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.

Laura E. de Vries[1,§], Mateo I. Sanchez[2,§], Katarzyna Groborz[3,§], Laurie Kuppens[4,§], Marcin Poreba[3], Christine Lehmann[4], Fang Yuan[5], Shirin Arastu-Kapur[5], Martin Horn[6], Michael Mares[6], Matthew Bogyo[5], Marcin Drag[3], and Edgar Deu[4,§,*] .

[1]Chemical Biology Approaches to Malaria Laboratory, The Francis Crick Institute, London, England, United Kingdom.

[2]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands.

[3]Department of Genetics, Stanford School of Medicine, Stanford, California, United States.

[4]Division of Bioorganic Chemistry, Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland.

[5]Department of Pathology, Stanford University School of Medicine, Stanford, United States.

[6]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic.

[§] These authors contributed equally to this work.

*Corresponding author:

e-mail: edgar.deu@crick.ac.uk; Tel: +44 (0) 20 3796 1412

Supplementary Results

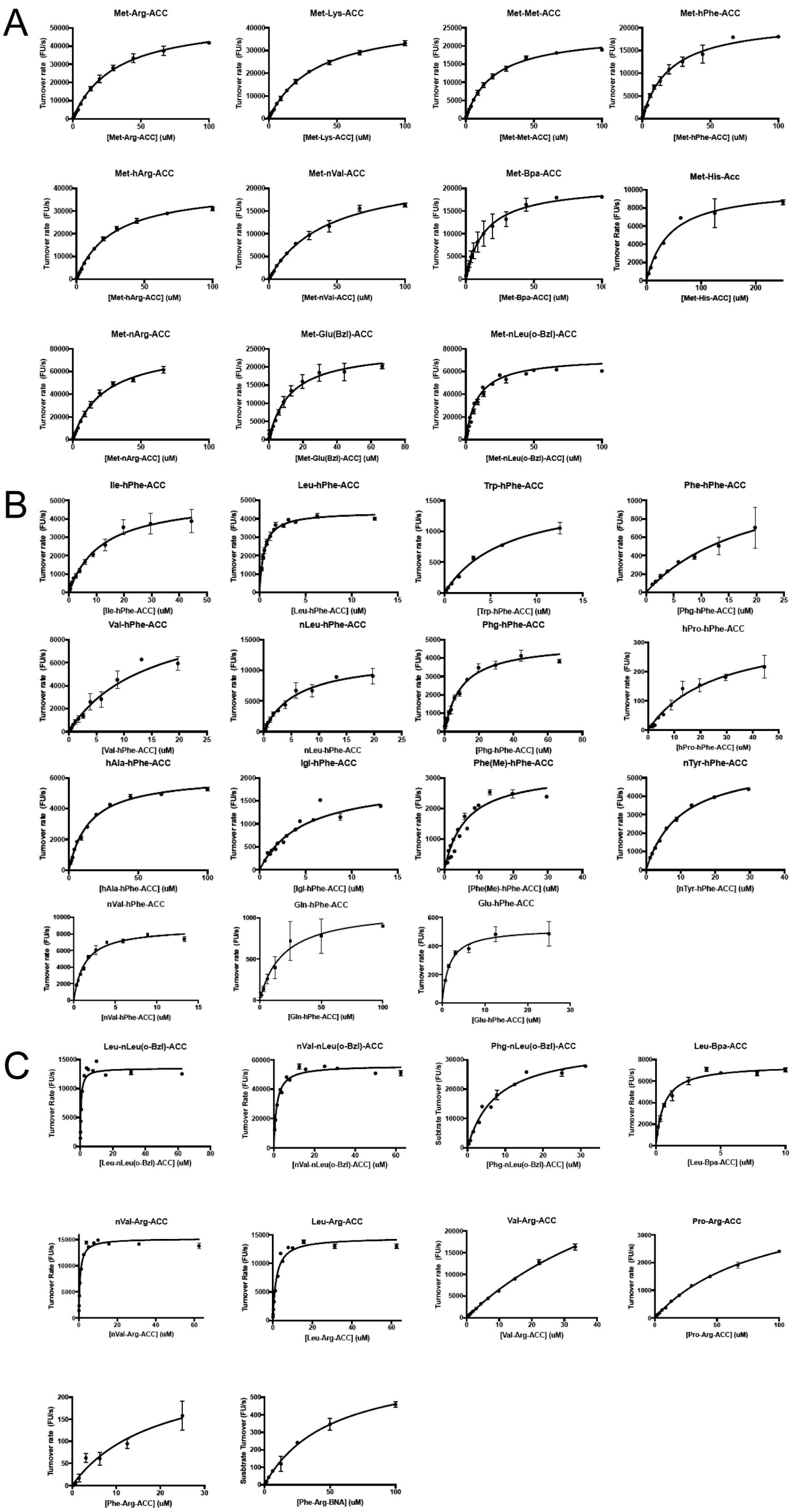


Figure S1. Michaelis Menten fits for rDPAP3. The turnover rate of the indicated substrates was measured at different concentrations and 1 nM rDPAP3 in assay buffer. Data was fitted in Prism to a Michaelis Menten model. K_m, k_{cat}, and k_{cat}/K_m values are reported in Table 1. (**A**) Substrates with P2 Met. (**B**) Substrates with P1 hPhe. (**C**) Substrates with selected P1 and P2 residues.

Figure S2. 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 lest 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.

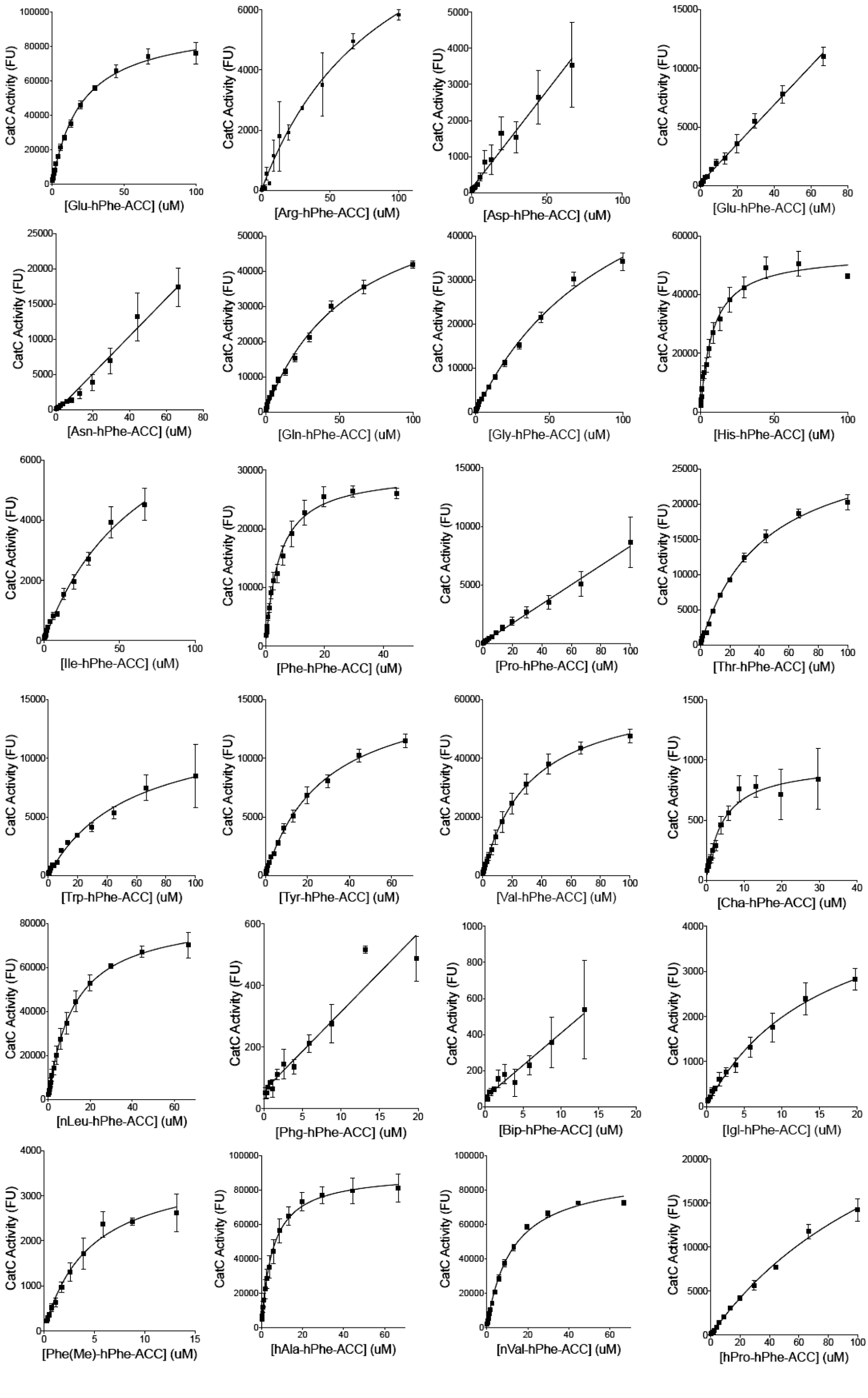


Figure S3. Michaelis Menten fits for CatC. The turnover rate of the indicate substrates was measured at different concentrations and 1 nM CatC in assay buffer. Data was fitted in Prism to a Michaelis Menten model. K_m, k_{cat}, and k_{cat}/K_m values are reported in Table S1.

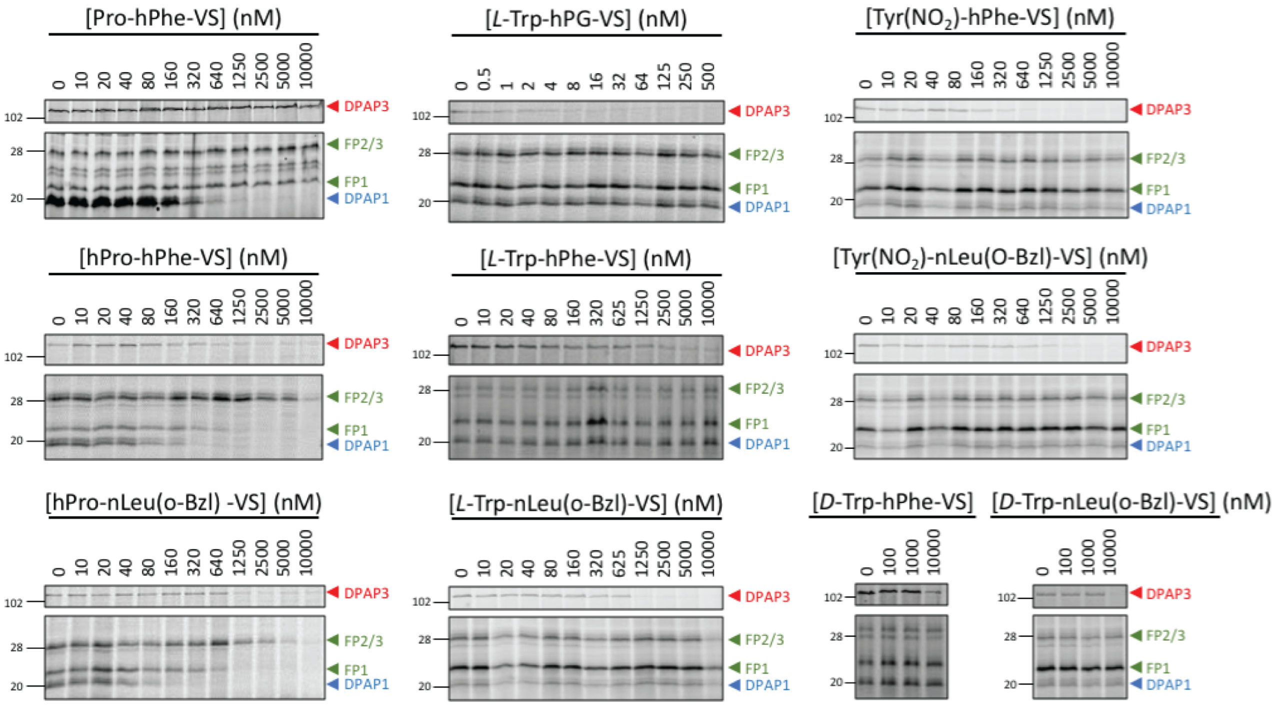


Figure S4. Selective inhibition of DPAP1 or DPAP3 in live parasites. Intact mature schizonts were pre-treated for 30 min with a dose response of inhibitor followed by 1 h treatment with FY01 to label the residual level of DPAPs and FPs activities. Fluorescents bands corresponding to the different cysteine proteases labelled by the probe are indicated by arrows. Dose response curves are shown in Fig. 4 and the IC_{50} values for each protease and inhibitor reported in Table 4.

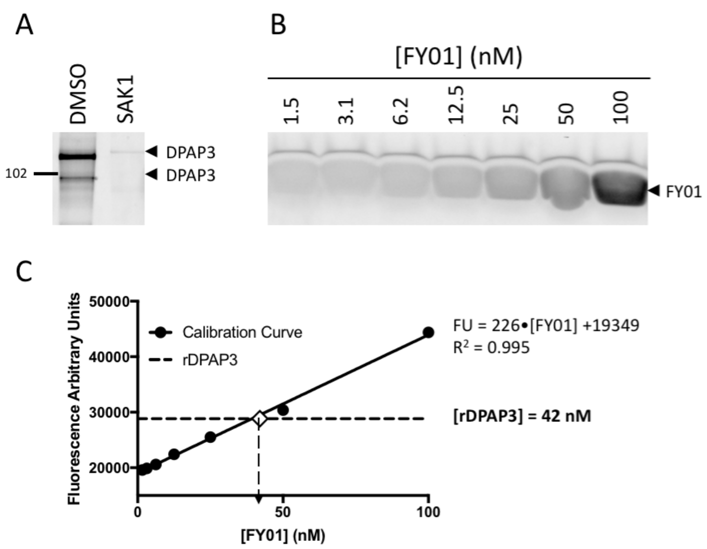


Figure S5. Active site titration of rDPAP3 using activity-based probes. (**A**) Labelling of purified rDPAP3 by FY01 in the presence or absence of SAK1. Our stock of rDPAP3 was diluted 20-fold in assay buffer, treated with DMSO or 1 μM SAK1 for 30 min, and labelled with FY01 for 1 h. Samples were run 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 H_2N-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 DICI (1.85 mmol, 242μl) 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 P_2O_5 and divided into eight equal portions (0.09 mmol per portion). Each portion of the H_2N-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 µl) 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 DICI (0.225 mmol, 30 µl) in DMF, added to the cartridge wells containing 1 eq of H_2N-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 P_2O_5 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.

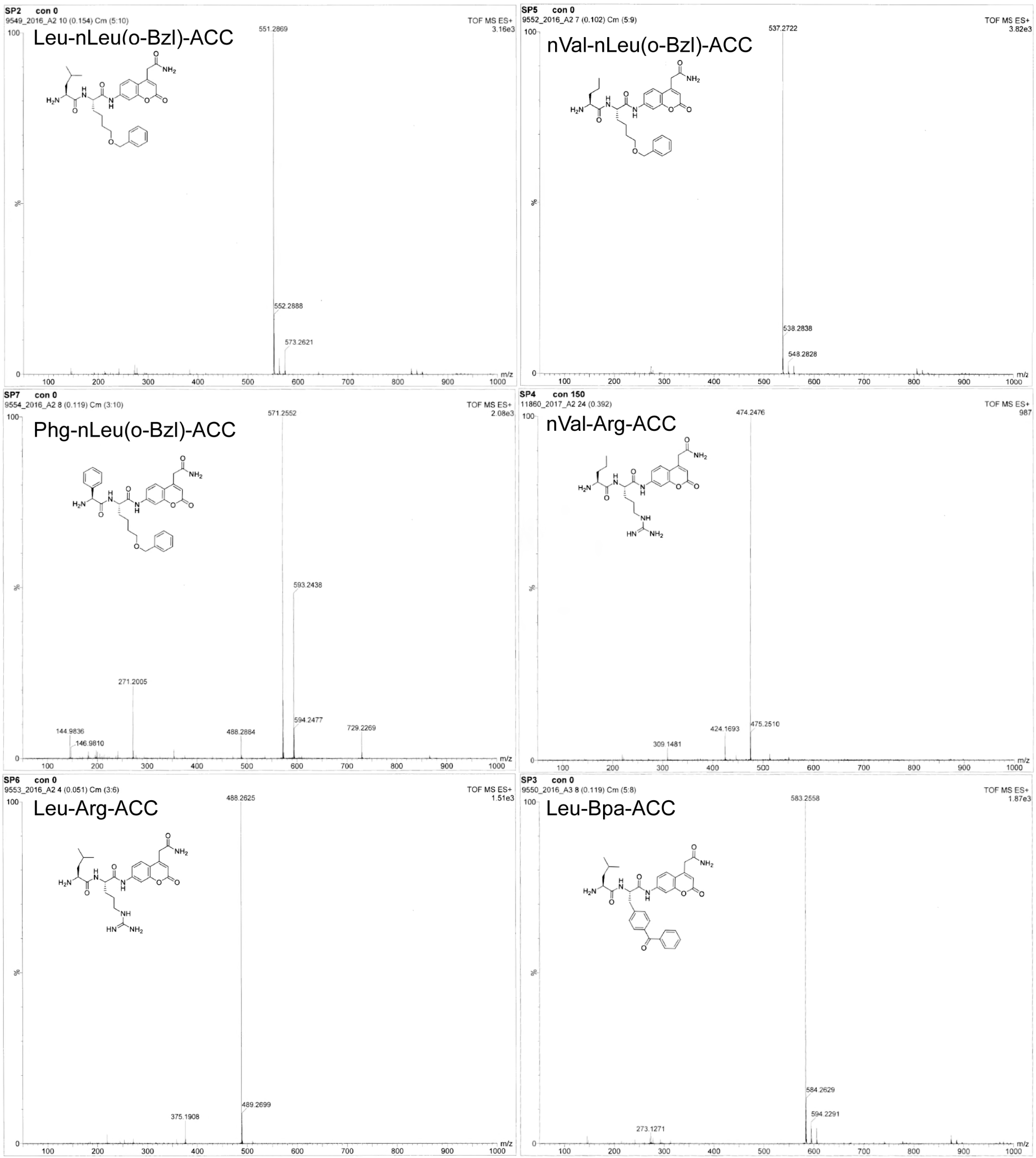
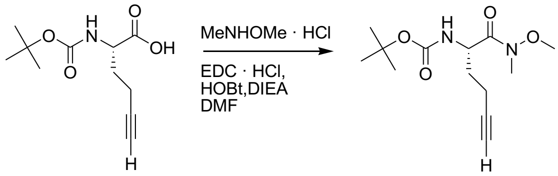


Figure S7. High resolution mass spectra of DPAP fluorogenic substrates.

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 $LiAlH_4$. With the carbonyl at hand, in Horner-Wadsworth-Emmons (HWE) type reaction with diethyl phenylsulfonylmethyl-phosphonate, 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



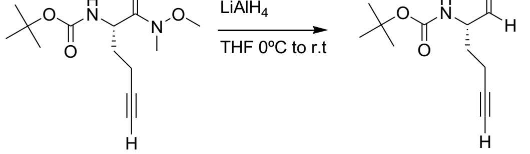
To a solution of (S)-2-(Boc-amino)-5-hexynoic acid (0.6 g, 2.64 mmol) in dry DMF (14 mL) at 0 °C was added EDC (607 mg, 3.17 mmol), HOBt (427 mg, 3.17 mmol), N,O-dimethylhydroxylamine hydrochloride (308 mg, 3.17 mmol) and DIPEA (0.7 mL, 7.92 mmol). The reaction was stirred at rt for 12 h. The resulting solution was evaporated in vacuo to give a light yellow oil, which was diluted with aqueous NH_4Cl (10 wt%) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried over Na_2SO_4, 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%).

^{1}H NMR (*$DCCl_3$ 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).

^{13}C NMR (*$DCCl_3$ δ*): 15.0 (CH_2), 28.3 (CH_3), 32.0 (CH_2), 32.2 (CH_3), 49.8 (CH), 61.6 (CH_3), 68.7 (CH), 79.7 (C), 83.2 (C), 155.4 (C), 172.4 (C).

ESI-MS: $[M+H]^+$ calcd. for $C_{13}H_{22}N_2O_4Na$ = 293.1772 found 293.1478. (M.W. 270.3248)

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



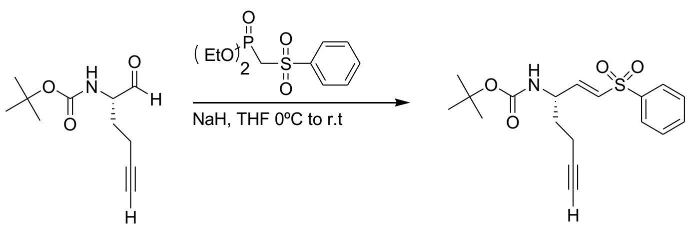
To a solution of *tert*-Butyl (S)-1-(N-methoxy-N-methylcarbamoyl)pent-4-ynylcarbamate (0.62 g, 2.3 mmol) in dry THF (23 mL) at 0 °C was added $LiAlH_4$ (0.1 g, 2.76 mmol) over 10 min, with vigorous stirring. The mixture was stirred for an additional 20 min at 0 °C, whereupon cold water was carefully 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 H_2O (150 mL) and extraction with EtOAc (3 x 75 mL), the combined organic extracts dried over Na_2SO_4, 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%).

^{1}H 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).

^{13}C NMR (*DCCl$_3$ δ*): 14.5 (CH_2), 27.9 (CH_2), 28.1 (CH_3), 59.0 (CH), 70.0 (CH), 80.2 (C), 82.5 (C), 155.3 (C), 199.0 (CH)

ESI-MS: $[M+H]^+$ calcd. for $C_{11}H_{17}NO_3Na$ = 234.1101 found 234.1105. (M.W. 211.2576)

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



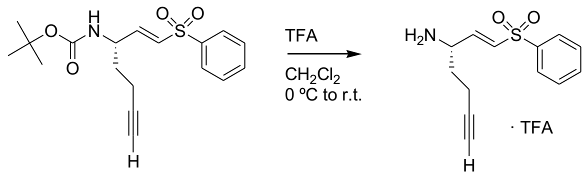
To a cooled (0°C) suspension of hexane-washed NaH (60% in mineral oil; 0.1 g, 2.38 mmol) in dry THF (10 mL) was added drop-wise diethyl[benzenesulfonyl)methyl]phosphonate (0.64 g, 2.18 mmol) in dry THF (10 mL) via syringe. The mixture was stirred for an additional 30 min at 0 °C and *tert*-butyl (S)-1-formylpent-4-ynylcarbamate (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% NH_4Cl 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 Na_2SO_4, 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%).

^{1}H 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).

^{13}C NMR (*DCCl$_3$ δ*): 15.1 (CH_2), 28.2 (CH_3), 32.7 (CH_2), 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 $C_{18}H_{23}NO_4Na$ = 372.1240 found 372.1254. (M.W. 349.4445)

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

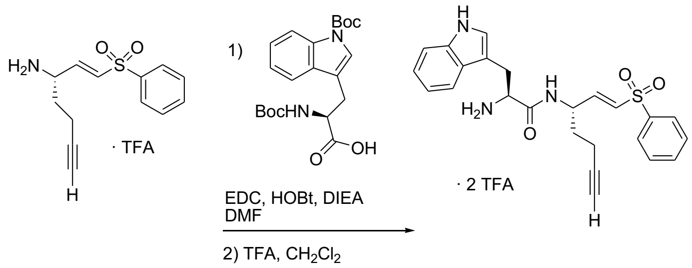


^{1}H NMR (*MeOD-d_4 δ*): 1.90-2.07 (m, 2H), 2.20-2.31 (m, 2H), 2.40 (t, J = 2.6 Hz, 1H), 4.14 (dt, J = 7.9, 6.0 Hz, 1H), 6.88 (dd, J = 15.2, 7.4 Hz, 1H), 7.02 (d, J = 15.2 Hz, 1H), 7.61-7.74 (m, 3H), 7.92-7.95 (m, 2H).

^{13}C NMR (*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: $[M+H]^+$ calcd. for $C_{13}H_{16}NO_2S$ = 250.0896 found 250.0890. (M.W. 363.3520)

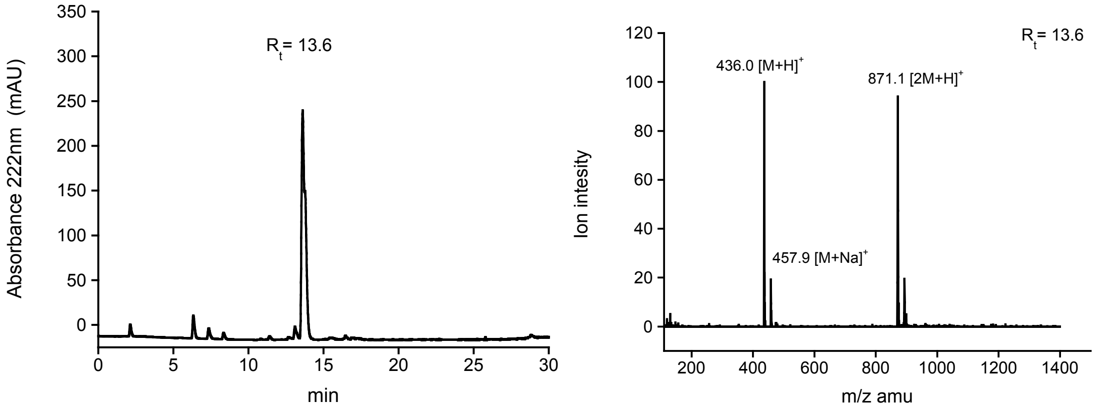
(2S)-2-Amino-3-(1H-indol-3-yl)-N-((S,E)-1-(phenylsulfonyl)hept-1-en-6-yn-3-yl)propanamide (Trp-hPG-VS)



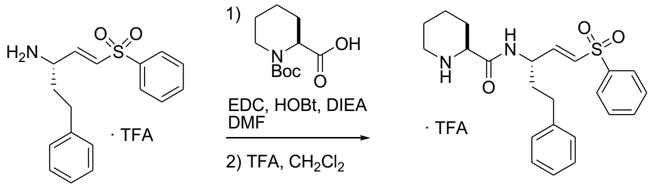
^{1}H NMR (*$MeOD_4$ δ*): 1.73-1.86 (m, 2H), 2.21 (dt, *J* = 7.2, 2.6 Hz, 2H),2.28 (bs, 1H), 3.20-3.35 (m, 2H, partial overlapping with MeOD signal), 4.12 (dd, *J* = 7.80, 7.15 Hz, 1H), 4.70 (dd, *J* = 13.3, 4.8 Hz, 1H), 6.15 (dd, *J* = 15.1, 1.5 Hz, 1H), 6.78 (dd, *J* = 15.1, 5.4 Hz, 1H), 7.05-7.08 (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}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: $[M+H]^+$ calcd. for $C_{24}H_{26}N_3O_3S$ = 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)



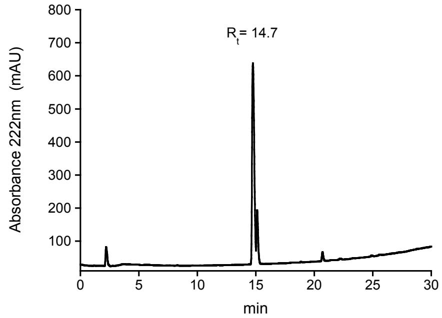
To a solution of (S)-N-Boc-Piperidine-2-carboxylic acid (211 mg, 0.92 mmol) in DMF (3 mL) was added EDC/HCl (177 mg, 0.92 mmol), HOBt (125 mg,0.92 mmol) and DIEA (0.6 mL, 3.1 mmol). After 10 min, (S)-3-benzenesulfonyl-1-phenethylallylamine trifluoroacetate (TFA•HphVSPh, X) (320 mg, 0.77 mmol) in DMF (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 NH_4Cl (10 wt%) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried over Na_2SO_4, 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.

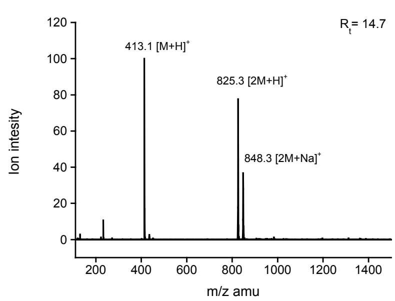
To a solution of this intermediate in CH_2Cl_2 (5 mL) at 0 °C was added trifluoroacetic acid (5 mL). The solution was stirred at room temperature overnight, then evaporated to dryness in vacuo and finally and freeze-dried yielding a white solid that was identified as the trifluoroacetic salt of the desired product (216 mg, 0.41 mmol, 52%).

^{1}H NMR (*MeOD-d_4 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).

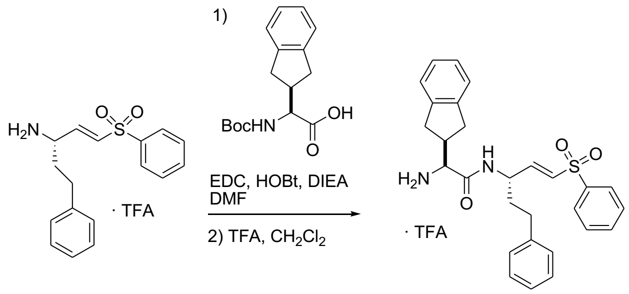
^{13}C NMR (*MeOD-d_4 δ*): 22.8 (CH_2), 23.1 (CH_2), 28.7 (CH_2), 33.0 (CH_2), 36.2 (CH_2), 44.9 (CH_2), 50.8 (CH), 59.0 (CH), 127.3 (CH), 128.7 (CH), 129.5 (CH), 129.6 (CH), 130.7 (CH), 132.4 (CH), 134.9 (CH), 141.7 (C), 141.9 (C), 146.8 (CH), 169.8 (C).

ESI-MS: $[M+H]^+$ calcd. for $C_{23}H_{29}N_2O_3S$ = 413.1893 found 413.1896. (M.W. 526.5684)





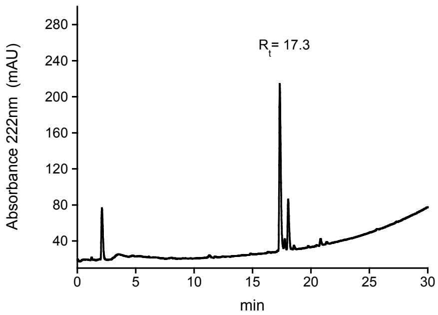
(2S)-2-amino-2-(2,3-dihydro-1H-inden-2-yl)-N-((S,E)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)acetamide Lm1msed44 **(Igl-hPhe-VS)**

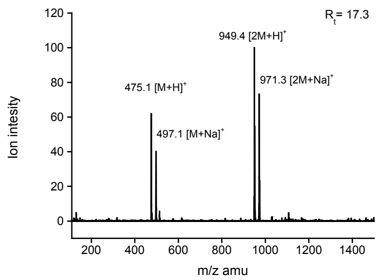


^{1}H NMR (*MeOD-d$_4$ δ*): 1.96 (td, *J* = 15.3, 7.5 Hz, 2H), 2.66 (dd, *J* = 15.6, 8.3 Hz, 2H), 2.88 (t, *J* = 5.60 Hz, 4H), 3.00-3.06 (m, 1H), 3.95 (dd, *J* = 5.4, 1.2 Hz, 1H), 4.60 (q, *J* = 6.8 Hz, 1H), 6.64 (d, *J* = 15.2 Hz, 1H), 6.89 (dd, *J* = 15.1, 6.6 Hz, 1H), 7.16 (bs, 7H), 7.24 (d, *J* = 7.1 Hz, 2H), 7.54-7.58 (m, 2H), 7.62-7.68 (m, 1H), 7.85 (d, *J* = 7.68 Hz, 2H).

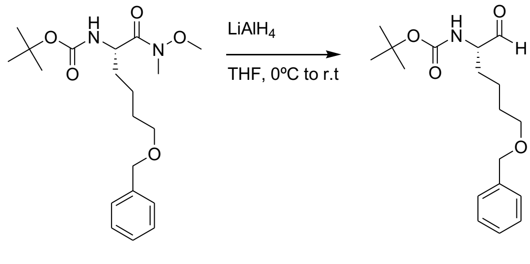
^{13}C NMR (*MeOD-d$_4$ δ*): 32.9 (CH_2), 36.2 (CH_2), 36.3 (CH_2), 36.4 (CH_2), 43.1 (CH), 51.2 (CH), 57.8 (CH), 125.5 (CH), 127.3 (CH), 128.0 (CH), 128.7 (CH), 129.5 (CH), 129.6 (CH), 130.7 (CH), 133.2 (CH), 134.9 (CH), 141.6 (C), 142.0 (C), 142.1 (C), 142.2 (C), 146.0 (CH), 169.2 (C).

ESI-MS: $[M+H]^+$ calcd. for $C_{28}H_{31}N_2O_3S$ = 475.2050 found 475.2049. (M.W. 588.6377)





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



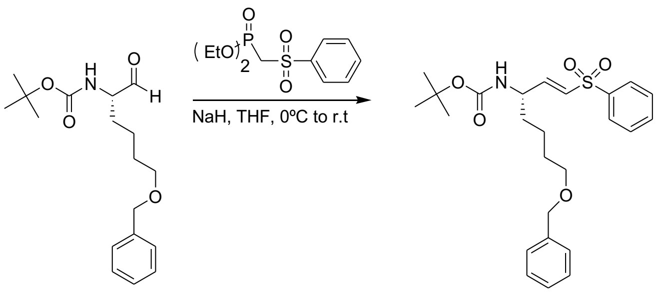
To a solution of *tert*-butyl (S)-1-(N-methoxy-N-methylcarbamoyl) -5-(benzyloxy) pentylcarbamate (2.2 g, 5.8 mmol) in dry THF (50 mL) at 0 °C was added $LiAlH_4$ (0.26 g, 7 mmol) over 10 min, with vigorous stirring. The mixture was stirred for an additional 20 min at 0 °C, whereupon cold water was carefully 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 H_2O (150 mL) and extraction with EtOAc (3 x 75 mL), the combined organic extracts dried over Na_2SO_4, 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%).

^{1}H 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).

^{13}C NMR (*DCCl$_3$*): 22.0 (CH_2), 28.2 (CH_3), 28.9 (CH_2), 29.3 (CH_2), 59.7 (CH), 69.7 (CH_2), 72.9 (CH_2), 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 $C_{18}H_{27}N_1O_4Na$ = 344.1832 found 344.1832. (M.W. 321.4113)

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



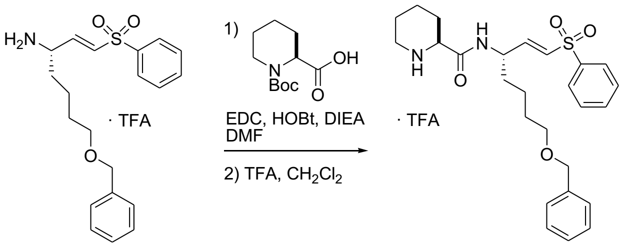
To a cooled (0°C) suspension of hexane-washed NaH (60% in mineral oil; 0.36 g, 9 mmol) in dry THF (50 mL) was added drop-wise diethyl[(benzenesulfonyl)methyl]phosphonate (2.4 g, 8.2 mmol) in dry THF (10 mL) via syringe. The mixture was stirred for an additional 30 min at 0 °C and *tert*-butyl (S)-5-(benzyloxy)-1-formylpentylcarbamate (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% NH_4Cl 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 Na_2SO_4, 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%).

^{1}H 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).

^{13}C NMR (*DCCl$_3$*): 22.4 (CH_2), 28.2 (CH_3), 29.2 (CH_2), 33.9 (CH_2), 50.9 (CH), 69.6 (CH_2), 72.9 (CH_2), 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 $C_{20}H_{32}N_2O_5Na$ = 482.1972 found 482.1972. (M.W. 459.5982)

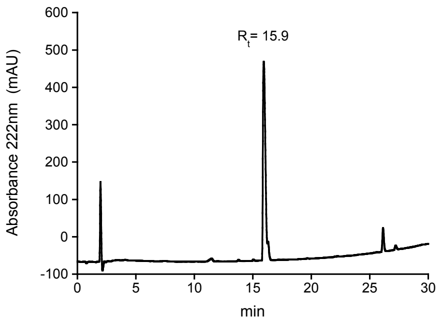
(2S)-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)piperidine-2-carboxamide Lm1msed55 **(hPro-nLeu(O-Bzl)-VS)**

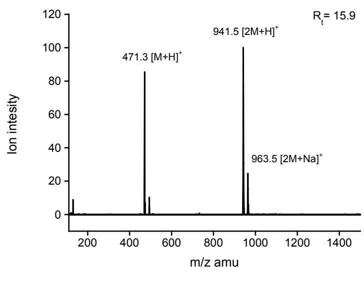


^{1}H NMR (*MeOD-d$_4$ δ*):1.35-1.45 (m, 2H) , 1.56-1.69 (m, 7H), 1.82-1.88 (m, 2H), .2.14 (d, J = 9.5 Hz, 1H), 2.97 (t, J = 12.4 Hz, 1H), 3.36 (d, J = 12.9 Hz, 1H), 3.45 (t, J = 6.3 Hz, 2H), 3.78 (dd, J = 11.2, 2.9 Hz, 1H), 4.46 (s, 2H), 4.56 (dd, J = 13.3, 5.9 Hz, 1H), 6.56 (d, J = 15.2 Hz, 1H), 6.89 (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).

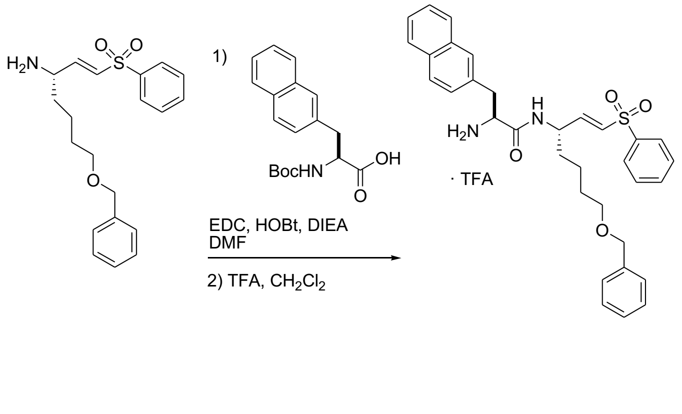
^{13}C NMR (*MeOD-d$_4$ δ*): 22.7 (CH_2), 22.9 (CH_2), 23.6 (CH_2), 28.6 (CH_2), 30.0 (CH_2), 33.9 (CH_2), 44.9 (CH_2), 51.2 (CH), 58.9 (CH), 70.9 (CH_2), 73.8 (CH_2), 128.6 (CH), 128.7 (CH), 128.9 (CH), 129.4 (CH), 130.6 (CH), 132.0 (CH), 134.9 (CH), 139.7 (C), 141.5 (C), 147.2 (CH), 169.8 (C).

ESI-MS: $[M+H]^+$ calcd. for $C_{26}H_{35}N_2O_4S$ = 471.2312 found 471.2314. (M.W. 584.6481)





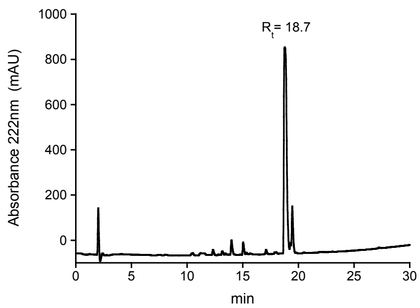
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(naphthalen-2-yl)propanamide Lm1msed56 **(2Nal-nLeu(O-Bzl)-VS)**

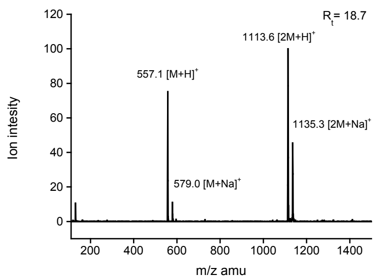


^{1}H NMR (*MeOD-d_4* $\boldsymbol{\delta}$): 1.29-1.37 (m, 2H), 1.50-1.61 (m, 4H), 3.18-3.28 (m, 3H), 3.41 (t, J = 6.3 Hz, 1H), 4.15 (t, J = 7.3 Hz, 1H), 4.53 (q, J = 6.4 Hz, 1H), 4.40 (s, 2H), 6.27 (d, J = 15.2 Hz, 1H), 6.74 (dd, J = 15.2, 5.8 Hz, 1H), 7.30 (bs, 6H), 7.51 (bs, 4H), 7.63 (t, J = 7.4 Hz, 1H), 7.69-7.74 (m 3H), 7.83-7.87 (m, 3H).

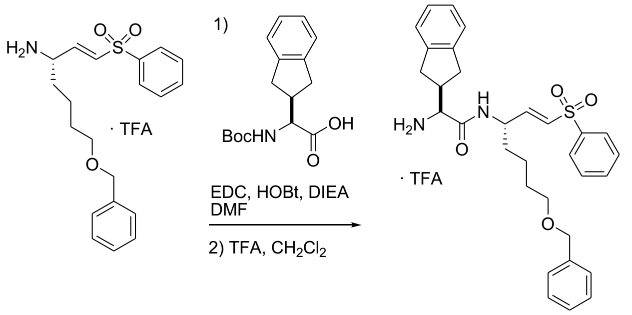
^{13}C NMR (*MeOD-d_4* $\boldsymbol{\delta}$): 23.5 (CH_2), 30.2 (CH_2), 34.1 (CH_2), 38.8 (CH_2), 51.4 (CH), 55.7 (CH), 70.9 (CH_2), 73.9 (CH_2), 127.3 (CH), 127.6 (CH), 128.1 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 128.9 (CH), 129.0 (CH), 129.4 (CH), 129.6 (CH), 130.1 (CH), 130.6 (CH), 132.2 (CH), 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 $C_{33}H_{37}N_2O_4S$ = 557.2469 found 557.2464. (M.W. 670.7383)





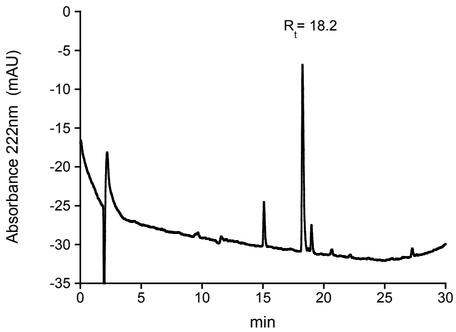
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-2-(2,3-dihydro-1H-inden-2-yl)acetamide Lm1msed57 **(Igl-nLeu(O-Bzl)-VS)**

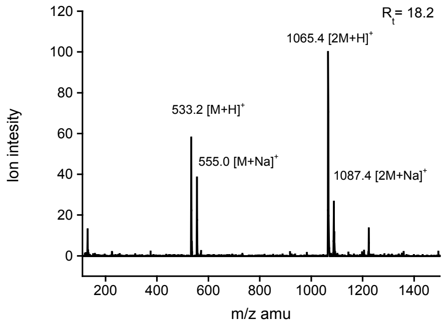


^{1}H NMR (*MeOD-d_4* $\boldsymbol{\delta}$): 1.37-1.47 (m, 2H), 1.58-1.70 (m, 4H), 2.84 (dd, J = 18.7, 5.2 Hz, 4H), 2.99-3.04 (m, 1H), 3.47 (t, J = 6.2 Hz, 2H), 3.89 (d, J = 6.8 Hz, 1H), 4.47 (s, 2H), 4.58 (dd, J = 13.9, 6.8 Hz, 1H), 6.63 (d, J = 15.1 Hz, 1H), 6.86 (dd, J = 15.1, 6.5 Hz, 1H), 7.16 (bs, 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).

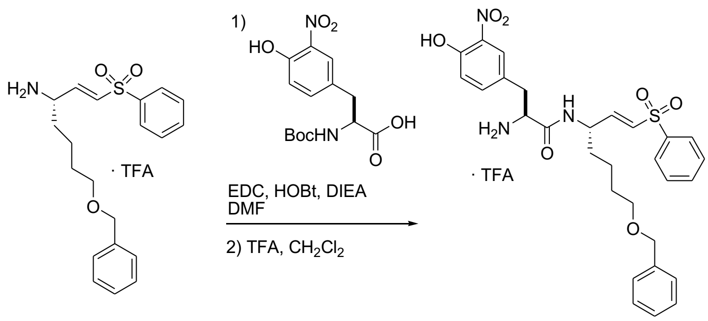
^{13}C NMR (*MeOD-d_4* $\boldsymbol{\delta}$): 23.7 (CH_2), 30.2 (CH_2), 34.0 (CH_2), 36.3 (CH_2), 43.1 (CH), 51.5 (CH), 57.8 (CH), 71.0 (CH_2), 73.9 (CH_2), 125.5 (CH), 128.0 (CH), 128.6 (CH), 128.7 (CH), 128.9 (CH), 129.4 (CH), 130.7 (CH), 132.9 (CH), 134.9 (CH), 139.6 (C), 142.1 (C), 142.2 (C), 146.4 (CH), 169.2 (C).

ESI-MS: $[M+H]^+$ calcd. for $C_{31}H_{37}N_2O_4S$ = 533.2469 found 533.2463. (M.W. 646.7169)





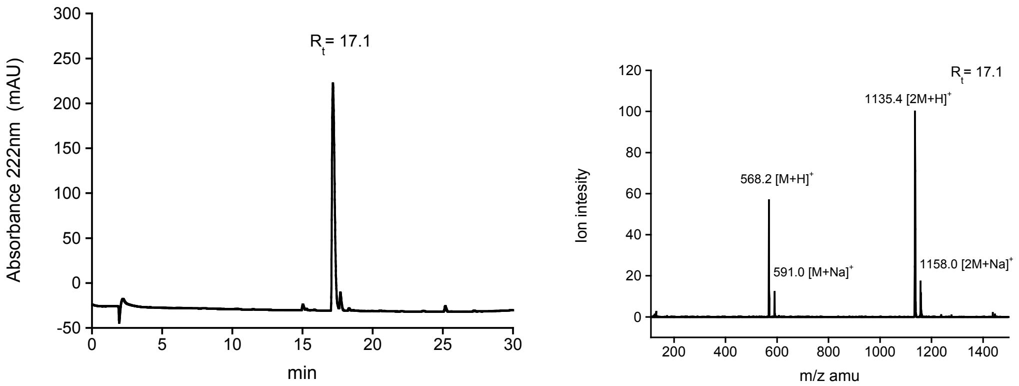
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(4-hydroxy-3-nitrophenyl)propanamide Lm1msed58 **(Tyr(NO2)-nLeu(O-Bzl)-VS)**



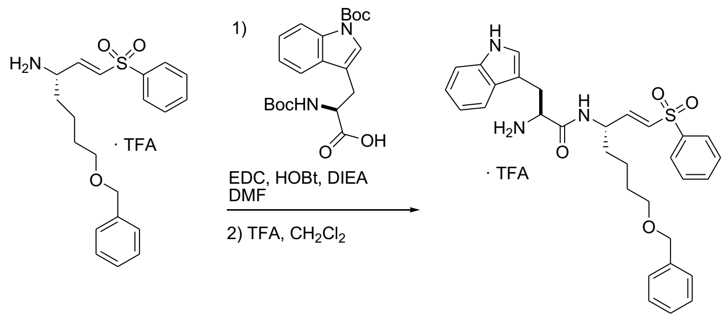
^{1}H NMR (*MeOD-d_4* $\boldsymbol{\delta}$): 1.35-1.41 (m, 2H), 1.54-1.63 (m, 4H), 3.10 (dq, *J* = 14.1, 7.4 Hz, 2H), 3.45 (t, *J* = 6.2 Hz, 2H), 4.05 (t, *J* = 7.3 Hz, 1H), 4.45 (s, 2H), 4.55 (dd, *J* = 12.9, 6.3 Hz, 1H), 6.25 (d, *J* = 15.2 Hz, 1H), 6.72 (dd, *J* = 15.2, 5.8 Hz, 1H), 7.15 (d, *J* = 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).

^{13}C NMR (*MeOD-d_4* $\boldsymbol{\delta}$): 23.5 (CH_2), 30.2 (CH_2), 34.2 (CH_2), 37.3 (CH_2), 51.3 (CH), 55.3 (CH), 70.9 (CH_2), 73.9 (CH_2), 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 $C_{29}H_{34}N_3O_7S$ = 568.2112 found 568.2102. (M.W. 681.6766).



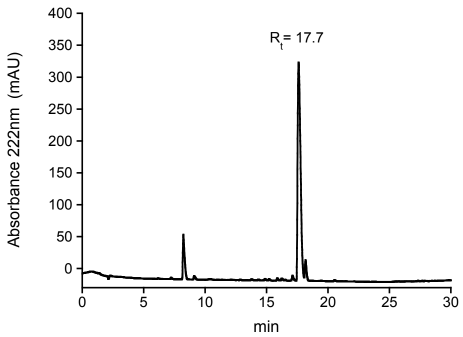
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(1H-indol-3-yl)propanamide Lm1msed68 (**Trp-nLeu(O-Bzl)-VS)**

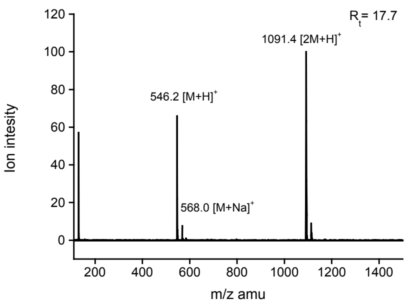


^{1}H NMR (*MeOD-d$_4$ δ*): 1.31-1.37 (m, 2H), 1.49-1.59 (m, 4H), 3.20 (dd, J = 14.5, 7.0 Hz, 1H), 3.34 (d, J = 7.8 Hz, 1H), 3.43 (t, J = 6.2 Hz, 2H), 4.09 (t, J = 7.4 Hz, 1H),4.44 (s, 2H), 4.52 (q, J = 6.2 Hz, 1H), 6.12 (d, J = 15.2 Hz, 1H), 6.73 (dd, J = 15.2, 5.5 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 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).

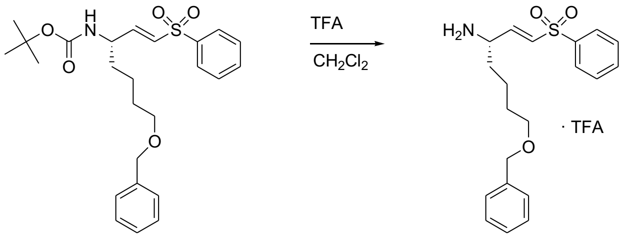
^{13}C NMR (*MeOD-d$_4$ δ*): 23.5 (CH_2), 29.0 (CH_2), 30.2 (CH_2), 34.2 (CH_2), 51.2 (CH), 54.9 (CH), 71.0 (CH_2), 73.9 (CH_2),108.0 (C), 112.9 (CH), 119.1 (CH), 120.4 (CH), 123.0 (CH), 125.5 (CH), 128.1 (C), 128.7 (CH), 128.8 (C), 128.9 (CH), 129.4 (CH), 130.6 (CH), 132.0 (CH), 134.8 (CH), 138.2 (C), 139.7 (C), 141.6 (C), 146.8 (CH), 169.8 (C).

ESI-MS: $[M+H]^+$ calcd. for $C_{31}H_{36}N_3O_4S$ = 546.2421 found 546.2438. (M.W. 773.7390)





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



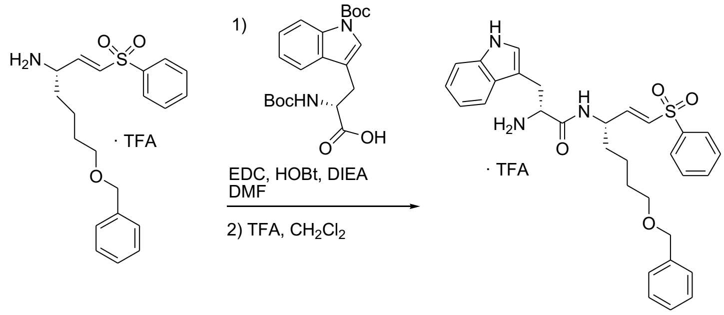
To a cooled (0°C) solution of *tert*-butyl (S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-ylcarbamate (0.2 g, 0.43 mmol) in CH_2Cl_2 (5 mL) was added drop-wise TFA (1 mL) via syringe and stirred for 30 min. After checking by HPLC-MS that all the starting material was consumed, the solvent was removed 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}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}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: $[M+H]^+$ calcd. for $C_{20}H_{25}NO_3SNa$ = 382.1447 found 382.1458. (M.W. 473.5057)

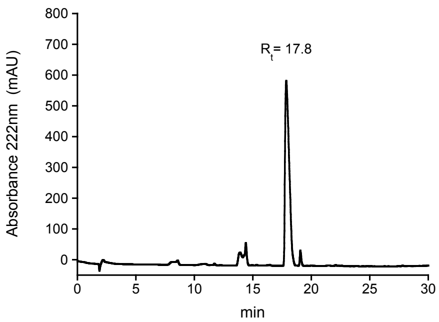
(2R)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(1H-indol-3-yl)propanamide Lm1msed71 **(*L*-Trp-nLeu(O-Bzl)-VS)**

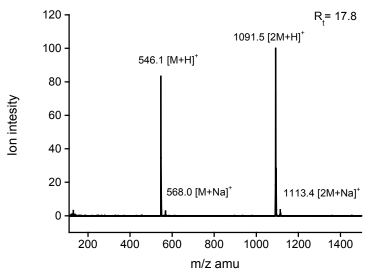


^{1}H NMR (*MeOD-d_4 δ*): 0.95-1.02 (m, 2H), 1.09-1.18 (m, 1H), 1.28-1.35 (m, 1H), 1.37-1.45 (m, 2H), 3.21 (dd, *J* = 14.4, 7.2 Hz, 1H), 3.33-3.38 (m, 3H), 4.05 (t, *J* = 7.6 Hz, 1H), 4.43 (bs, 3H), 6.61 (d, *J* = 15.2 Hz, 1H), 6.81 (dd, *J* = 15.1, 5.2 Hz, 1H), 7.04 (t, *J* = 7.4 Hz, 1H), 7.11 (t, *J* = 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).

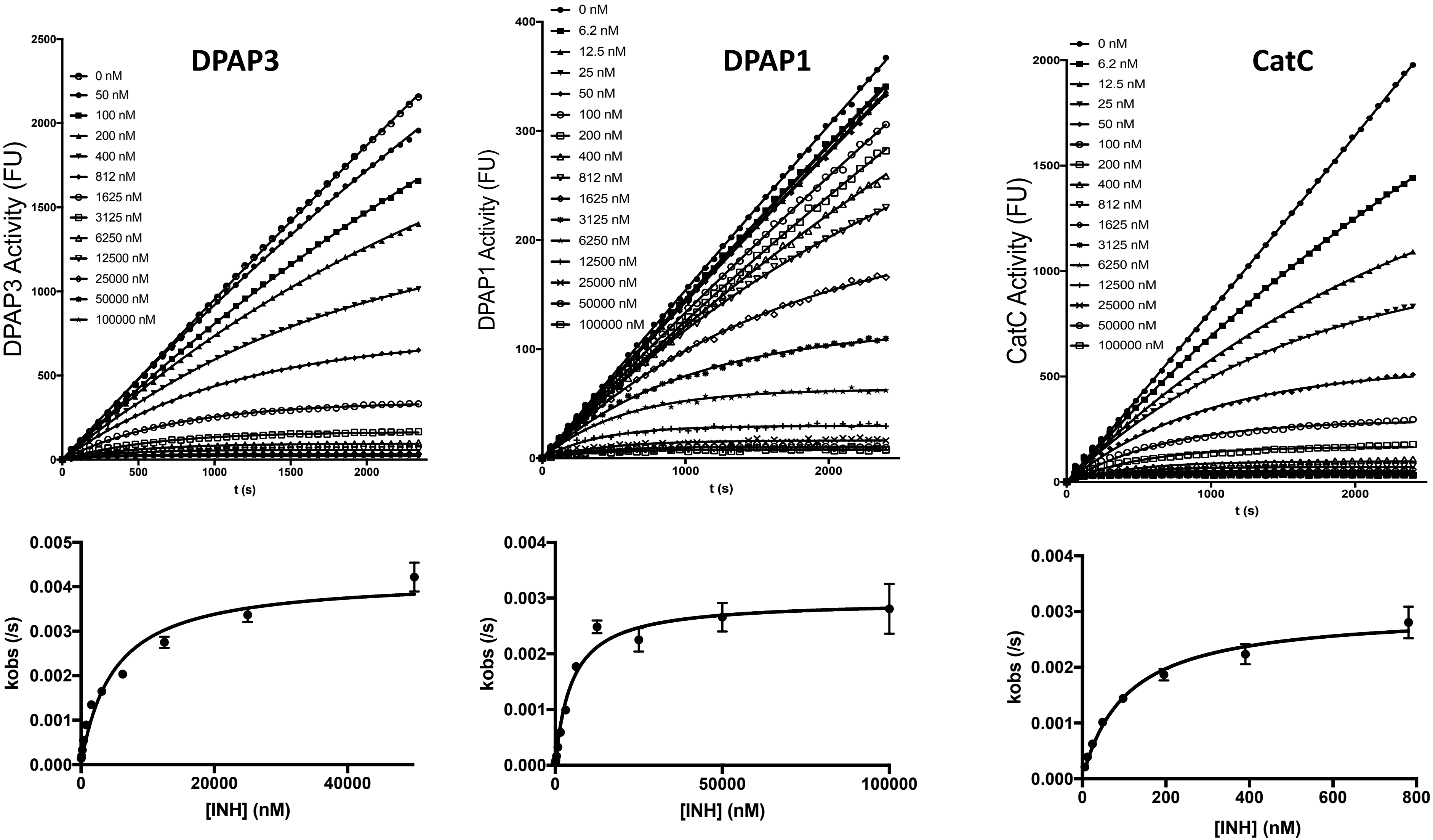
^{13}C NMR (*MeOD-d_4 δ*): 23.2 (CH_2), 29.0 (CH_2), 30.1 (CH_2) 33.9 (CH_2), 51.3 (CH), 55.3 (CH), 70.9 (CH_2), 73.9 (CH_2), 108.2 (C), 112.7 (CH), 119.1 (CH), 120.3 (CH) 122.9 (CH), 125.5 (CH), 128.4 (C), 128.7 (CH), 128.8 (CH), 129.4 (CH), 130.6 (CH), 132.0 (CH), 134.8 (CH), 138.2 (C), 139.8 (C),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

ESI-MS: $[M+H]^+$ calcd. for $C_{31}H_{36}N_3O_4S$ = 546.2421 found 546.2411. (M.W. 773.7390)





P2: Gly

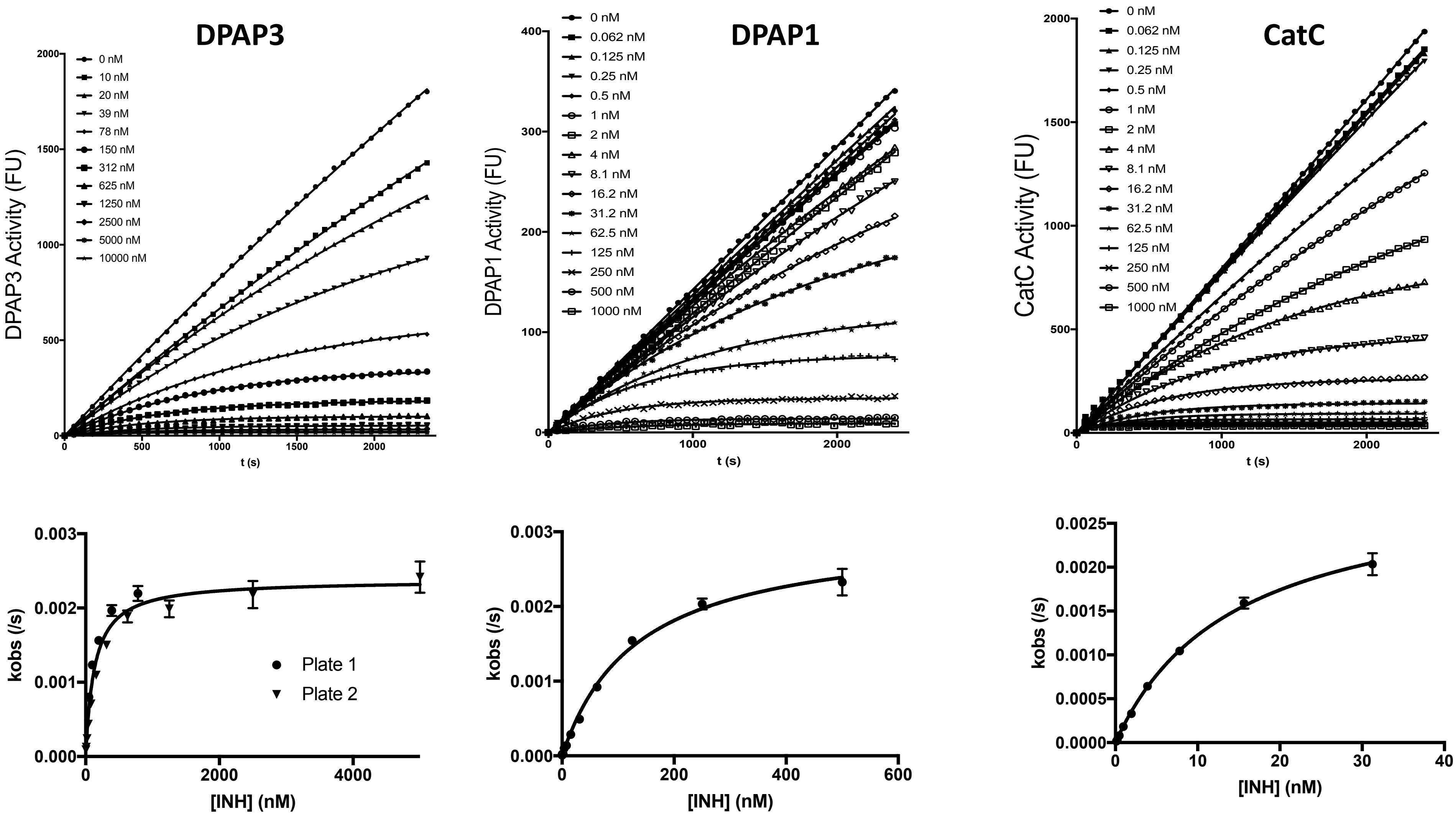


k_{inact} = 0.0042 ± 0.0003 s^{-1}
K_i = 3,300 ± 750 nM
k_{inact}/K_i = 1,300 ± 250 $M^{-1}s^{-1}$

k_{inact} = 0.0030 ± 0.0001 s^{-1}
K_i = 3,300 ± 500 nM
k_{inact}/K_i = 900 ± 100 $M^{-1}s^{-1}$

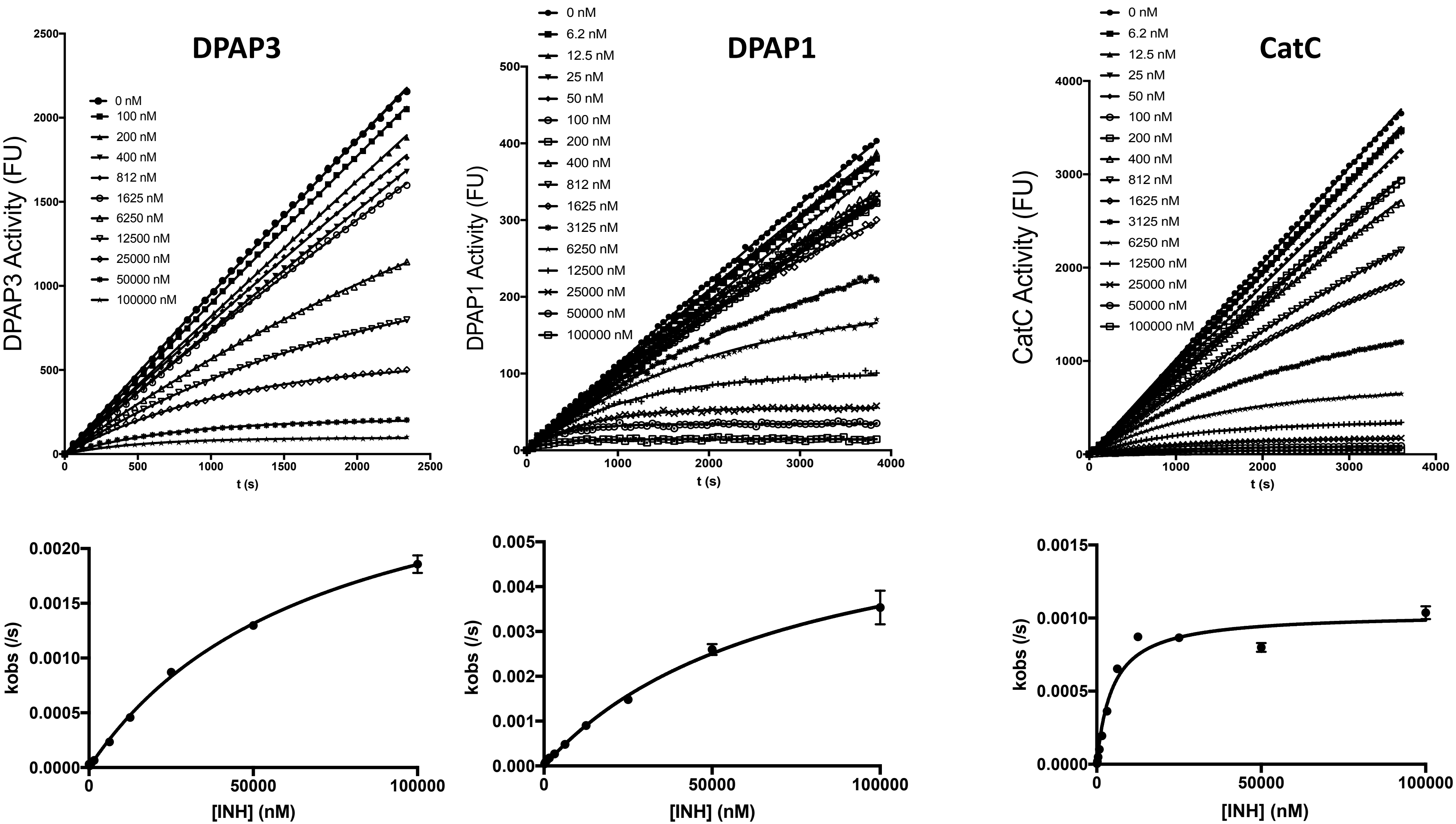
k_{inact} = 0.0030 ± 0.0002 s^{-1}
K_i = 70 ± 9 nM
k_{inact}/K_i = 43,000 ± 4,000 $M^{-1}s^{-1}$

P2: Ala



k_{inact} = 0.0024 ± 0.0001 s^{-1}	k_{inact} = 0.00303 ± 0.00009 s^{-1}	k_{inact} = 0.00300 ± 0.00007 s^{-1}
K_i = 88 ± 13 nM	K_i = 90 ± 7 nM	K_i = 9.7 ± 0.4 nM
k_{inact}/K_i = 27,000 ± 3,000 $M^{-1}s^{-1}$	k_{inact}/K_i = 34,000 ± 2,000 $M^{-1}s^{-1}$	k_{inact}/K_i = 310,000 ± 8,000 $M^{-1}s^{-1}$

P2: Acpc

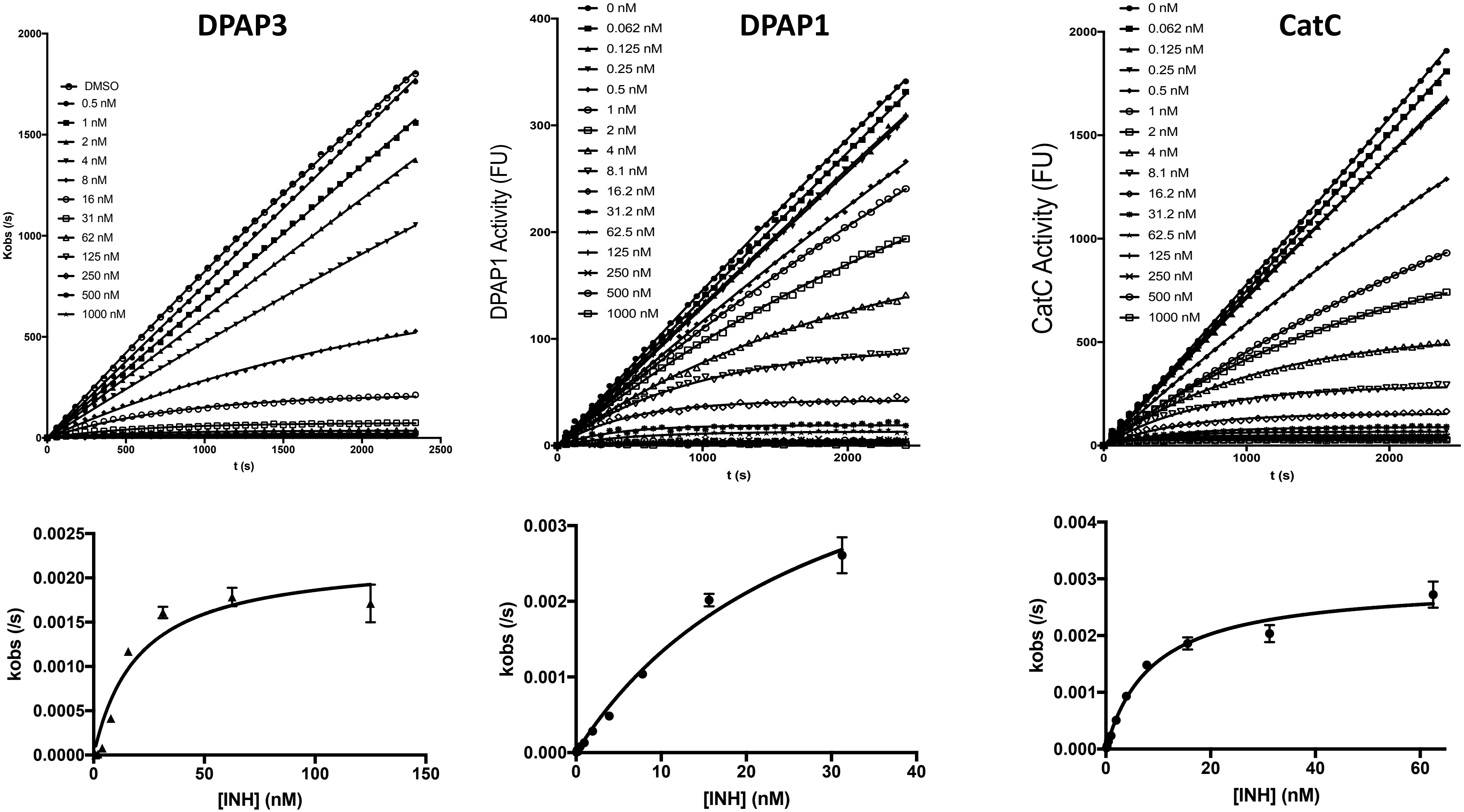


k_{inact} = 0.0032 ± 0.0001 s^{-1}
K_i = 47,000 ± 3,000 nM
k_{inact}/K_i = 67.7± 0.1 $M^{-1}s^{-1}$

k_{inact} = 0.0062 ± 0.0003 s^{-1}
K_i = 49,000 ± 4,000 nM
k_{inact}/K_i = 127 ± 6 $M^{-1}s^{-1}$

k_{inact} = 0.00103 ± 0.00006 s^{-1}
K_i = 3,200 ± 700 nM
k_{inact}/K_i = 330 ± 60 $M^{-1}s^{-1}$

P2: hAla



DPAP3

k_{inact} = 0.0022 ± 0.0003 s^{-1}

K_i = 13.5 ± 6 nM

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

DPAP1

k_{inact} = 0.0052 ± 0.0007 s^{-1}

K_i = 19 ± 4 nM

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

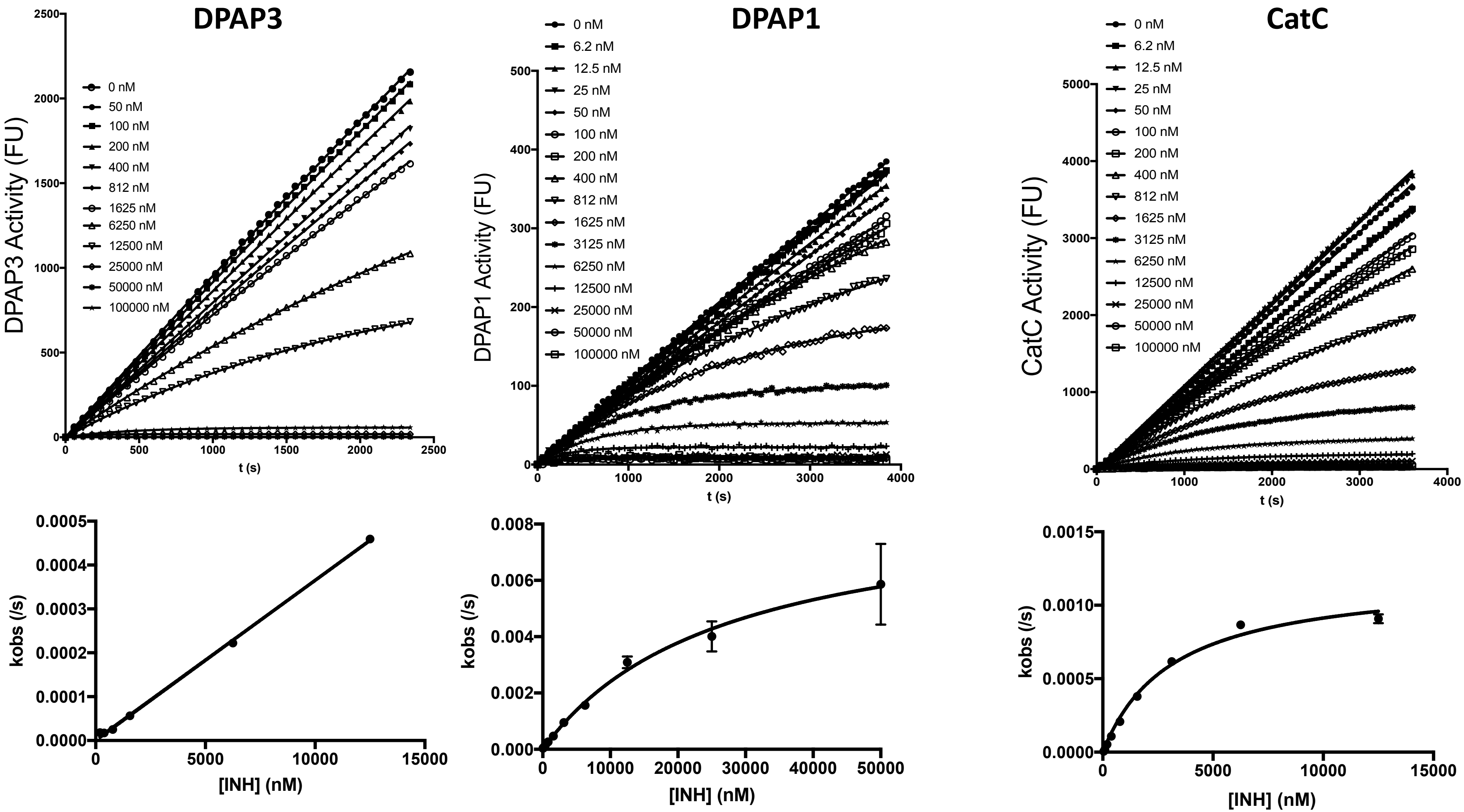
CatC

k_{inact} = 0.0029 ± 0.0002 s^{-1}

K_i = 5.9 ± 0.9 nM

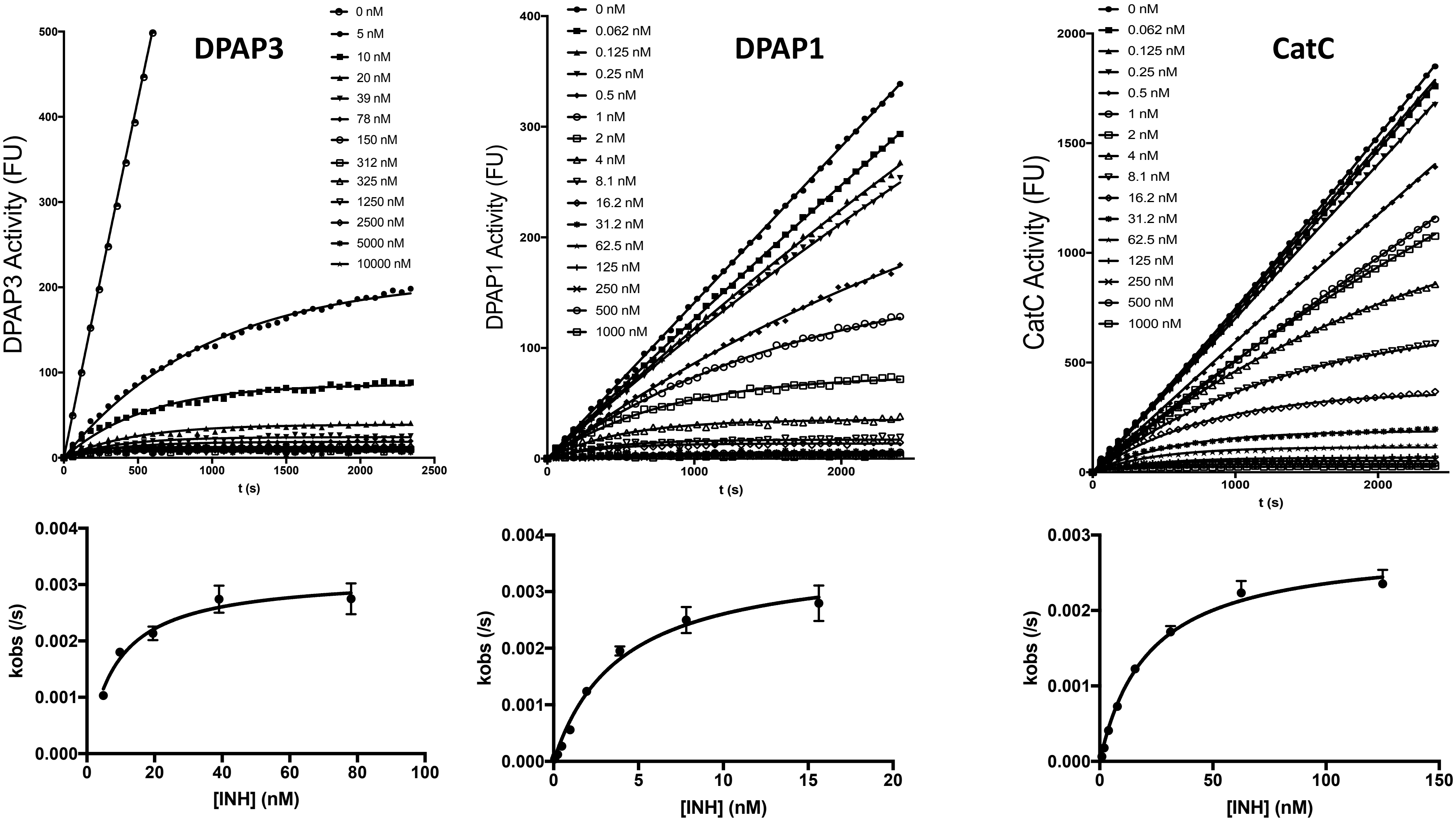
k_{inact}/K_i = 500,000 ± 50,000 $M^{-1}s^{-1}$

P2: Aib



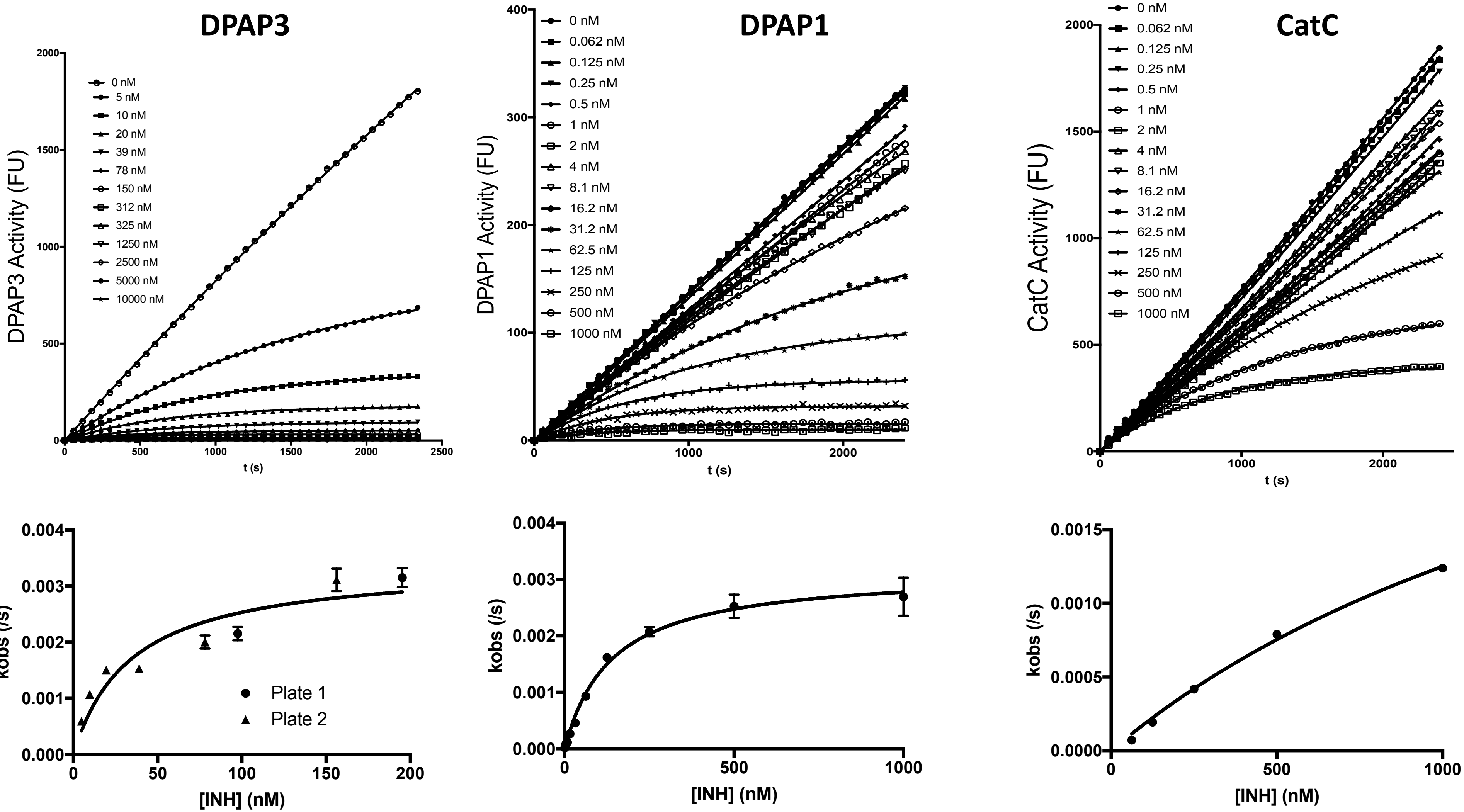
DPAP3	DPAP1	CatC
k_{inact} = N.S.	k_{inact} = 0.0089 ± 0.0005 s^{-1}	k_{inact} = 0.00120 ± 0.00007 s^{-1}
K_i = N.S.	K_i = 18,000 ± 2,000 nM	K_i = 2,100 ± 300 nM
K_{inact}/k_i = 36.3 ± 0.6 $M^{-1}s^{-1}$	k_{inact}/K_i = 490 ± 30 $M^{-1}s^{-1}$	k_{inact}/K_i = 570 ± 60 $M^{-1}s^{-1}$

P2: Val



DPAP3	DPAP1	CatC
k_{inact} = 0.0032 ± 0.0002 s^{-1}	k_{inact} = 0.0036 ± 0.0002 s^{-1}	k_{inact} = 0.0029 ± 0.0001 s^{-1}
K_i = 5.7 ± 1.1 nM	K_i = 2.6 ± 0.4 nM	K_i = 14 ± 2 nM
k_{inact}/K_i = 554,000 ± 84,000 $M^{-1}s^{-1}$	k_{inact}/K_i = 1, 380,000 ± 140,000 $M^{-1}s^{-1}$	k_{inact}/K_i = 200,000 ± 15,000 $M^{-1}s^{-1}$

P2: Ile

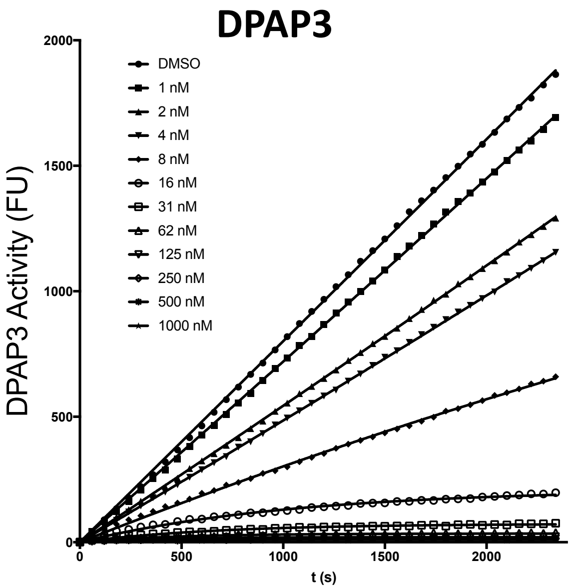


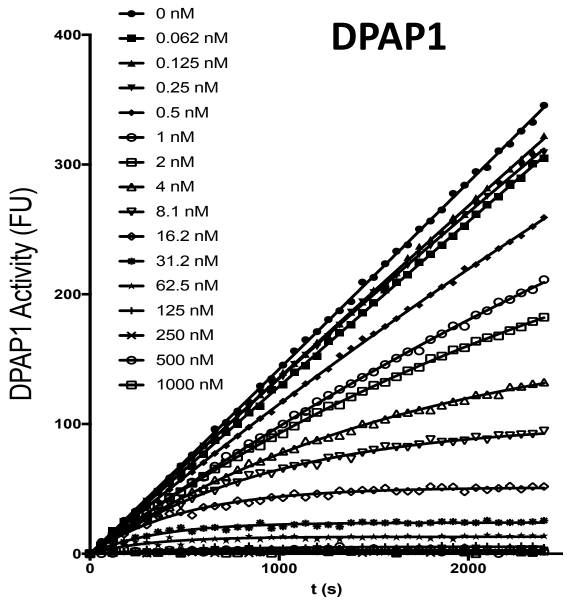
k_{inact} = 0.0034 ± 0.0004 s^{-1}
K_i = 23 ± 9 nM
k_{inact}/K_i = 148,000 ± 45,000 $M^{-1}s^{-1}$

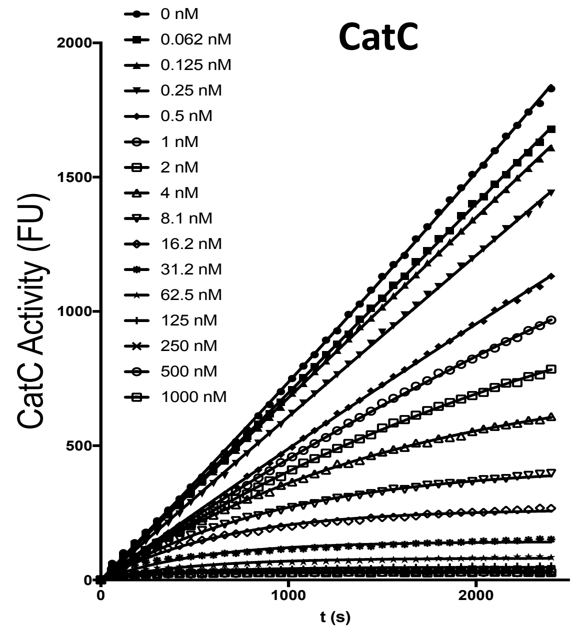
k_{inact} = 0.0032 ± 0.0001 s^{-1}
K_i = 92 ± 8 nM
k_{inact}/K_i = 34,000 ± 3,000 $M^{-1}s^{-1}$

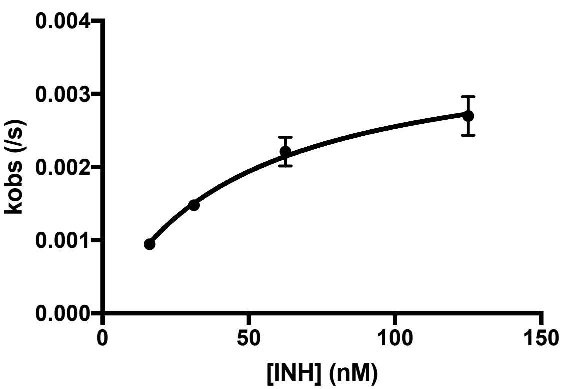
k_{inact} = 0.0037 ± 0.0008 s^{-1}
K_i = 1,300 ± 400 nM
k_{inact}/K_i = 2,900 ± 300 $M^{-1}s^{-1}$

P2: nLeu

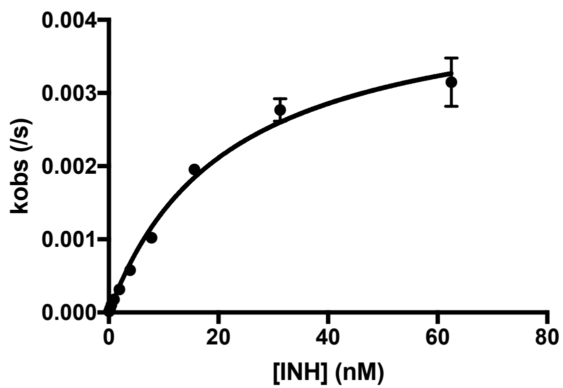




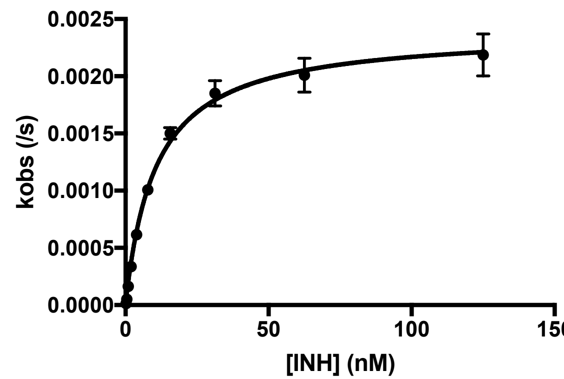




k_{inact} = 0.0037 ± 0.0002 s^{-1}
K_i = 31 ± 3 nM
k_{inact}/K_i = 120,000 ± 7,500 $M^{-1}s^{-1}$

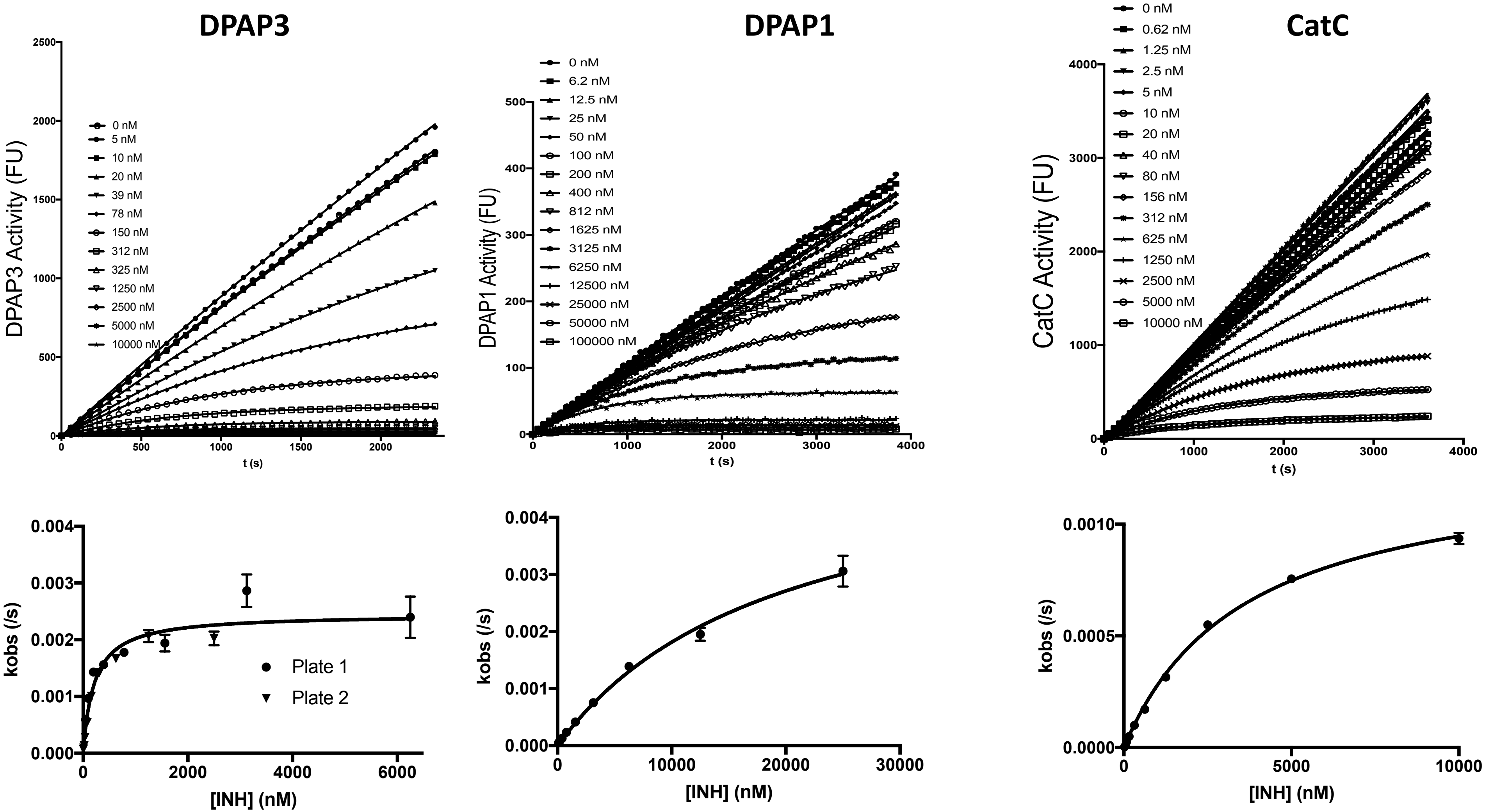


k_{inact} = 0.0044 ± 0.0002 s^{-1}
K_i = 14 ± 2 nM
k_{inact}/K_i = 305,000 ± 24,000 $M^{-1}s^{-1}$



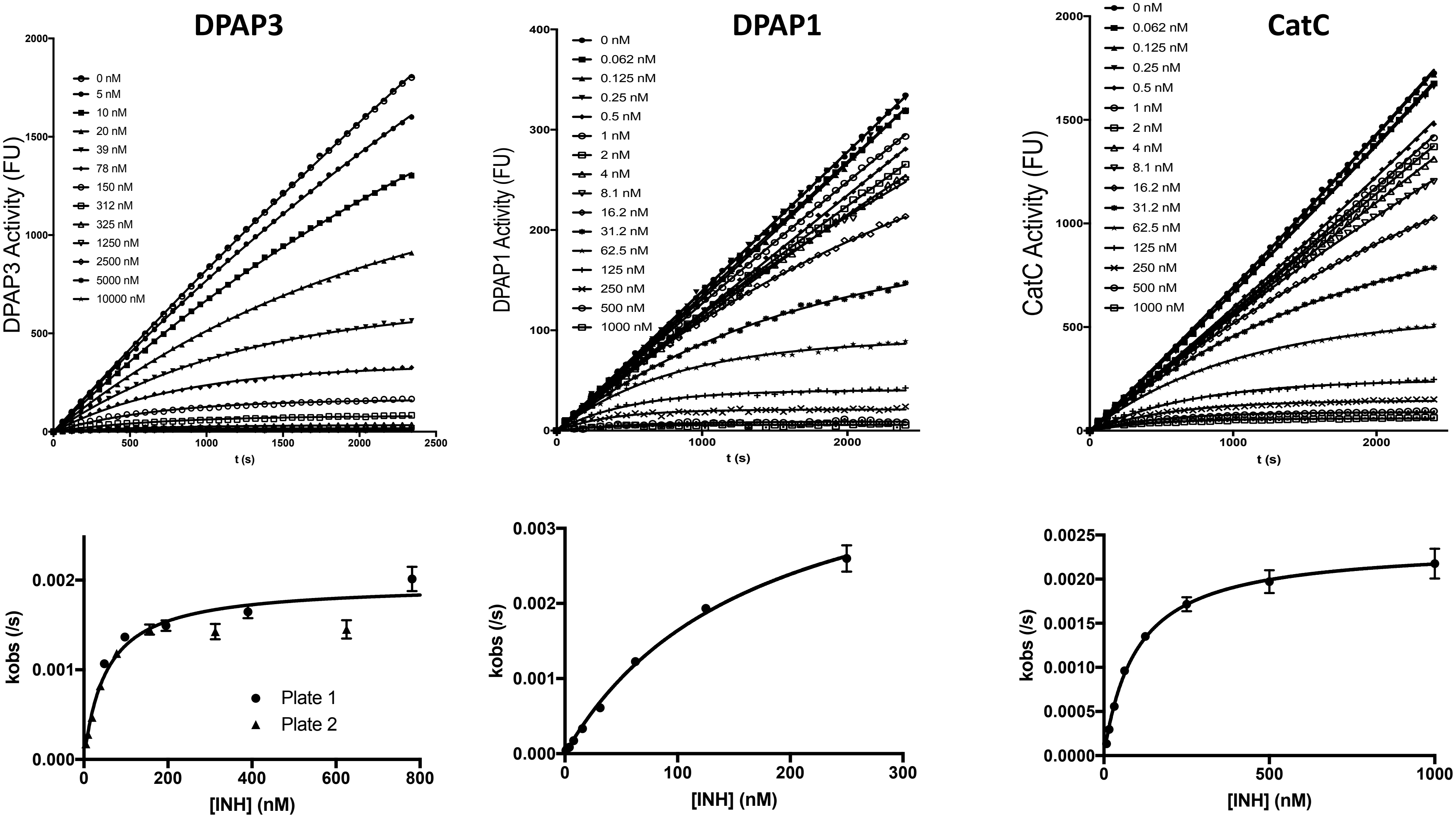
k_{inact} = 0.0024 ± 0.00005 s^{-1}
K_i = 7.0 ± 0.5 nM
k_{inact}/K_i = 340,000 ± 20,000 $M^{-1}s^{-1}$

P2: Cba



	DPAP3	DPAP1	CatC
k_{inact}	= 0.0025 ± 0.0001 s^{-1}	= 0.0054 ± 0.0004 s^{-1}	= 0.00129 ± 0.00003 s^{-1}
K_i	= 140 ± 25 nM	= 13,000 ± 2,000 nM	= 2,400 ± 140 nM
k_{inact}/K_i	= 17,000 ± 2,500 $M^{-1}s^{-1}$	= 410 ± 30 $M^{-1}s^{-1}$	= 530 ± 20 $M^{-1}s^{-1}$

P2: Thr



DPAP3

k_{inact} = 0.0020± 0.0001 s^{-1}

K_i = 36 ± 8 nM

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

DPAP1

k_{inact} = 0.0044 ± 0.0002 s^{-1}

K_i = 112 ± 10 nM

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

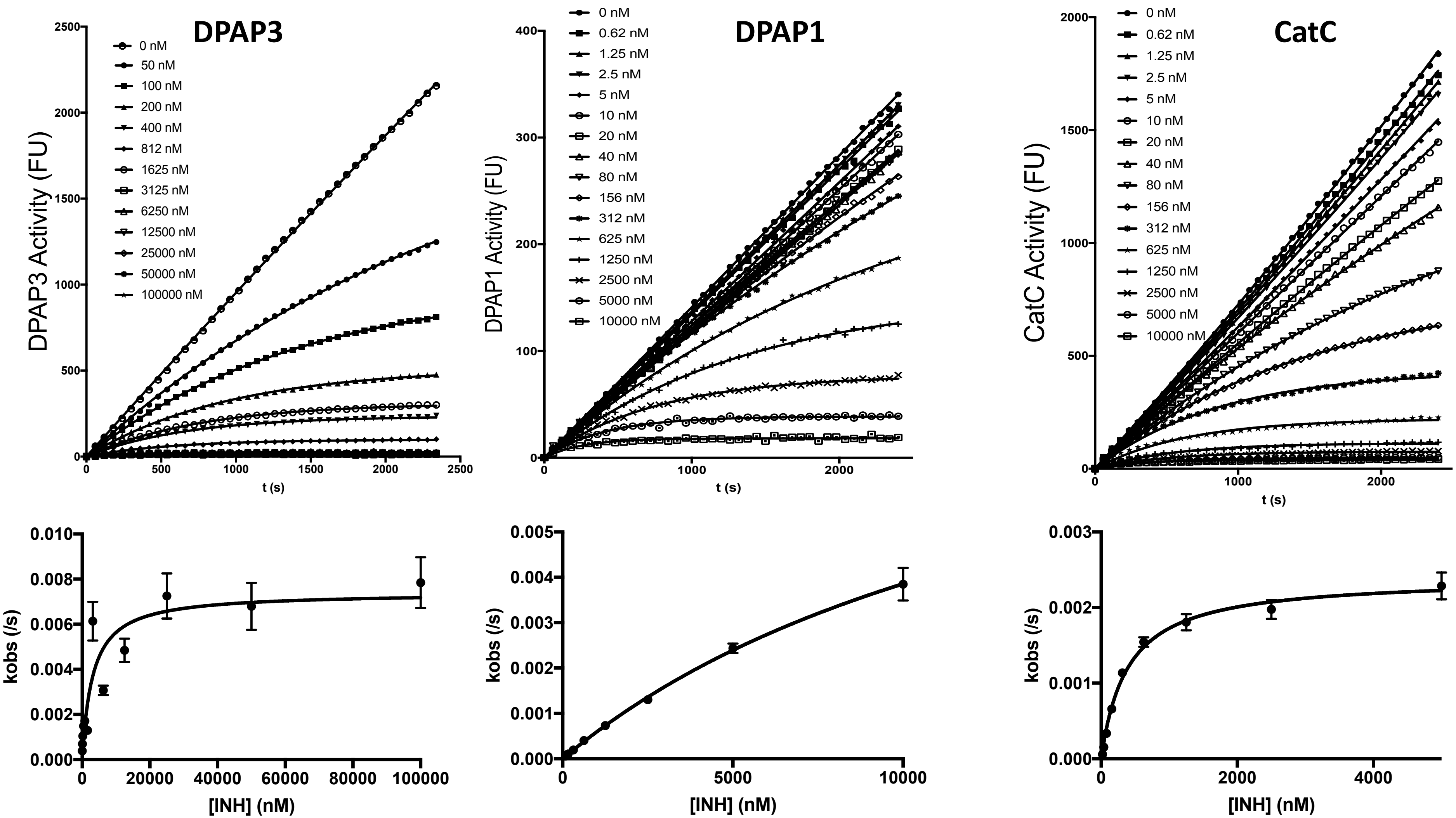
CatC

k_{inact} = 0.00238 ± 0.00003 s^{-1}

K_i = 65 ± 3 nM

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

P2: Asn

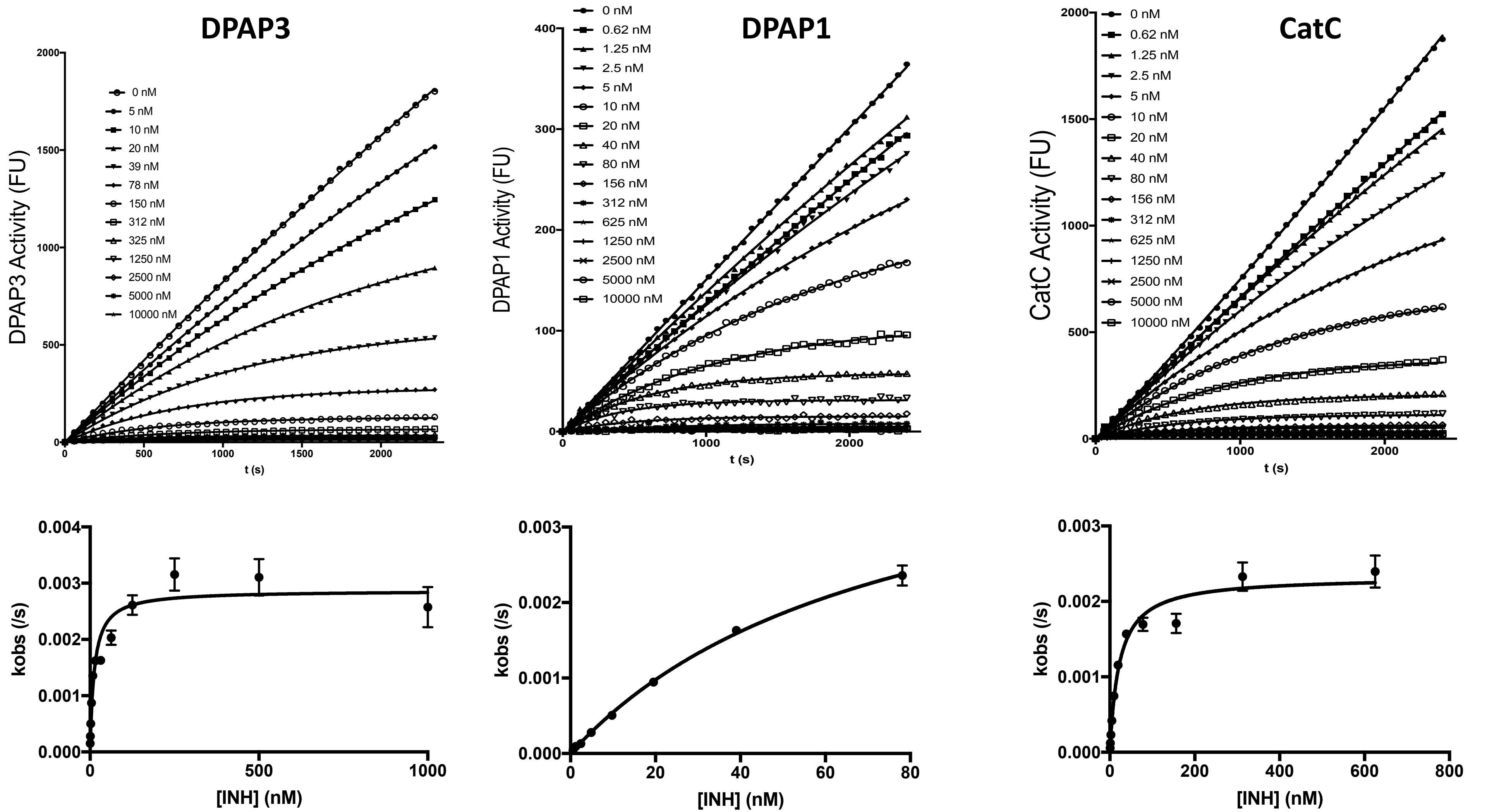


k_{inact} = 0.0074 ± 0.0008 s^{-1}
K_i = 2.1 ± 1 μM
k_{inact}/K_i = 3,500 ± 1,400 $M^{-1}s^{-1}$

k_{inact} = 0.0099 ± 0.0005 s^{-1}
K_i = 10,500 ± 750 nM
k_{inact}/K_i = 950 ± 20 $M^{-1}s^{-1}$

k_{inact} = 0.00240 ± 0.00007 s^{-1}
K_i = 260 ± 30 nM
k_{inact}/K_i = 9,200 ± 700 $M^{-1}s^{-1}$

P2: Gln



DPAP3

k_{inact} = 0.0029 ± 0.0002 s^{-1}

K_i = 80 ± 20 nM

k_{inact}/K_i = 35,000 ± 8,000 $M^{-1}s^{-1}$

DPAP1

k_{inact} = 0.0047 ± 0.0002 s^{-1}

K_i = 50 ± 3 nM

k_{inact}/K_i = 93,000 ± 3,000 $M^{-1}s^{-1}$

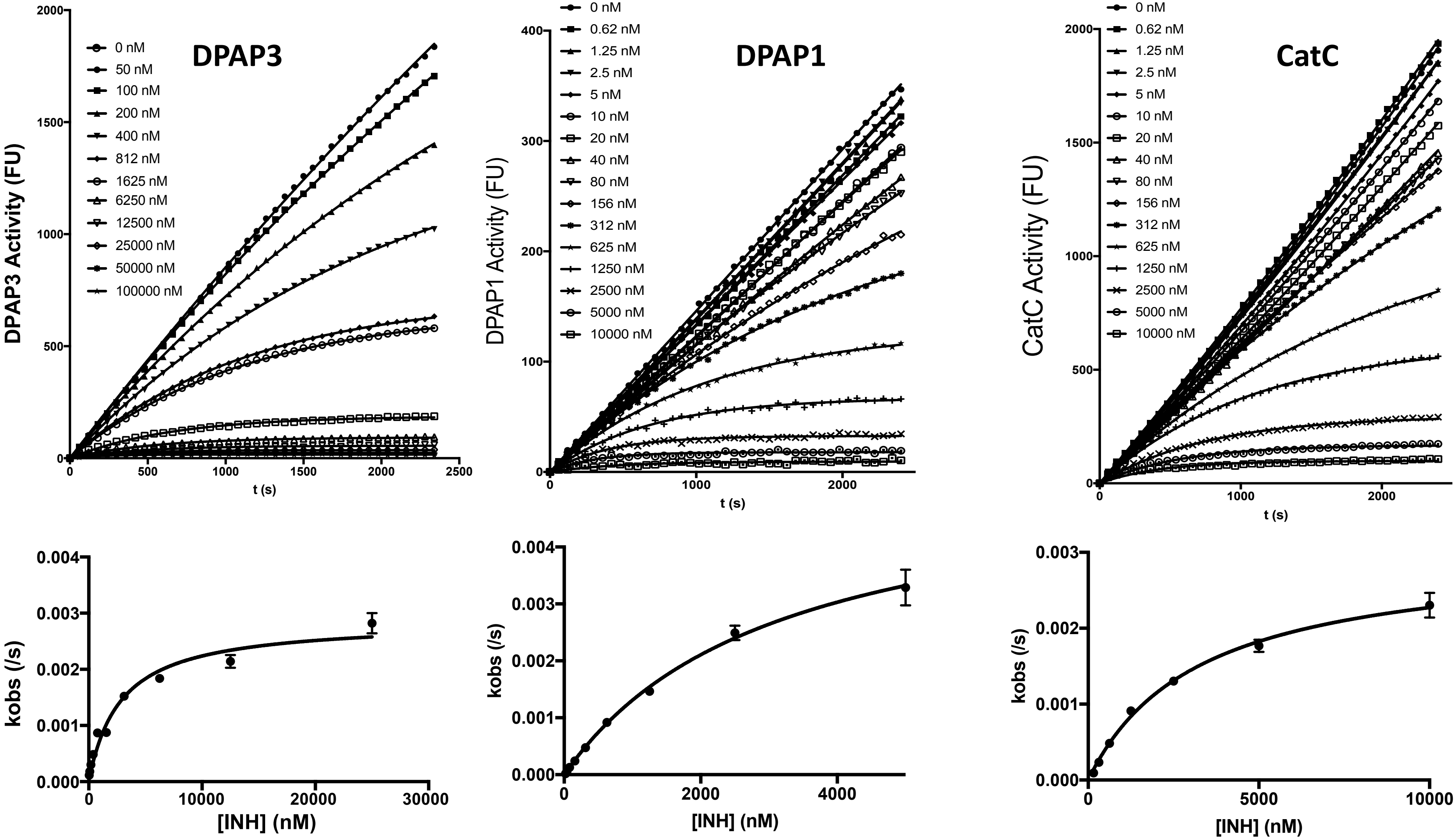
CatC

k_{inact} = 0.0023 ± 0.0001 s^{-1}

K_i = 15 ± 3 nM

k_{inact}/K_i = 160,000 ± 25,000 $M^{-1}s^{-1}$

P2: Asp

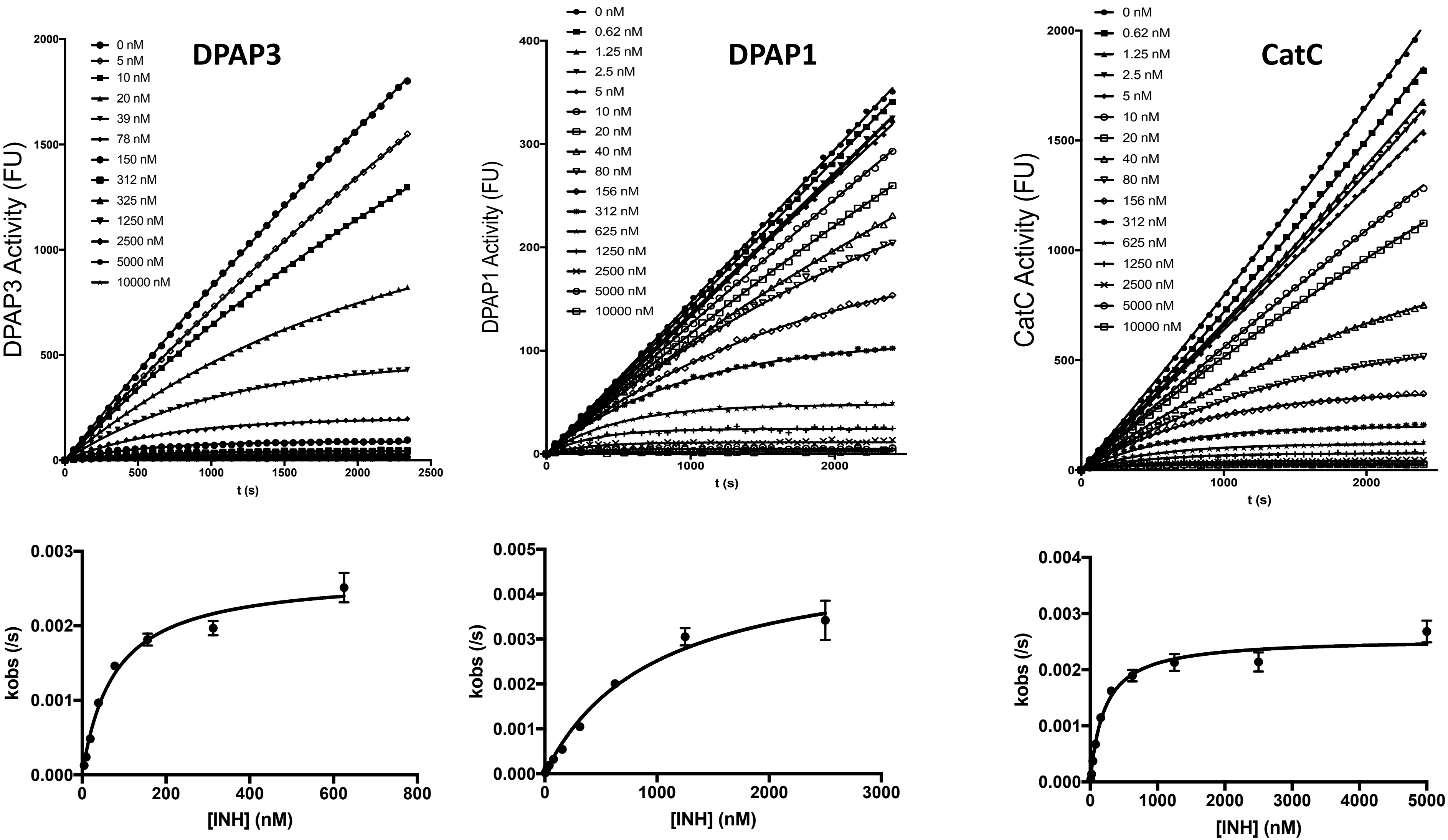


i_{inact} = 0.0029 ± 0.0002 s^{-1}
K_i = 1,900 ± 400 nM
k_{inact}/K_i = 1,500 ± 200 $M^{-1}s^{-1}$

k_{inact} = 0.0054 ± 0.0002 s^{-1}
K_i = 2,100 ± 200 nM
k_{inact}/K_i = 2,600 ± 100 $M^{-1}s^{-1}$

k_{inact} = 0.0030 ± 0.0001 s^{-1}
K_i = 2,200 ± 200 nM
k_{inact}/K_i = 1,400 ± 200 $M^{-1}s^{-1}$

P2: Glu

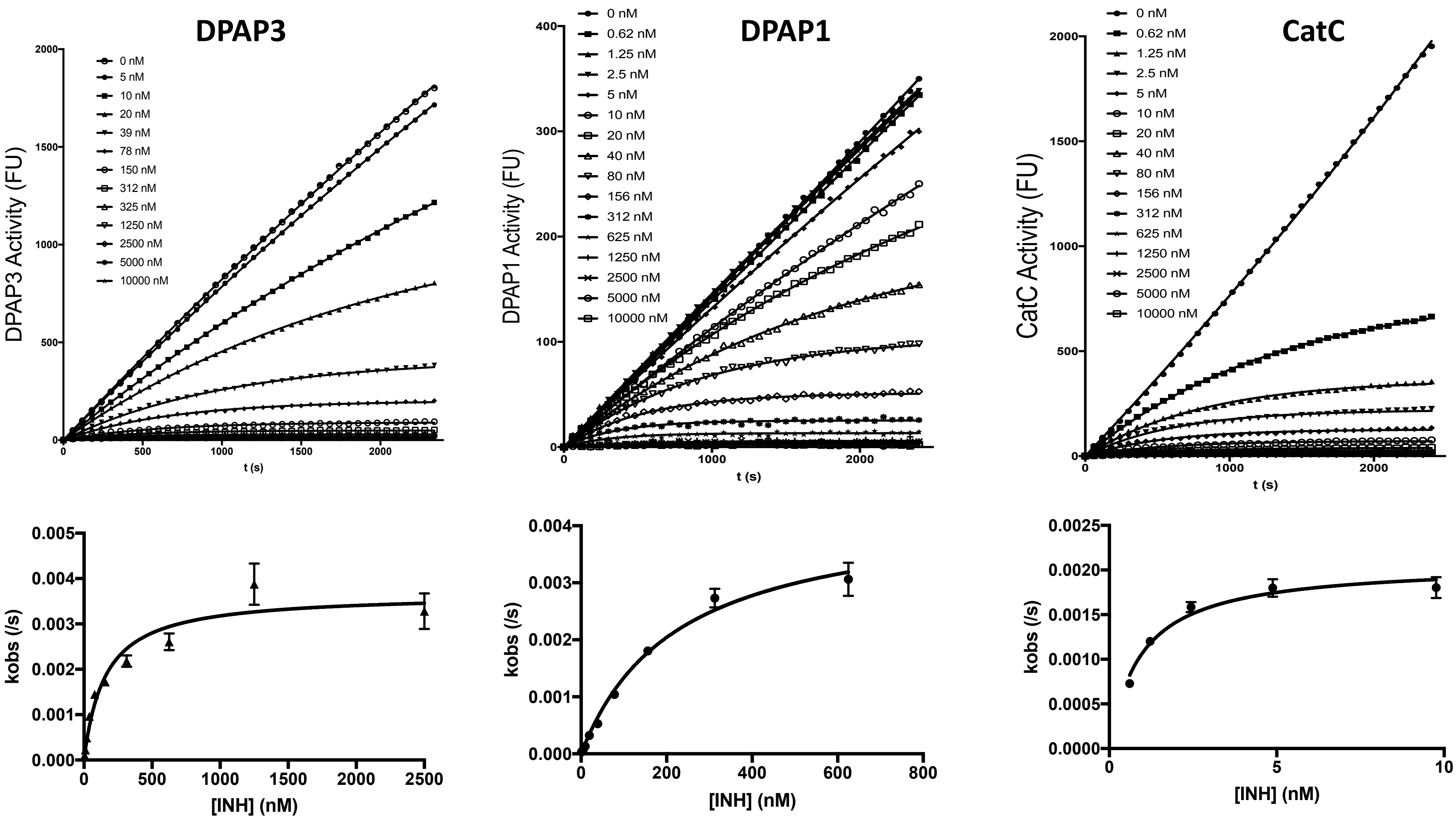


DPAP3:
k_{inact} = 0.0027 ± 0.0001 s^{-1}
K_i = 51 ± 8 nM
k_{inact}/K_i = 53,000 ± 6,000 $M^{-1}s^{-1}$

DPAP1:
k_{inact} = 0.0050 ± 0.0004 s^{-1}
K_i = 650 ± 100 nM
k_{inact}/K_i = 7,600 ± 750 $M^{-1}s^{-1}$

CatC:
k_{inact} = 0.0026 ± 0.0001 s^{-1}
K_i = 140 ± 20 nM
k_{inact}/K_i = 18,000 ± 2,000 $M^{-1}s^{-1}$

P2: His

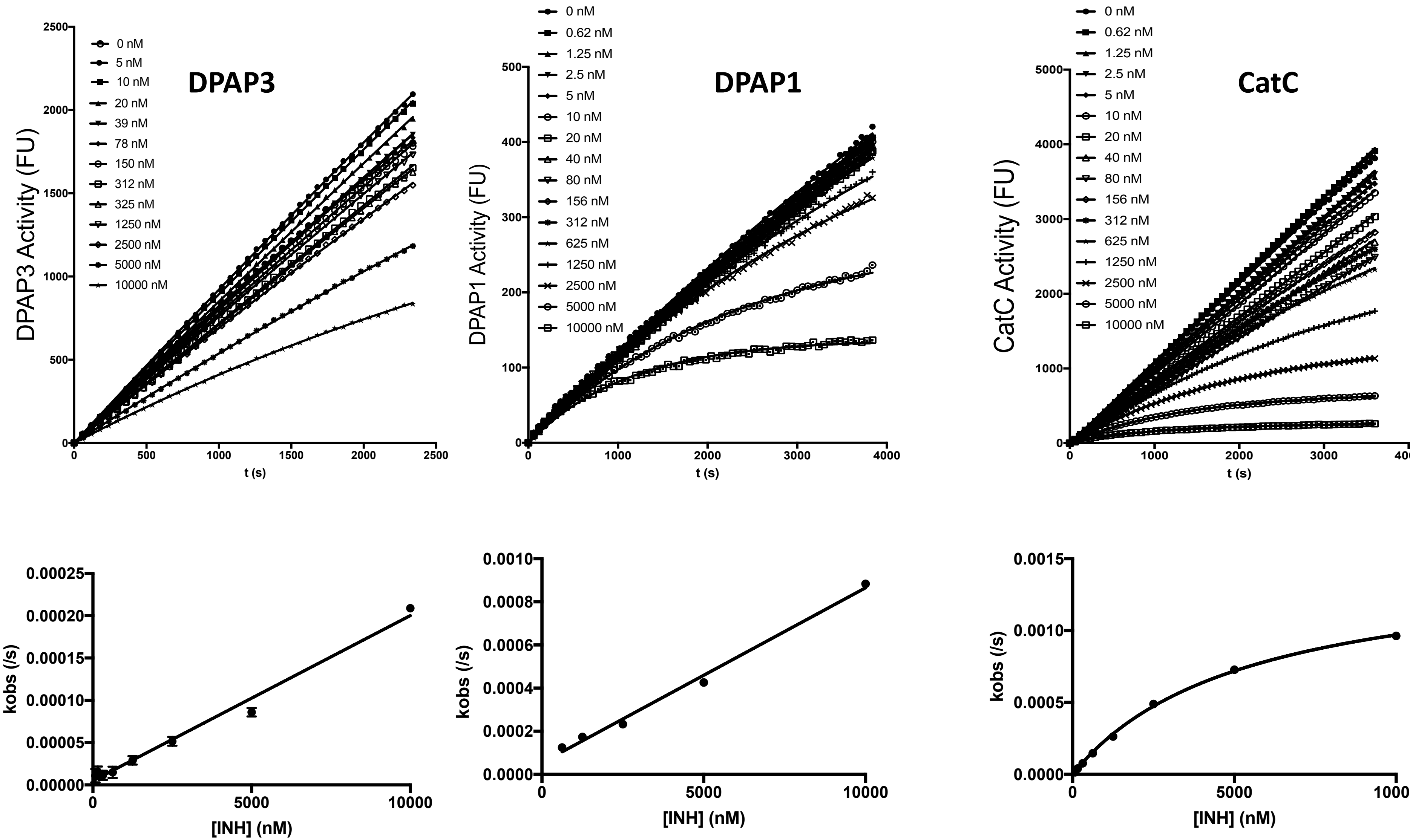


k_{inact}	= 0.0037 ± 0.0003 s^{-1}
K_i	= 100 ± 25 nM
k_{inact}/K_i	= 35,500 ± 7,500 $M^{-1}s^{-1}$

k_{inact}	= 0.0043 ± 0.0003 s^{-1}
K_i	= 150 ± 20 nM
k_{inact}/K_i	= 29,000 ± 3,000 $M^{-1}s^{-1}$

k_{inact}	= 0.0021 ± 0.0001 s^{-1}
K_i	= 0.6 ± 0.1 nM
k_{inact}/K_i	=3,350,000 ± 500,000 $M^{-1}s^{-1}$

P2: Lys

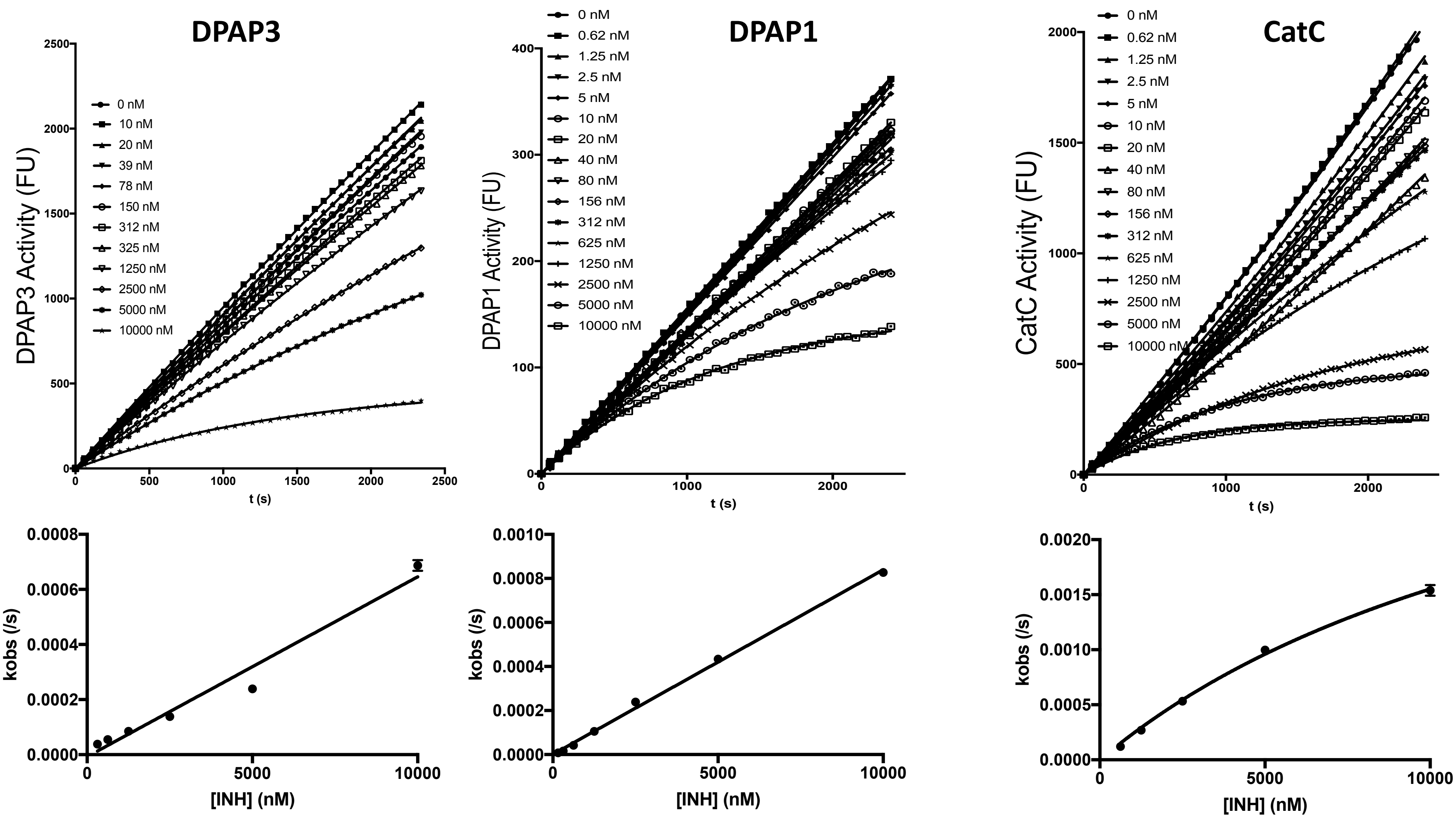


k_{inact} = N.S.
K_i = N.S.
K_{inact}/k_i = 20 ± 1 $M^{-1}s^{-1}$

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

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

P2: Arg

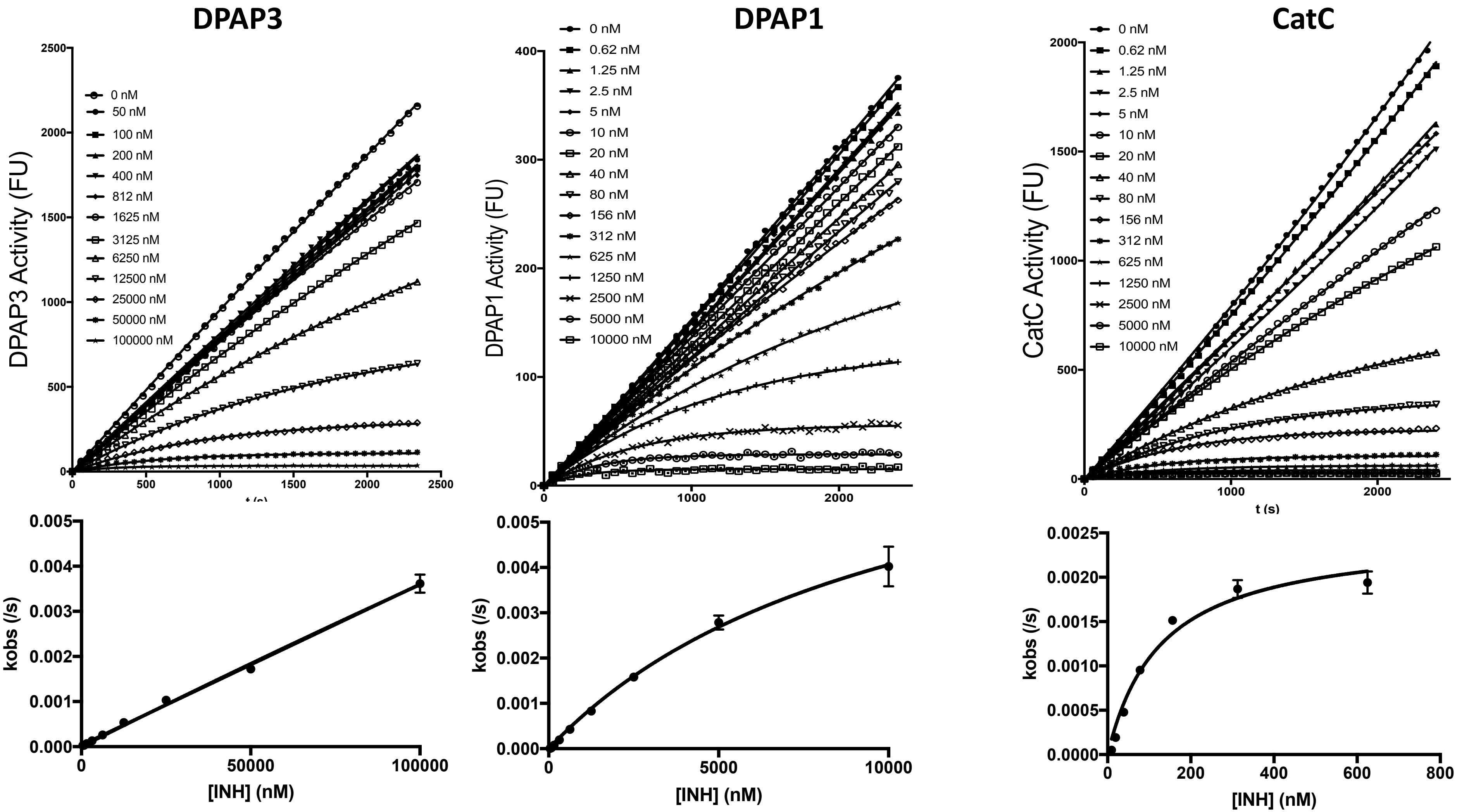


k_{inact} = N.S.
K_i = N.S.
K_{inact}/k_i = 65 ± 6 $M^{-1}s^{-1}$

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

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

P2: Phg

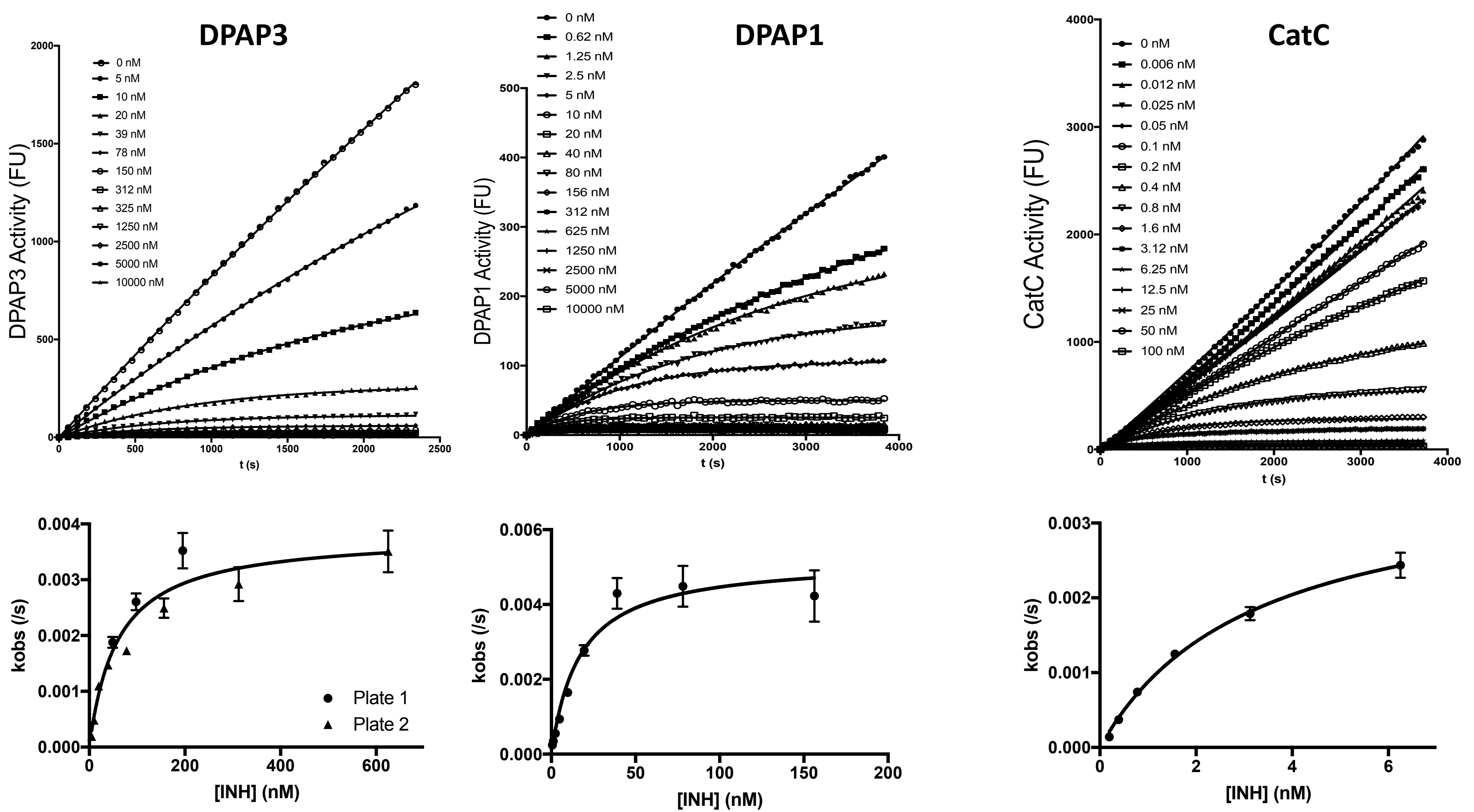


k_{inact} = N.S.
K_i = N.S.
K_{inact}/k_i = 35.7 ± 0.7 $M^{-1}s^{-1}$

k_{inact} = 0.0083 ± 0.0005 s^{-1}
K_i = 7,000 ± 650 nM
k_{inact}/K_i = 1,190 ± 50 $M^{-1}s^{-1}$

k_{inact} = 0.0025 ± 0.0002 s^{-1}
K_i = 86 ± 20 nM
k_{inact}/K_i = 29,000 ± 5,000 $M^{-1}s^{-1}$

P2: 2fa

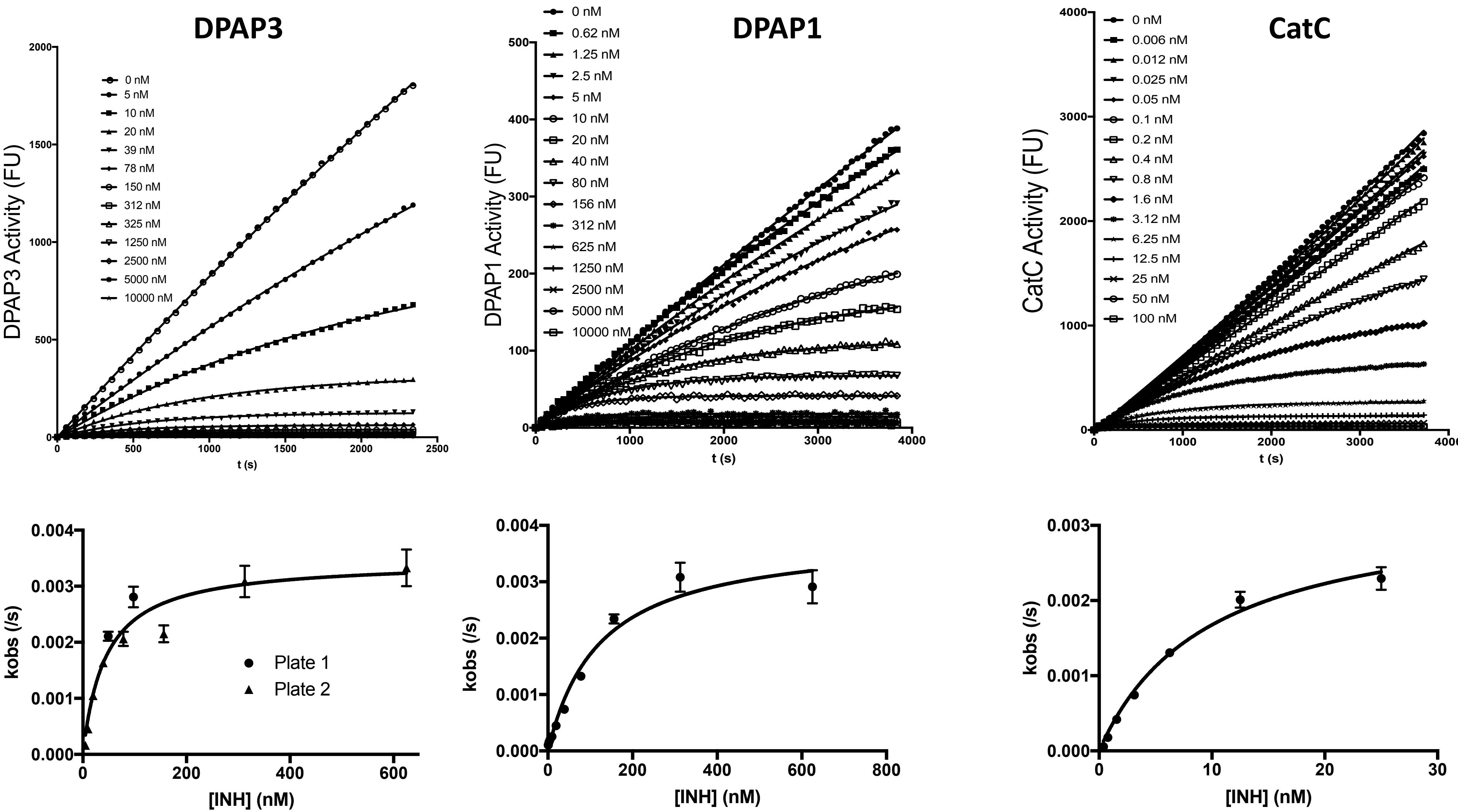


k_{inact} = 0.0038 ± 0.0003 s^{-1}
K_i = 39 ± 10 nM
k_{inact}/K_i = 97,000 ± 18,000 $M^{-1}s^{-1}$

k_{inact} = 0.0052 ± 0.0004 s^{-1}
K_i = 11 ± 3 nM
k_{inact}/K_i = 470,000 ± 90,000 $M^{-1}s^{-1}$

k_{inact} = 0.0037 ± 0.0002 s^{-1}
K_i = 2.1 ± 0.2 nM
k_{inact}/K_i = 1,720,000 ± 90,000 $M^{-1}s^{-1}$

P2: 2ta

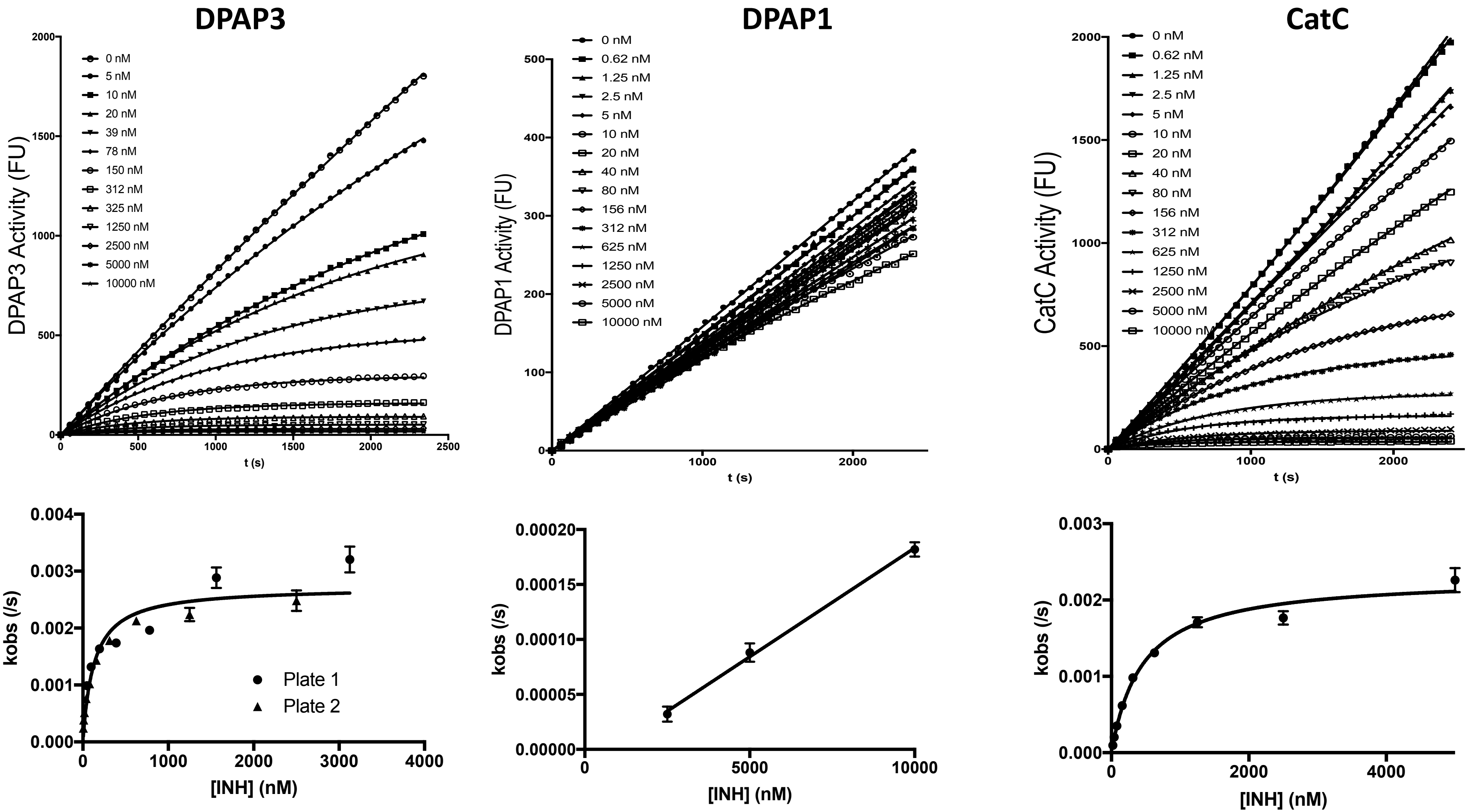


k_{inact} = 0.0034 ± 0.0003 s^{-1}
K_i = 29 ± 7 nM
k_{inact}/K_i = 118,000 ± 23,000 $M^{-1}s^{-1}$

k_{inact} = 0.0038 ± 0.0003 s^{-1}
K_i = 82 ± 20 nM
k_{inact}/K_i = 47,000 ± 7,000 $M^{-1}s^{-1}$

k_{inact} = 0.0033 ± 0.0003 s^{-1}
K_i = 6.5 ± 1 nM
k_{inact}/K_i = 510,000 ± 55,000 $M^{-1}s^{-1}$

P2: Cha

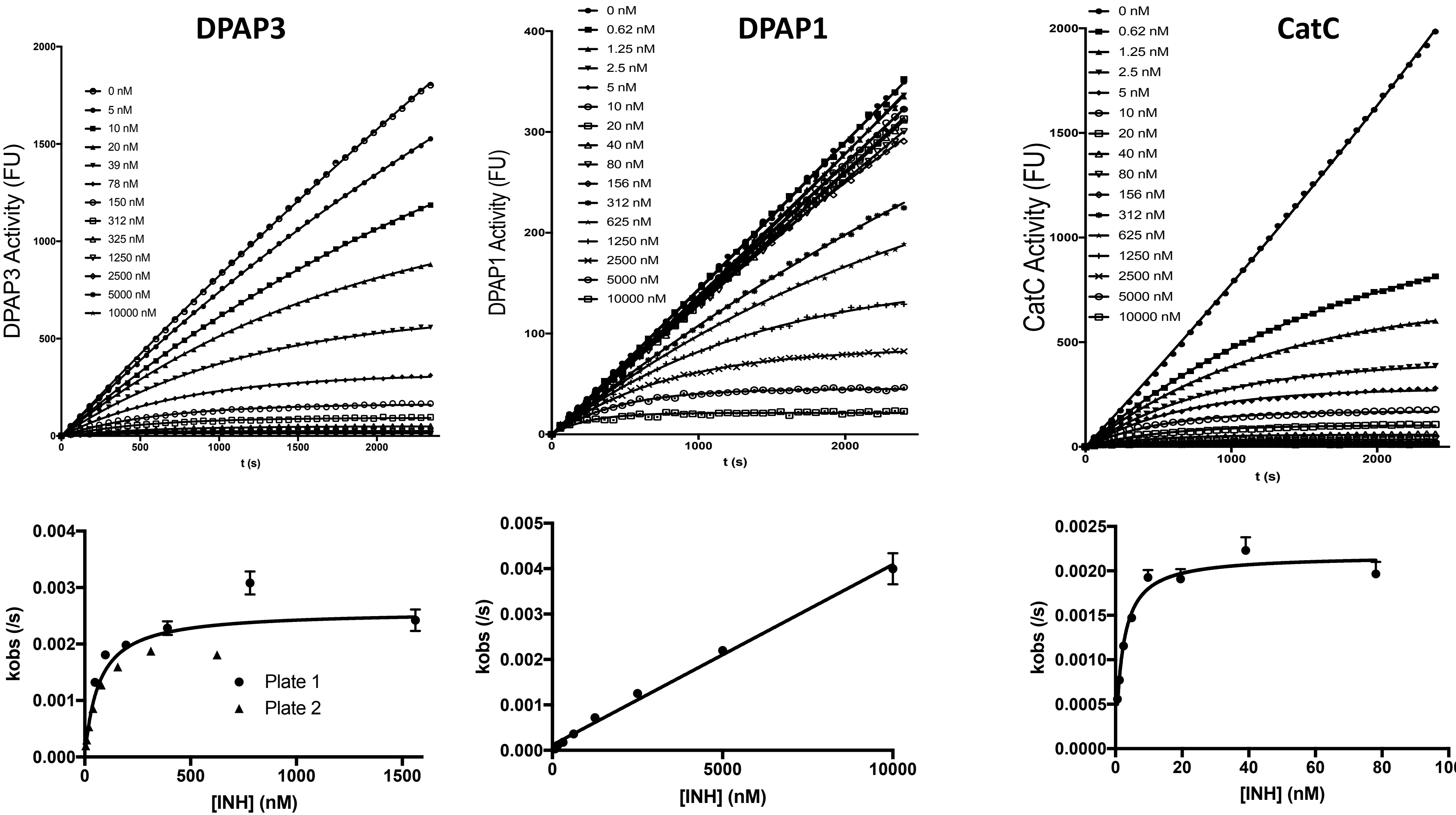


k_{inact} = 0.0027 ± 0.0002 s^{-1}
K_i = 90 ± 20 nM
k_{inact}/K_i = 31,600 ± 5,500 $M^{-1}s^{-1}$

k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 20 ± 1 $M^{-1}s^{-1}$

k_{inact} = 0.0023 ± 0.0001 s^{-1}
K_i = 300 ± 40 nM
k_{inact}/K_i = 7,700 ± 800 $M^{-1}s^{-1}$

P2: Phe

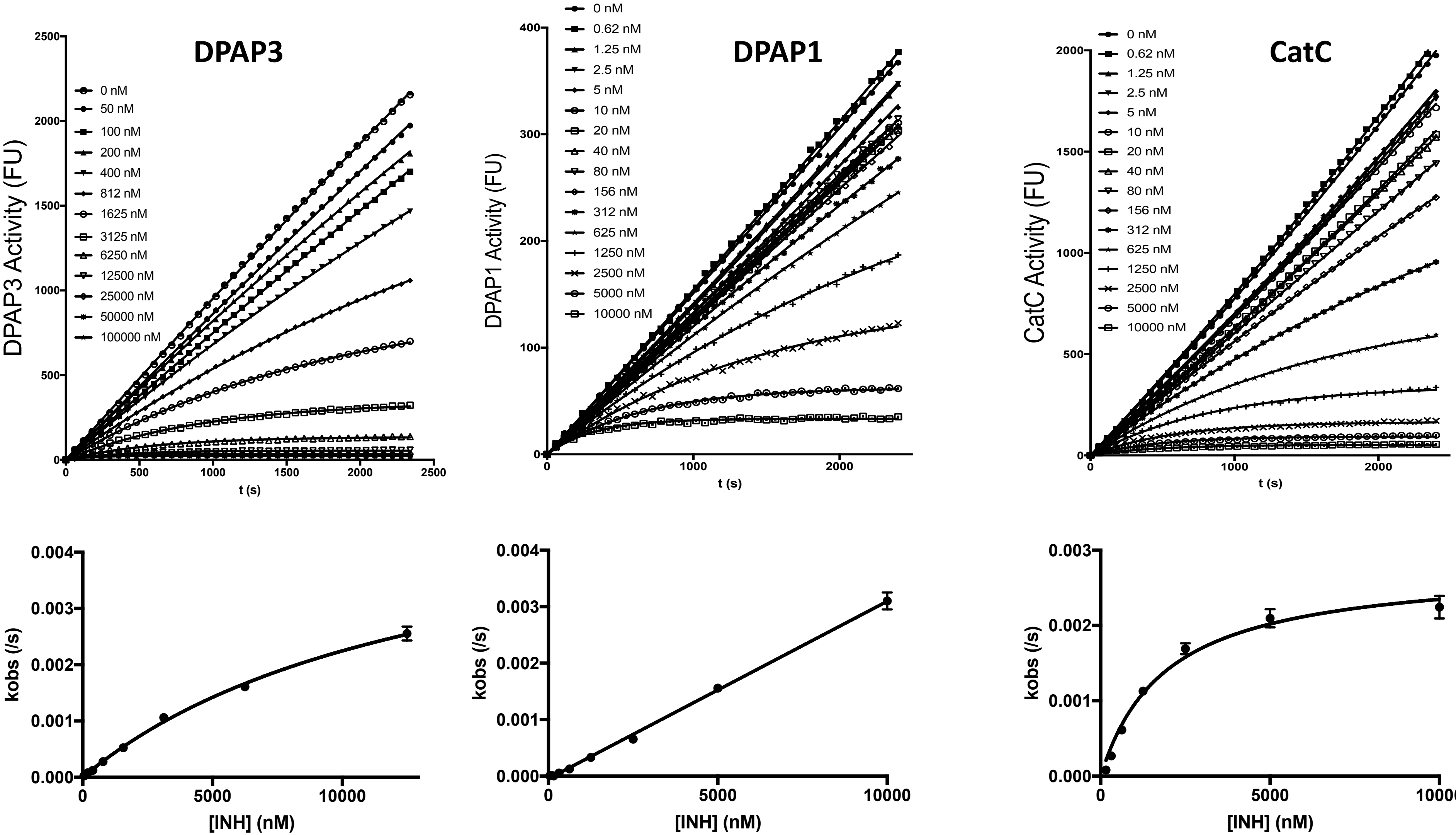


k_{inact} = 0.0026 ± 0.0002 s^{-1}
K_i = 45 ± 13 nM
k_{inact}/K_i = 58,000 ± 13,000 $M^{-1}s^{-1}$

k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 400 ± 10 $M^{-1}s^{-1}$

k_{inact} = 0.00218 ± 0.00008 s^{-1}
K_i = 1.4 ± 0.2 nM
k_{inact}/K_i = 1,600,000 ± 200,000 $M^{-1}s^{-1}$

P2: Phe(Me)

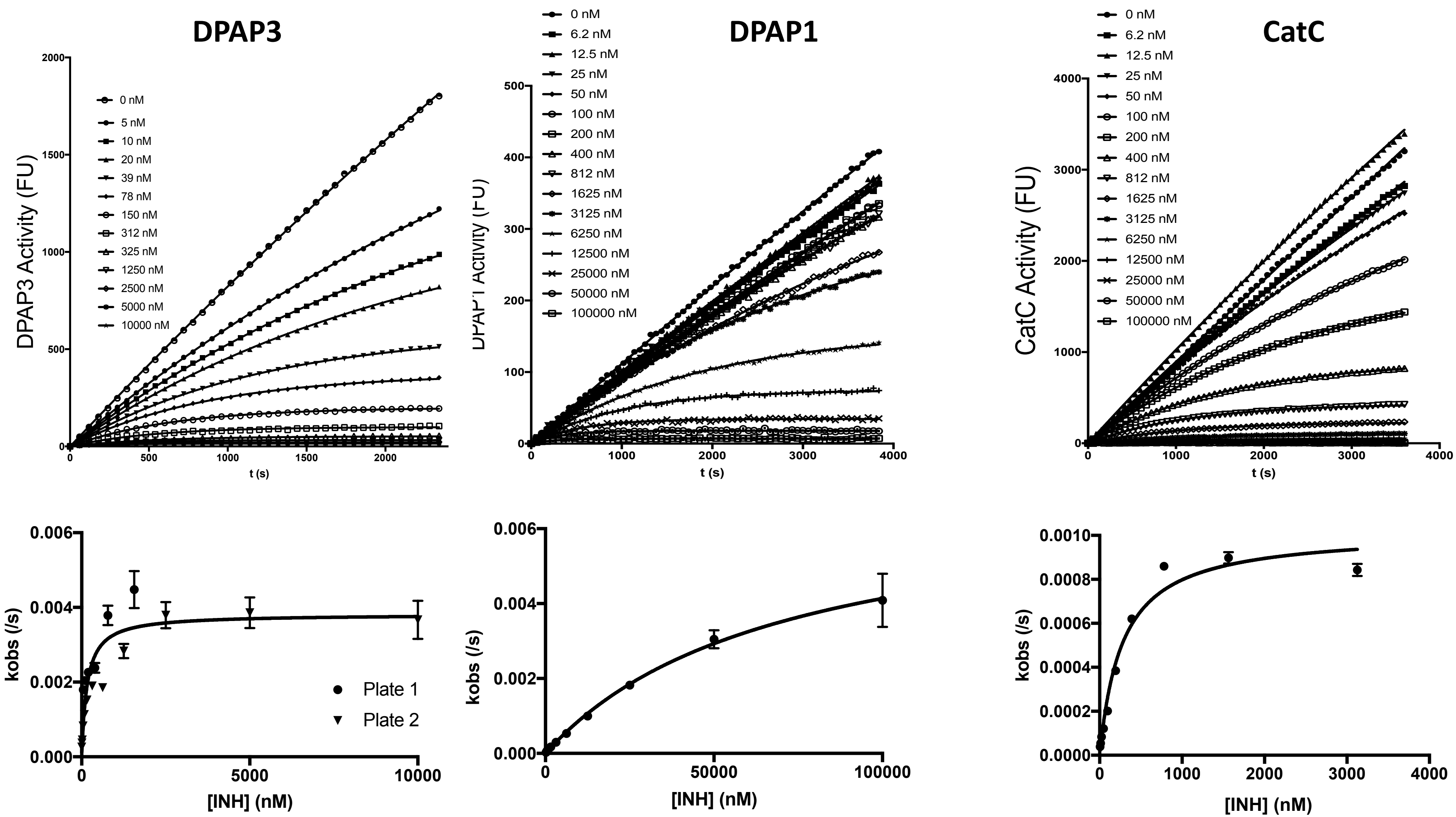


k_{inact} = 0.054 ± 0.004 s^{-1}
K_i = 9,200 ± 300 nM
k_{inact}/K_i = 580 ± 25 $M^{-1}s^{-1}$

k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 314 ± 5 $M^{-1}s^{-1}$

k_{inact} = 0.0028 ± 0.0002 s^{-1}
K_i = 1,300 ± 200 nM
k_{inact}/K_i = 2,200 ± 300 $M^{-1}s^{-1}$

P2: Phe(I)

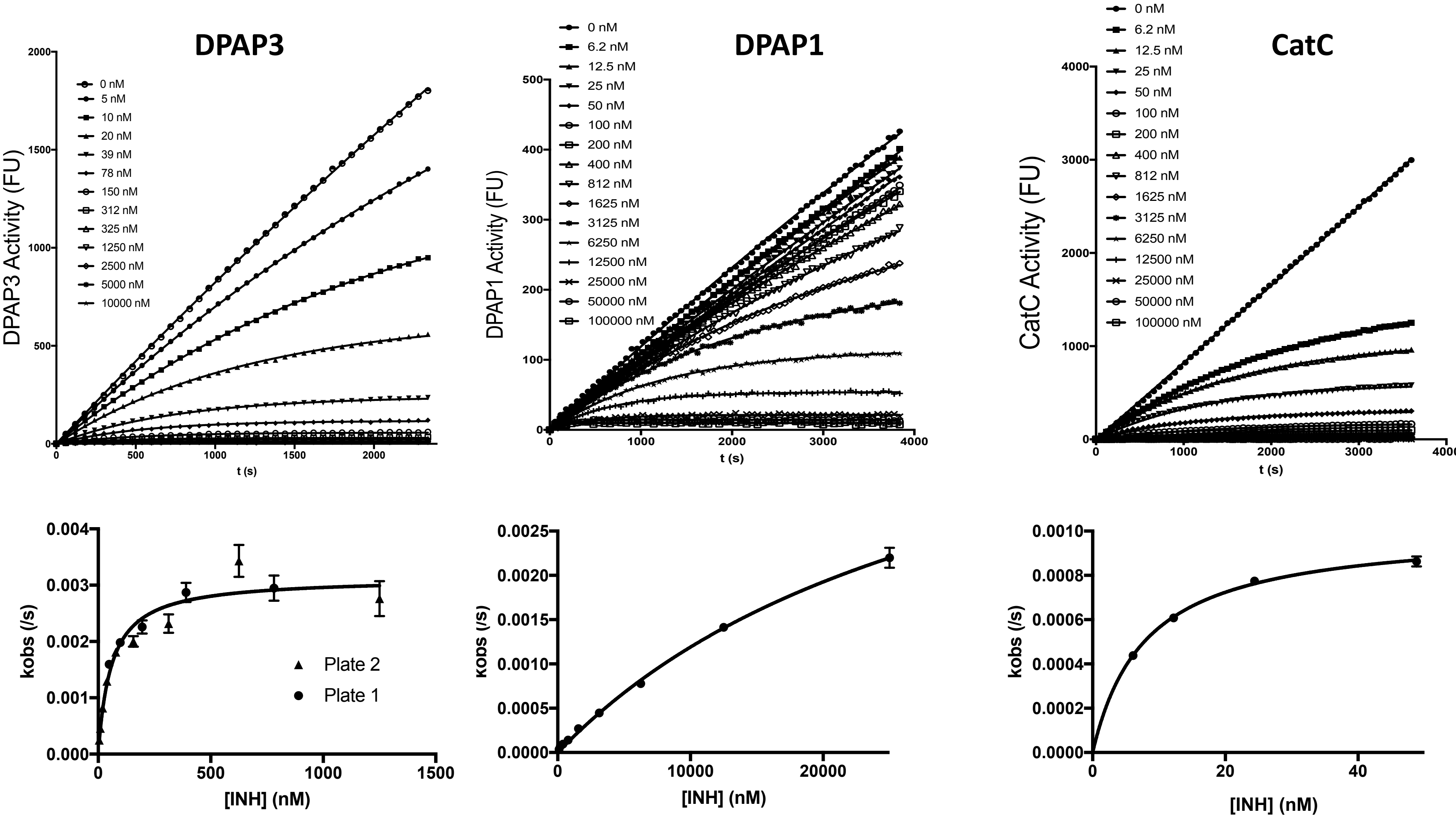


k_{inact} = 0.0038 ± 0.0003 s^{-1}
K_i = 110 ± 30 nM
k_{inact}/K_i = 36,000 ± 10,000 $M^{-1}s^{-1}$

k_{inact} = 0.0070 ± 0.0003 s^{-1}
K_i = 47,000 ± 3,500 nM
k_{inact}/K_i = 151 ± 6 $M^{-1}s^{-1}$

k_{inact} = 0.00102 ± 0.00006 s^{-1}
K_i = 185 ± 40 nM
k_{inact}/K_i = 5,500 ± 900 $M^{-1}s^{-1}$

P2: Phe(NH_2)



DPAP3

k_{inact} = 0.0031 ± 0.0002 s^{-1}

K_i = 39 ± 7 nM

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

DPAP1

k_{inact} = 0.0050 ± 0.0004 s^{-1}

K_i = 22,000 ± 2,500 nM

k_{inact}/K_i = 230 ± 10 $M^{-1}s^{-1}$

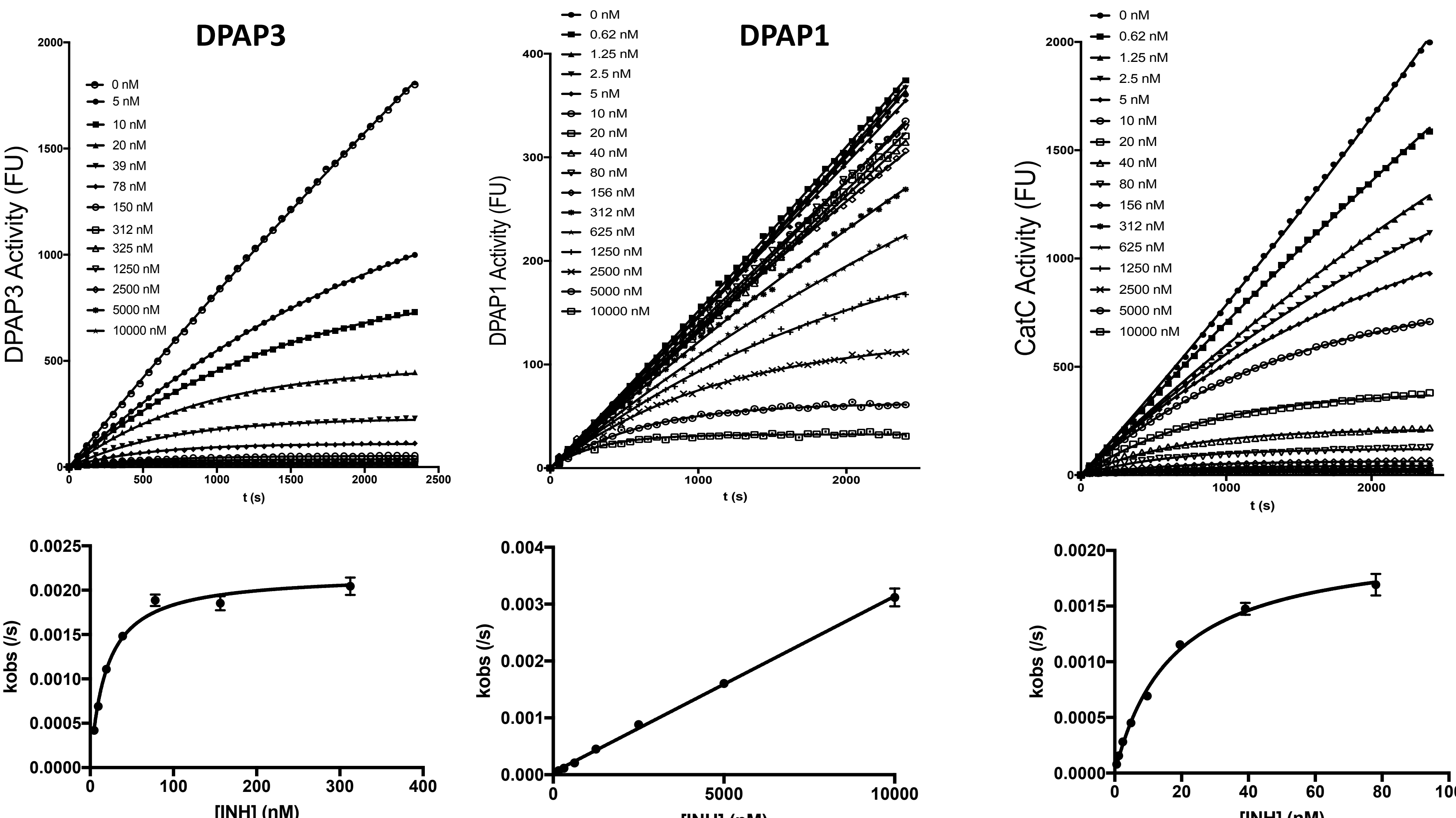
CatC

k_{inact} = 0.00101 ± 0.00002 s^{-1}

K_i = 5.3 ± 0.3 nM

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

P2: Tyr

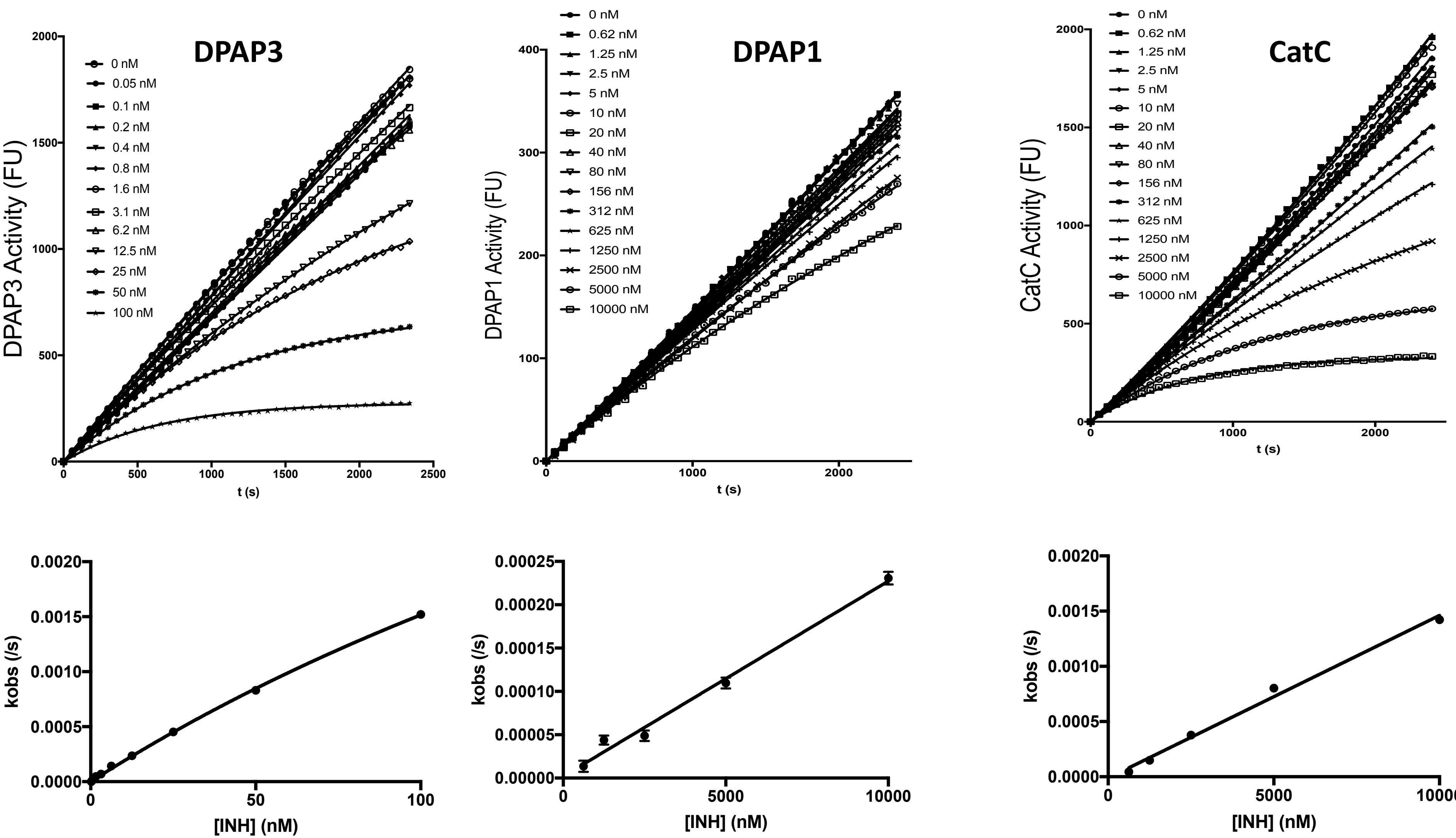


k_{inact} = 0.00218 ± 0.00007 s^{-1}
K_i = 12.7 ± 1.5 nM
k_{inact}/K_i = 172,000 ± 16,000 $M^{-1}s^{-1}$

k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 310 ± 5 $M^{-1}s^{-1}$

k_{inact} = 0.00210 ± 0.00007 s^{-1}
K_i = 11.5 ± 0.9 nM
k_{inact}/K_i = 182,000 ± 10,000 $M^{-1}s^{-1}$

P2: Tyr(NO_2)



DPAP3

k_{inact} = 0.0072 ± 0.0009 s^{-1}
K_i = 250 ± 40 nM
k_{inact}/K_i = 28,800 ± 800 $M^{-1}s^{-1}$

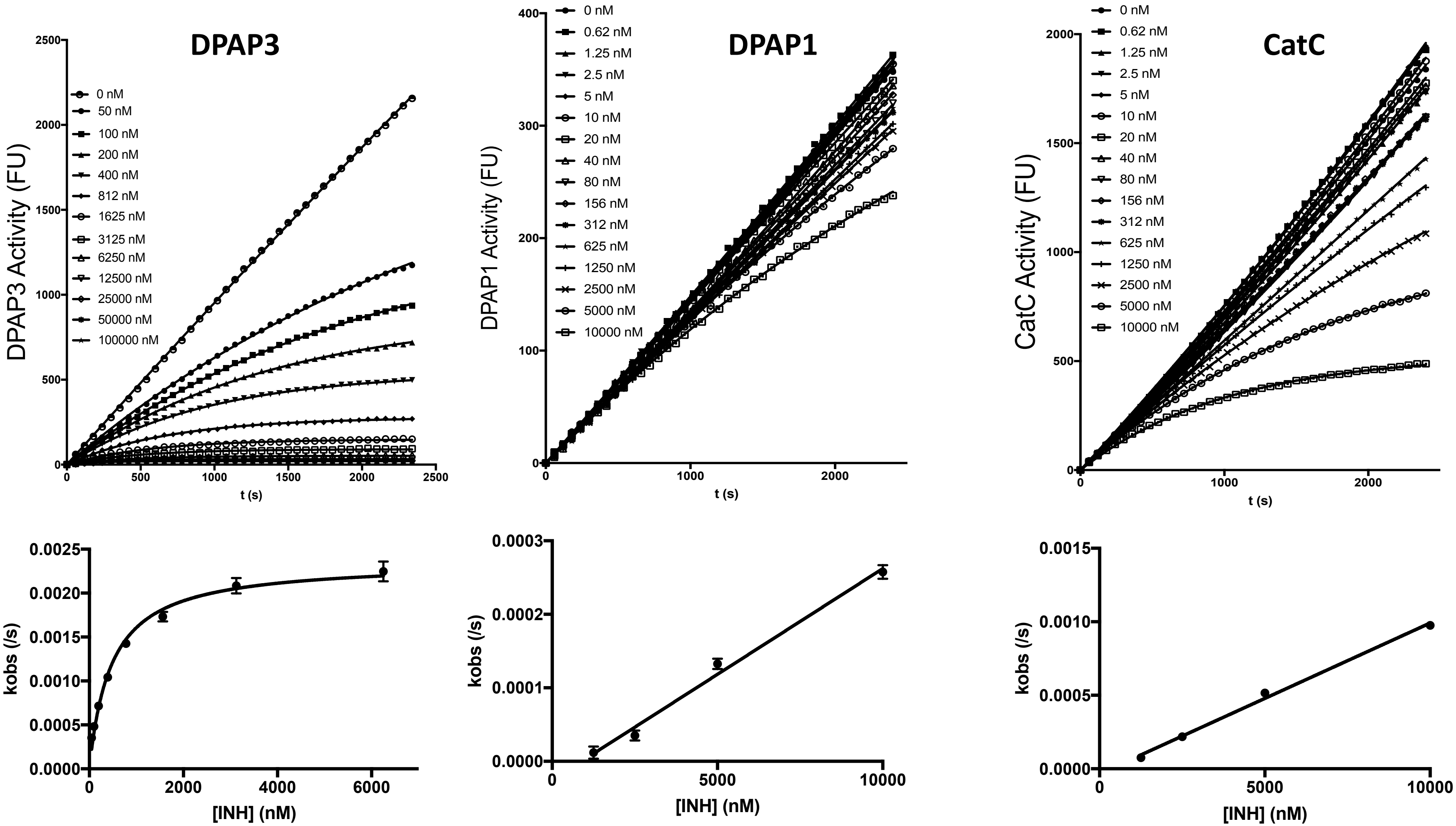
DPAP1

k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 23 ± 2 $M^{-1}s^{-1}$

CatC

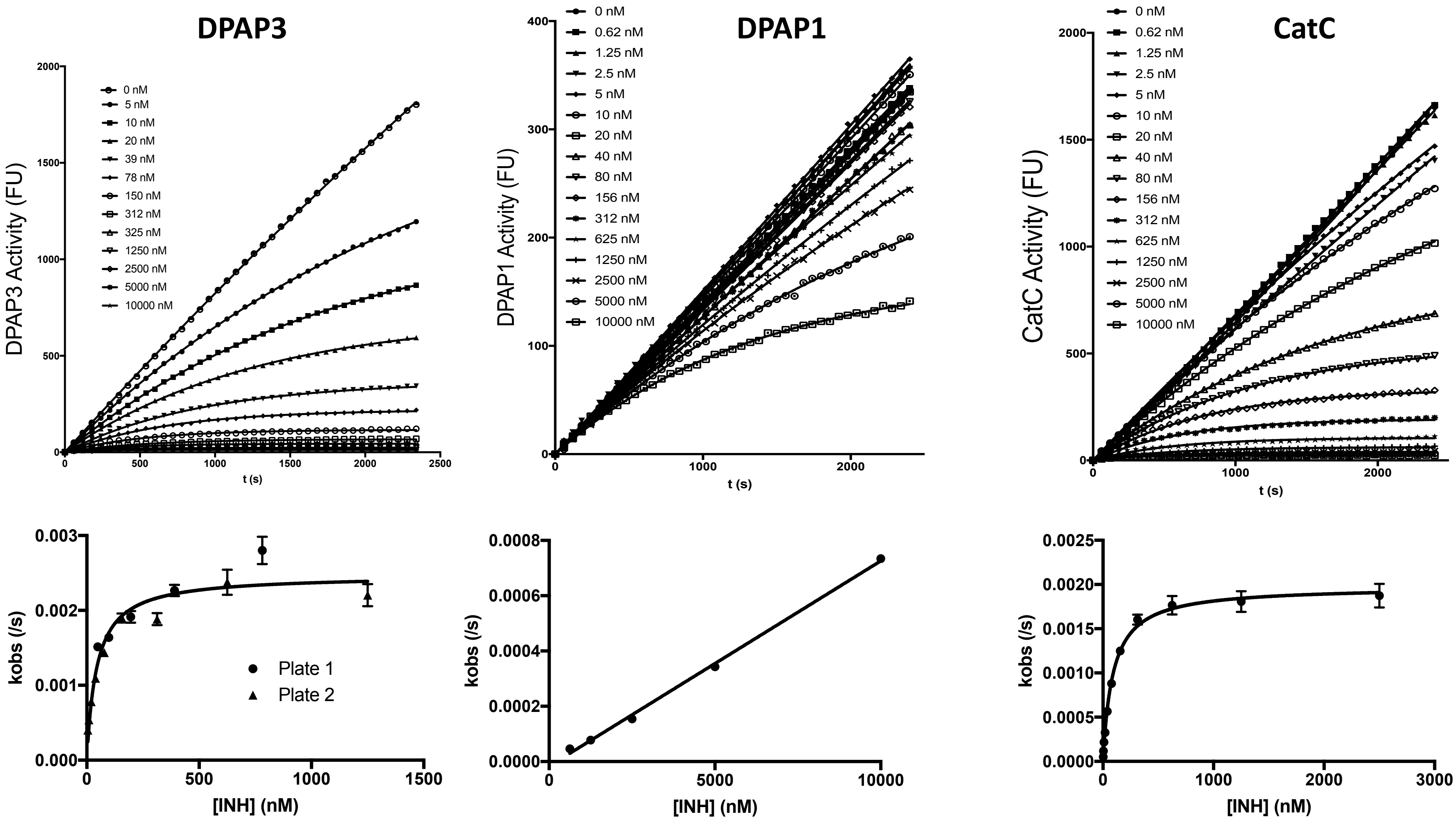
k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 147 ± 8 $M^{-1}s^{-1}$

P2: hPhe



DPAP3	DPAP1	CatC
k_{inact} = 0.00236 ± 0.00008 s^{-1}	k_{inact} = N.S.	k_{inact} = N.S.
K_i = 310 ± 30 nM	K_i = N.S.	K_i = N.S.
k_{inact}/K_i = 7,600 ± 700 $M^{-1}s^{-1}$	k_{inact}/K_i = 29 ± 2 $M^{-1}s^{-1}$	k_{inact}/K_i = 103 ± 5 $M^{-1}s^{-1}$

P2: Trp

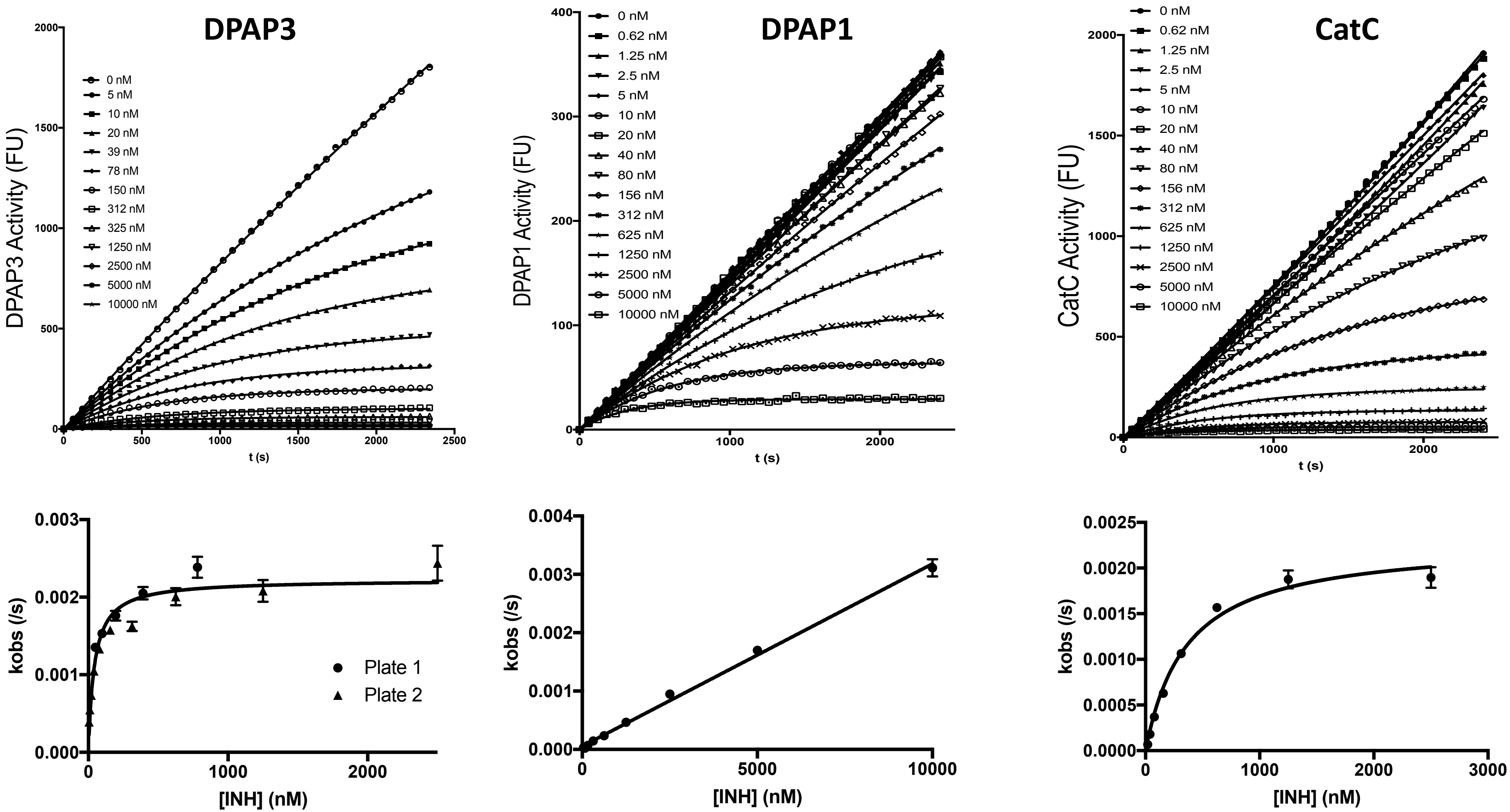


k_{inact} = 0.0025 ± 0.0001 s^{-1}
K_i = 30 ± 6 nM
k_{inact}/K_i = 84,000 ± 14,000 $M^{-1}s^{-1}$

k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 74 ± 2 $M^{-1}s^{-1}$

k_{inact} = 0.00198 ± 0.00003 s^{-1}
K_i = 61 ± 4 nM
k_{inact}/K_i = 32,000 ± 2,000 $M^{-1}s^{-1}$

P2: Igl



DPAP3

k_{inact} = 0.0022 ± 0.0001 s^{-1}

K_i = 29 ± 5 nM

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

DPAP1

k_{inact} = N.S.

K_i = N.S.

k_{inact}/K_i = 313 ± 7 $M^{-1}s^{-1}$

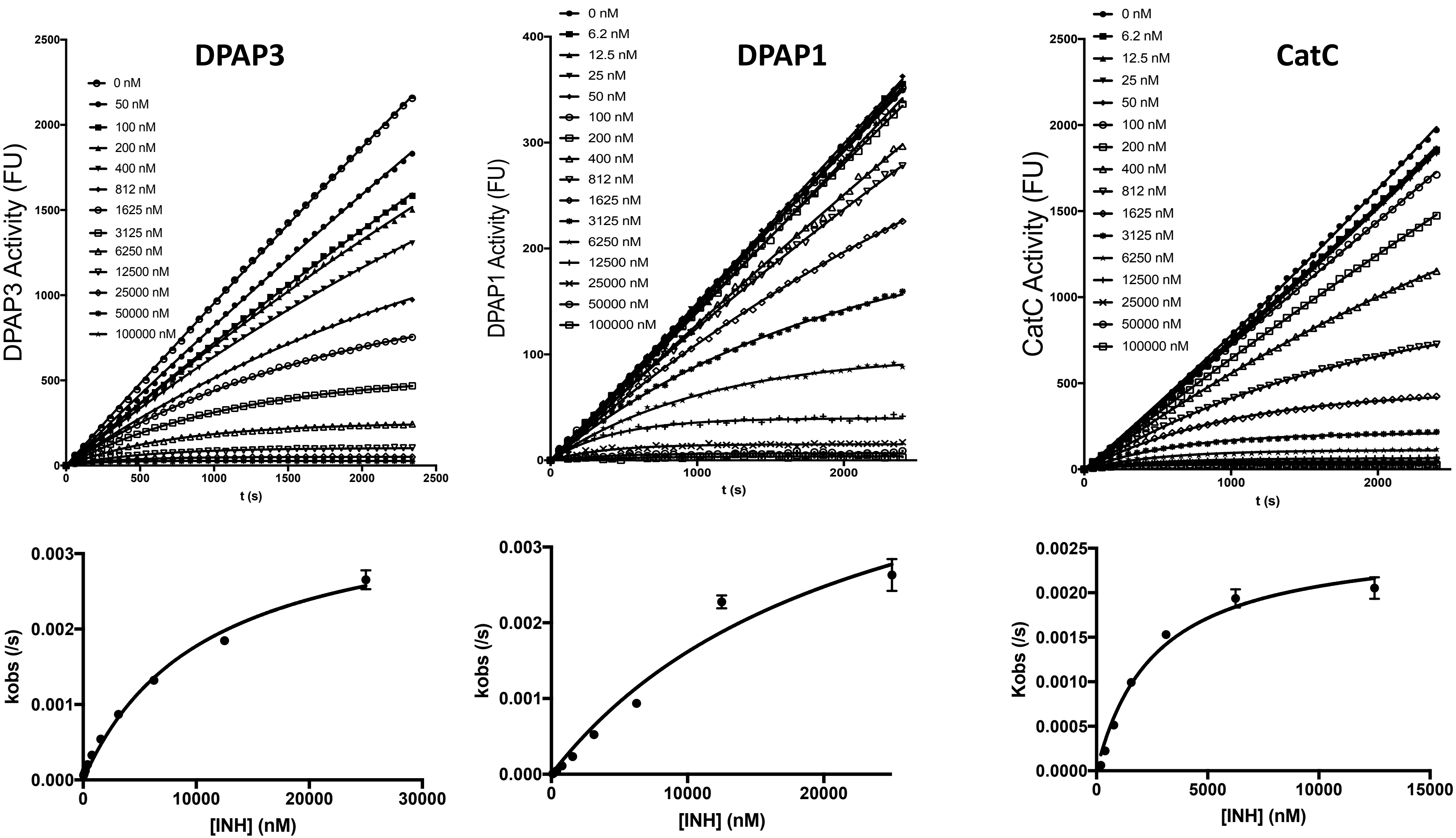
CatC

k_{inact} = 0.0023 ± 0.0001 s^{-1}

K_i = 240 ± 40 nM

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

P2: 2Nal

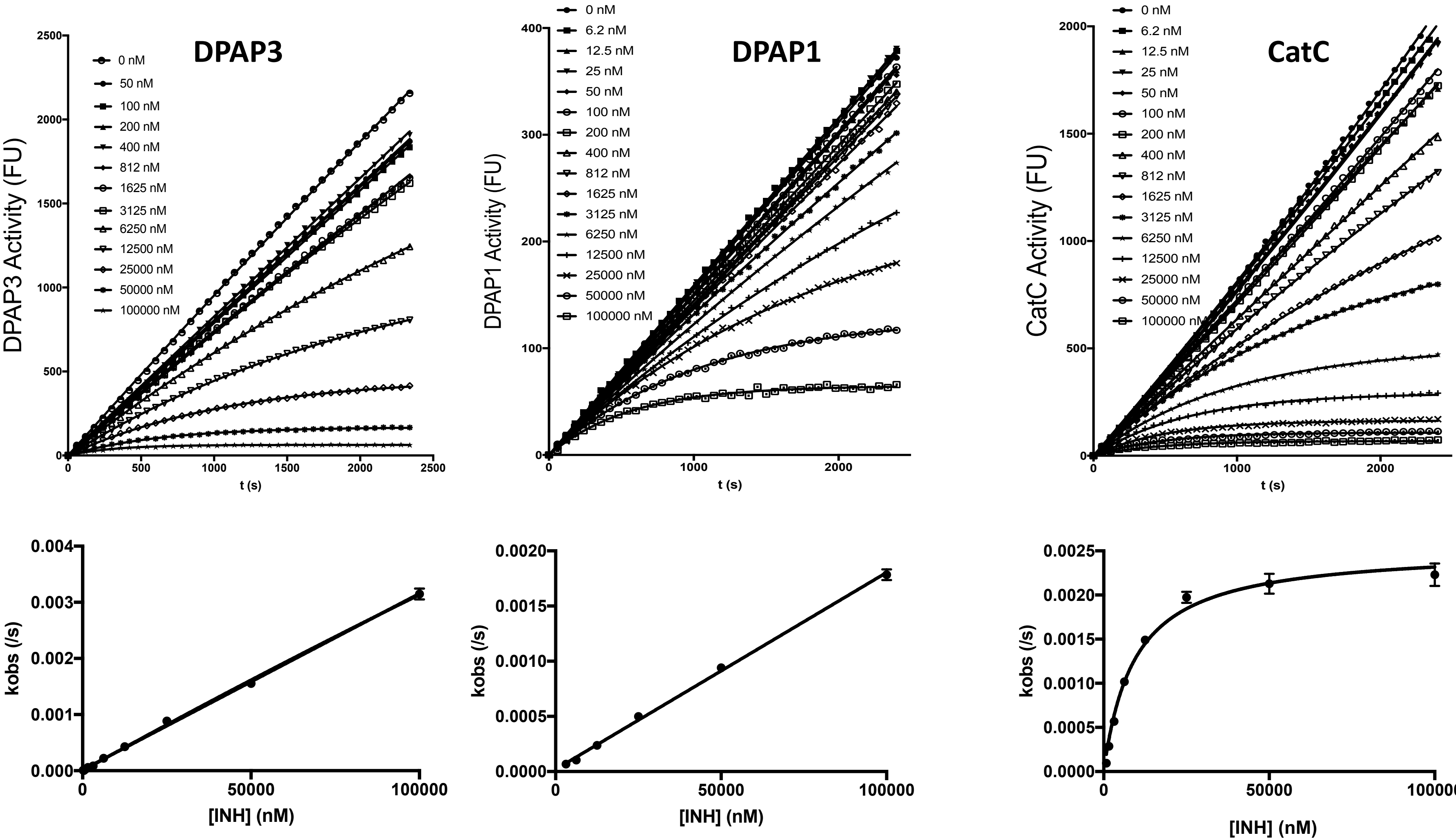


k_{inact} = 0.0037 ± 0.0003 s^{-1}
K_i = 7,100 ± 1,000 nM
k_{inact}/K_i = 510 ± 40 $M^{-1}s^{-1}$

k_{inact} = 0.0053 ± 0.0013 s^{-1}
K_i = 15,200 ± 6,500 nM
k_{inact}/K_i = 350 ± 70 $M^{-1}s^{-1}$

k_{inact} = 0.0026 ± 0.0002 s^{-1}
K_i = 1,800 ± 400 nM
k_{inact}/K_i = 1,500 ± 400 $M^{-1}s^{-1}$

P2: Bip

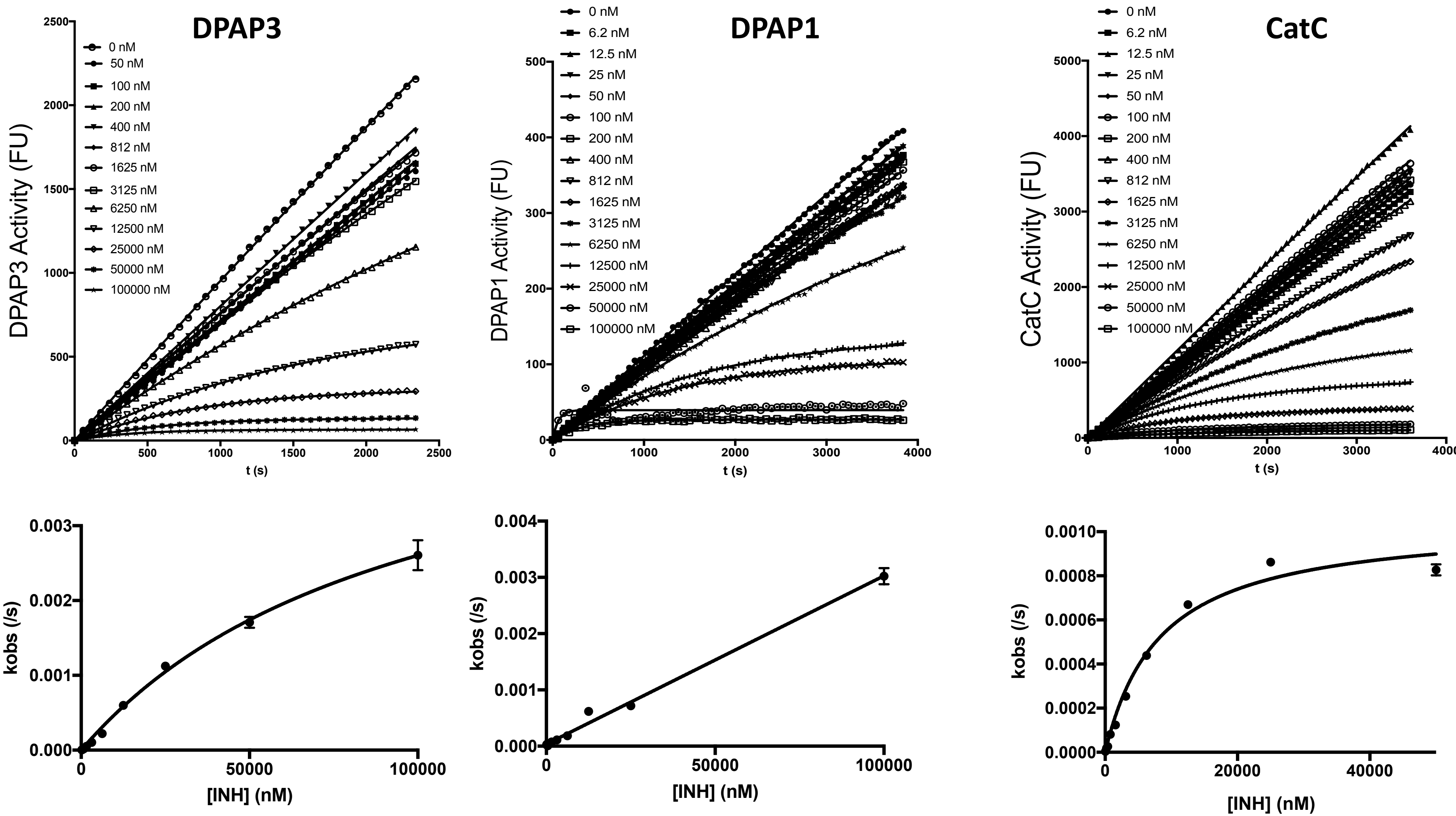


k_{inact} = N.S.
K_i = N.S.
k_{inact}/K_i = 31.4 ± 0.4 $M^{-1}s^{-1}$

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

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

P2: Inp



DPAP3

k_{inact} = 0.0052 ± 0.0004 s^{-1}

K_i = 65,000 ± 8,000 nM

k_{inact}/K_i = 79 ± 4 $M^{-1}s^{-1}$

DPAP1

k_{inact} = N.S.

K_i = N.S.

k_{inact}/K_i = 30 ± 1 $M^{-1}s^{-1}$

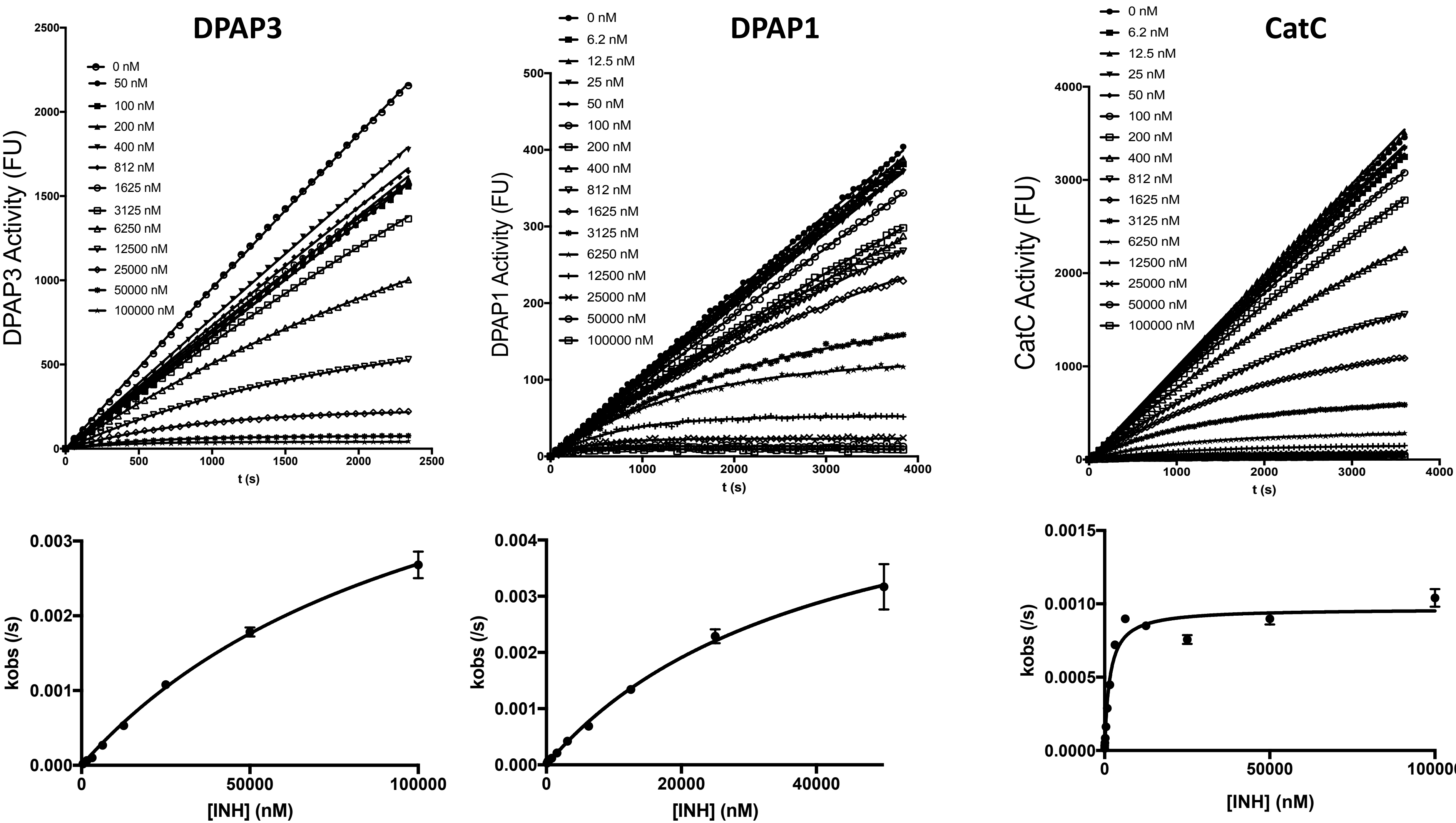
CatC

k_{inact} = 0.00105 ± 0.00006 s^{-1}

K_i = 5,600 ± 900 nM

k_{inact}/K_i = 190 ± 20 $M^{-1}s^{-1}$

P2: Amc

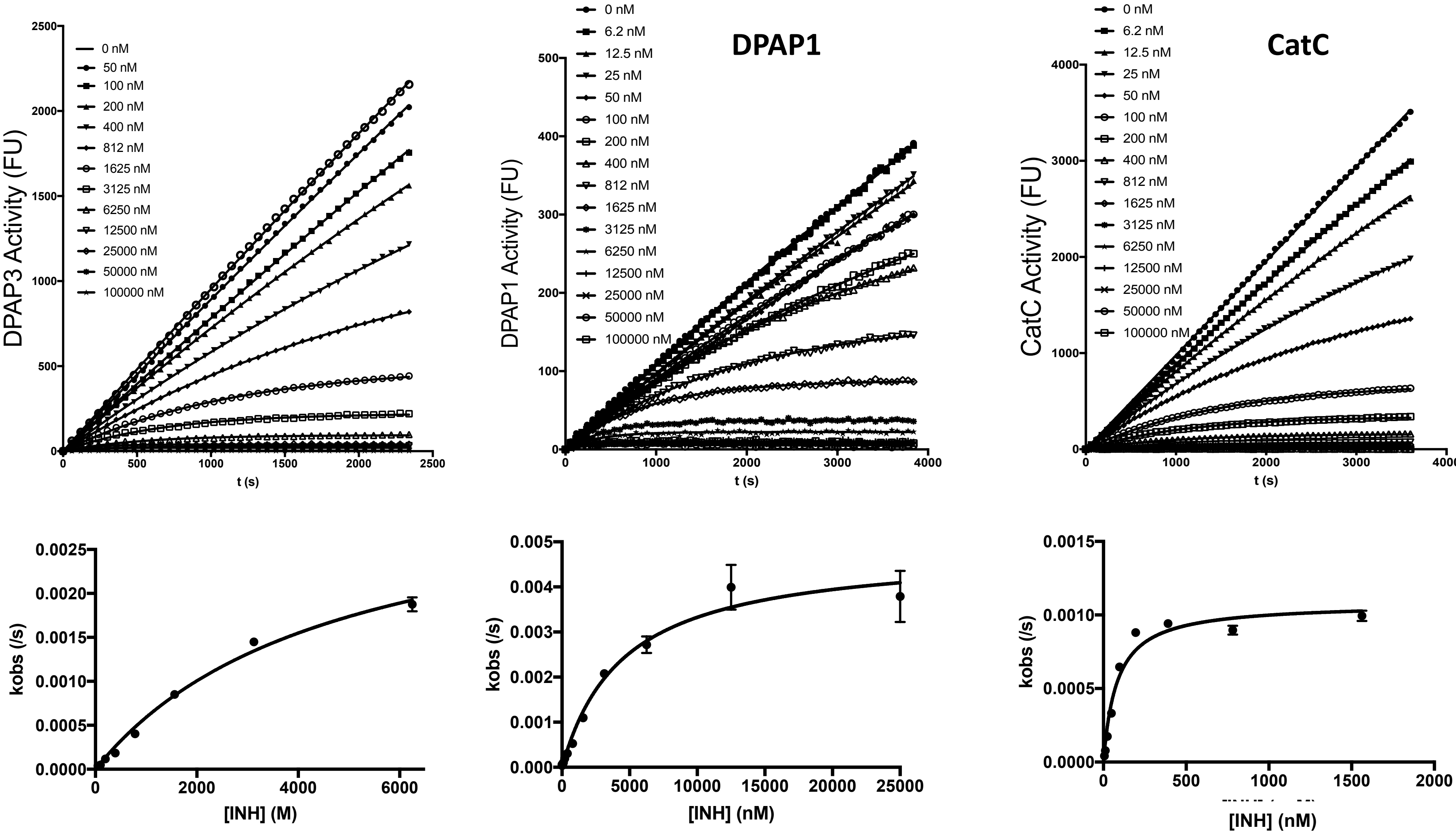


k_{inact} = 0.0057 ± 0.0003 s^{-1}
K_i = 75,000 ± 7,000 nM
k_{inact}/K_i = 76 ± 3 $M^{-1}s^{-1}$

k_{inact} = 0.0058 ± 0.0003 s^{-1}
K_i = 27,000 ± 2,500 nM
k_{inact}/K_i = 213 ± 9 $M^{-1}s^{-1}$

k_{inact} = 0.00097 ± 0.00005 s^{-1}
K_i = 1,000 ± 200 nM
k_{inact}/K_i = 940 ± 170 $M^{-1}s^{-1}$

P2: 3Abz

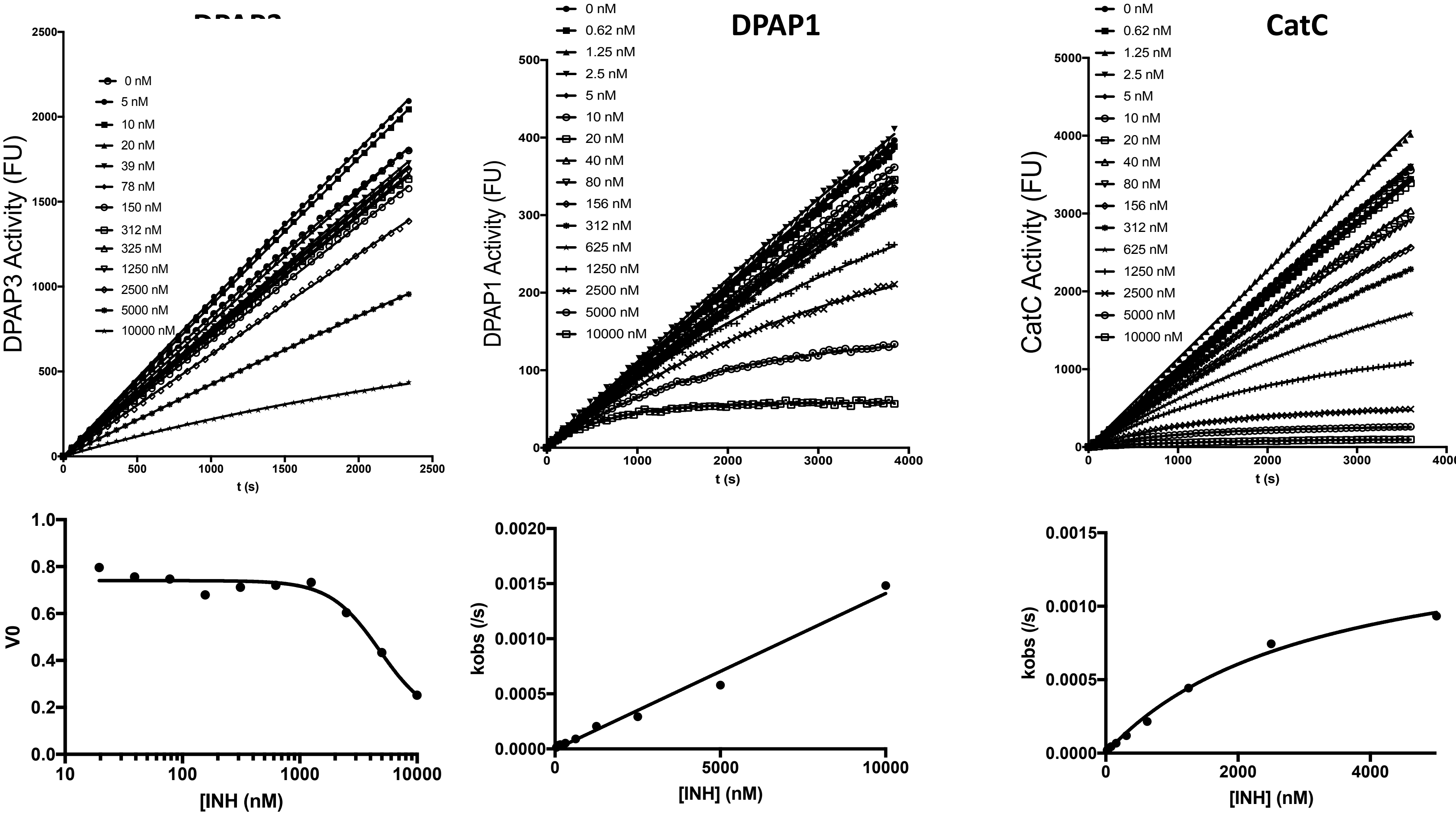


k_{inact} = 0.0034 ± 0.0004 s^{-1}
K_i = 3,100 ± 600 nM
k_{inact}/K_i = 1,100 ± 100 $M^{-1}s^{-1}$

k_{inact} = 0.0048 ± 0.0003 s^{-1}
K_i = 3,000 ± 500 nM
k_{inact}/K_i = 1,600 ± 200 $M^{-1}s^{-1}$

k_{inact} = 0.00108 ± 0.00007 s^{-1}
K_i = 55 ± 12 nM
k_{inact}/K_i = 20,000 ± 4,000 $M^{-1}s^{-1}$

P2: Amb

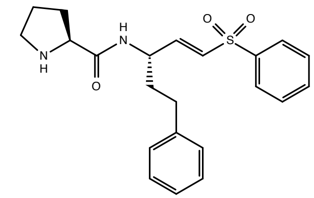


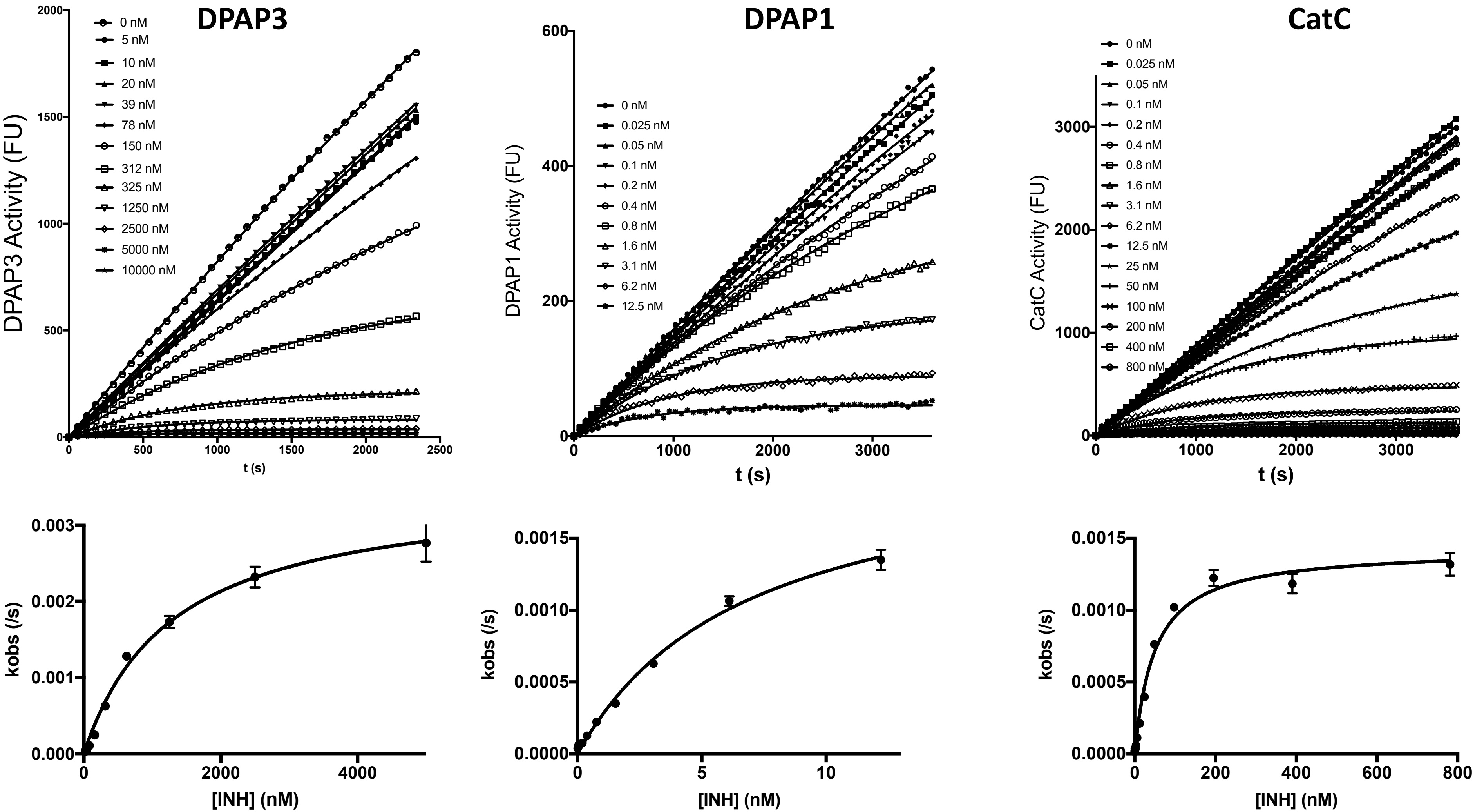
k_{inact} = N/A
K_i = 3,200 ± 100 nM
k_{inact}/K_i = N/A

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

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

P2: Pro; P1: hPhe



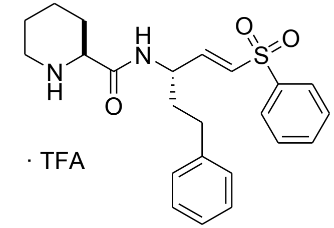


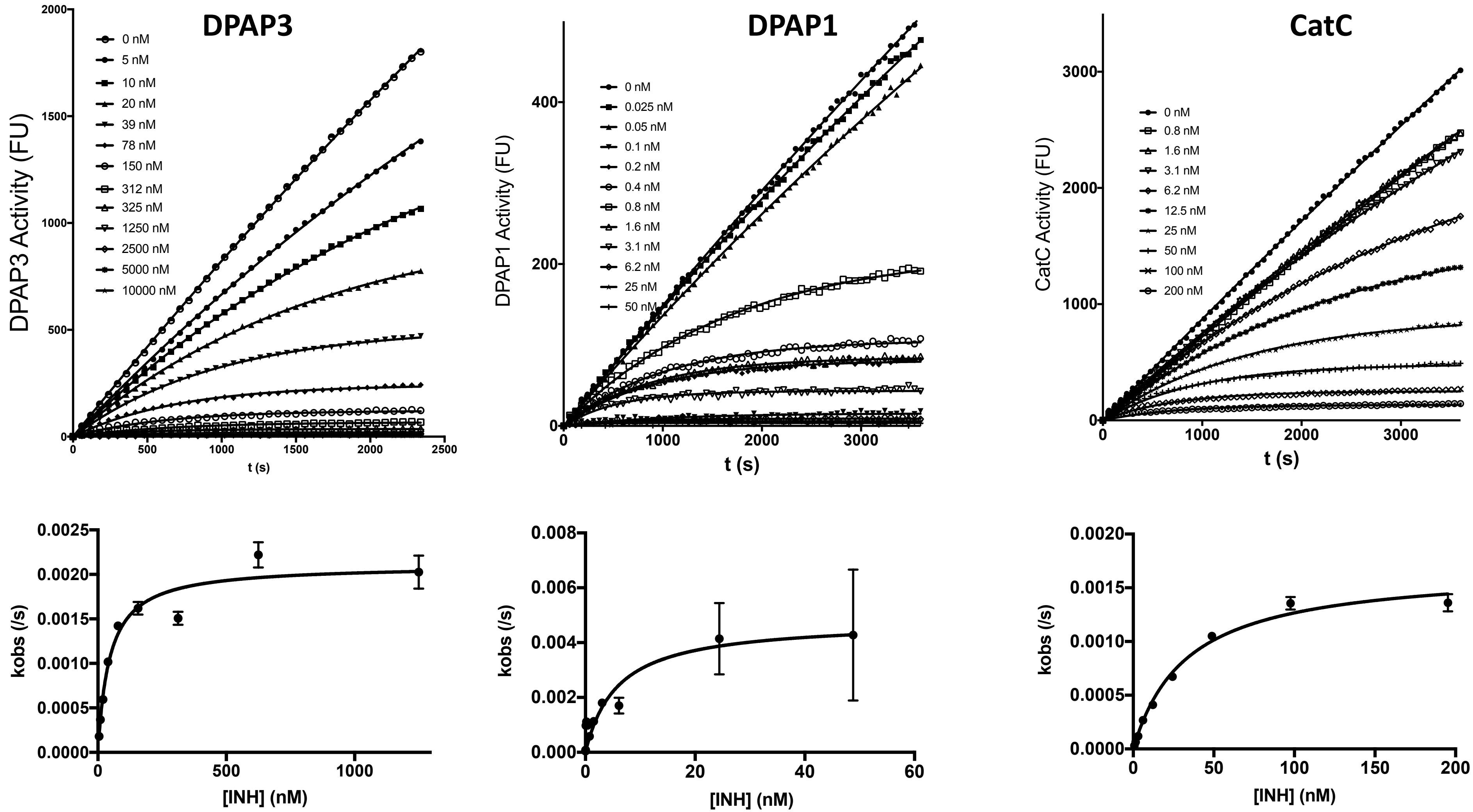
k_{inact} = 0.0035 ± 0.0002 s^{-1}
K_i = 860 ± 100 nM
k_{inact}/K_i = 4,100 ± 300 $M^{-1}s^{-1}$

k_{inact} = 0.0021 ± 0.0002 s^{-1}
K_i = 4.6 ± 0.7 nM
k_{inact}/K_i = 470,000 ± 35,000 $M^{-1}s^{-1}$

k_{inact} = 0.00145 ± 0.00007 s^{-1}
K_i = 34 ± 5 nM
k_{inact}/K_i = 42,000 ± 5,000 $M^{-1}s^{-1}$

P2: hPro; P1: hPhe



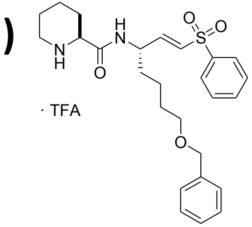


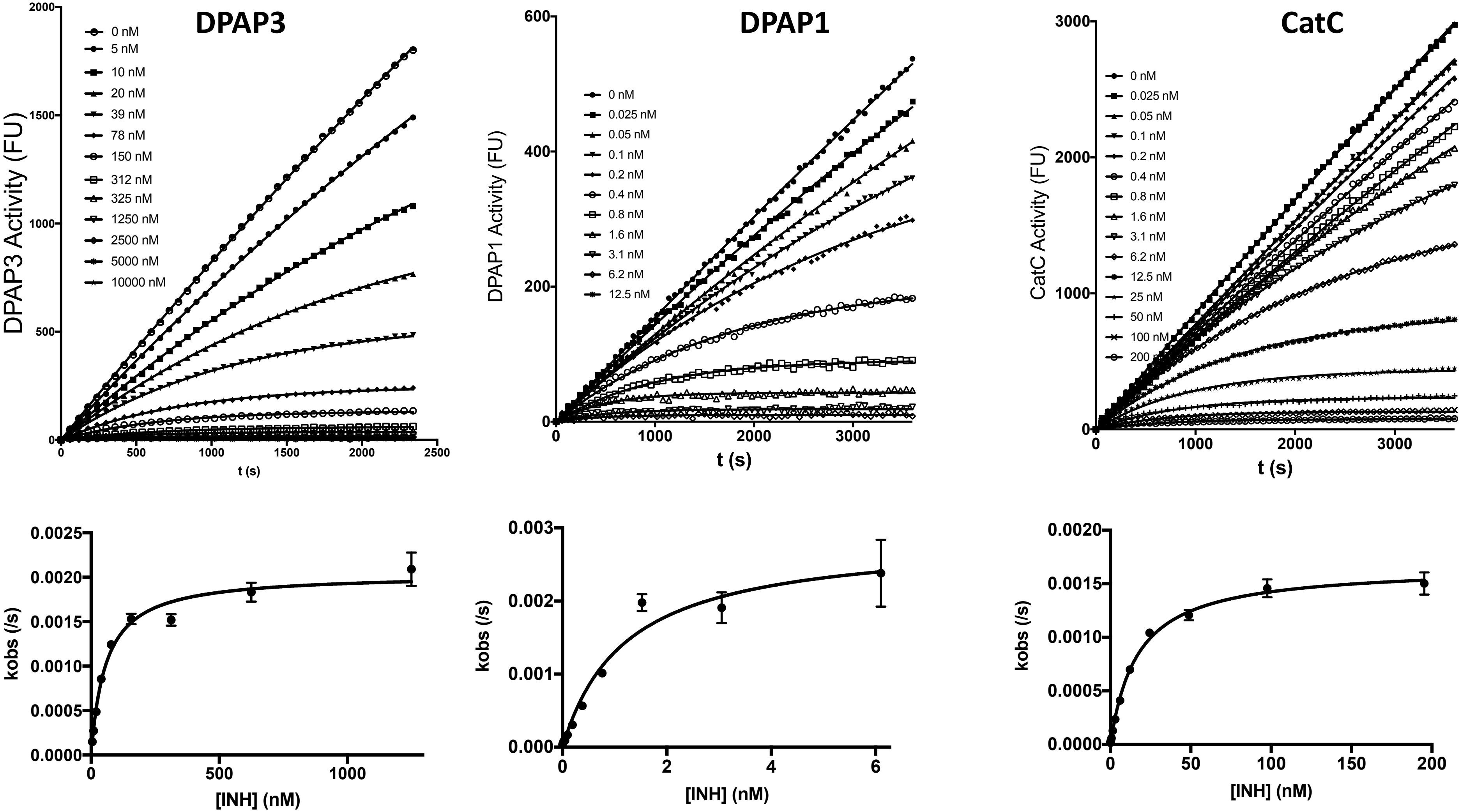
k_{inact} = 0.0021 ± 0.0001 s^{-1}
K_i = 30 ± 7 nM
k_{inact}/K_i = 69,000 ± 13,000 $M^{-1}s^{-1}$

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

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

P2: hPro; P1: nLeu(oBzl)





DPAP3

k_{inact} = 0.0020 ± 0.0001 s^{-1}

K_i = 39 ± 6 nM

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

DPAP1

k_{inact} = 0.0029 ± 0.0003 s^{-1}

K_i = 0.8 ± 0.2 nM

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

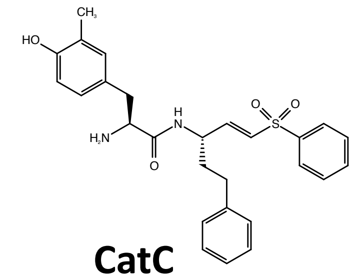
CatC

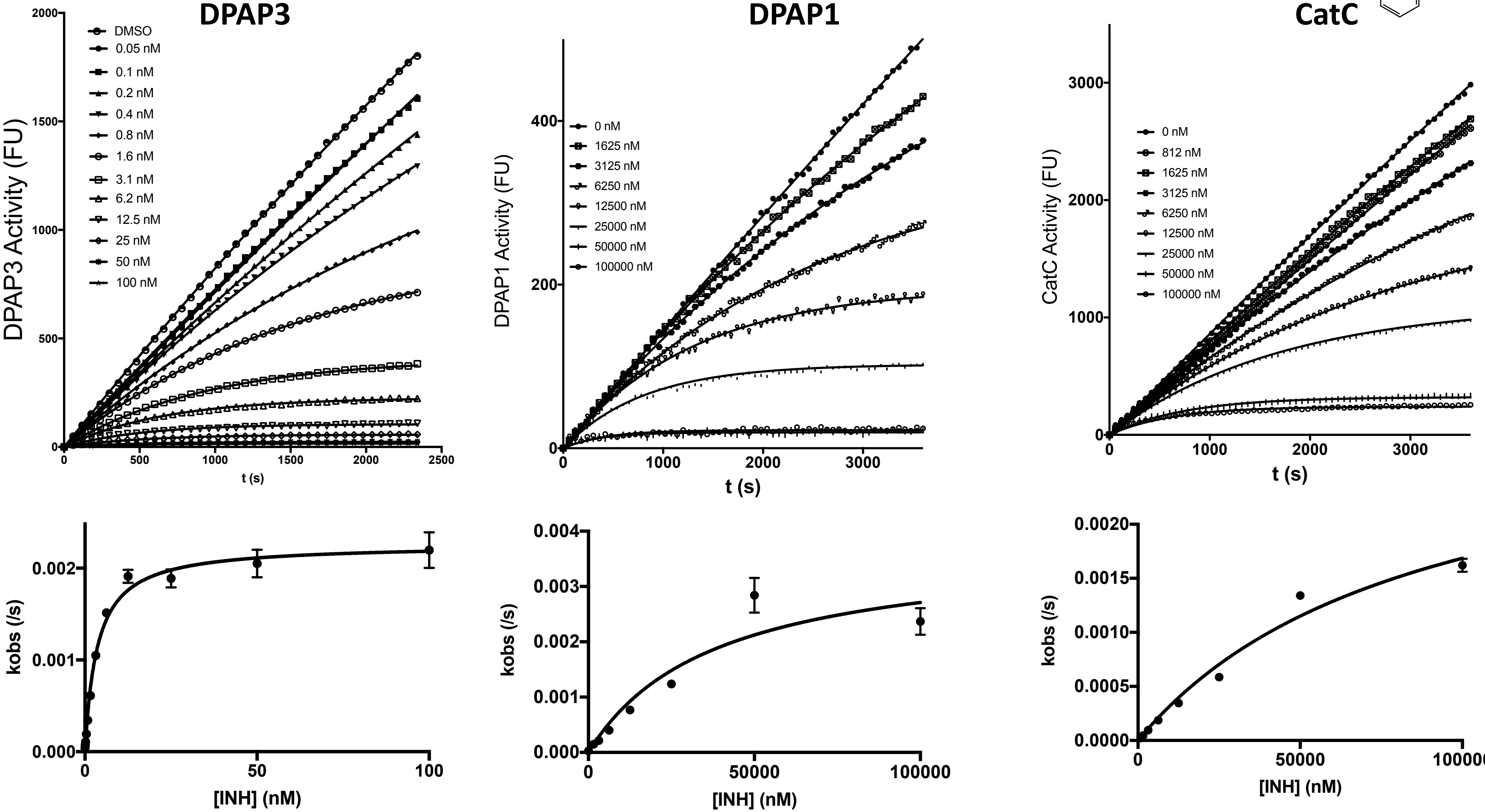
k_{inact} = 0.00167 ± 0.00003 s^{-1}

K_i = 11.2 ± 0.6 nM

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

P2: Tyr(NO_2); P1: hPhe



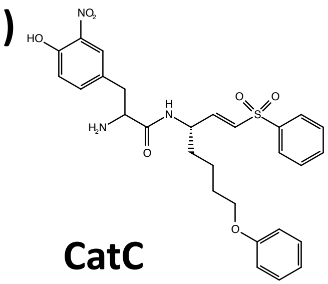


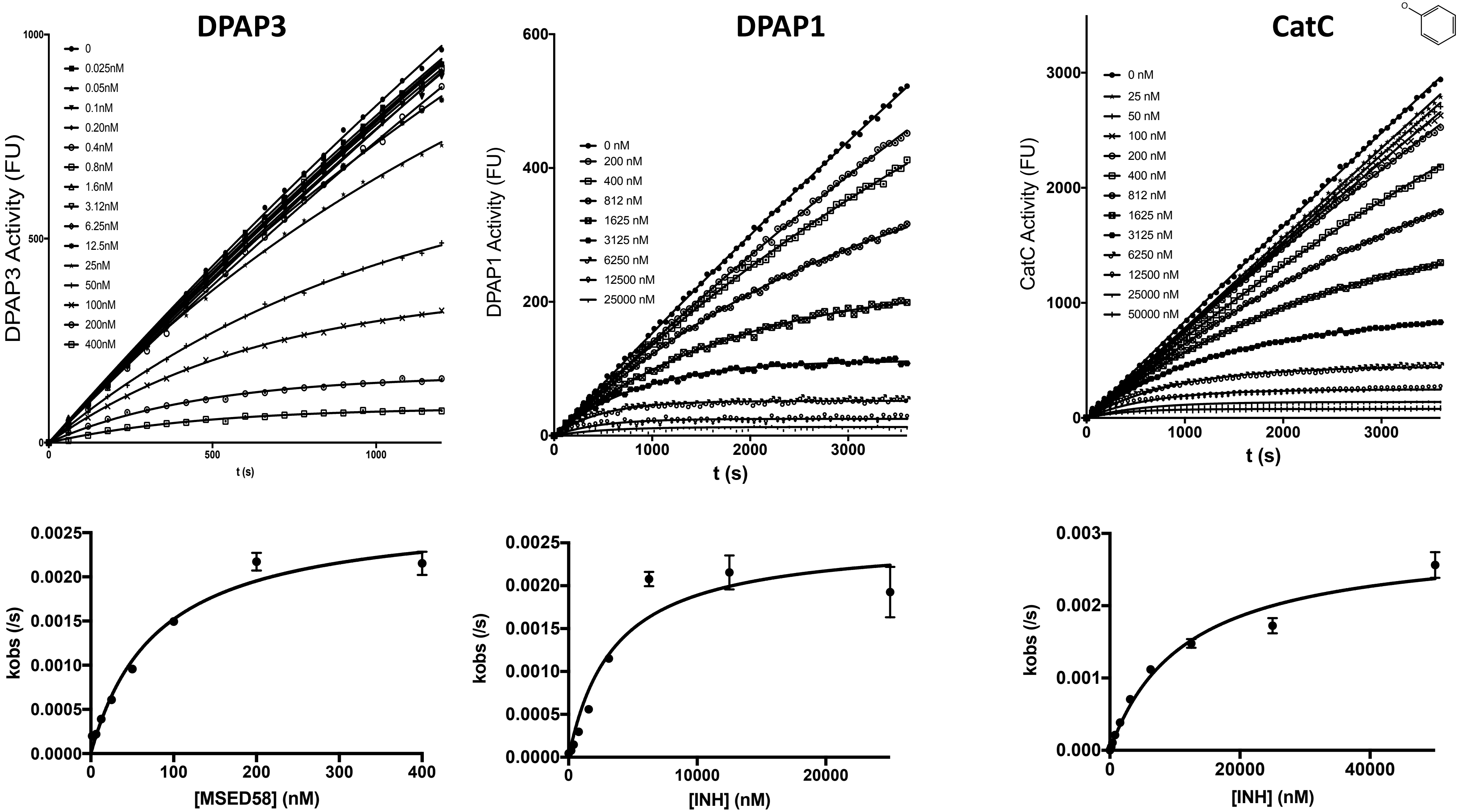
k_{inact} = 0.00226 ± 0.00005 s^{-1}
K_i = 2.4 ± 0.2 nM
k_{inact}/K_i = 950,000 ± 74,000 $M^{-1}s^{-1}$

k_{inact} = 0.0038 ± 0.001 s^{-1}
K_i = 25,000 ± 1,400 nM
k_{inact}/K_i = 150 ± 50 $M^{-1}s^{-1}$

k_{inact} = 0.0032 ± 0.0006 s^{-1}
K_i = 58,000 ± 20,000 nM
k_{inact}/K_i = 54 ± 8 $M^{-1}s^{-1}$

P2: Tyr(NO_2); P1: nLeu(o-Bzl)



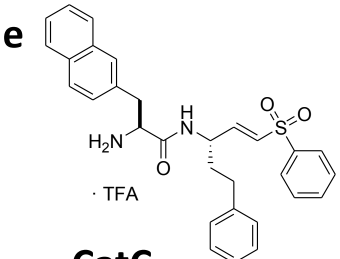


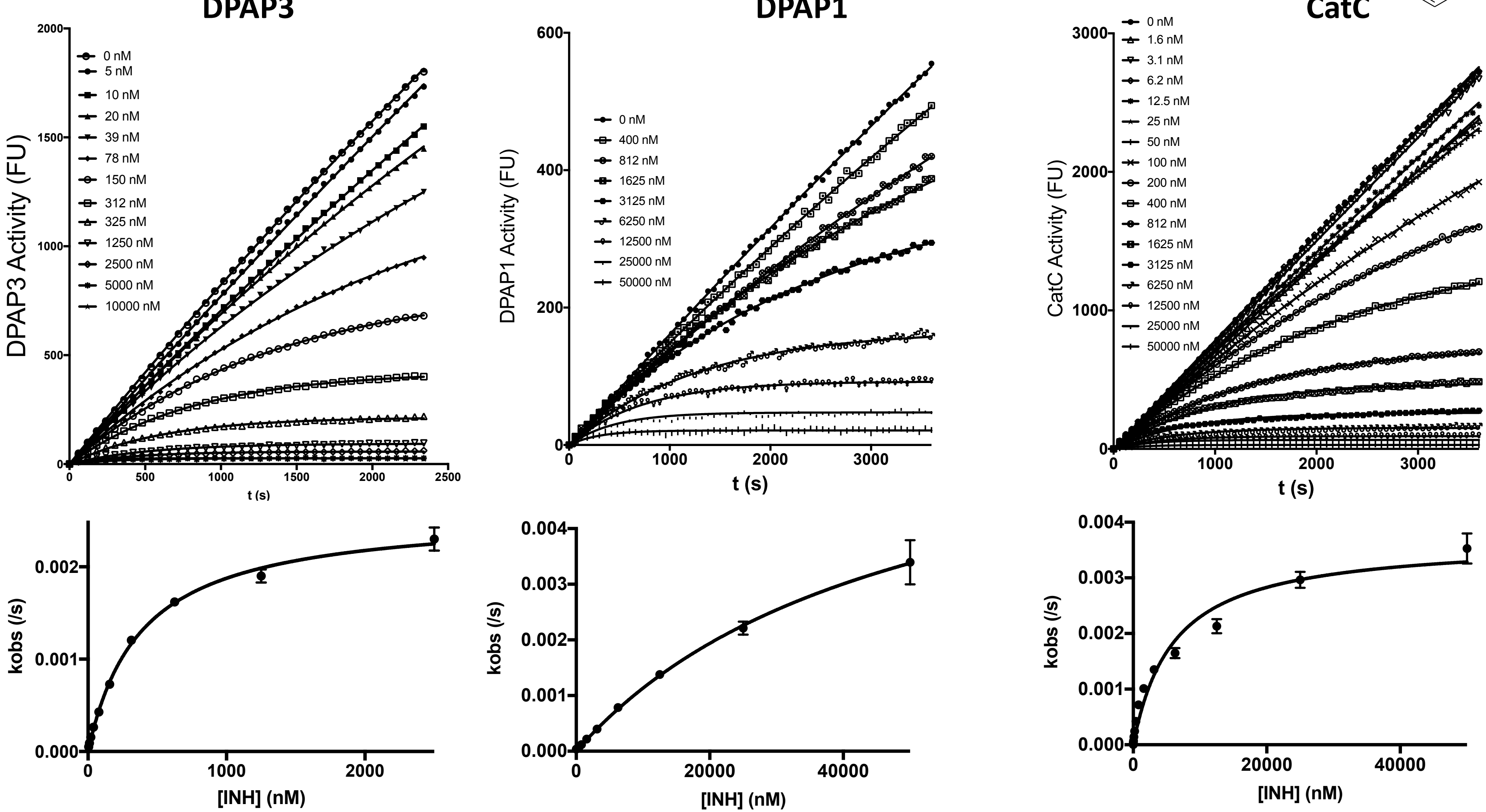
k_{inact} = 0.0027 ± 0.0002 s^{-1}
K_i = 53 ± 10 nM
k_{inact}/K_i = 51,000 ± 7,000 $M^{-1}s^{-1}$

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

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

P2: 2Nal; P1:hPhe





DPAP3

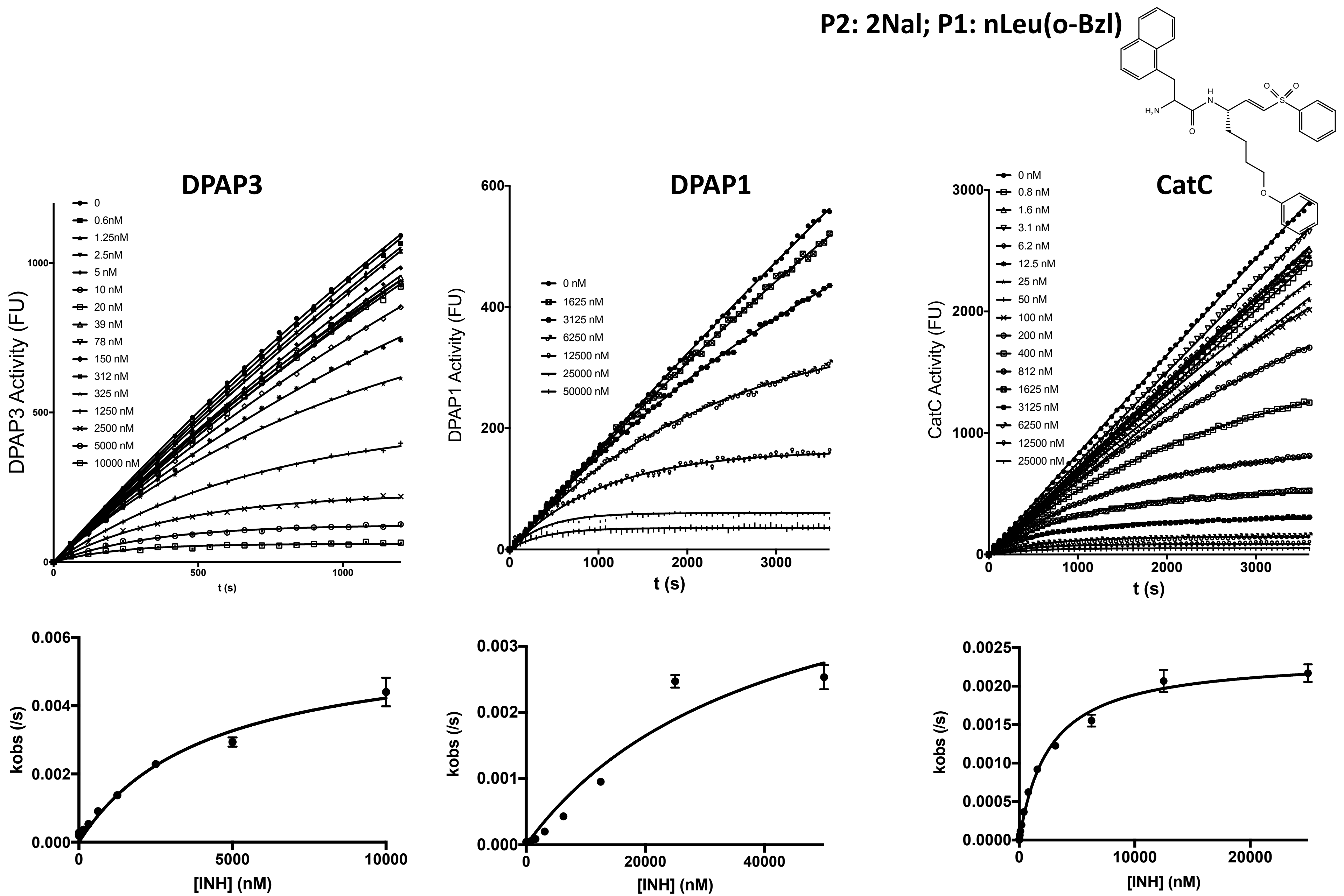
k_{inact} = 0.00259 ± 0.00007 s^{-1}
K_i = 254 ± 16 nM
k_{inact}/K_i = 10,200 ± 500 $M^{-1}s^{-1}$

DPAP1

k_{inact} = 0.0067 ± 0.0002 s^{-1}
K_i = 33,000 ± 2,000 nM
k_{inact}/K_i = 200 ± 5 $M^{-1}s^{-1}$

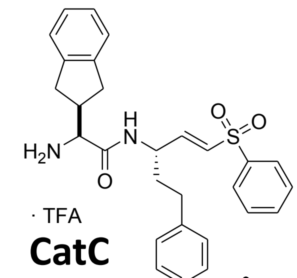
CatC

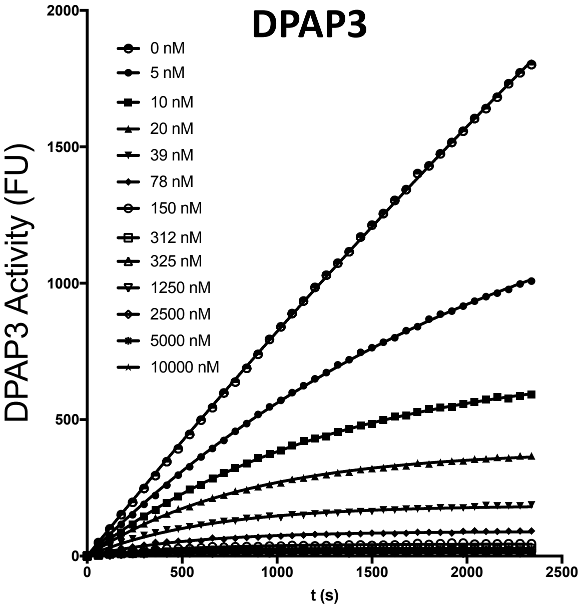
k_{inact} = 0.0037 ± 0.0002 s^{-1}
K_i = 4,000 ± 700 nM
k_{inact}/K_i = 900 ± 100 $M^{-1}s^{-1}$

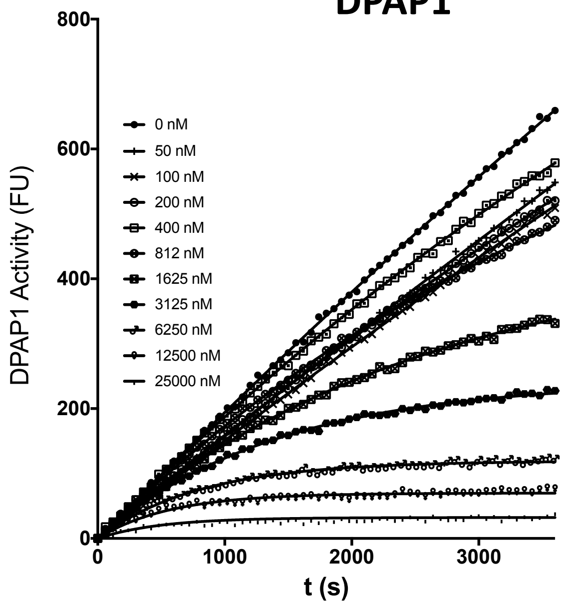


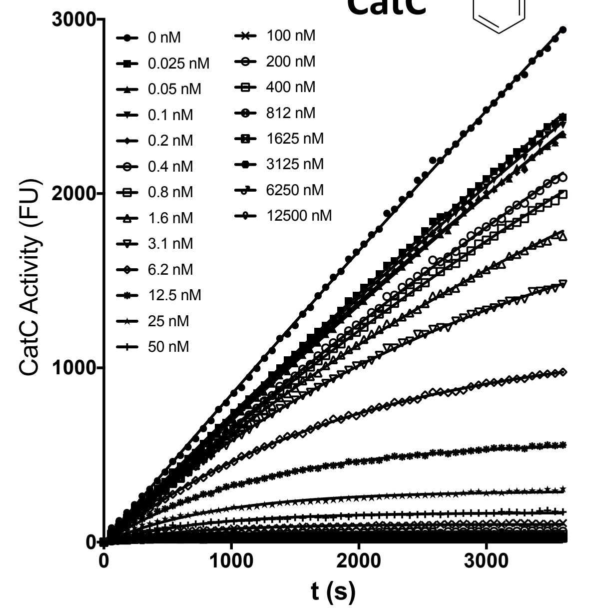
	DPAP3	DPAP1	CatC
k_{inact}	= 0.0060 ± 0.0007 s^{-1}	= 0.005 ± 0.002 s^{-1}	= 0.0024 ± 0.0006 s^{-1}
K_i	= 2,800 ± 600 nM	= 27,000 ± 16,000 nM	= 1,740 ± 150 nM
k_{inact}/K_i	= 2,200 ± 300 $M^{-1}s^{-1}$	= 190 ± 50 $M^{-1}s^{-1}$	= 1370 ± 90 $M^{-1}s^{-1}$

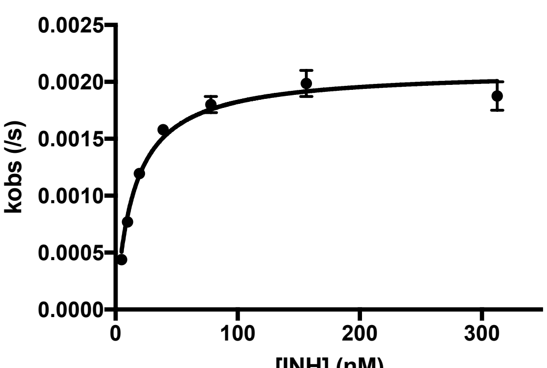
P2: Igl; P1: hPhe







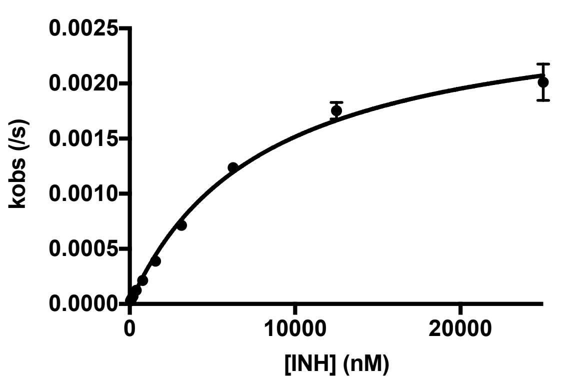




k_{inact} = 0.0021 ± 0.00007 s^{-1}

K_i = 10 ± 1 nM

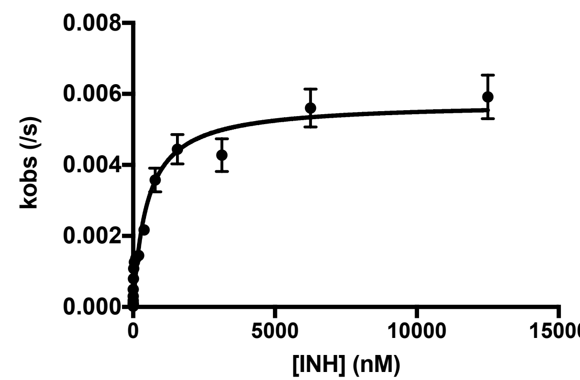
k_{inact}/K_i = 205,000 ± 22,000 $M^{-1}s^{-1}$



k_{inact} = 0.0027 ± 0.0001 s^{-1}

K_i = 5.400 ± 500 nM

k_{inact}/K_i = 500 ± 30 $M^{-1}s^{-1}$

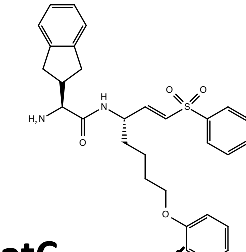


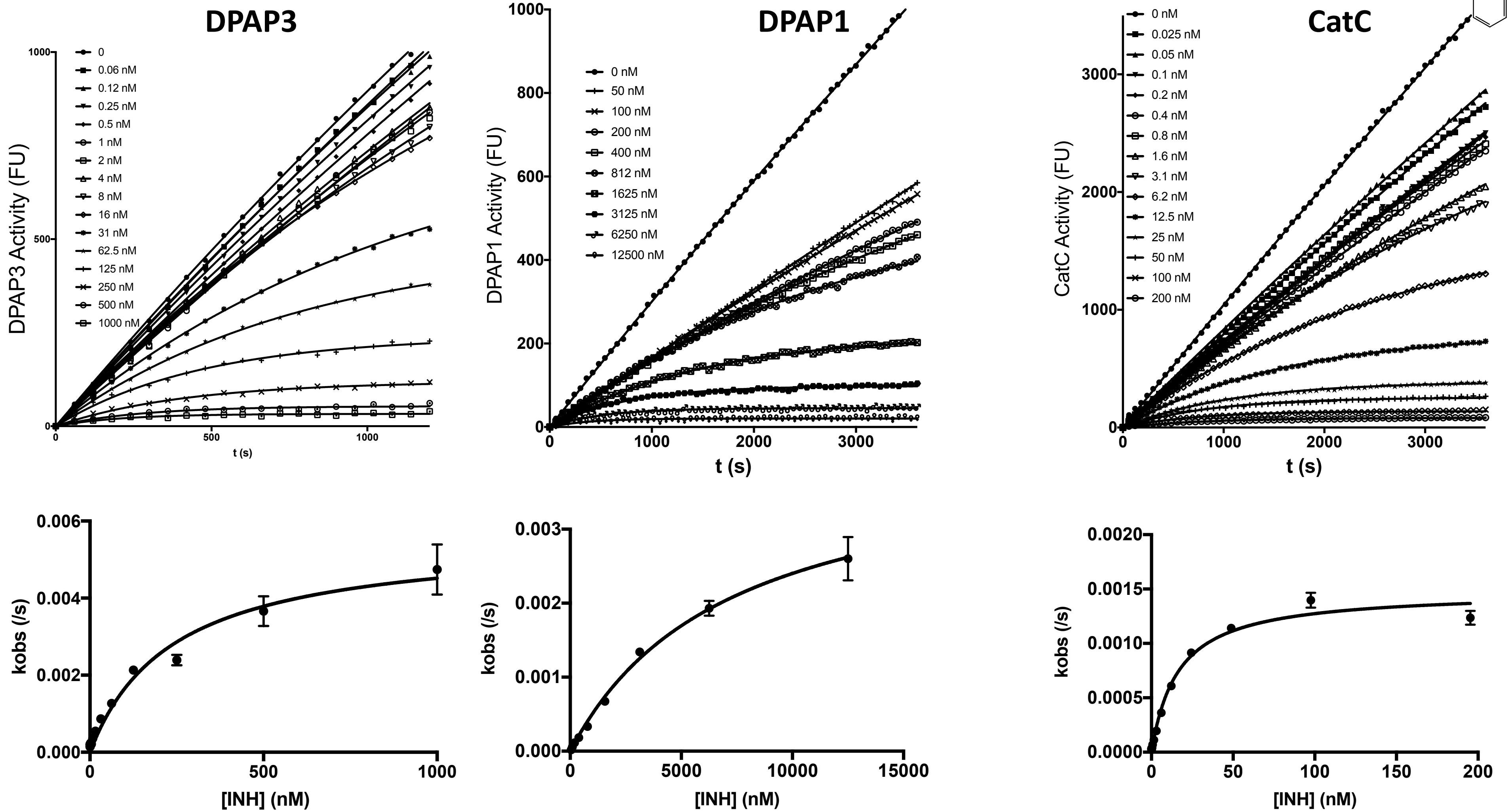
k_{inact} = 0.0058 ± 0.0003 s^{-1}

K_i = 320 ± 70 nM

k_{inact}/K_i = 18,000 ± 300 $M^{-1}s^{-1}$

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





DPAP3:

k_{inact} = 0.0056 ± 0.0004 s^{-1}

K_i = 160 ± 30 nM

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

DPAP1:

k_{inact} = 0.0041 ± 0.0002 s^{-1}

K_i = 4,800 ± 500 nM

k_{inact}/K_i = 860 ± 50 $M^{-1}s^{-1}$

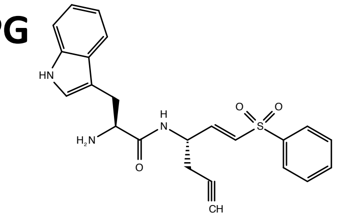
CatC:

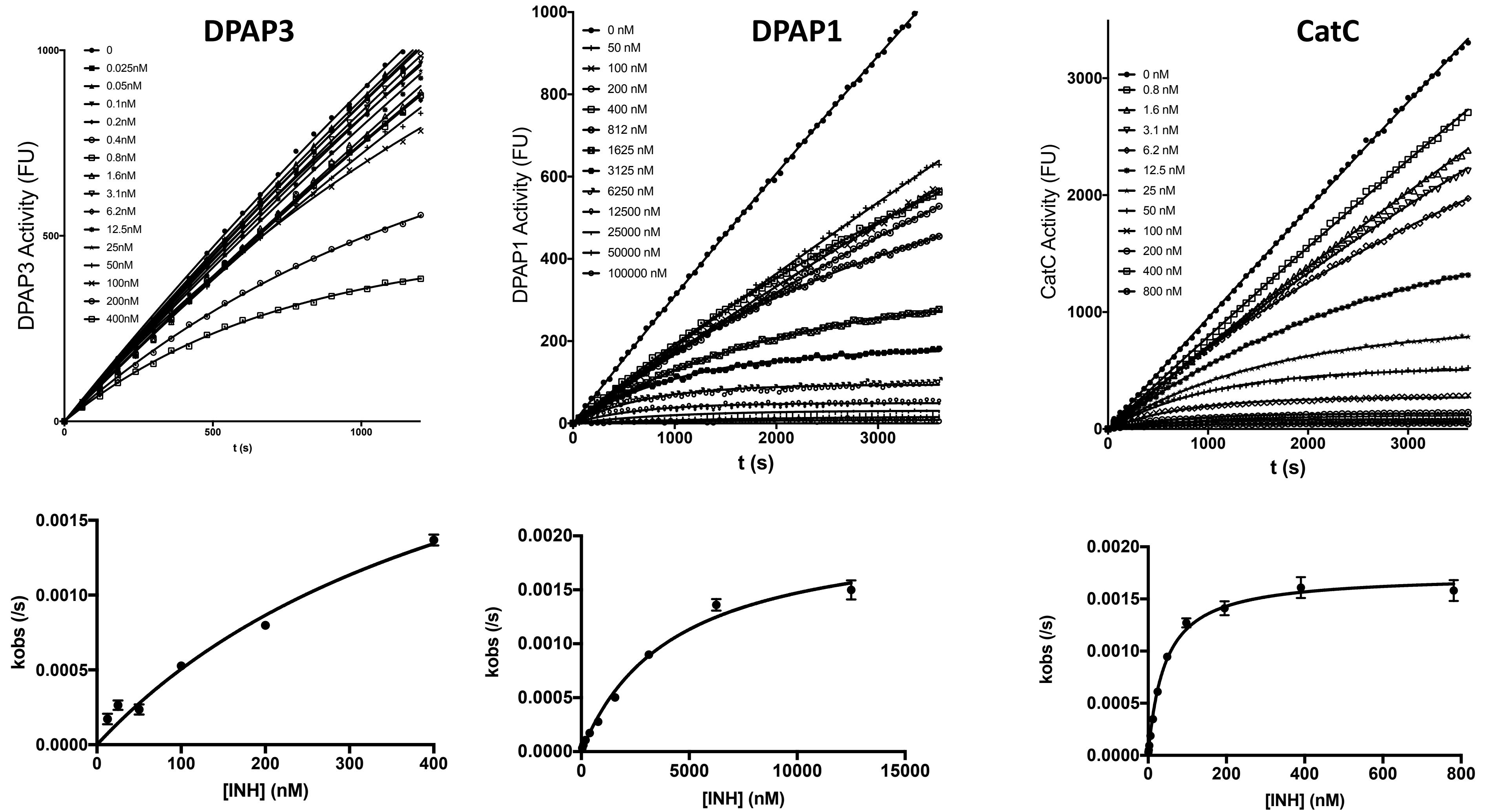
k_{inact} = 0.00148 ± 0.00006 s^{-1}

K_i = 11 ± 2 nM

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

P2: Trp; P1: hPG



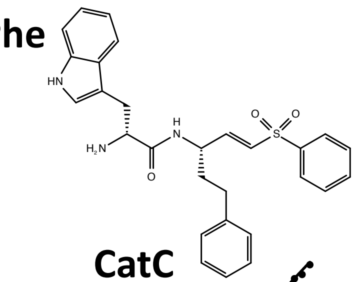


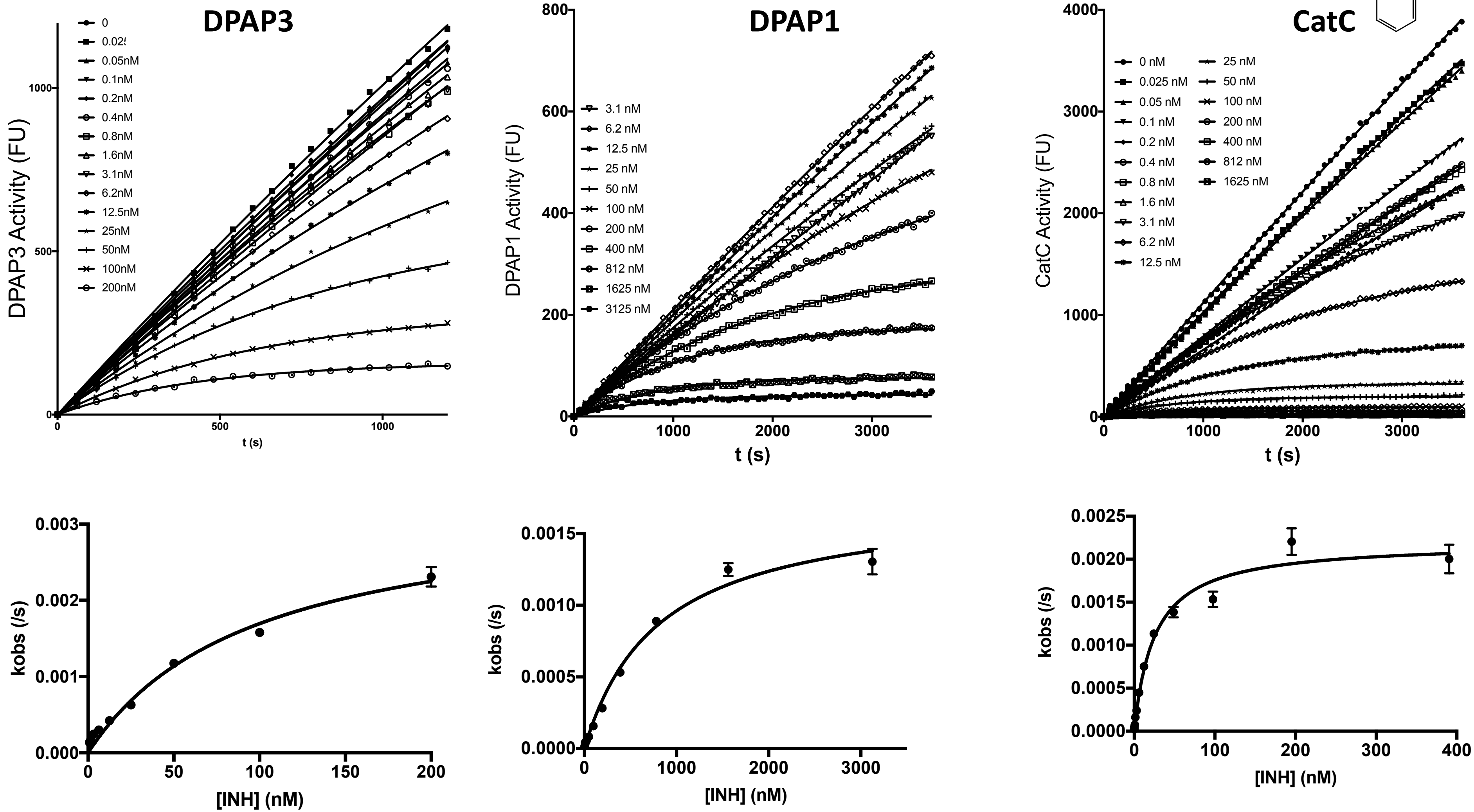
k_{inact} = 0.003 ± 0.001 s^{-1}
K_i = 330 ± 150 nM
k_{inact}/K_i = 9,100 ± 1,500 $M^{-1}s^{-1}$

k_{inact} = 0.0021 ± 0.0002 s^{-1}
K_i = 2,800 ± 450 nM
k_{inact}/K_i = 740 ± 70 $M^{-1}s^{-1}$

k_{inact} = 0.00173 ± 0.00003 s^{-1}
K_i = 28 ± 2 nM
k_{inact}/K_i = 61,000 ± 4,000 $M^{-1}s^{-1}$

P2: Trp; P1: hPhe



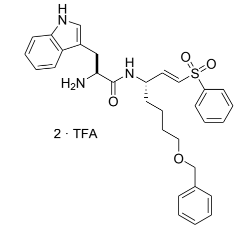


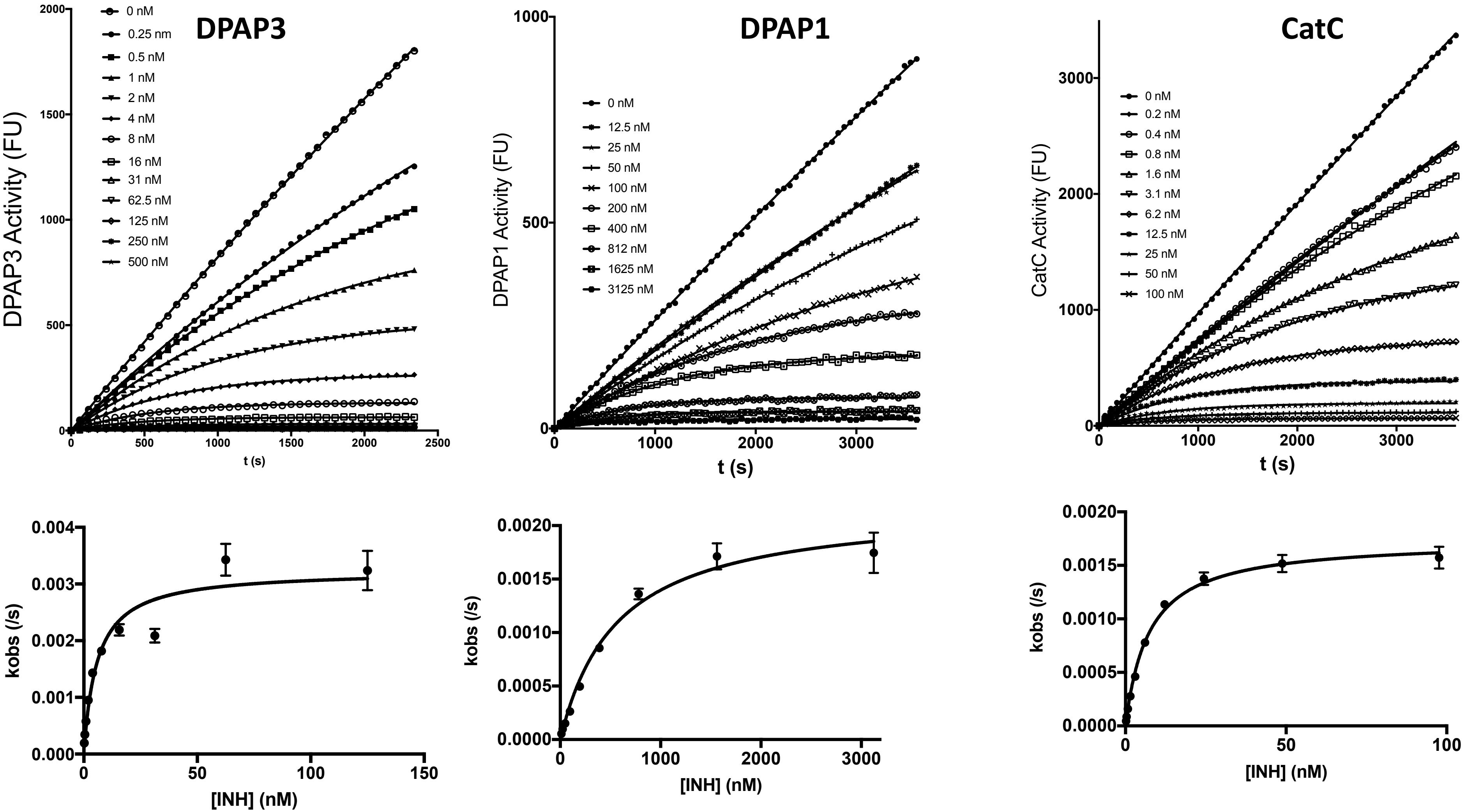
k_{inact} = 0.0033 ± 0.0004 s^{-1}
K_i = 65 ± 15 nM
k_{inact}/K_i = 52,000 ± 7,000 $M^{-1}s^{-1}$

k_{inact} = 0.0017 ± 0.0001 s^{-1}
K_i = 540 ± 80 nM
k_{inact}/K_i = 3,200 ± 300 $M^{-1}s^{-1}$

k_{inact} = 0.0022 ± 0.00009 s^{-1}
K_i = 16 ± 3 nM
k_{inact}/K_i = 131,000 ± 15,000 $M^{-1}s^{-1}$

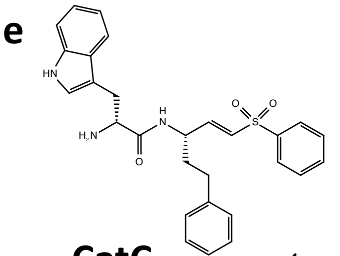
P2: Trp; P1: nLeu(o-Bzl)

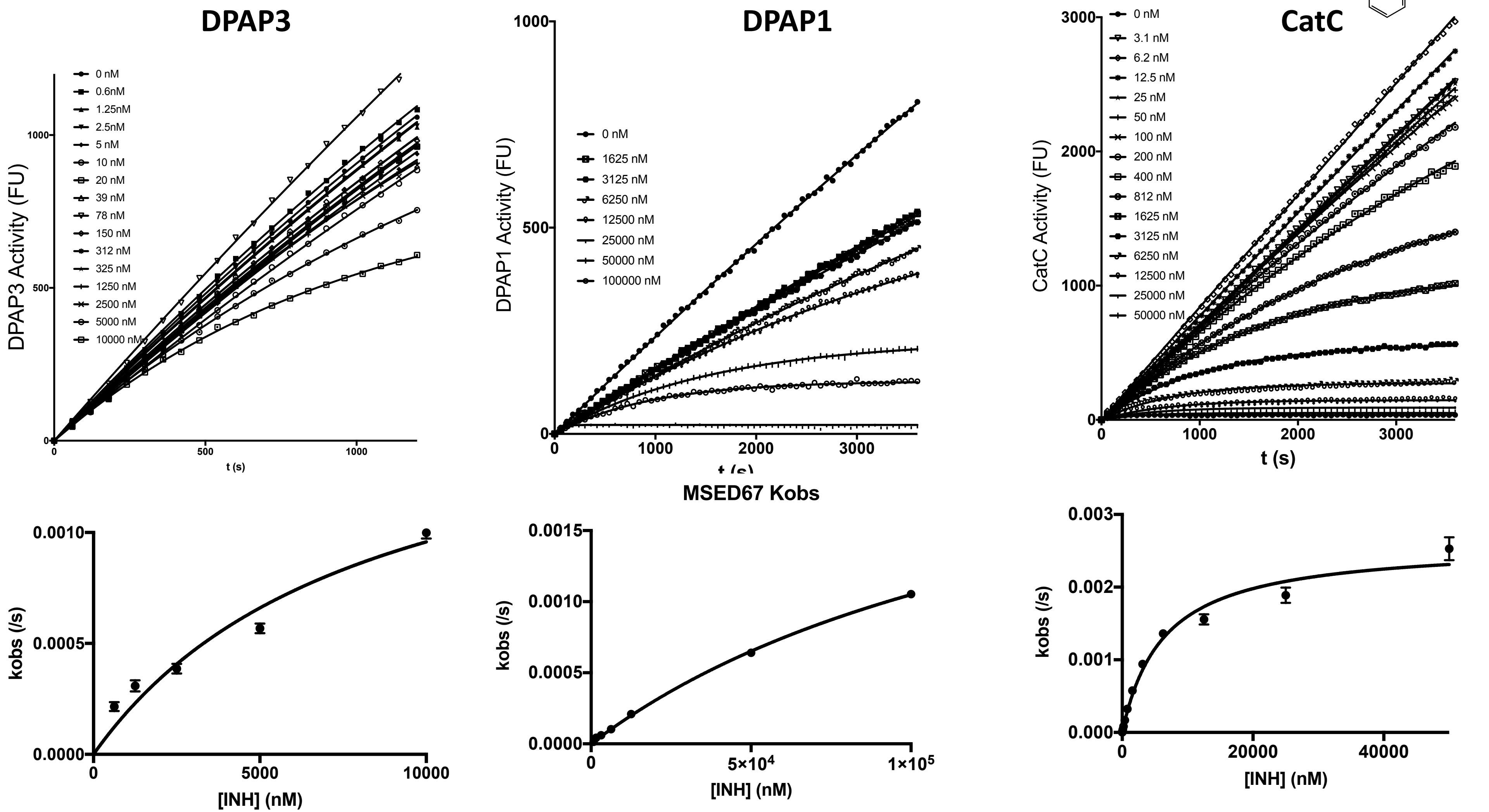




k_{inact} = 0.0032 ± 0.0003 s^{-1}	k_{inact} = 0.0022 ± 0.0001 s^{-1}	k_{inact} = 0.00174 ± 0.00003 s^{-1}
K_i = 4 ± 1 nM	K_i = 380 ± 60 nM	K_i = 4.9 ± 0.3 nM
k_{inact}/K_i = 826,000 ± 193,000 $M^{-1}s^{-1}$	k_{inact}/K_i = 5,800 ± 600 $M^{-1}s^{-1}$	k_{inact}/K_i = 355,000 ± 18,000 $M^{-1}s^{-1}$

P2: *D*-Trp; P1: hPhe



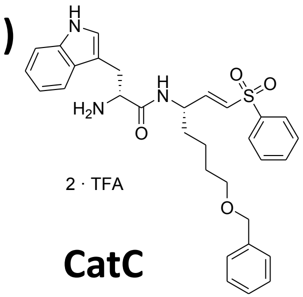


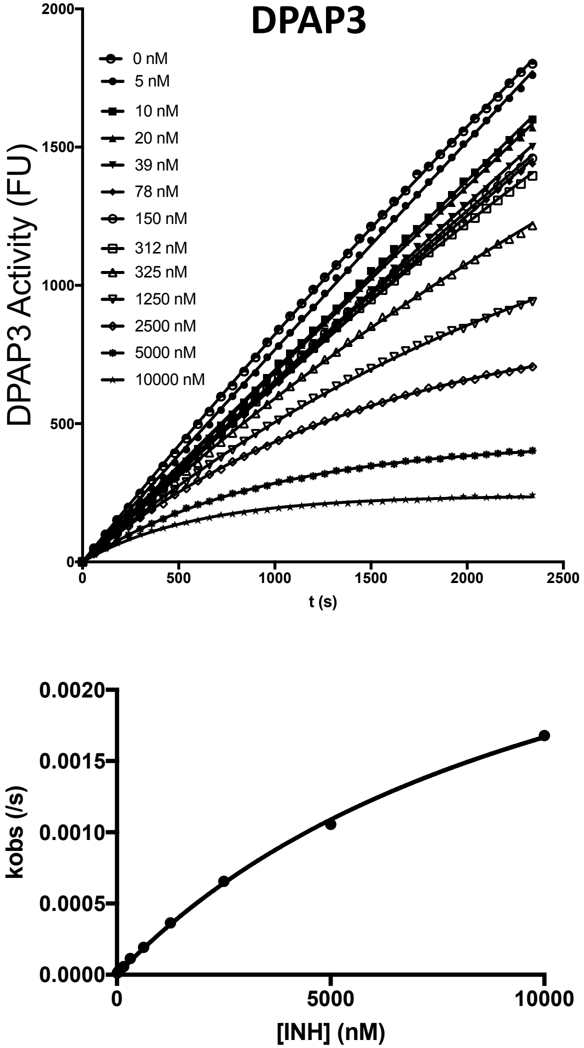
k_{inact} = 0.0017 ± 0.0006 s^{-1}
K_i = 5,500 ± 3,000 nM
k_{inact}/K_i = 320 ± 80 $M^{-1}s^{-1}$

k_{inact} = 0.0027 ± 0.0002 s^{-1}
K_i = 105,000 ± 10,000 nM
k_{inact}/K_i = 26 ± 1 $M^{-1}s^{-1}$

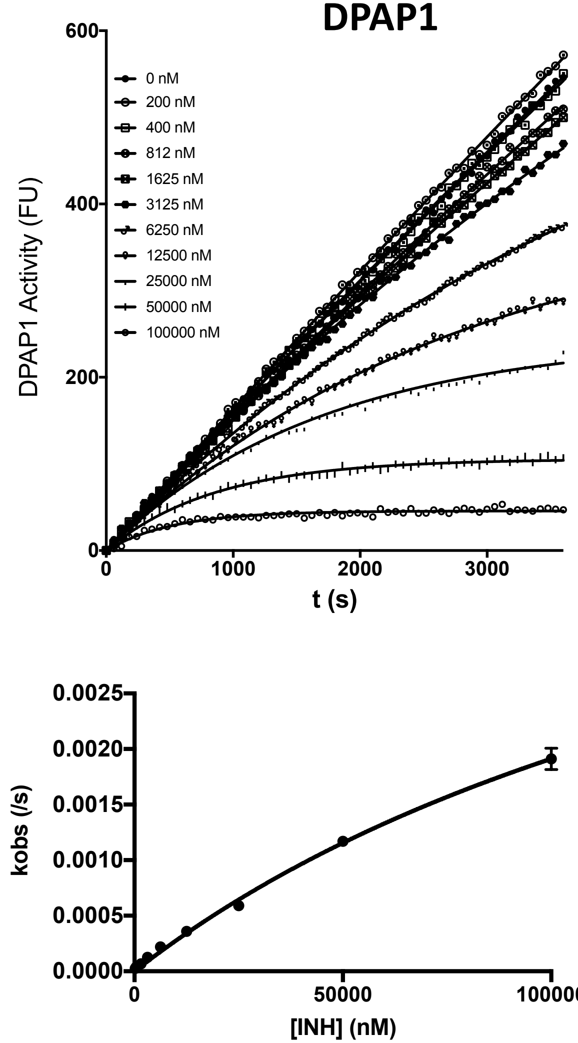
k_{inact} = 0.0026 ± 0.0001 s^{-1}
K_i = 4,200 ± 600 nM
k_{inact}/K_i = 620 ± 70 $M^{-1}s^{-1}$

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

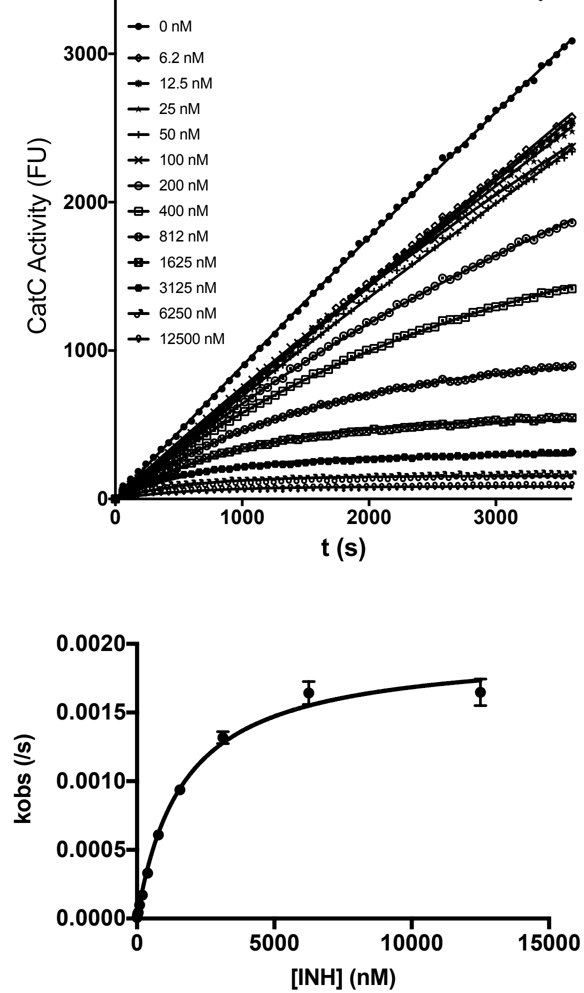




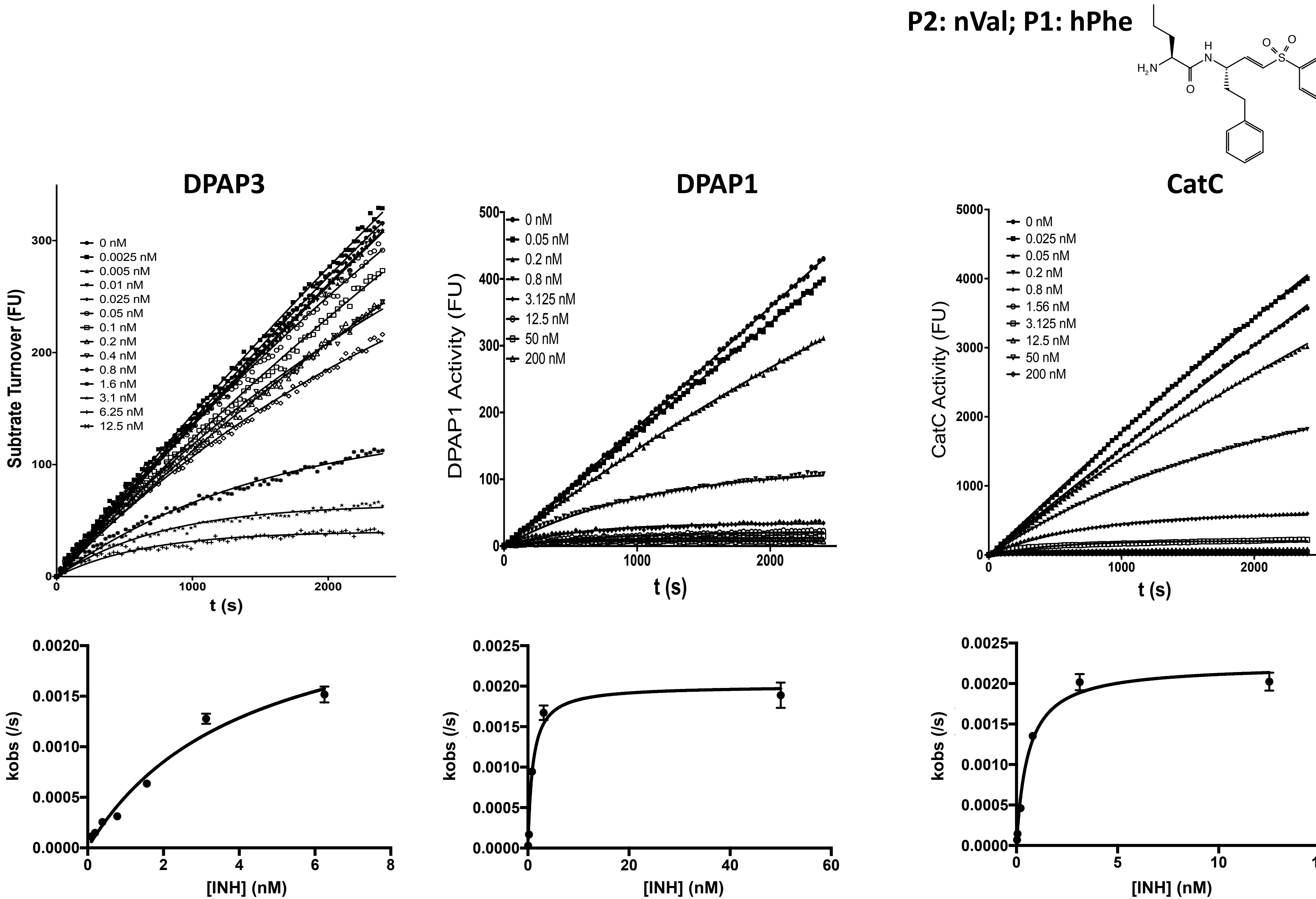
k_{inact} = 0.0036 ± 0.0002 s^{-1}
K_i = 7,600 ± 600 nM
k_{inact}/K_i = 470 ± 13 $M^{-1}s^{-1}$



k_{inact} = 0.0055 ± 0.0007 s^{-1}
K_i = 127,000 ± 20,000 nM
k_{inact}/K_i = 44 ± 2 $M^{-1}s^{-1}$



k_{inact} = 0.00197 ± 0.00006 s^{-1}
K_i = 1,100 ± 100 nM
k_{inact}/K_i = 1700 ± 100 $M^{-1}s^{-1}$



DPAP3

k_{inact} = 0.0026 ± 0.0004 s^{-1}
K_i = 2.8 ± 0.9 nM
k_{inact}/K_i = 940,000 ± 160,000 $M^{-1}s^{-1}$

DPAP1

k_{inact} = 0.0020 ± 0.0001 s^{-1}
K_i = 0.6 ± 0.2 nM
k_{inact}/K_i = 3,160,000 ± 750,000 $M^{-1}s^{-1}$

CatC

k_{inact} = 0.0022 ± 0.0001 s^{-1}
K_i = 0.38 ± 0.08 nM
k_{inact}/K_i = 5,830,000 ± 970,000 $M^{-1}s^{-1}$