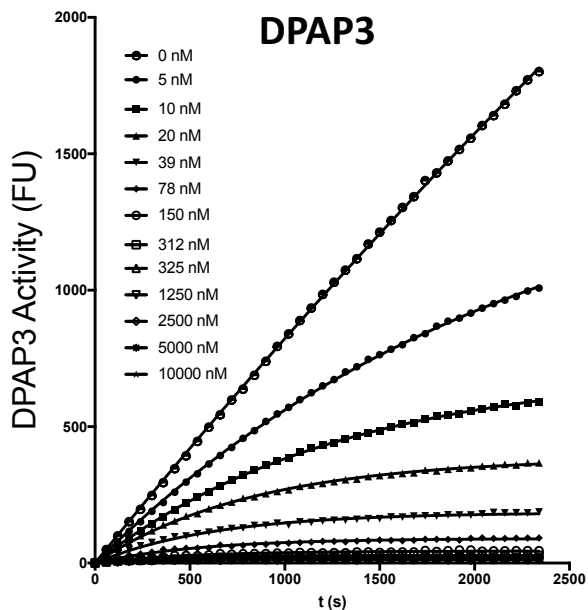
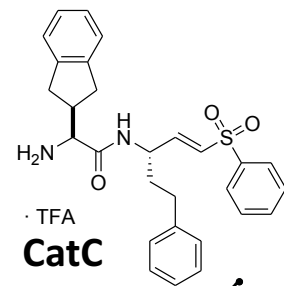
$$k_{\text{inact}}/K_i = 190 \pm 50 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0024 \pm 0.0006 \text{ s}^{-1}$$

$$K_i = 1,740 \pm 150 \text{ nM}$$

$$k_{\text{inact}}/K_i = 1370 \pm 90 \text{ M}^{-1}\text{s}^{-1}$$

P2: Igl; P1: hPhe

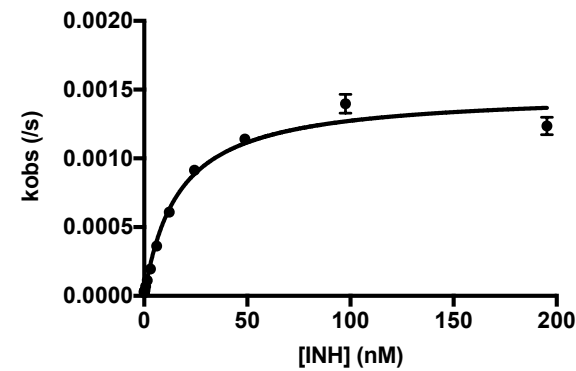
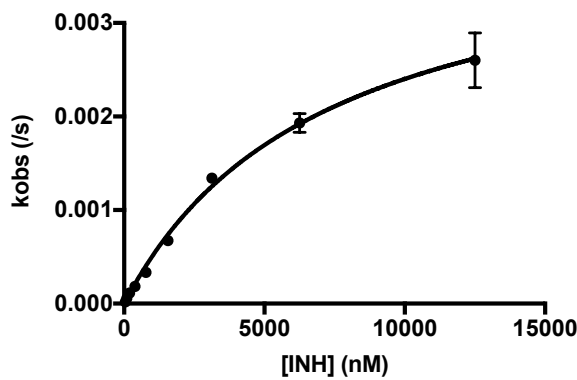
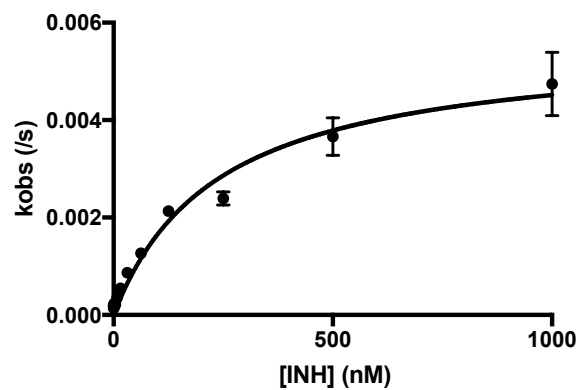
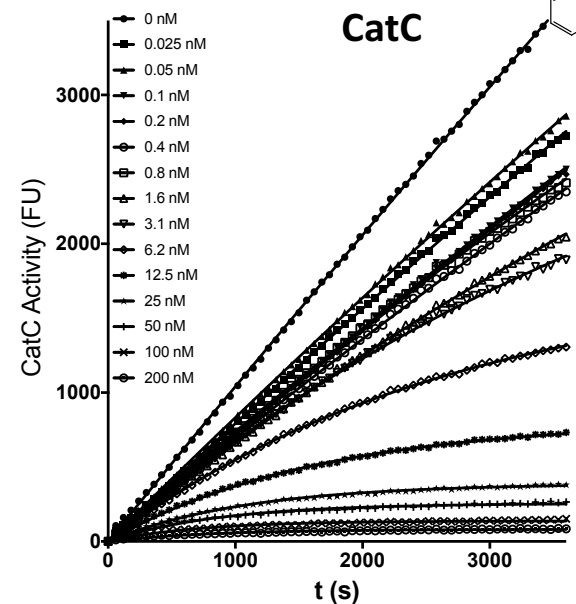
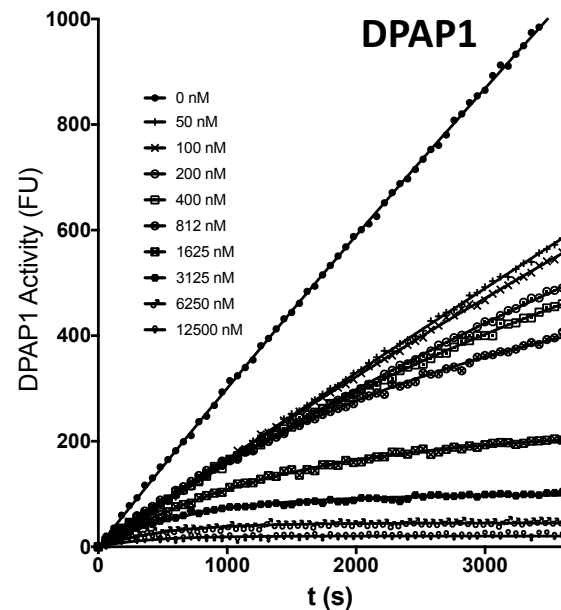
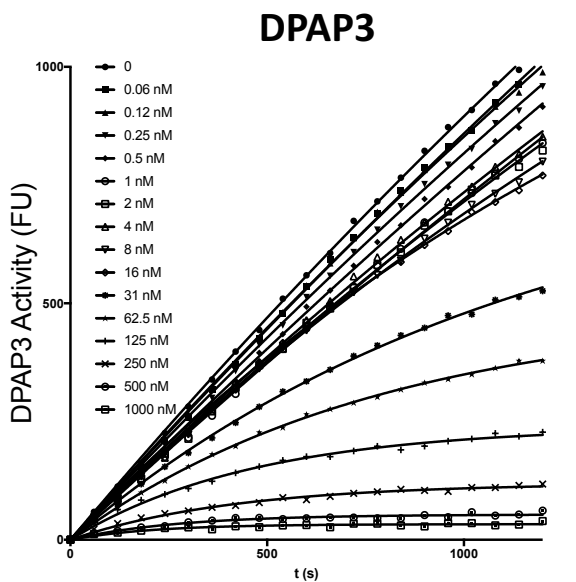
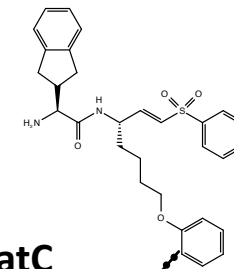


$k_{inact} = 0.0021 \pm 0.00007 \text{ s}^{-1}$
 $K_i = 10 \pm 1 \text{ nM}$
 $k_{inact}/K_i = 205,000 \pm 22,000 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.0027 \pm 0.0001 \text{ s}^{-1}$
 $K_i = 5.400 \pm 500 \text{ nM}$
 $k_{inact}/K_i = 500 \pm 30 \text{ M}^{-1}\text{s}^{-1}$

$k_{inact} = 0.0058 \pm 0.0003 \text{ s}^{-1}$
 $K_i = 320 \pm 70 \text{ nM}$
 $k_{inact}/K_i = 18,000 \pm 300 \text{ M}^{-1}\text{s}^{-1}$

P2: IgI; P1: nLeu(o-Bzl)



$$k_{inact} = 0.0056 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 160 \pm 30 \text{ nM}$$

$$k_{inact}/K_i = 35,000 \pm 4,500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0041 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 4,800 \pm 500 \text{ nM}$$

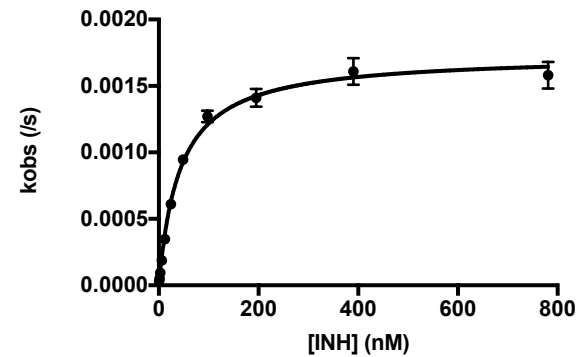
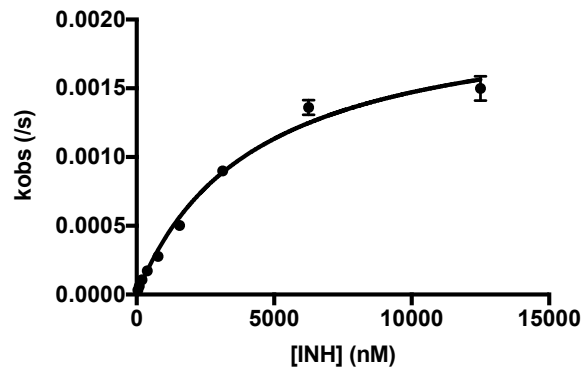
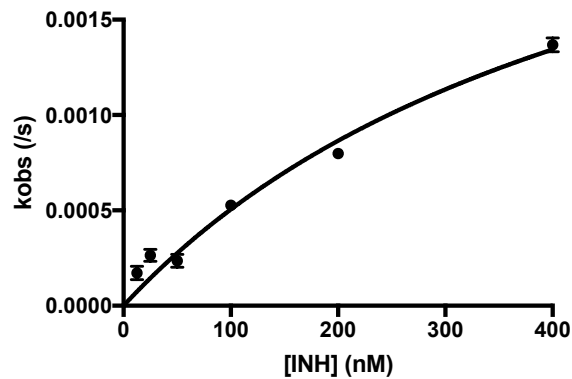
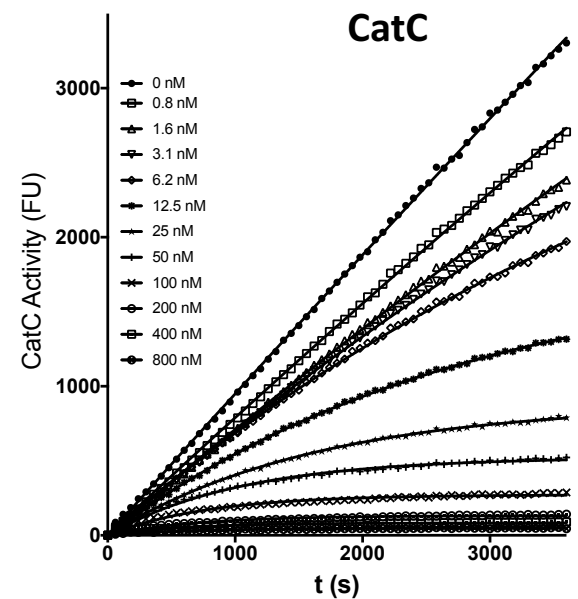
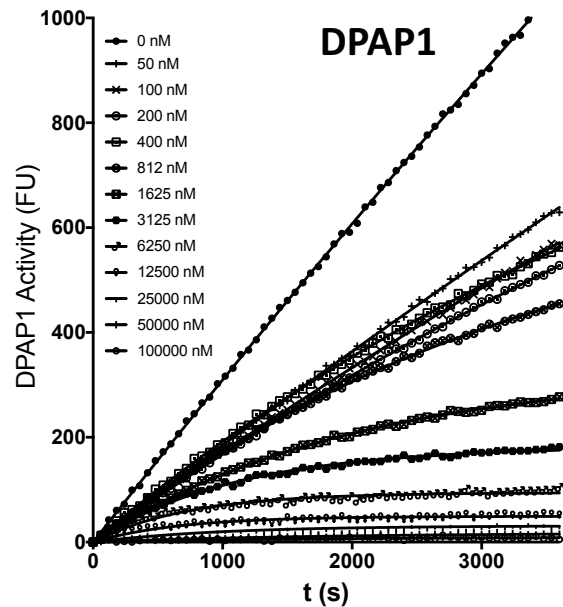
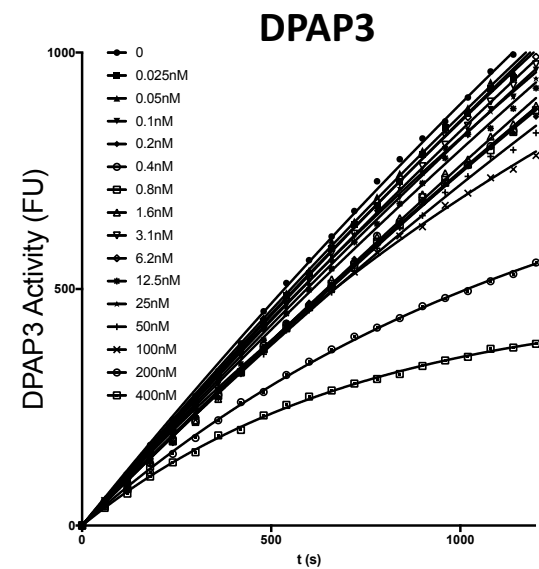
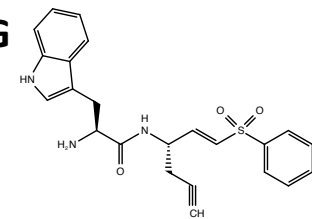
$$k_{inact}/K_i = 860 \pm 50 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00148 \pm 0.00006 \text{ s}^{-1}$$

$$K_i = 11 \pm 2 \text{ nM}$$

$$k_{inact}/K_i = 135,000 \pm 14,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Trp; P1: hPG



$$k_{inact} = 0.003 \pm 0.001 \text{ s}^{-1}$$

$$K_i = 330 \pm 150 \text{ nM}$$

$$k_{inact}/K_i = 9,100 \pm 1,500 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0021 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 2,800 \pm 450 \text{ nM}$$

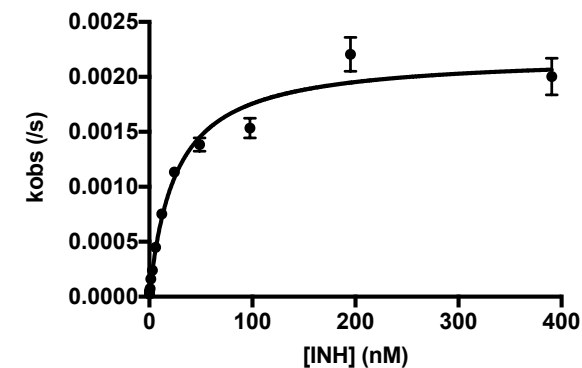
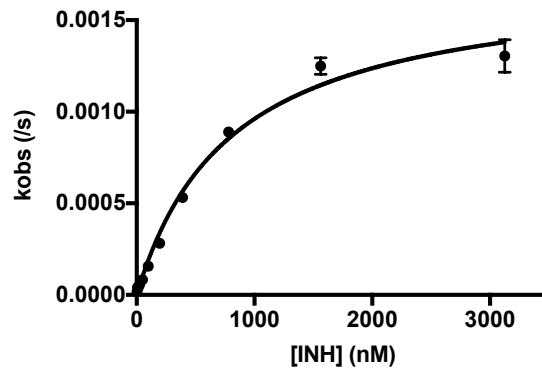
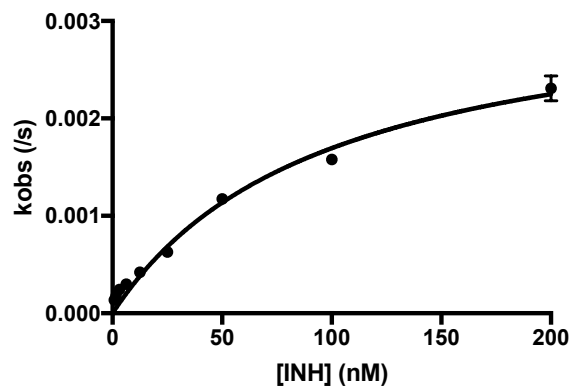
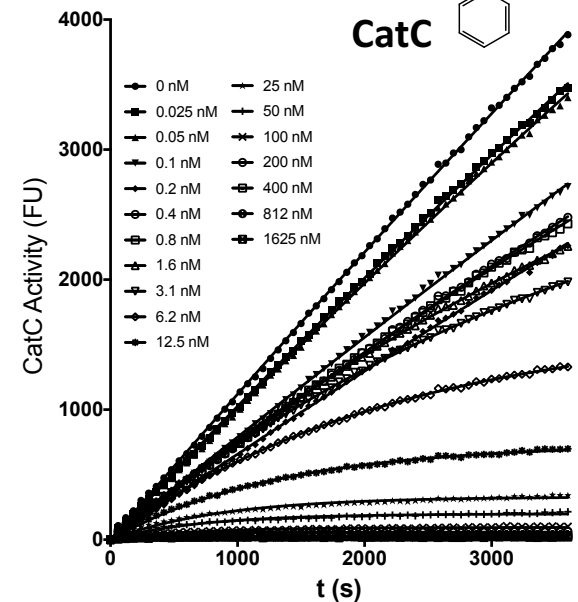
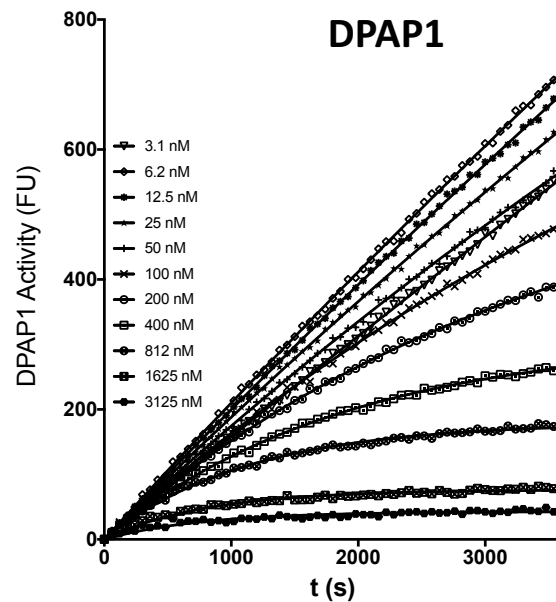
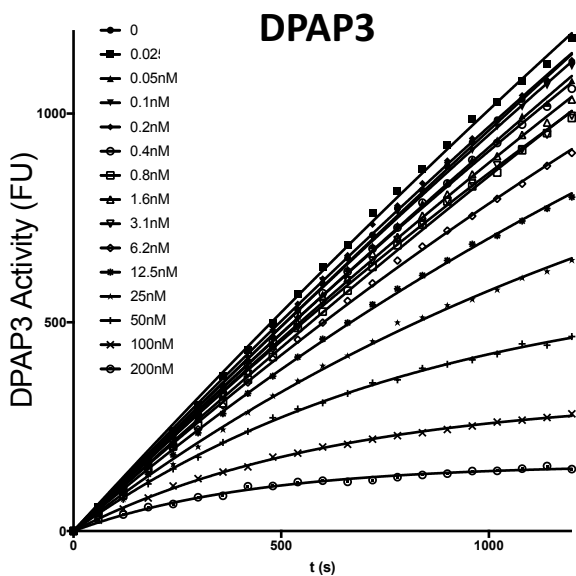
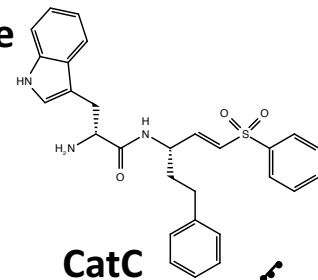
$$k_{inact}/K_i = 740 \pm 70 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00173 \pm 0.00003 \text{ s}^{-1}$$

$$K_i = 28 \pm 2 \text{ nM}$$

$$k_{inact}/K_i = 61,000 \pm 4,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Trp; P1: hPhe



$$k_{\text{inact}} = 0.0033 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 65 \pm 15 \text{ nM}$$

$$k_{\text{inact}}/K_i = 52,000 \pm 7,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0017 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 540 \pm 80 \text{ nM}$$

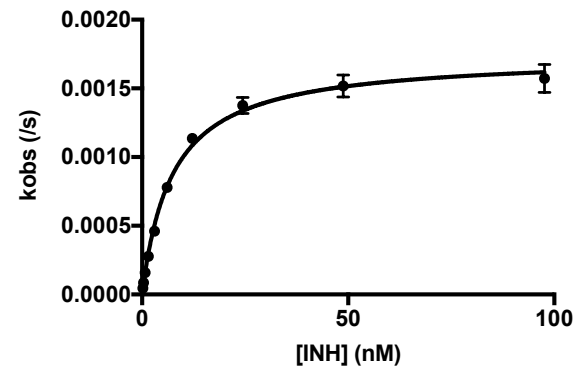
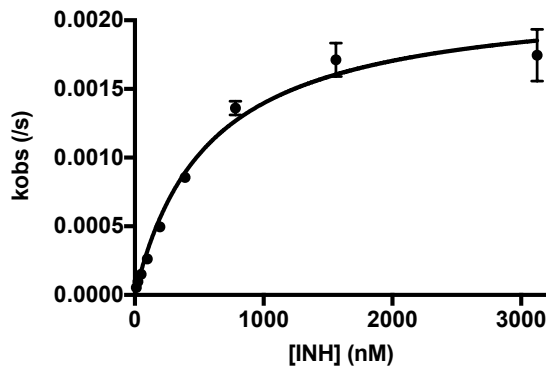
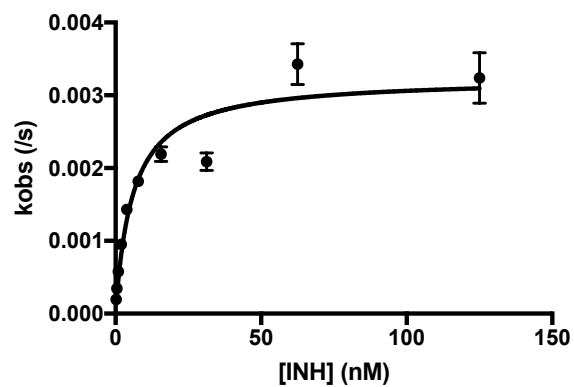
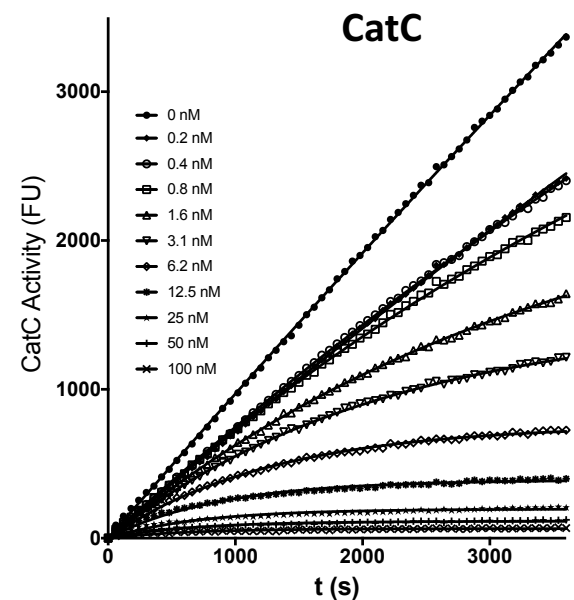
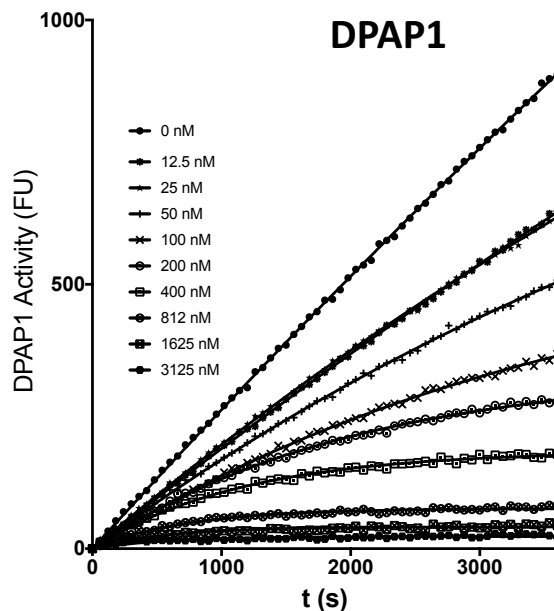
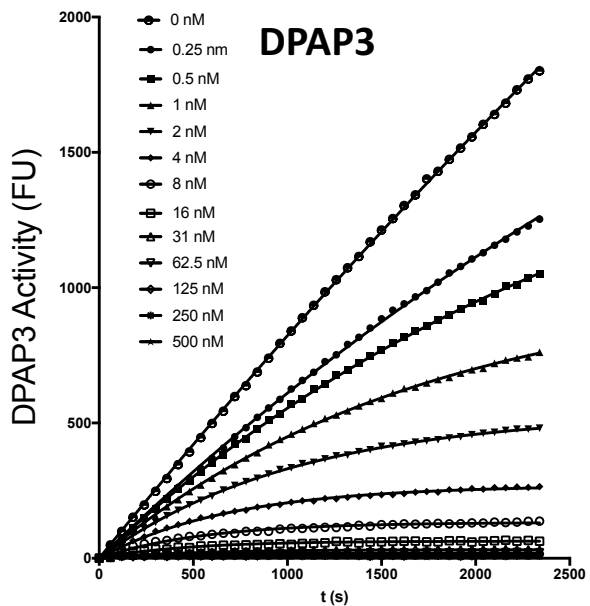
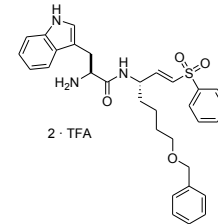
$$k_{\text{inact}}/K_i = 3,200 \pm 300 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0022 \pm 0.00009 \text{ s}^{-1}$$

$$K_i = 16 \pm 3 \text{ nM}$$

$$k_{\text{inact}}/K_i = 131,000 \pm 15,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: Trp; P1: nLeu(o-Bzl)



$$k_{\text{inact}} = 0.0032 \pm 0.0003 \text{ s}^{-1}$$

$$K_i = 4 \pm 1 \text{ nM}$$

$$k_{\text{inact}}/K_i = 826,000 \pm 193,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.0022 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 380 \pm 60 \text{ nM}$$

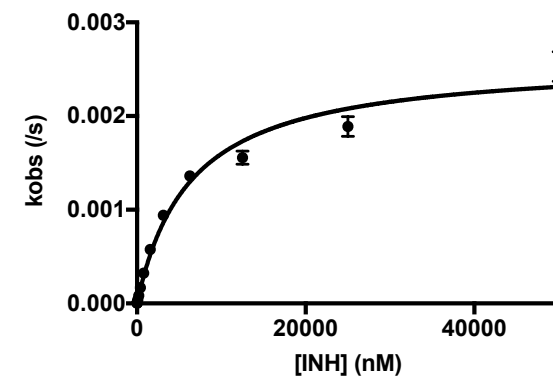
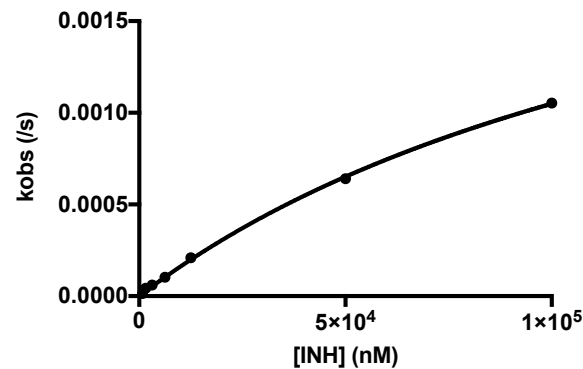
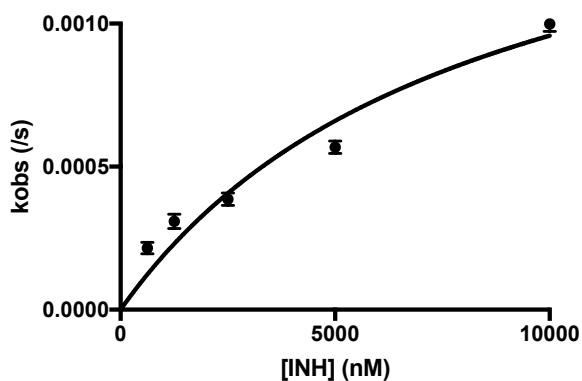
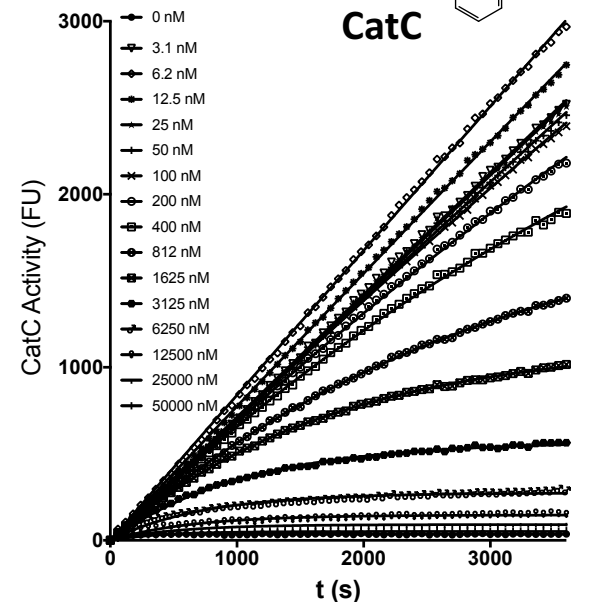
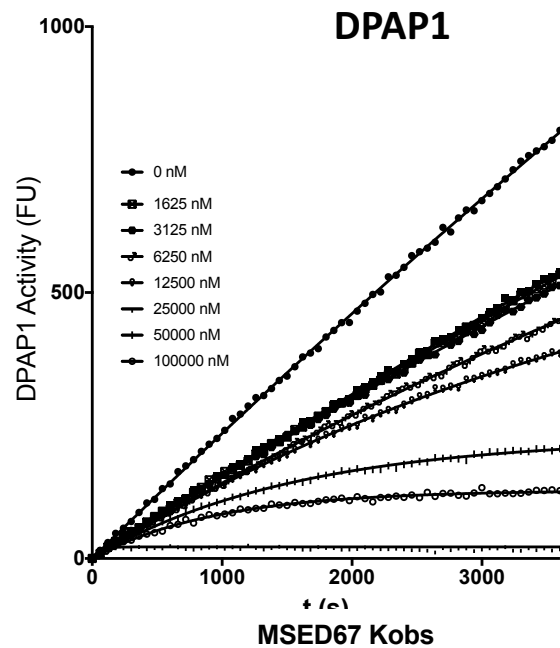
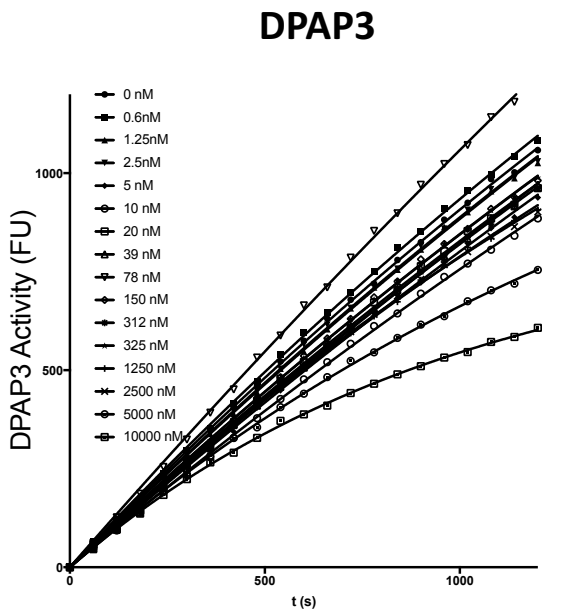
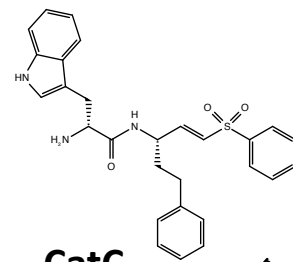
$$k_{\text{inact}}/K_i = 5,800 \pm 600 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{inact}} = 0.00174 \pm 0.00003 \text{ s}^{-1}$$

$$K_i = 4.9 \pm 0.3 \text{ nM}$$

$$k_{\text{inact}}/K_i = 355,000 \pm 18,000 \text{ M}^{-1}\text{s}^{-1}$$

P2: *D*-Trp; P1: hPhe



$$k_{inact} = 0.0017 \pm 0.0006 \text{ s}^{-1}$$

$$K_i = 5,500 \pm 3,000 \text{ nM}$$

$$k_{inact}/K_i = 320 \pm 80 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0027 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 105,000 \pm 10,000 \text{ nM}$$

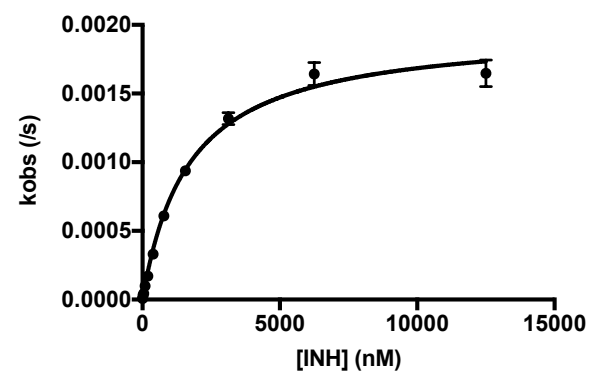
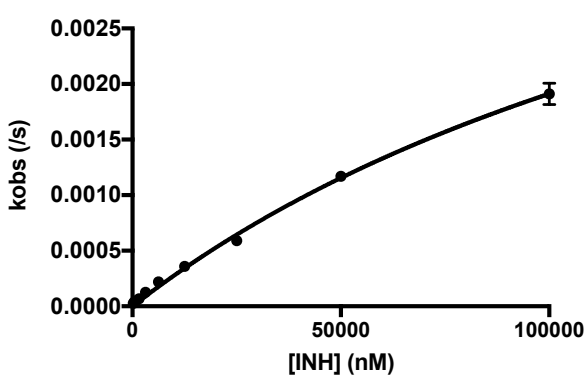
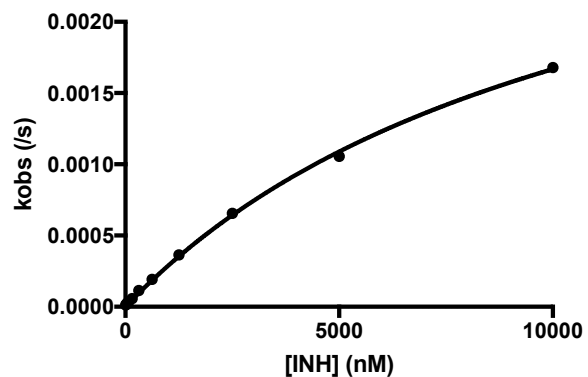
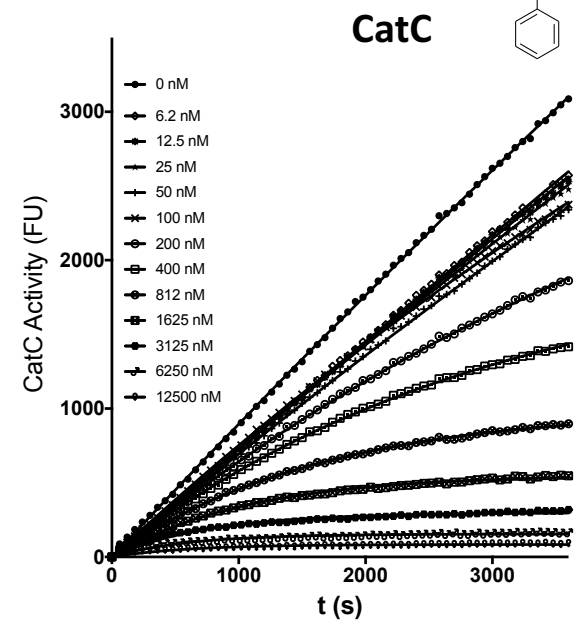
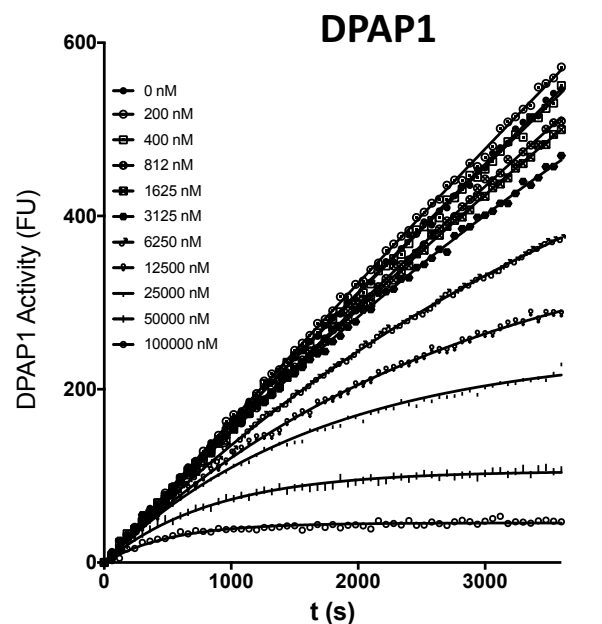
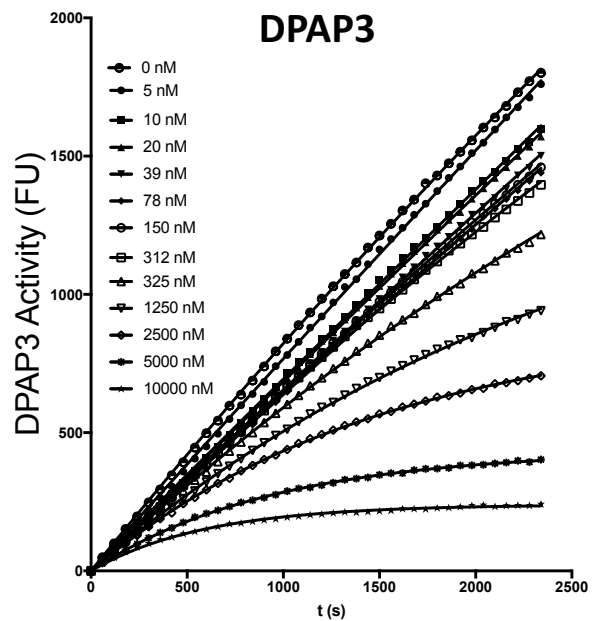
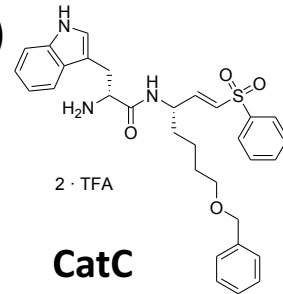
$$k_{inact}/K_i = 26 \pm 1 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0026 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 4,200 \pm 600 \text{ nM}$$

$$k_{inact}/K_i = 620 \pm 70 \text{ M}^{-1}\text{s}^{-1}$$

P2: *D*-Trp; P1: *n*Leu(*o*-Bzl)



$$k_{inact} = 0.0036 \pm 0.0002 \text{ s}^{-1}$$

$$K_i = 7,600 \pm 600 \text{ nM}$$

$$k_{inact}/K_i = 470 \pm 13 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0055 \pm 0.0007 \text{ s}^{-1}$$

$$K_i = 127,000 \pm 20,000 \text{ nM}$$

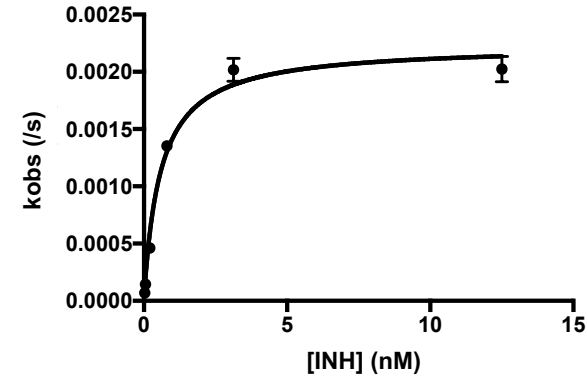
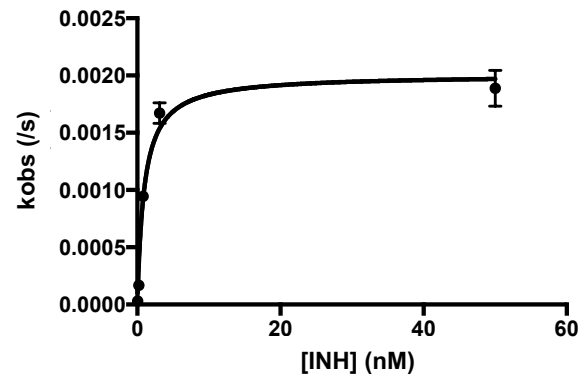
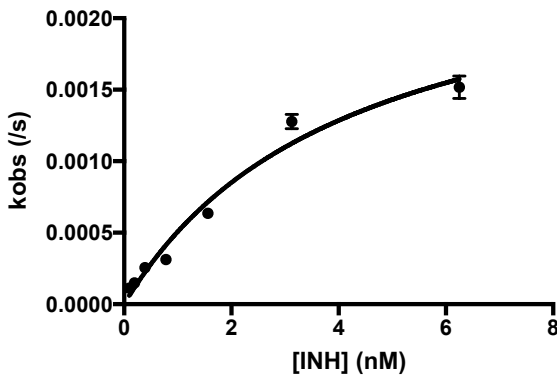
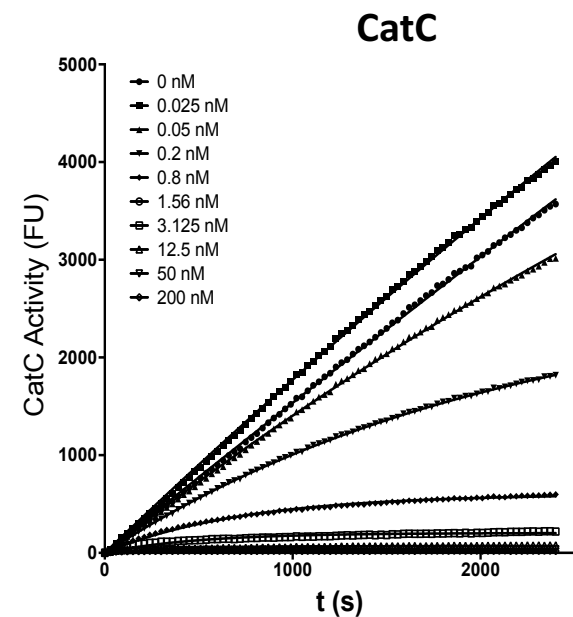
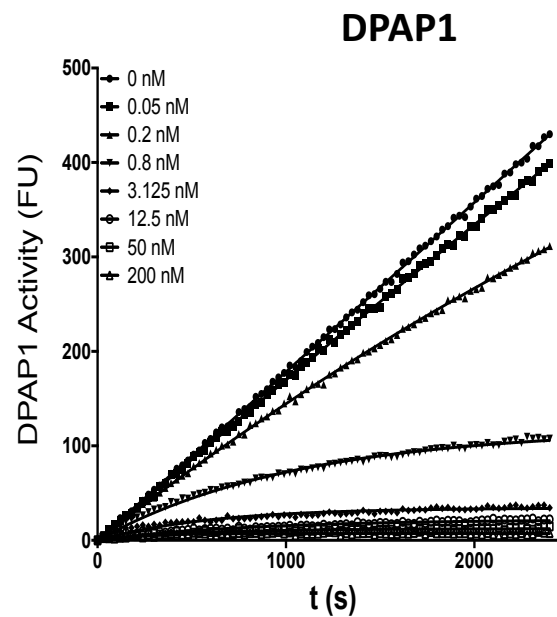
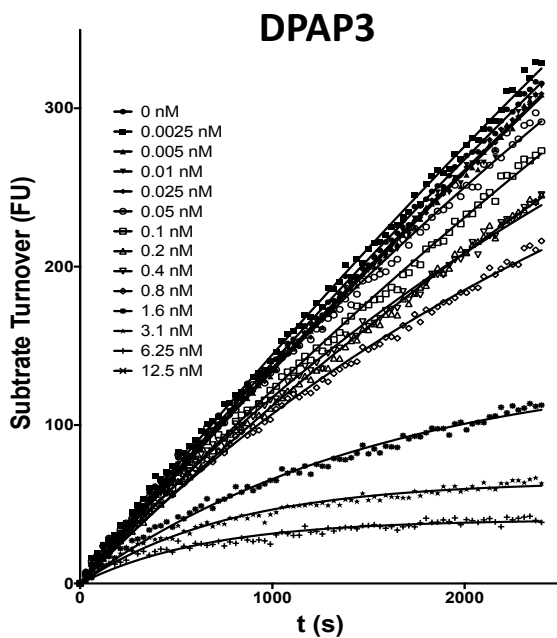
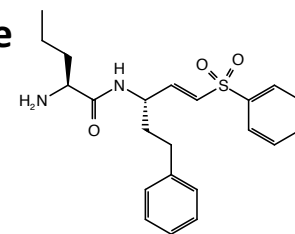
$$k_{inact}/K_i = 44 \pm 2 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.00197 \pm 0.00006 \text{ s}^{-1}$$

$$K_i = 1,100 \pm 100 \text{ nM}$$

$$k_{inact}/K_i = 1700 \pm 100 \text{ M}^{-1}\text{s}^{-1}$$

P2: nVal; P1: hPhe



$$k_{inact} = 0.0026 \pm 0.0004 \text{ s}^{-1}$$

$$K_i = 2.8 \pm 0.9 \text{ nM}$$

$$k_{inact}/K_i = 940,000 \pm 160,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0020 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 0.6 \pm 0.2 \text{ nM}$$

$$k_{inact}/K_i = 3,160,000 \pm 750,000 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{inact} = 0.0022 \pm 0.0001 \text{ s}^{-1}$$

$$K_i = 0.38 \pm 0.08 \text{ nM}$$

$$k_{inact}/K_i = 5,830,000 \pm 970,000 \text{ M}^{-1}\text{s}^{-1}$$