SUPPLEMENTARY MATERIALS

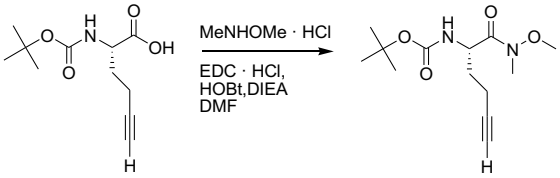
Supplementary Methods

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*-dmiethylformamide (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, 30µl) 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.

Synthesis of vinyl sulfone inhibitors.

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



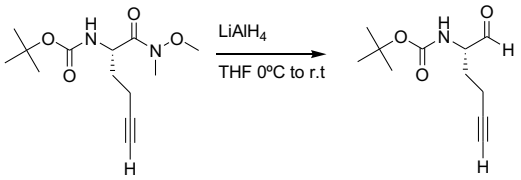
To a solution of (S)-2-(Boc-amino)-5-hexynoic acid (0.6 g, 2.64 mmol) in dry DMF (14 mL) at 0 °C was added EDC (607 mg, 3.17 mmol), HOBt (427 mg, 3.17 mmol), N,O-dimethylhydroxylamine hydrochloride (308 mg, 3.17 mmol) and DIPEA (0.7 mL, 7.92 mmol). The reaction was stirred at rt for 12 h. The resulting 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



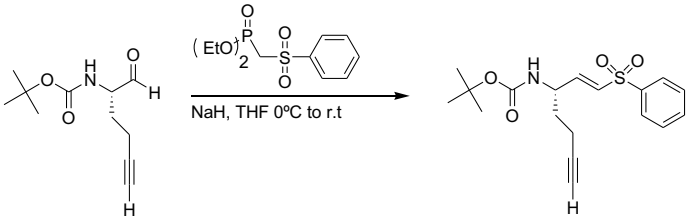
To a solution of *tert*-Butyl (S)-1-(N-methoxy-N-methylcarbamoyl)pent-4-ynylcarbamate (0.62 g, 2.3 mmol) in dry THF (23 mL) at 0 °C was added $LiAlH_4$ (0.1 g, 2.76 mmol) over 10 min, with vigorous stirring. The mixture was stirred for an additional 20 min at 0 °C, whereupon cold water was carefully 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**



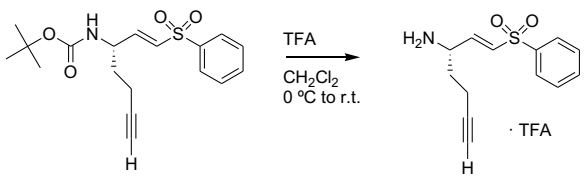
To a cooled (0°C) suspension of hexane-washed NaH (60% in mineral oil; 0.1 g, 2.38 mmol) in dry THF (10 mL) was added drop-wise diethyl[benzenesulfonyl)methyl]phosphonate (0.64 g, 2.18 mmol) in dry THF (10 mL) via syringe. The mixture was stirred for an additional 30 min at 0 °C and *tert*-butyl (S)-1-formylpent-4-ynylcarbamate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

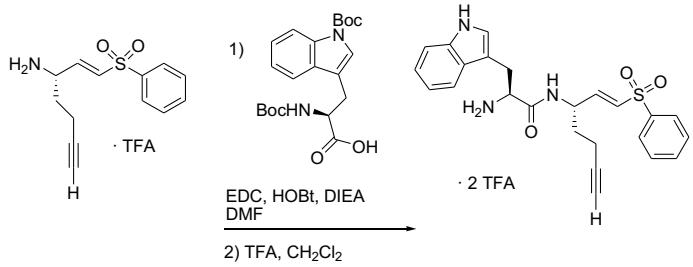


^{1}H NMR (*MeOD-d_4* δ): 1.90-2.07 (m, 2H), 2.20-2.31 (m, 2H), 2.40 (t, J = 2.6 Hz, 1H), 4.14 (dt, J = 7.9, 6.0 Hz, 1H), 6.88 (dd, J = 15.2, 7.4 Hz, 1H), 7.02 (d, J = 15.2 Hz, 1H), 7.61-7.74 (m, 3H), 7.92-7.95 (m, 2H).

^{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)

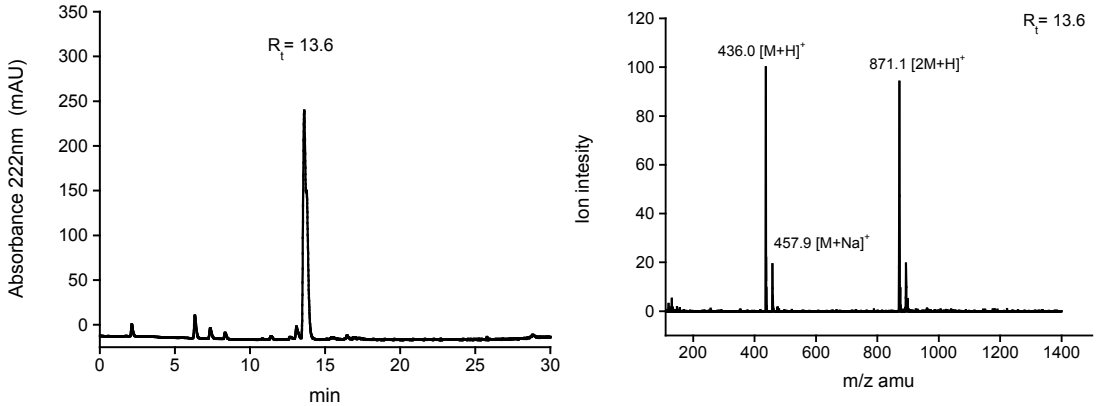
(2S)-2-Amino-3-(1H-indol-3-yl)-N-((S,E)-1-(phenylsulfonyl)hept-1-en-6-yn-3-yl)propanamide (Trp-hPG-VS)



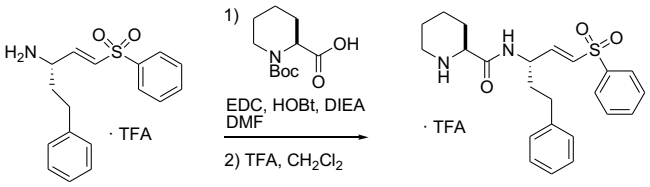
^{1}H NMR (*MeOD$_4$* δ): 1.73-1.86 (m, 2H), 2.21 (dt, *J* = 7.2, 2.6 Hz, 2H),2.28 (bs, 1H), 3.20-3.35 (m, 2H, partial overlapping with MeOD signal), 4.12 (dd, *J* = 7.80, 7.15 Hz, 1H), 4.70 (dd, *J* = 13.3, 4.8 Hz, 1H), 6.15 (dd, *J* = 15.1, 1.5 Hz, 1H), 6.78 (dd, *J* = 15.1, 5.4 Hz, 1H), 7.05-7.08 (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)



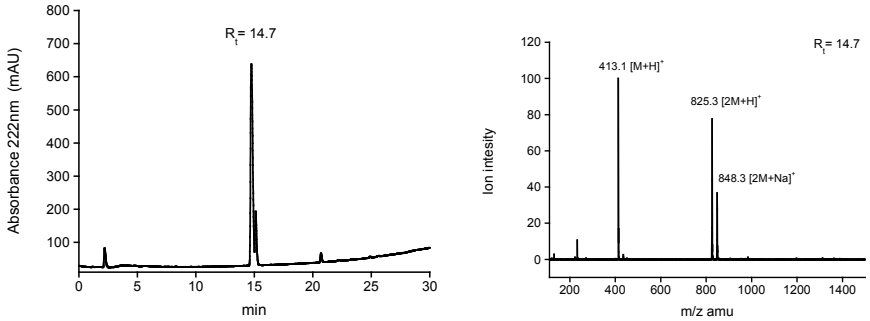
To a solution of (S)-N-Boc-Piperidine-2-carboxylic acid (211 mg, 0.92 mmol) in DMF (3 mL) was added EDC/HCl (177 mg, 0.92 mmol), HOBt (125 mg,0.92 mmol) and DIEA (0.6 mL, 3.1 mmol). After 10 min, (S)-3-benzenesulfonyl-1-phenethylallylamine trifluoroacetate (TFA·HphVSPh, X) (320 mg, 0.77 mmol) in DMF (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%).

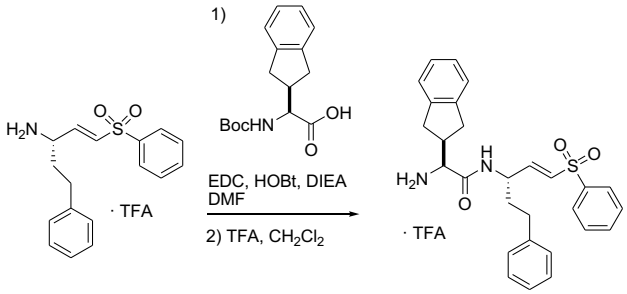
^{1}H NMR (*MeOD-d$_4$ δ*): 1.59-1.69 (m, 3H), 1.84-1.99 (m, 4H), 2.19 (d, *J* = 9.5 Hz, 1H),2.57-2.67 (m, 2H), 3.01 (t, *J* = 10.9 Hz, 1H), 3.38 (d, *J* = 12.6 Hz, 1H), 3.82 (dd, *J* = 11.1, 2.8 Hz, 1H), 4.57 (dd, *J* = 13.9, 5.7 Hz, 1H), 6.56 (d, *J* = 15.2 Hz, 1H), 6.91 (dd, *J* = 15.1, 5.9 Hz, 1H), 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)



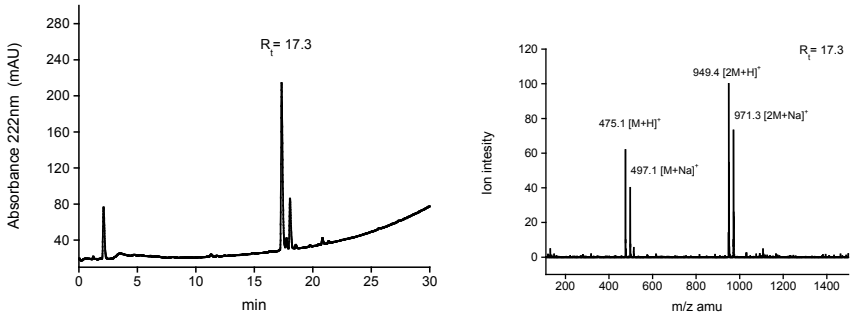
(2S)-2-amino-2-(2,3-dihydro-1H-inden-2-yl)-N-((S,E)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)acetamide Lm1msed44 **(Igl-hPhe-VS)**



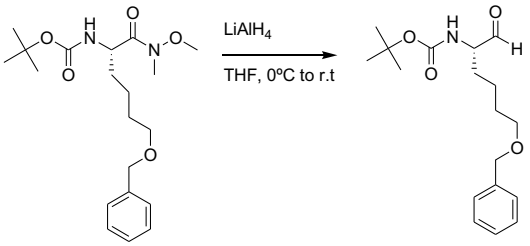
^{1}H NMR (*MeOD-d$_4$ δ):* 1.96 (td, *J* = 15.3, 7.5 Hz, 2H), 2.66 (dd, *J* = 15.6, 8.3 Hz, 2H), 2.88 (t, *J* = 5.60 Hz, 4H), 3.00-3.06 (m, 1H), 3.95 (dd, *J* = 5.4, 1.2 Hz, 1H), 4.60 (q, *J* = 6.8 Hz, 1H), 6.64 (d, *J* = 15.2 Hz, 1H), 6.89 (dd, *J* = 15.1, 6.6 Hz, 1H), 7.16 (bs, 7H), 7.24 (d, *J* =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

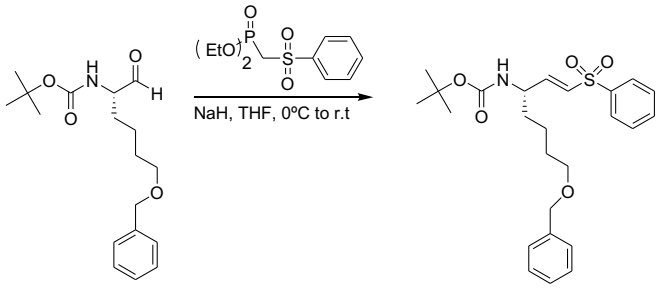
the gelatinous emulsion until pH 6~7. Upon dilution with H_2O (150 mL) and extraction with EtOAc (3 x 75 mL), the combined organic extracts dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by flash column chromatography (silica gel; using 40% EtOAc in hexanes) provided the product *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



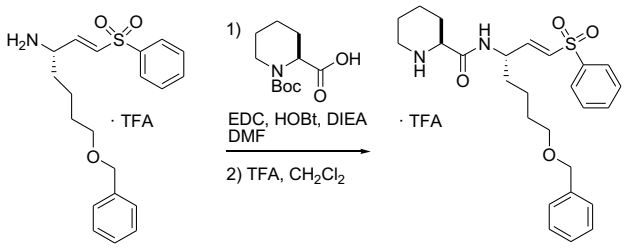
To a cooled (0°C) suspension of hexane-washed NaH (60% in mineral oil; 0.36 g, 9 mmol) in dry THF (50 mL) was added drop-wise DIETHYL[BENZENESULFONYL)METHYL]PHOSPHONATE (2.4 g, 8.2 mmol) in dry THF (10 mL) via syringe. The mixture was stirred for an additional 30 min at 0 °C and *tert*-butyl (S)-5-(benzyloxy)-1-formylpentylcarbamate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

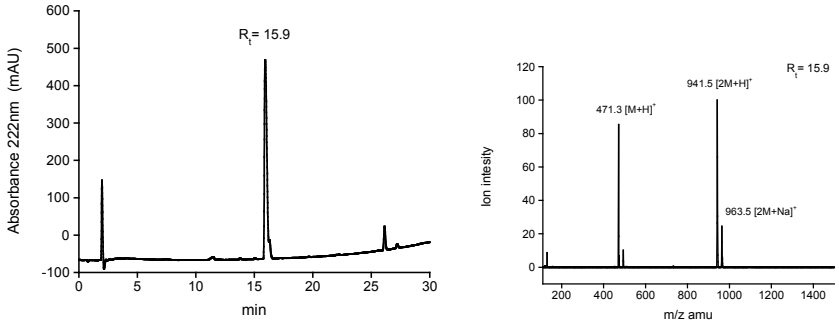
(2S)-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)piperidine-2-carboxamide Lm1msed55 **(hPro-nLeu(O-Bzl)-VS)**



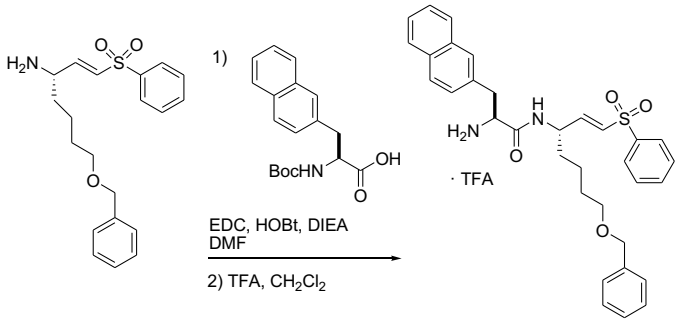
^{1}H NMR (*MeOD-d$_4$* δ):1.35-1.45 (m, 2H) , 1.56-1.69 (m, 7H), 1.82-1.88 (m, 2H), .2.14 (d, J = 9.5 Hz, 1H), 2.97 (t, J = 12.4 Hz, 1H), 3.36 (d, J = 12.9 Hz, 1H), 3.45 (t, J = 6.3 Hz, 2H), 3.78 (dd, J = 11.2, 2.9 Hz, 1H), 4.46 (s, 2H), 4.56 (dd, J = 13.3, 5.9 Hz, 1H), 6.56 (d, J = 15.2 Hz, 1H), 6.89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

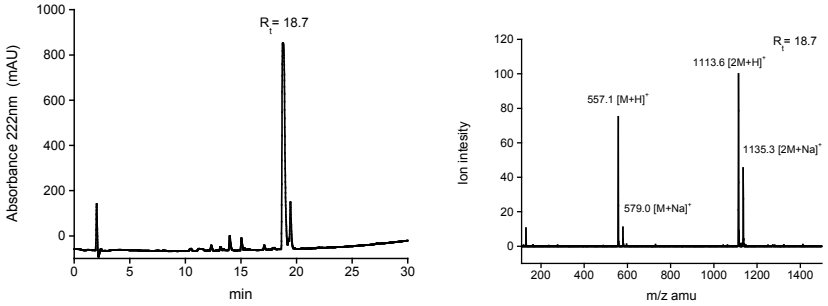


^{1}H NMR (*MeOD-d$_4$* δ): 1.29-1.37 (m, 2H), 1.50-1.61 (m, 4H), 3.18-3.28 (m, 3H), 3.41 (t, J = 6.3 Hz, 1H), 4.15 (t, J = 7.3 Hz, 1H), 4.53 (q, J = 6.4 Hz, 1H), 4.40 (s, 2H), 6.27 (d, J = 15.2 Hz, 1H), 6.74 (dd, J

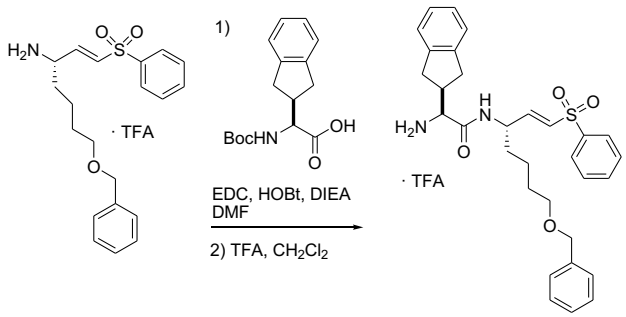
= 15.2, 5.8 Hz, 1H), 7.30 (bs, 6H), 7.51 (bs, 4H), 7.63 (t, J = 7.4 Hz, 1H), 7.69-7.74 (m 3H), 7.83-7.87 (m, 3H).

^{13}C NMR (*MeOD-d_4 δ*): 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)



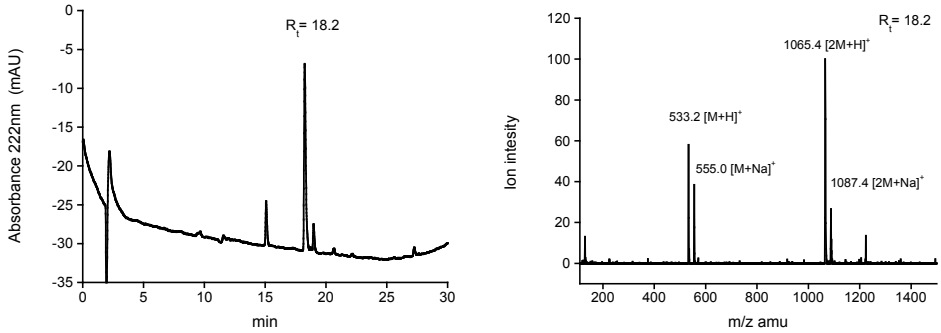
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-2-(2,3-dihydro-1H-inden-2-yl)acetamide Lm1msed57 **(Igl-nLeu(O-Bzl)-VS)**



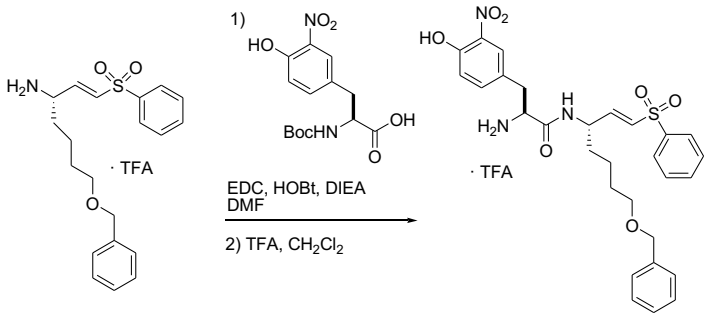
^{1}H NMR (*MeOD-d_4 δ*): 1.37-1.47 (m, 2H), 1.58-1.70 (m, 4H), 2.84 (dd, J = 18.7, 5.2 Hz, 4H), 2.99-3.04 (m, 1H), 3.47 (t, J = 6.2 Hz, 2H), 3.89 (d, J = 6.8 Hz, 1H), 4.47 (s, 2H), 4.58 (dd, J = 13.9, 6.8 Hz, 1H), 6.63 (d, J = 15.1 Hz, 1H), 6.86 (dd, J = 15.1, 6.5 Hz, 1H), 7.16 (bs, 4H), 7.32 (bs, 5H), 7.54 (t, J = 7.7 Hz, 2H), 7.60-7.67 (m, 1H), 7.84 (d, J = 7.8 Hz, 2H).

^{13}C NMR (*MeOD-d_4 δ*): 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)



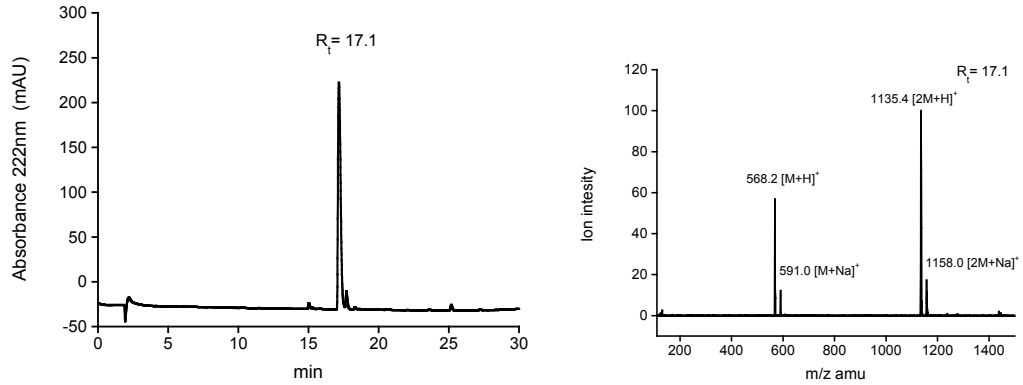
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(4-hydroxy-3-nitrophenyl)propanamide Lm1msed58 **(Tyr(NO2)-nLeu(O-Bzl)-VS)**



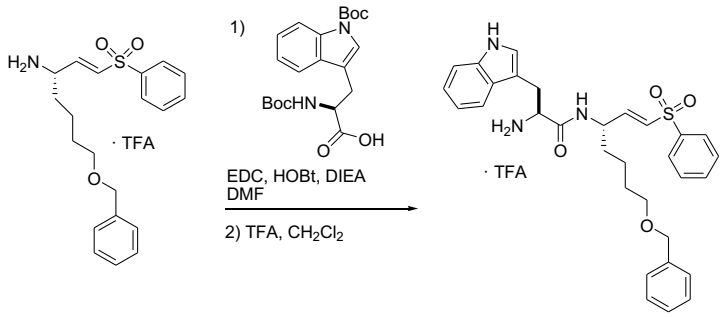
^{1}H NMR (*MeOD-d$_4$* δ): 1.35-1.41 (m, 2H), 1.54-1.63 (m, 4H), 3.10 (dq, *J* = 14.1, 7.4 Hz, 2H), 3.45 (t, *J* = 6.2 Hz, 2H), 4.05 (t, *J* = 7.3 Hz, 1H), 4.45 (s, 2H), 4.55 (dd, *J* = 12.9, 6.3 Hz, 1H), 6.25 (d, *J* = 15.2 Hz, 1H), 6.72 (dd, *J* = 15.2, 5.8 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 7.30 (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).

^{13}C NMR (*MeOD-d$_4$* δ): 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)



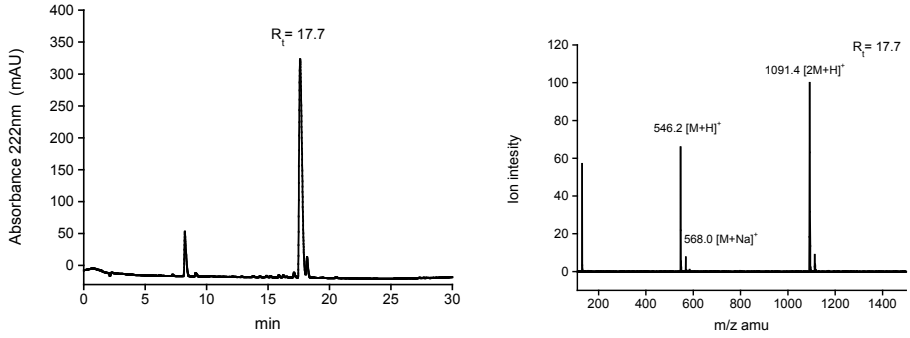
(2S)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(1H-indol-3-yl)propanamide Lm1msed68 **(Trp-nLeu(O-Bzl)-VS)**



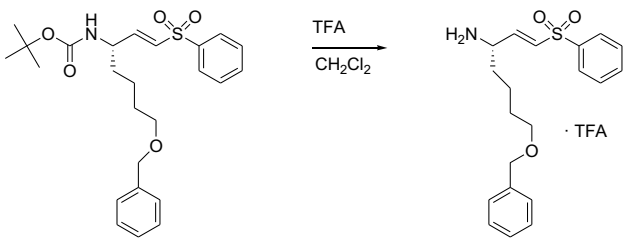
^{1}H NMR (*MeOD-d$_4$* δ): 1.31-1.37 (m, 2H), 1.49-1.59 (m, 4H), 3.20 (dd, J = 14.5, 7.0 Hz, 1H), 3.34 (d, J = 7.8 Hz, 1H), 3.43 (t, J = 6.2 Hz, 2H), 4.09 (t, J = 7.4 Hz, 1H),4.44 (s, 2H), 4.52 (q, J = 6.2 Hz, 1H), 6.12 (d, J = 15.2 Hz, 1H), 6.73 (dd, J = 15.2, 5.5 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H),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

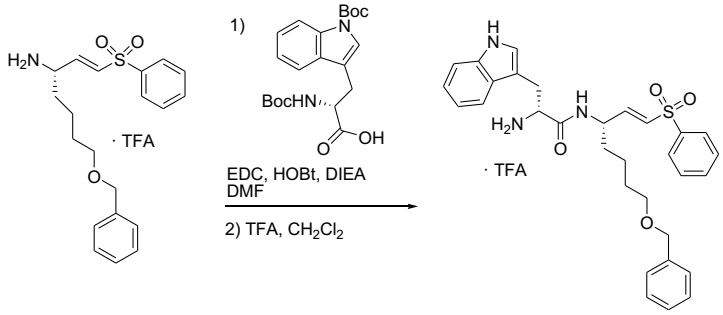
with CH_2Cl_2 and finally dried in vacuo to give (S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-amine as a trifluoroacetic salt (0.2 g; 0.43 mmol, 99%). This material was pure enough to be used in the next step without further purification.

^{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)

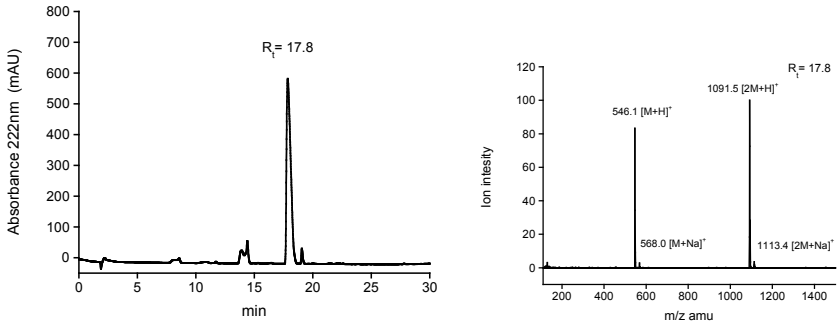
(2R)-2-amino-N-((S,E)-7-(benzyloxy)-1-(phenylsulfonyl)hept-1-en-3-yl)-3-(1H-indol-3-yl)propanamide Lm1msed71 **(*L*-Trp-nLeu(O-Bzl)-VS)**



^{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)



Suplemmentary Figures Legends.

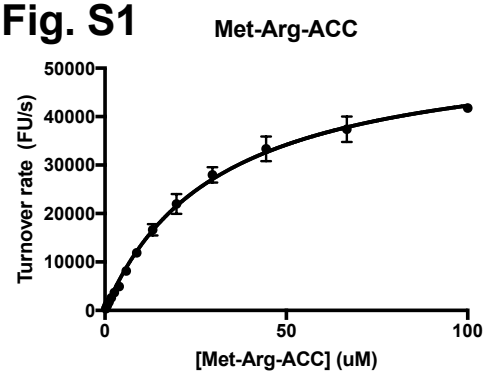
Figure S1. Michaelis Menten fits for rDPAP3. The turnover rate of the indicate substrate was measure at different substrate concentration and 1 nM rDPAP3 in assay buffer. Data was fitted in Prism to a Michaelis Menten model. K_m, k_{cat}, and k_{cat}/K_m values are reported in Table 1.

Figure S2. Irreversible inhibition fits for rDPAP3. The decrease in substrate turnover rate by rDPAP3 was measured over 40min at different concentration of the indicated inhibitor. Each progress at each inhibitor concentration was fitted to Eq. 2 to obtain k_{obs} value (upper left graph). These values were then fitted to Eqs 3 and 4 as a function of inhibitor concentration to obtain k_{inact}, K_i, and k_{inact}/K_i (Reported in Tables 2 and 3).

Figure S3. Irreversible inhibition fits for CatC and DPAP1. The decrease in substrate turnover rate by rDPAP3 was measured over 40min at different concentration of the indicated inhibitor. Each progress at each inhibitor concentration was fitted to Eq. 2 to obtain k_{obs} value (upper left graph). These values were then fitted to Eqs 3 and 4 as a function of inhibitor concentration to obtain k_{inact}, K_i, and k_{inact}/K_i (Reported in Tables 2 and 3).

Figure S4. High resolution mass spectra of DPAP fluorogenic substrates.

Fig. S1


Met-Arg-ACC
Turnover rate (FU/s)
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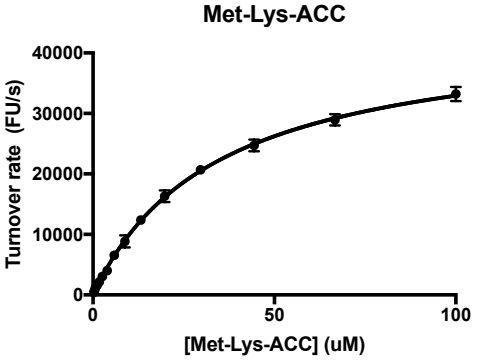
40000
30000
20000
10000
0
0
50
100
[Met-Arg-ACC] (uM)


Met-Lys-ACC
Turnover rate (FU/s)
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10000
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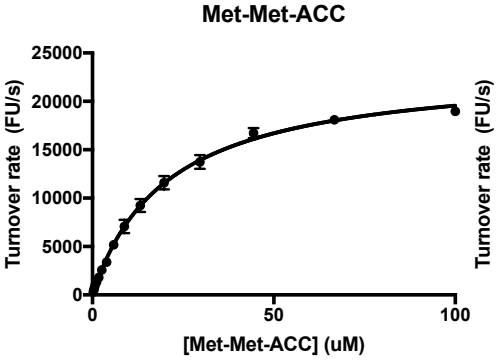
0
50
100
[Met-Lys-ACC] (uM)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Turnover rate (FU/s)
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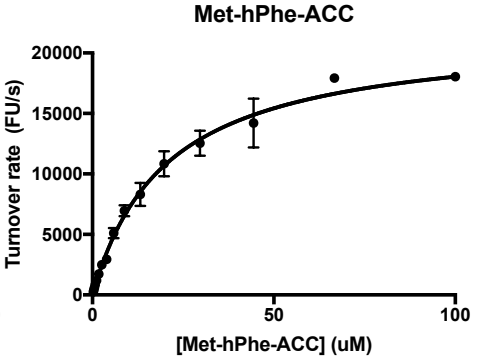
15000
10000
5000
0
0
50
100
[Met-Met-ACC] (uM)


Met-hPhe-ACC
Turnover rate (FU/s)
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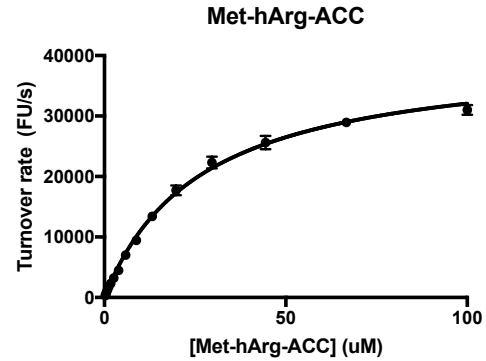
15000
10000
5000
0
0
50
100
[Met-hPhe-ACC] (uM)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Turnover rate (FU/s)
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20000
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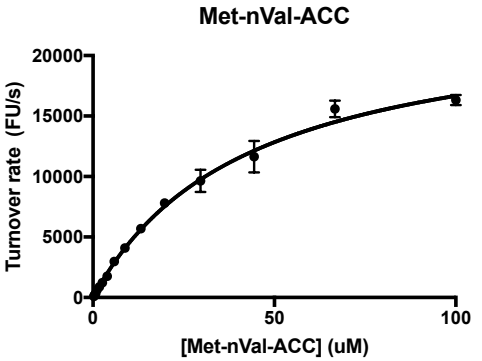
0
50
100
[Met-hArg-ACC] (uM)


Met-nVal-ACC
Turnover rate (FU/s)
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15000
10000
5000
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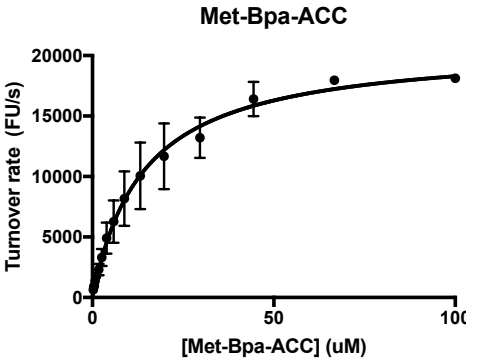
0
50
100
[Met-nVal-ACC] (uM)


Met-Bpa-ACC
Turnover rate (FU/s)
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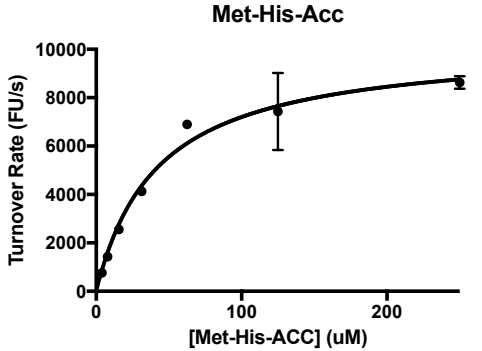
15000
10000
5000
0
0
50
[Met-Bpa-ACC] (uM)


Met-His-Acc
Turnover Rate (FU/s)
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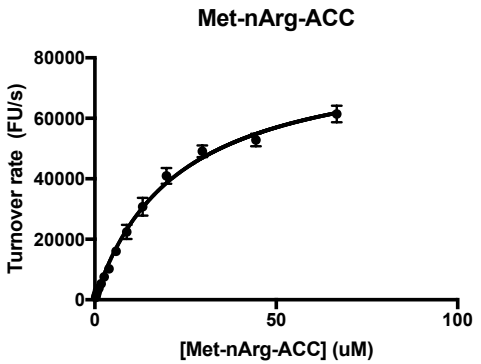
8000
6000
4000
2000
0
0
100
200
[Met-His-ACC] (uM)


Met-nArg-ACC
Turnover rate (FU/s)
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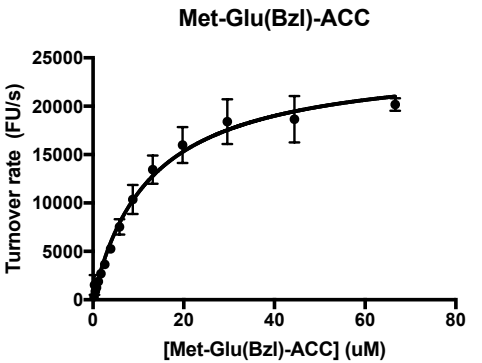
60000
40000
20000
0
0
50
100
[Met-nArg-ACC] (uM)


Met-Glu(Bzl)-ACC
Turnover rate (FU/s)
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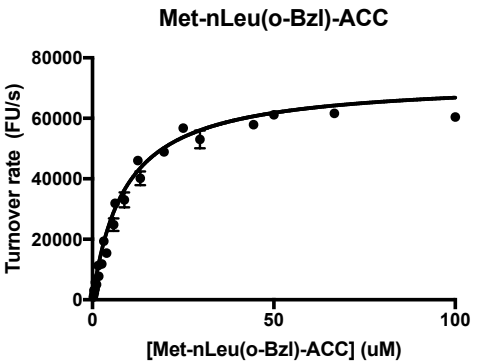
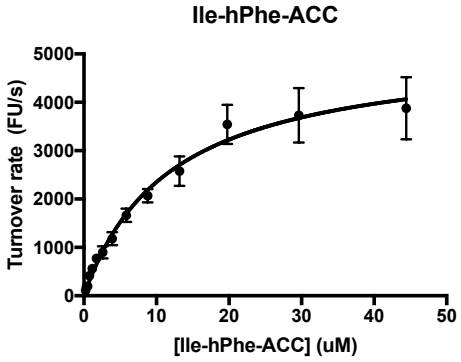
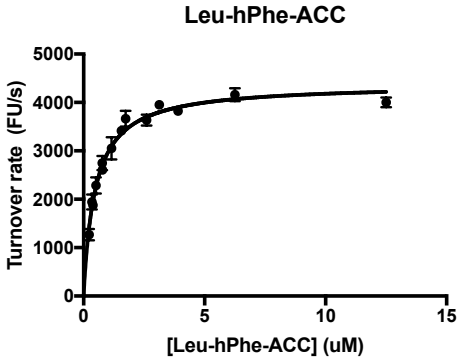
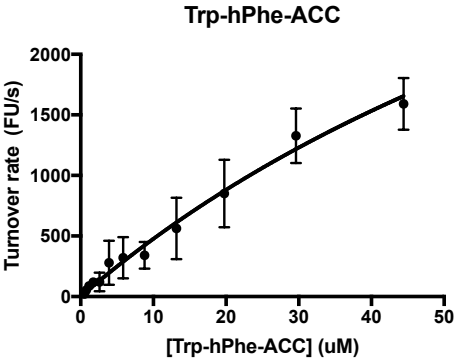
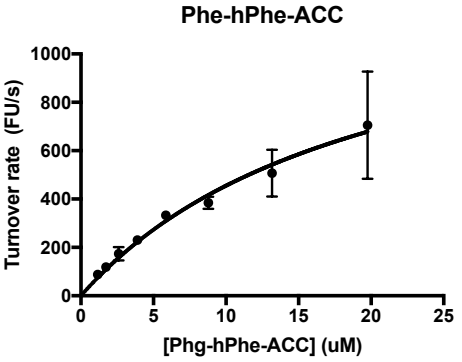
20000
15000
10000
5000
0
0
20
40
60
80
[Met-Glu(Bzl)-ACC] (uM)

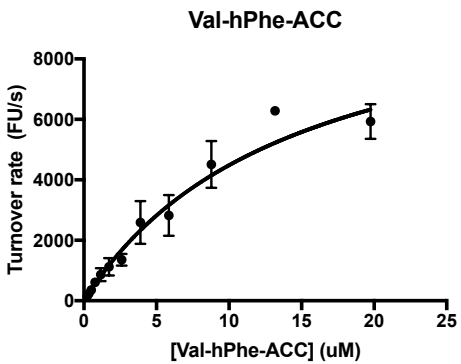

Met-nLeu(o-Bzl)-ACC
Turnover rate (FU/s)
80000
60000
40000
20000
0
0
50
100
[Met-nLeu(o-Bzl)-ACC] (uM)

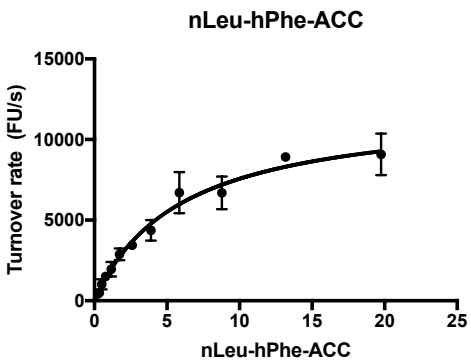

Ile-hPhe-ACC
Turnover rate (FU/s)
[Ile-hPhe-ACC] (uM)

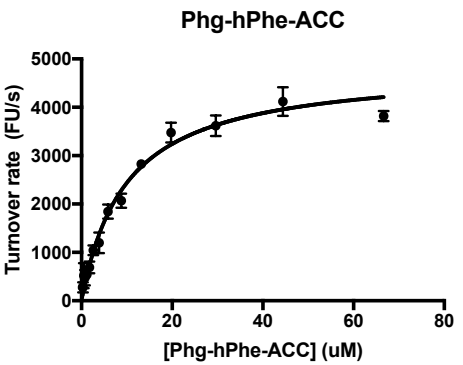

Leu-hPhe-ACC
Turnover rate (FU/s)
[Leu-hPhe-ACC] (uM)

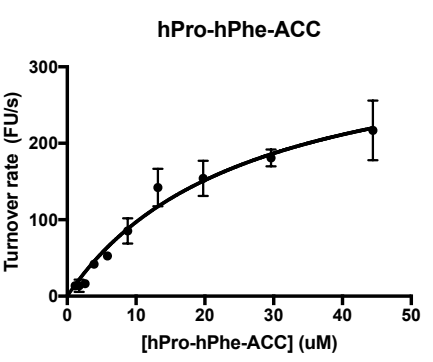

Trp-hPhe-ACC
Turnover rate (FU/s)
[Trp-hPhe-ACC] (uM)

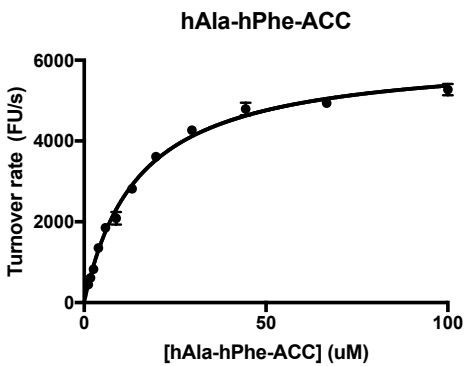

Phe-hPhe-ACC
Turnover rate (FU/s)
[Phg-hPhe-ACC] (uM)

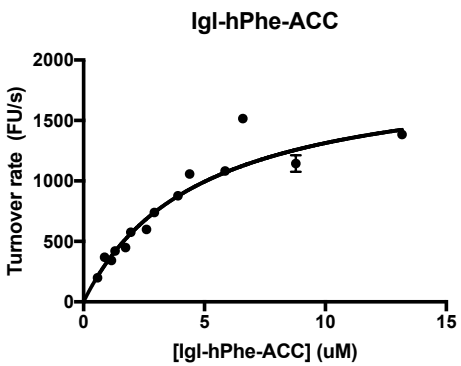

Val-hPhe-ACC
Turnover rate (FU/s)
[Val-hPhe-ACC] (uM)

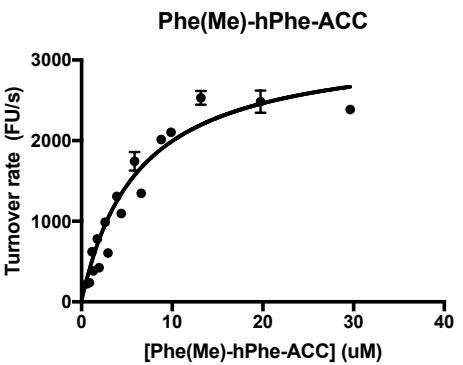

nLeu-hPhe-ACC
Turnover rate (FU/s)
nLeu-hPhe-ACC

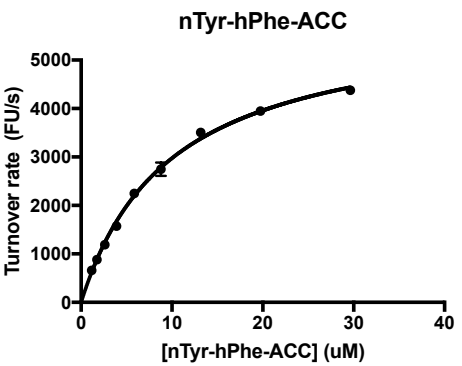

Phg-hPhe-ACC
Turnover rate (FU/s)
[Phg-hPhe-ACC] (uM)

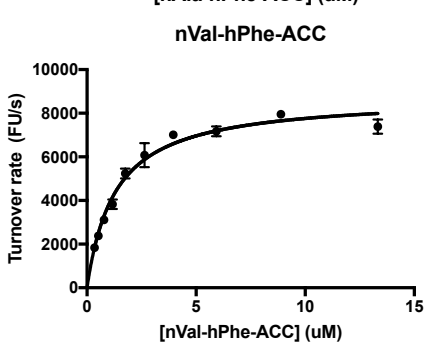

hPro-hPhe-ACC
Turnover rate (FU/s)
[hPro-hPhe-ACC] (uM)

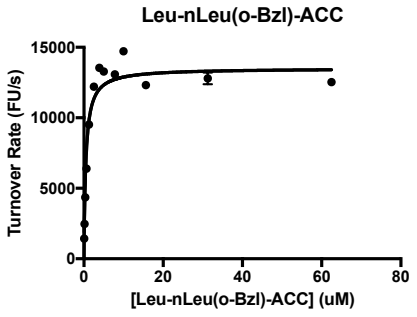

hAla-hPhe-ACC
Turnover rate (FU/s)
[hAla-hPhe-ACC] (uM)

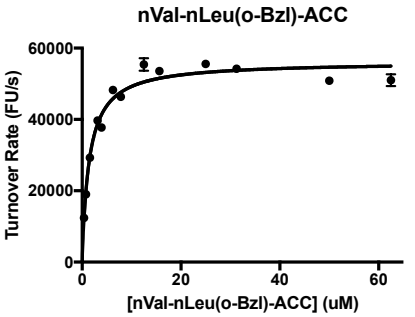

Igl-hPhe-ACC
Turnover rate (FU/s)
[Igl-hPhe-ACC] (uM)

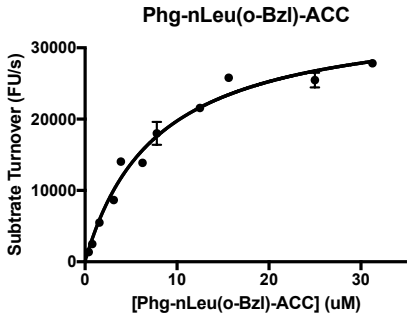

Phe(Me)-hPhe-ACC
Turnover rate (FU/s)
[Phe(Me)-hPhe-ACC] (uM)

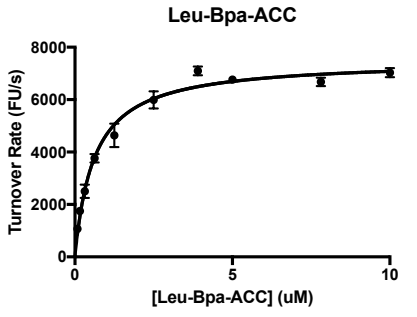

nTyr-hPhe-ACC
Turnover rate (FU/s)
[nTyr-hPhe-ACC] (uM)

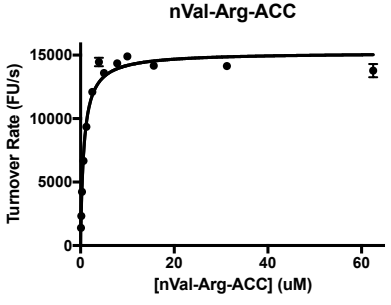

nVal-hPhe-ACC
Turnover rate (FU/s)
[nVal-hPhe-ACC] (uM)

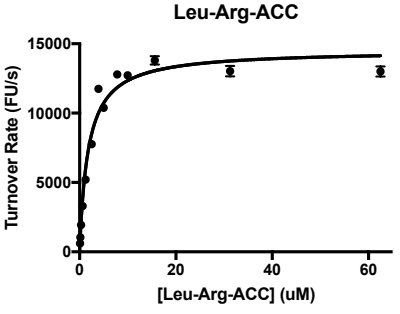

Leu-nLeu(o-Bzl)-ACC
Turnover Rate (FU/s)
15000
10000
5000
0
0
20
40
60
80
[Leu-nLeu(o-Bzl)-ACC] (uM)

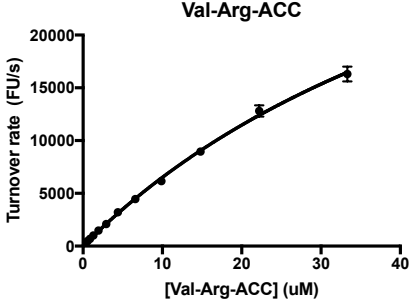

nVal-nLeu(o-Bzl)-ACC
Turnover Rate (FU/s)
60000
40000
20000
0
0
20
40
60
[nVal-nLeu(o-Bzl)-ACC] (uM)

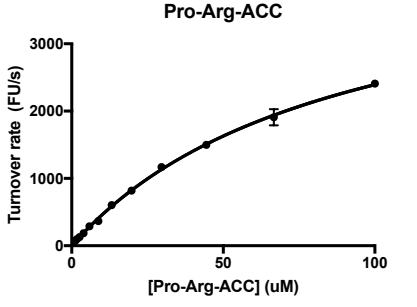

Phg-nLeu(o-Bzl)-ACC
Subtrate Turnover (FU/s)
30000
20000
10000
0
0
10
20
30
[Phg-nLeu(o-Bzl)-ACC] (uM)

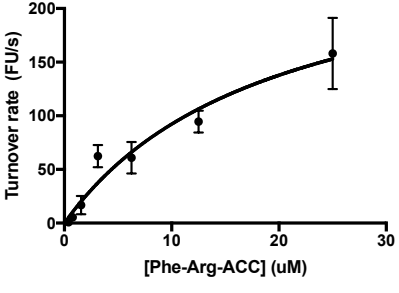

Leu-Bpa-ACC
Turnover Rate (FU/s)
8000
6000
4000
2000
0
0
5
10
[Leu-Bpa-ACC] (uM)


nVal-Arg-ACC
Turnover Rate (FU/s)
15000
10000
5000
0
0
20
40
60
[nVal-Arg-ACC] (uM)


Leu-Arg-ACC
Turnover Rate (FU/s)
15000
10000
5000
0
0
20
40
60
[Leu-Arg-ACC] (uM)


Val-Arg-ACC
Turnover rate (FU/s)
20000
15000
10000
5000
0
0
10
20
30
40
[Val-Arg-ACC] (uM)


Pro-Arg-ACC
Turnover rate (FU/s)
3000
2000
1000
0
0
50
100
[Pro-Arg-ACC] (uM)


Turnover rate (FU/s)
200
150
100
50
0
0
10
20
30
[Phe-Arg-ACC] (uM)

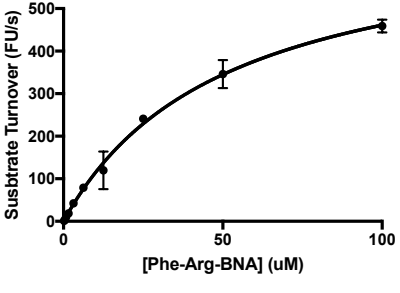
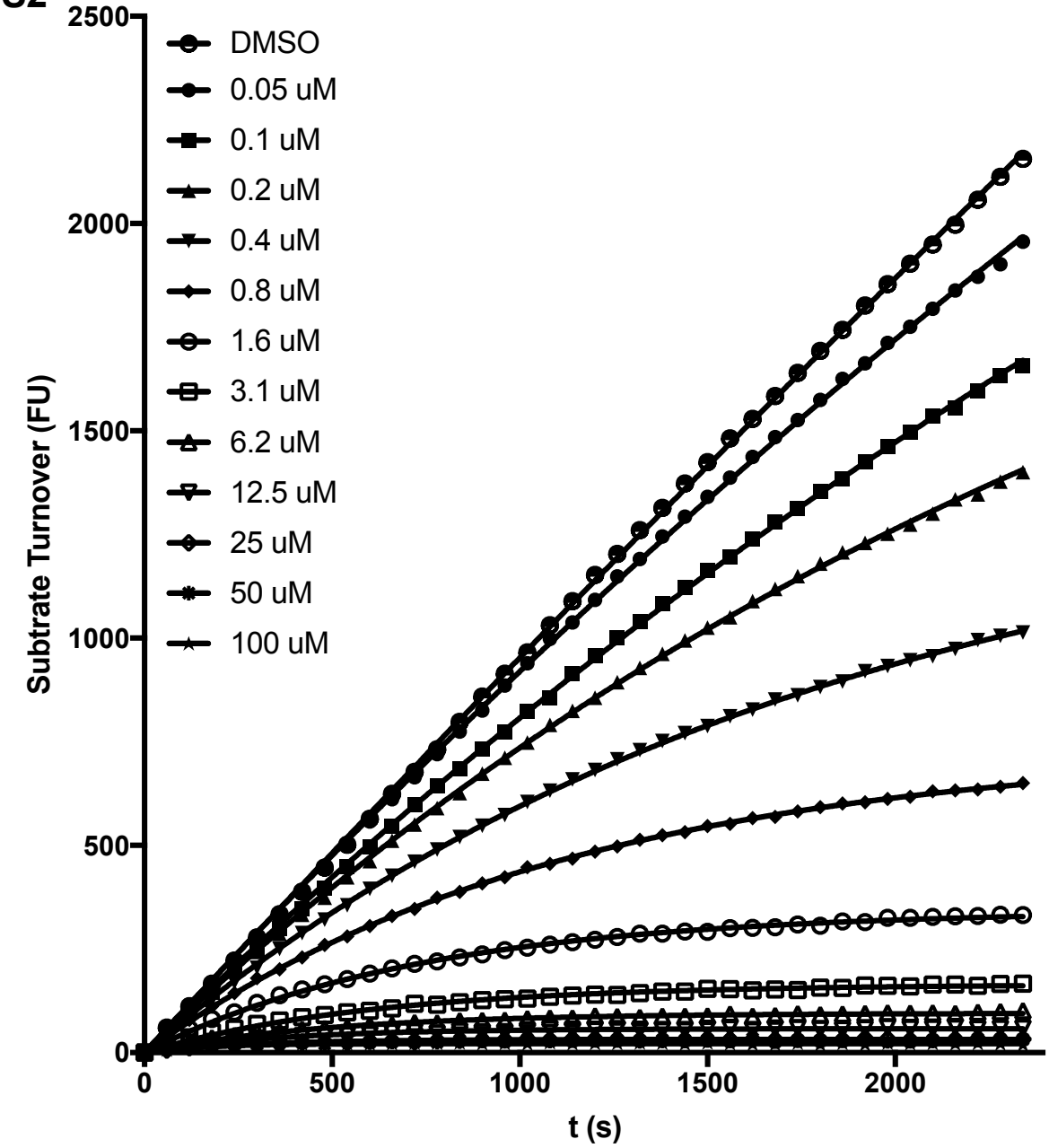
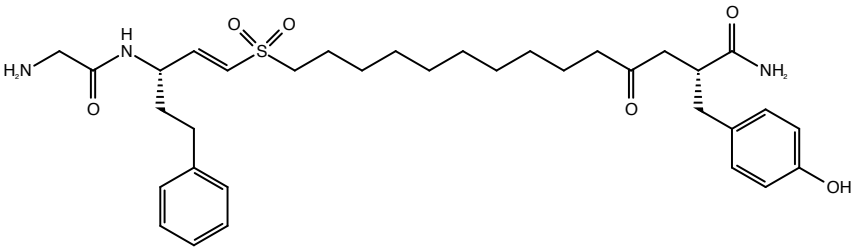
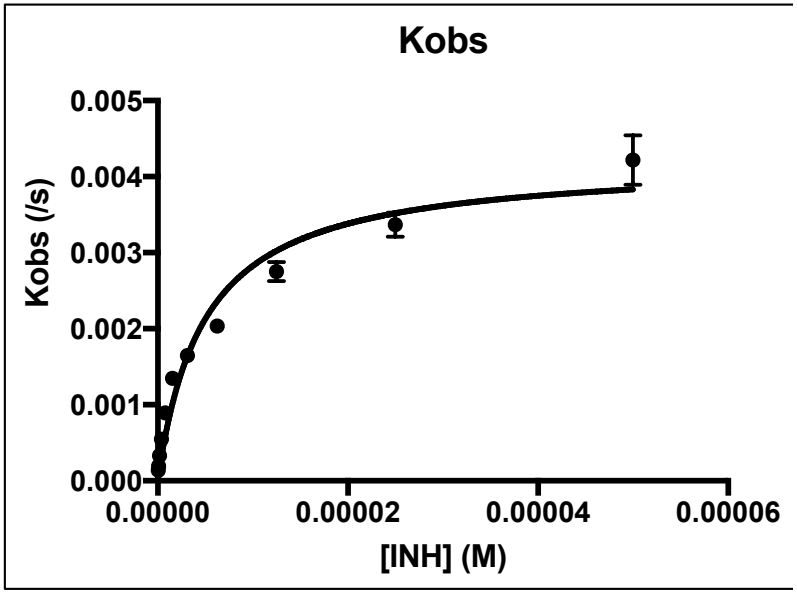

Susbtrate Turnover (FU/s)
500
400
300
200
100
0
0
50
100
[Phe-Arg-BNA] (uM)

Fig. S2

P2: Gly





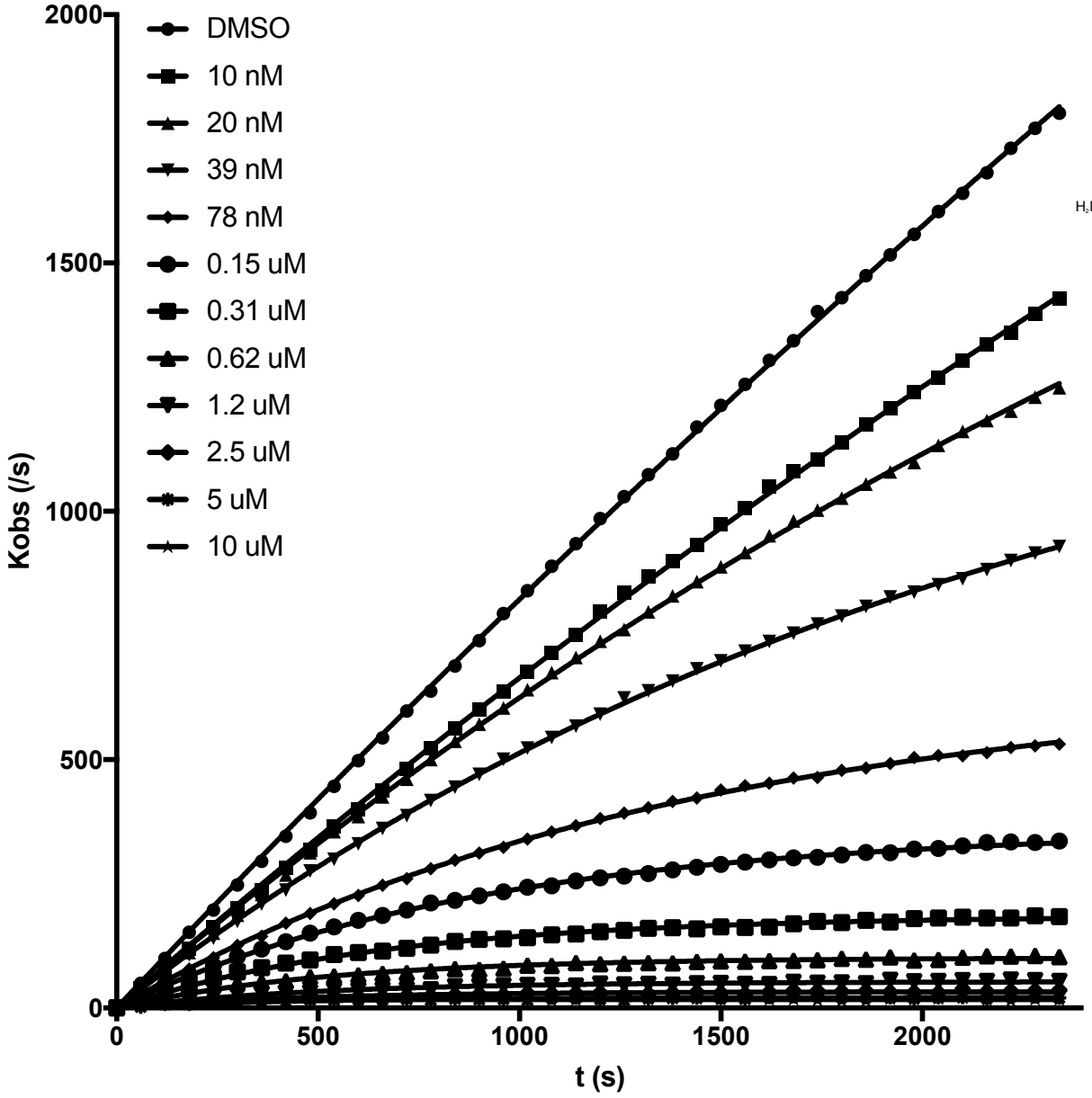


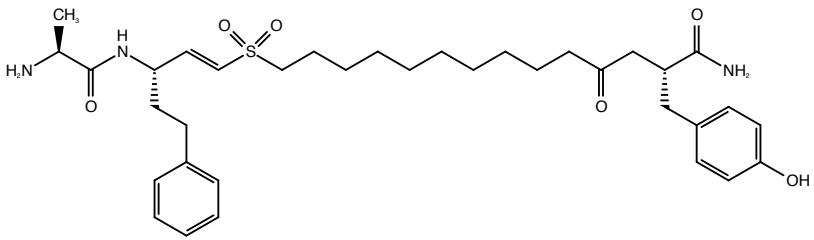
k_{inact} = 0.0042 ± 0.0003 s^{-1}

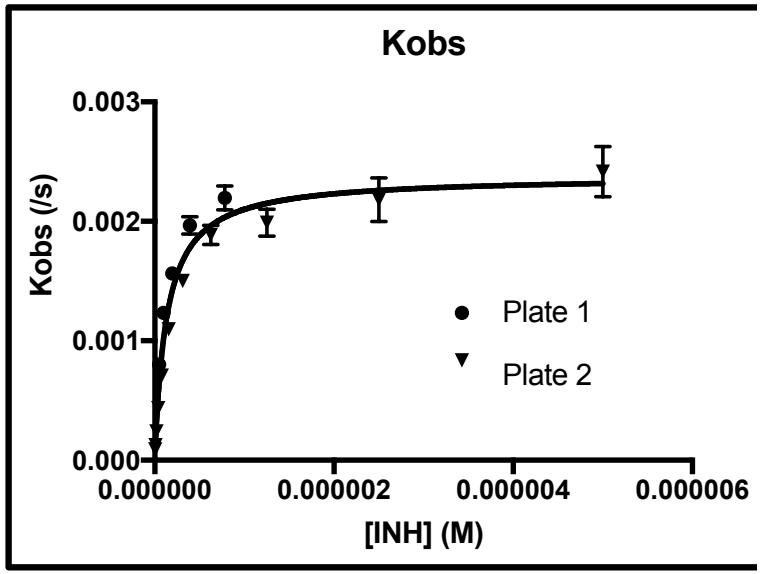
K_i = 3.3 ± 0.7 μM

K_{inact}/K_i = 1300 ± 230 $M^{-1}s^{-1}$

P2: Ala





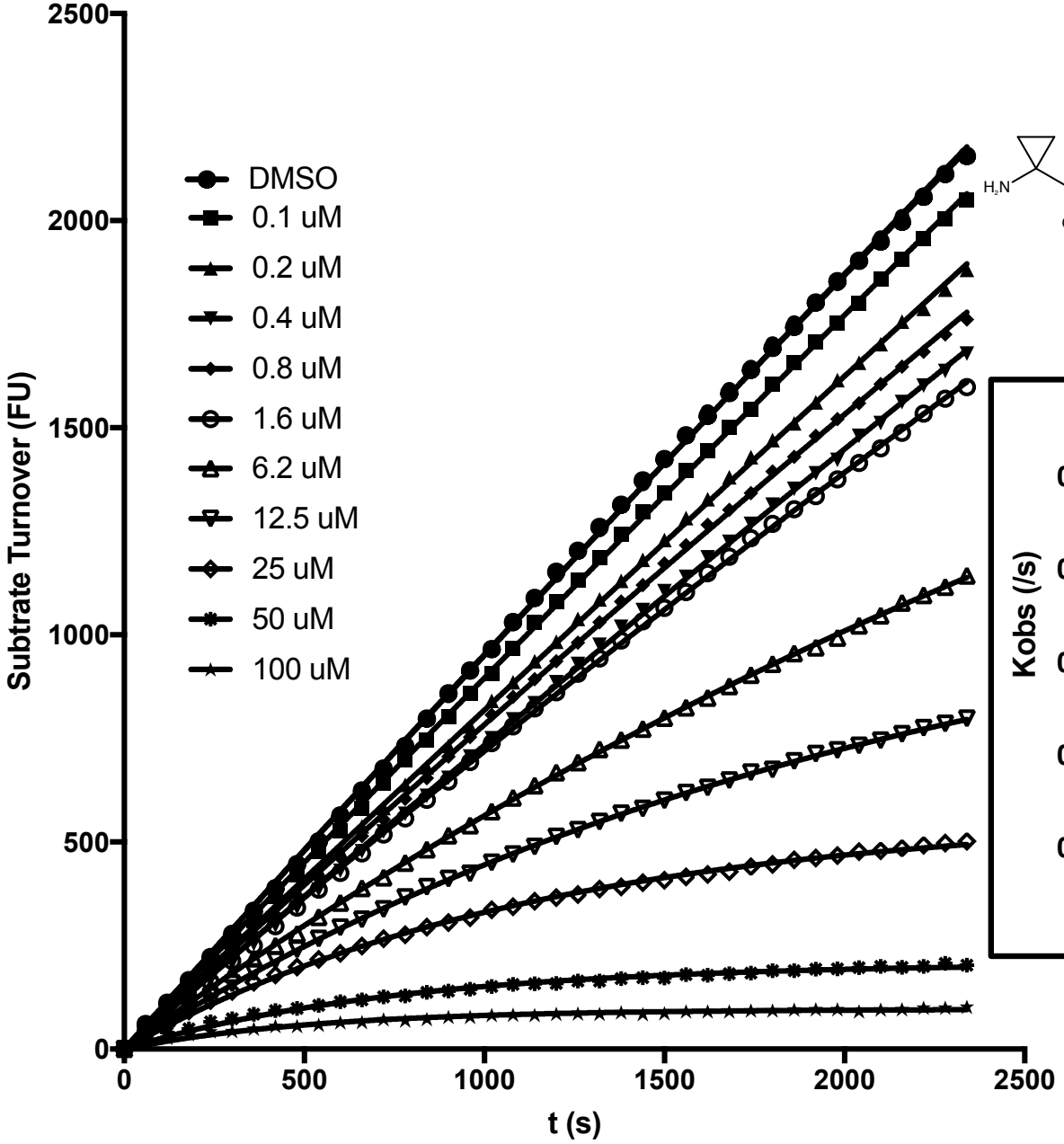


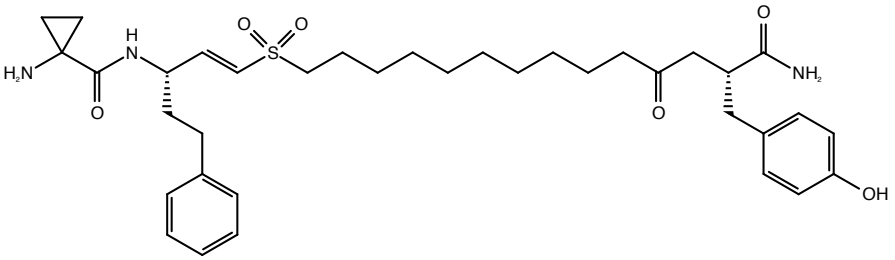
k_{inact} = 0.0024 ± 0.0001 s^{-1}

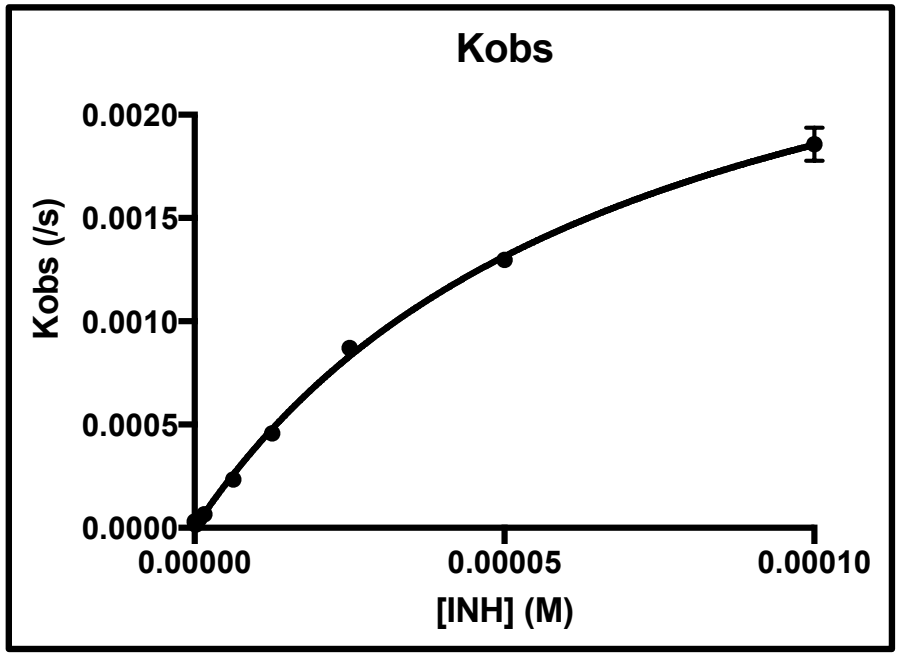
K_i = 88 ± 13 nM

K_{inact}/K_i = 27000 ± 3100 $M^{-1}s^{-1}$

P2: ACPC





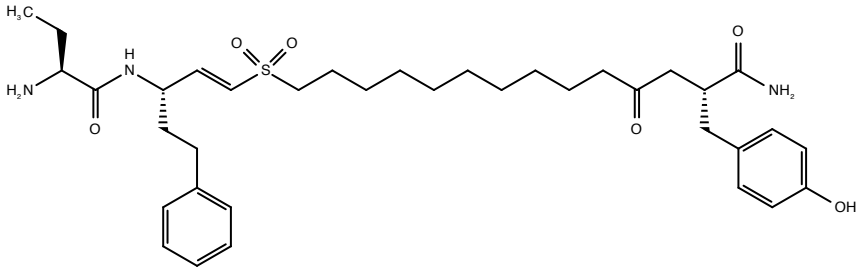


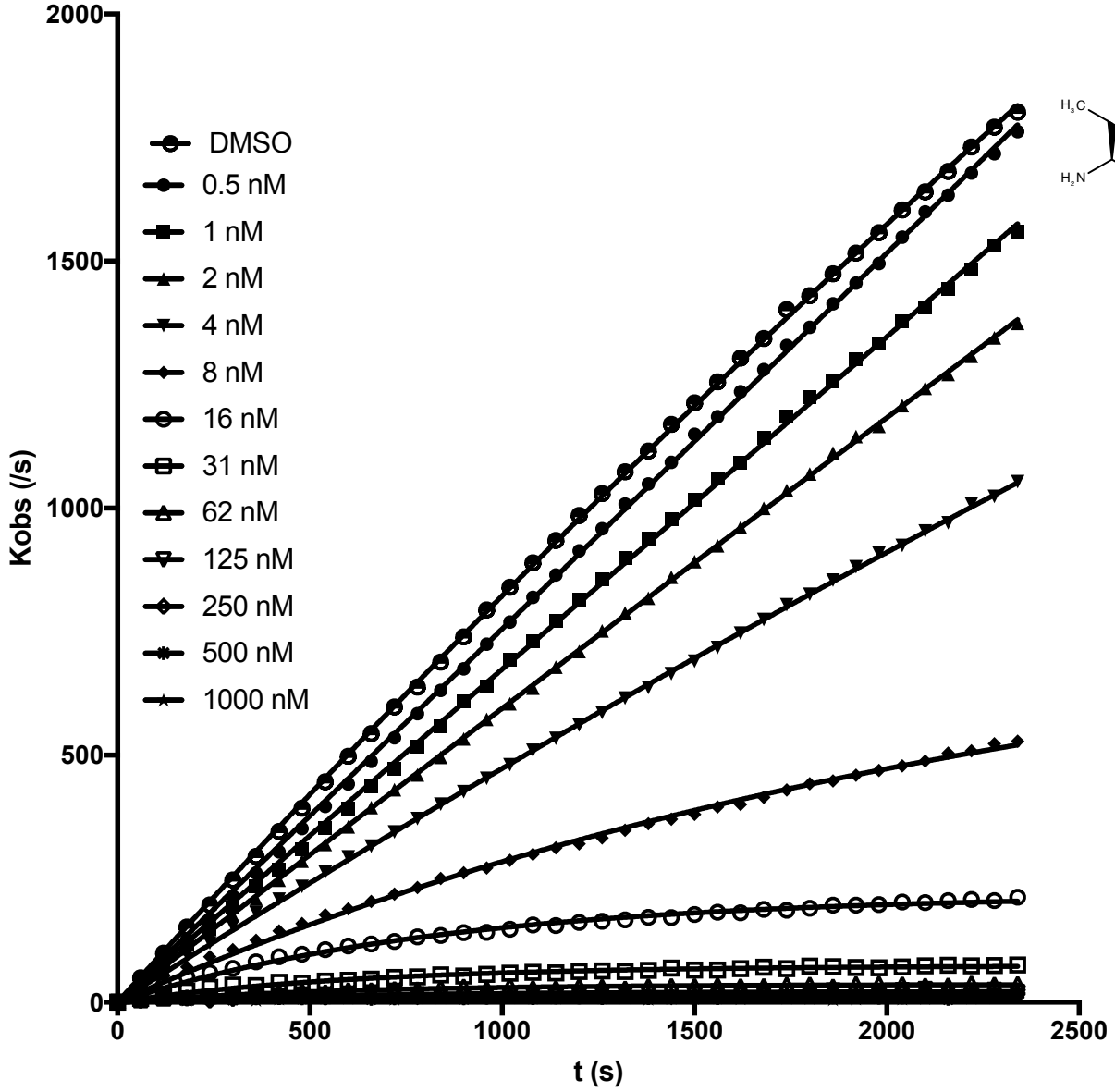
k_{inact} = 0.00315 ± 0.0001 s^{-1}

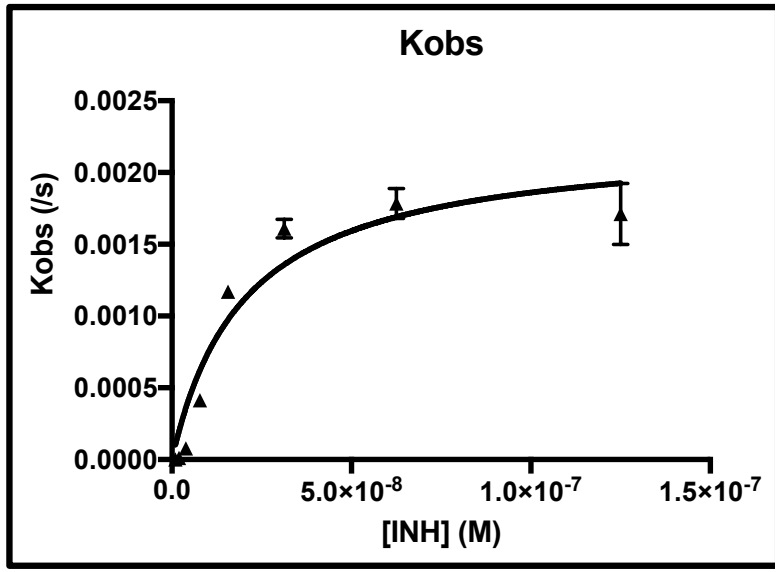
K_i = 47 ± 3 uM

K_{inact}/K_i = 67.7± 0.1 $M^{-1}s^{-1}$

P2: 2Abu





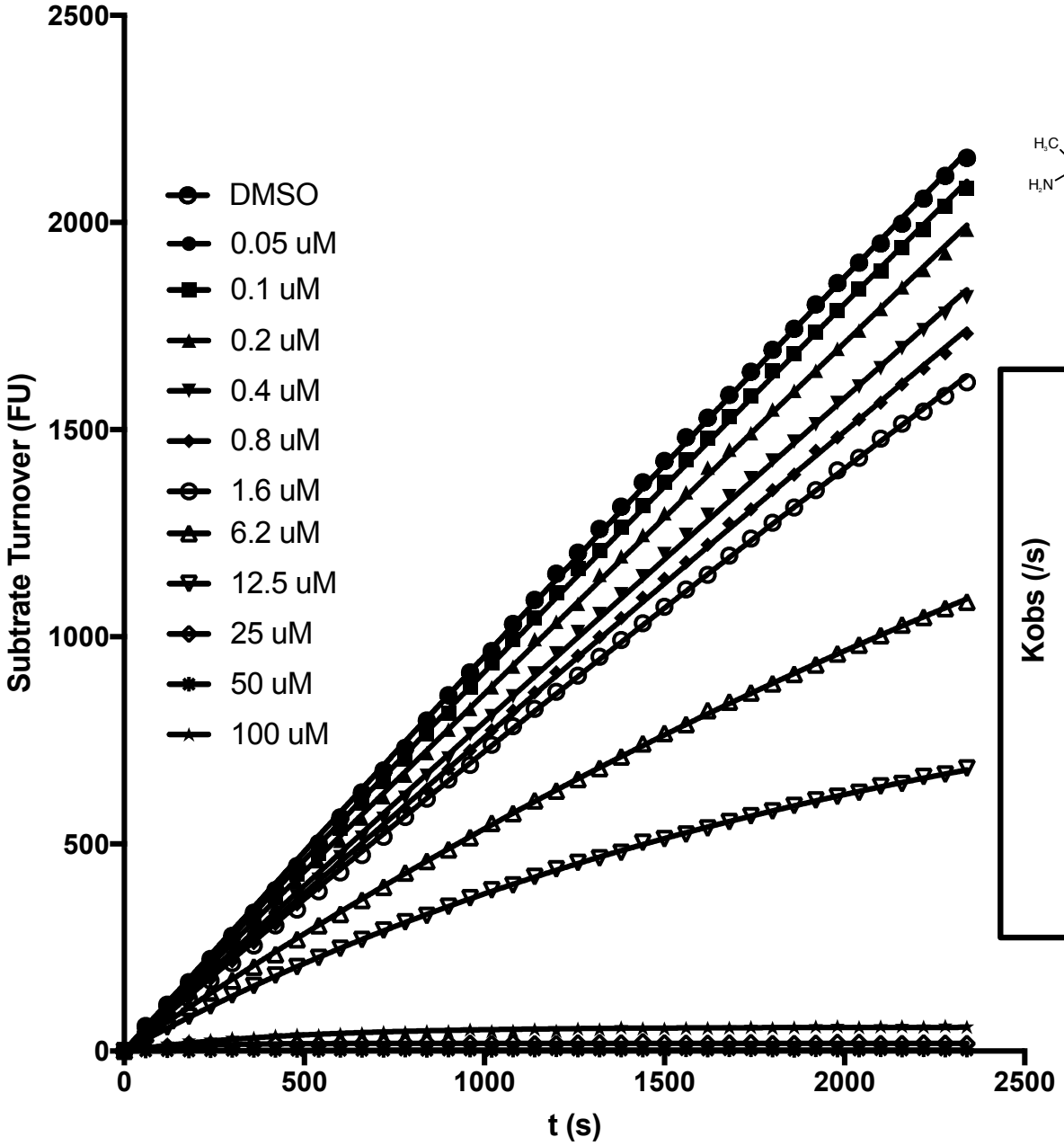


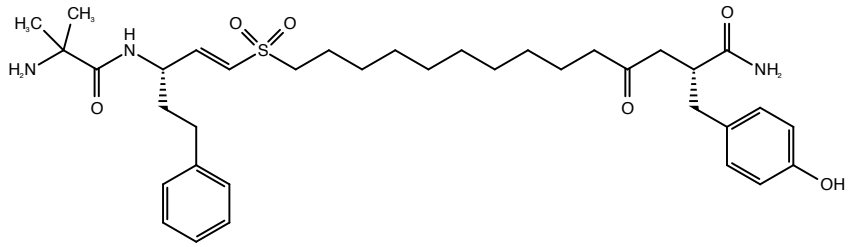
k_{inact} = 0.0022 ± 0.0003 s^{-1}

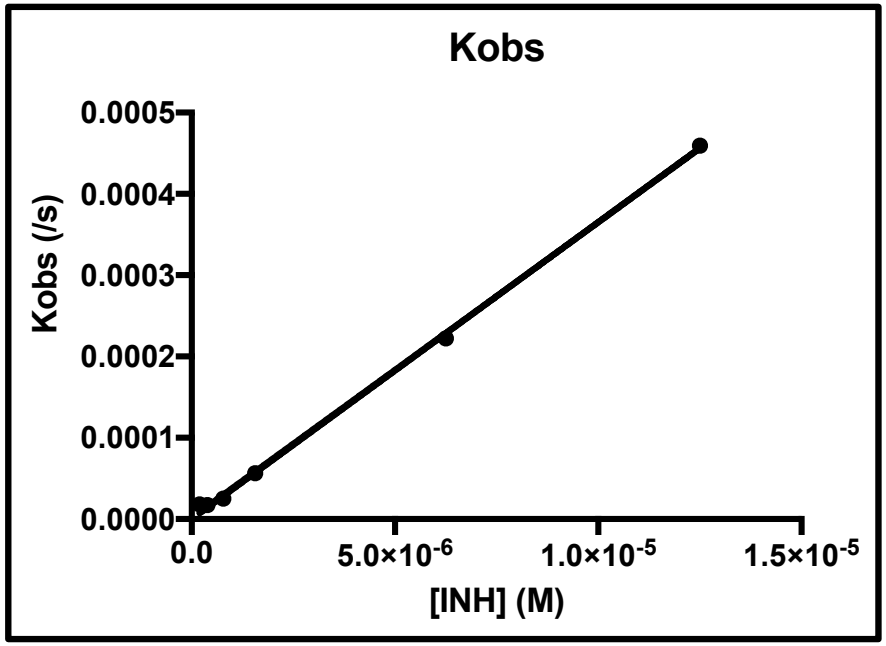
K_i = 13.5 ± 6 nM

K_{inact}/K_i = 166000 ± 50000 $M^{-1}s^{-1}$

P2: Aib





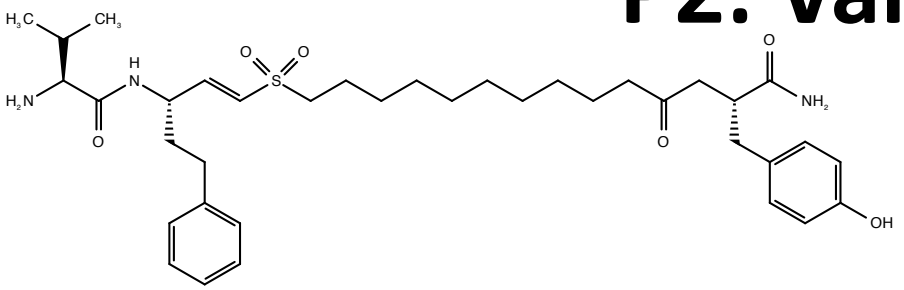


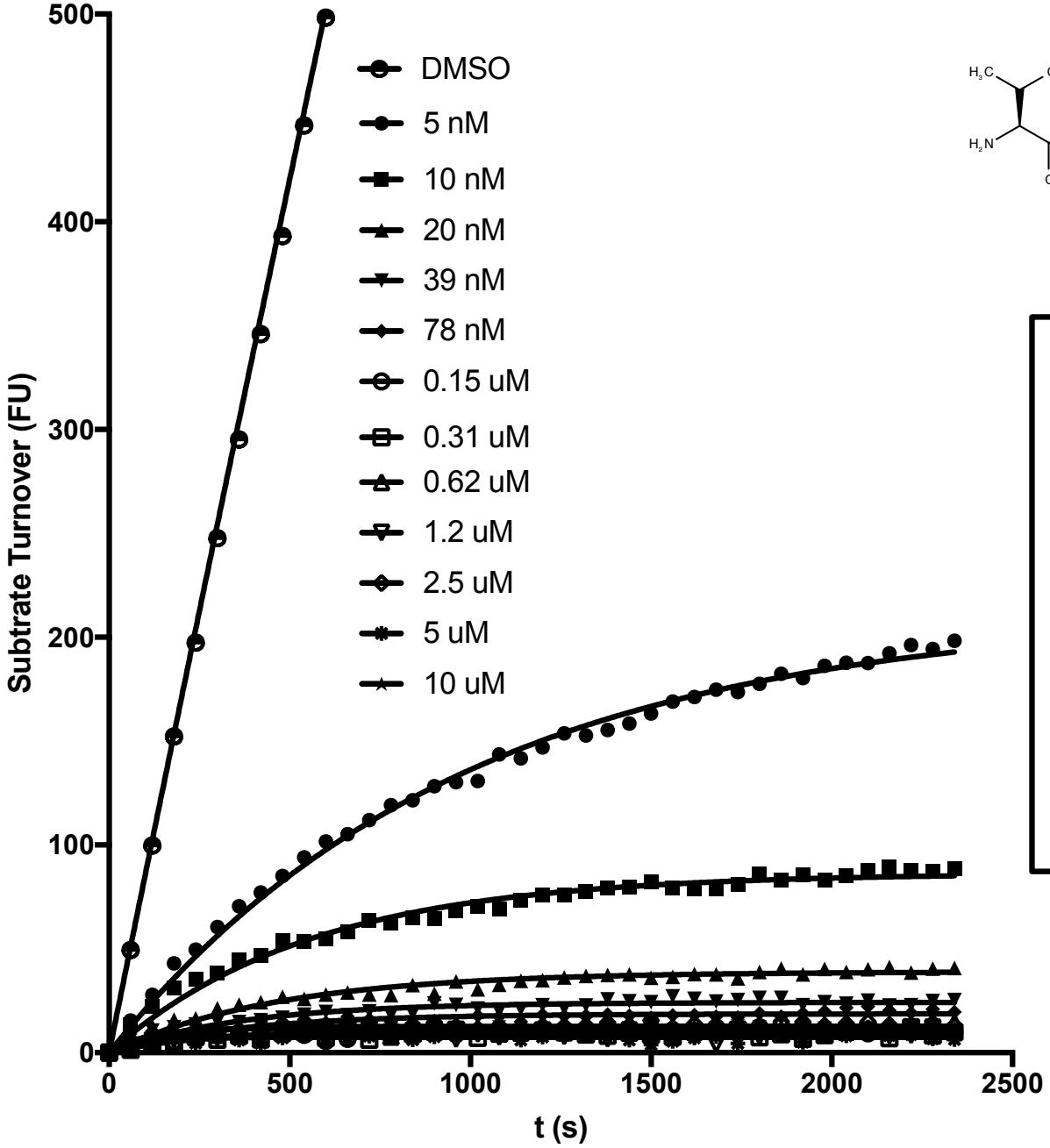
k_{inact} = N/A

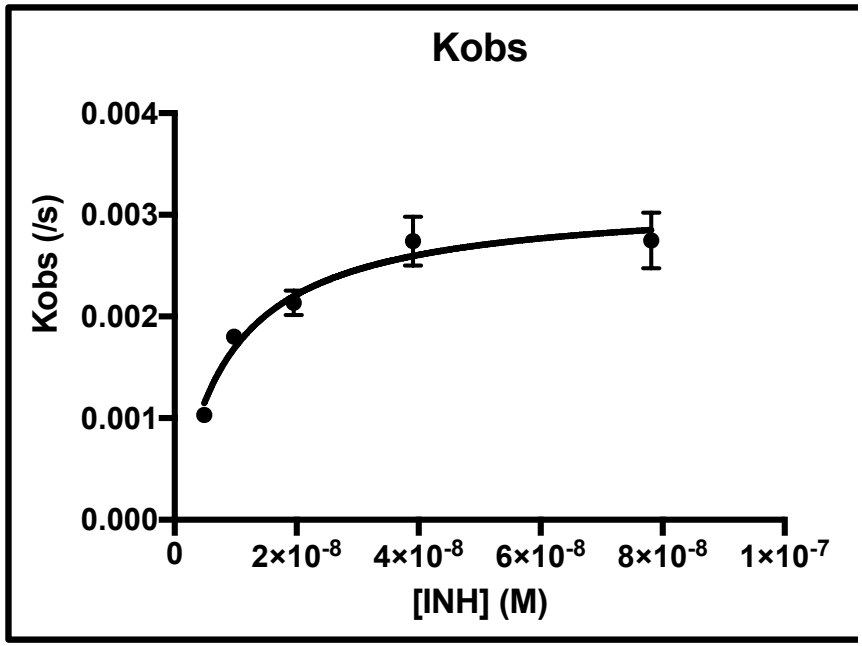
K_i = N/A

K_{inact}/K_i = 36.3 ± 0.6 $M^{-1}s^{-1}$

P2: Val





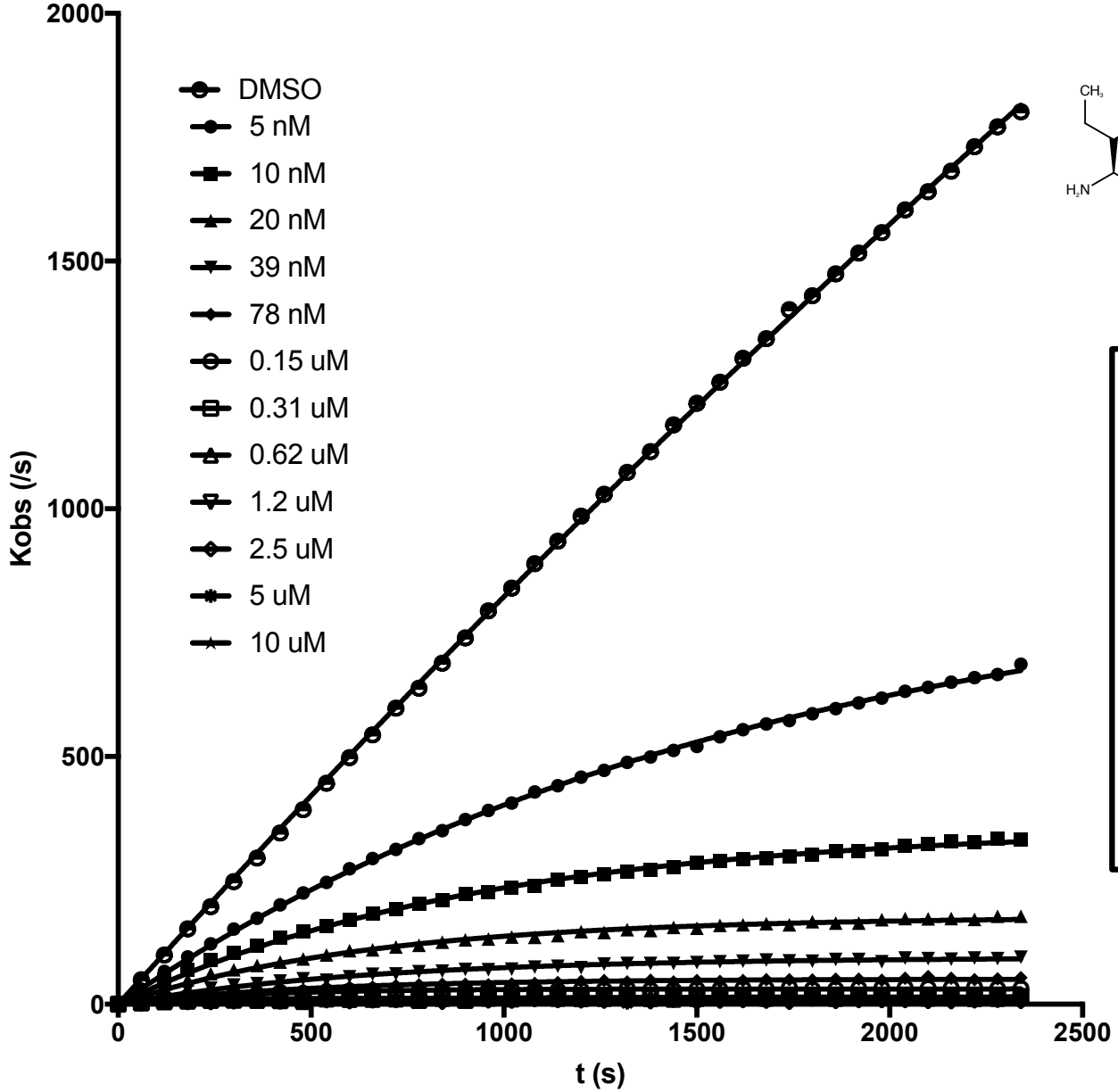


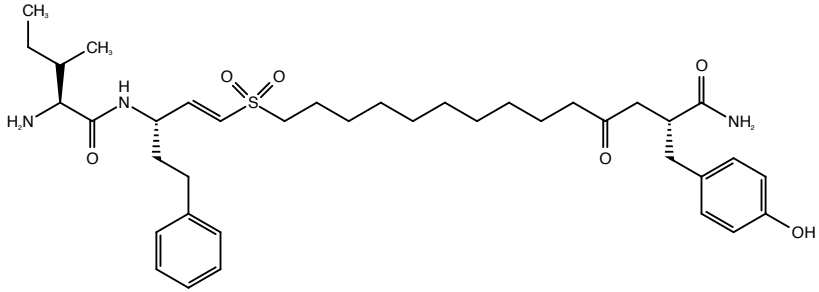
k_{inact} = 0.0032 ± 0.0002 s^{-1}

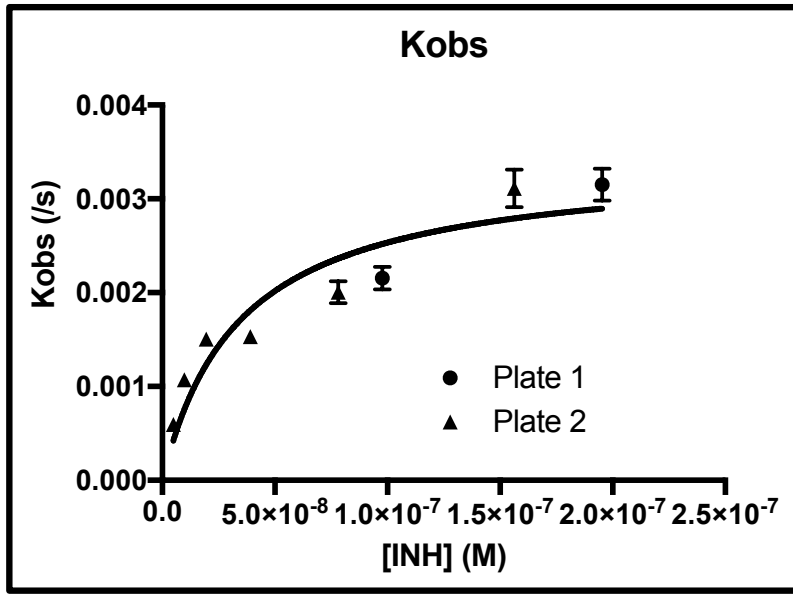
K_i = 5.7 ± 1.1 nM

K_{inact}/K_i = 554000 ± 84000 $M^{-1}s^{-1}$

P2: Ile





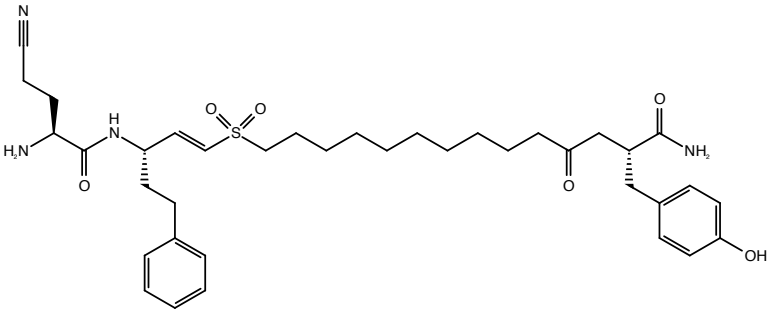


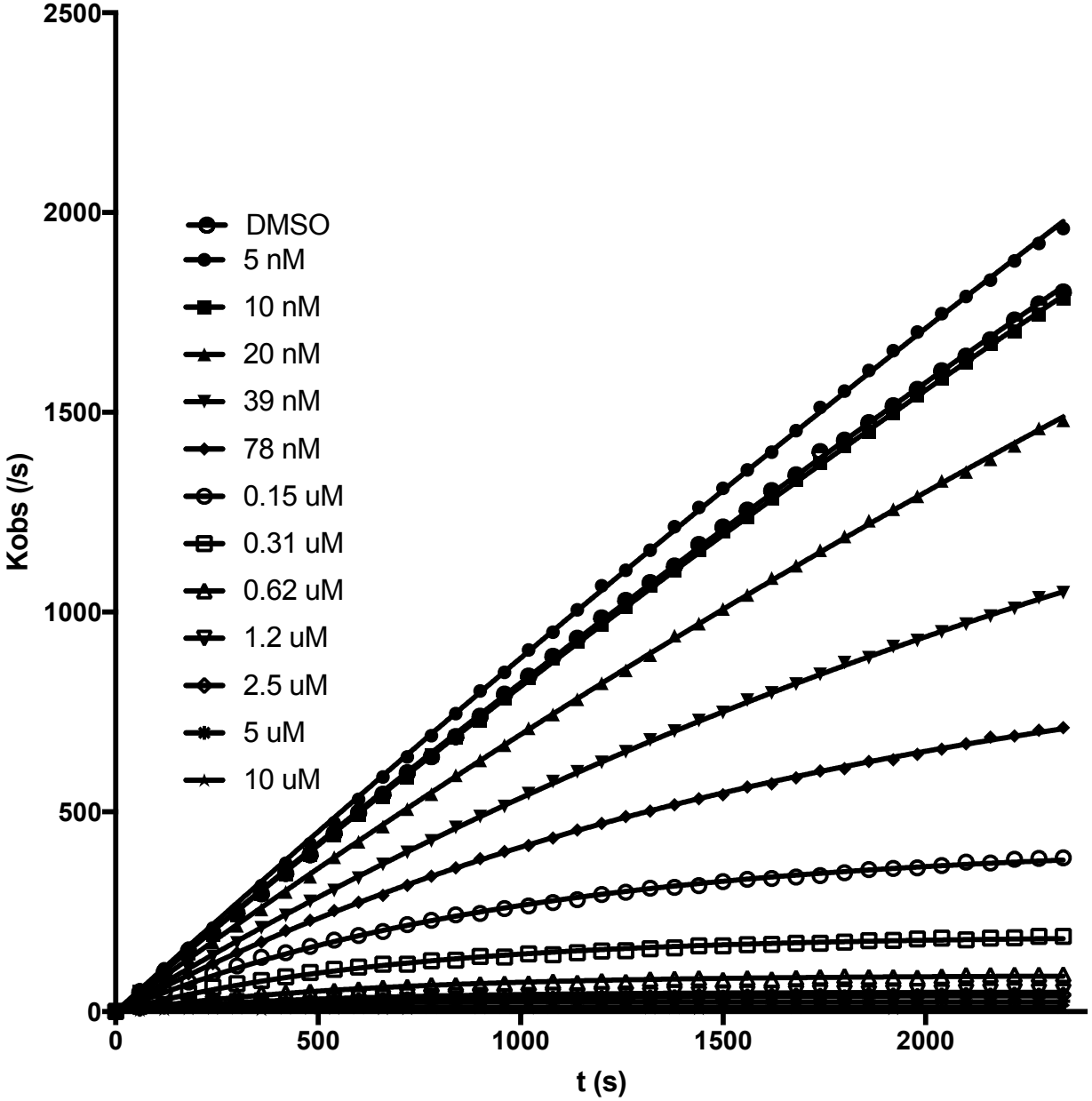
k_{inact} = 0.0034 ± 0.0004 s^{-1}

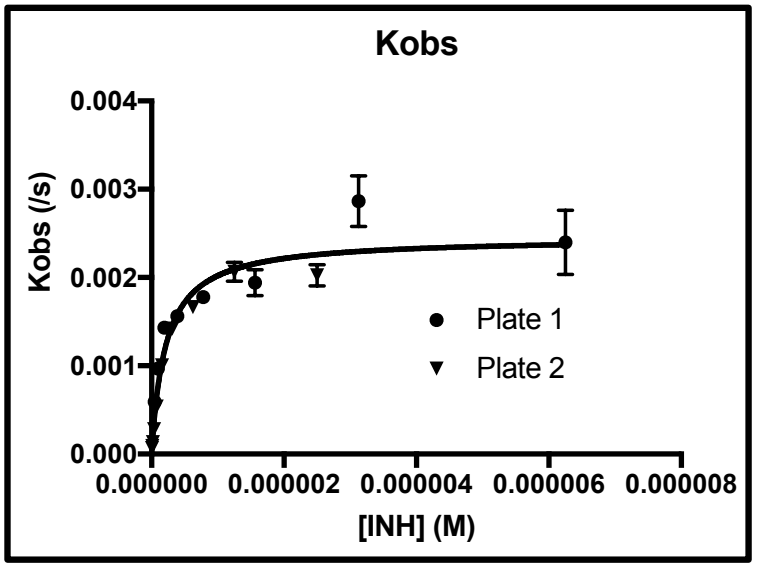
K_i = 23 ± 9 nM

K_{inact}/K_i = 148400 ± 44000 $M^{-1}s^{-1}$

P2: Cba





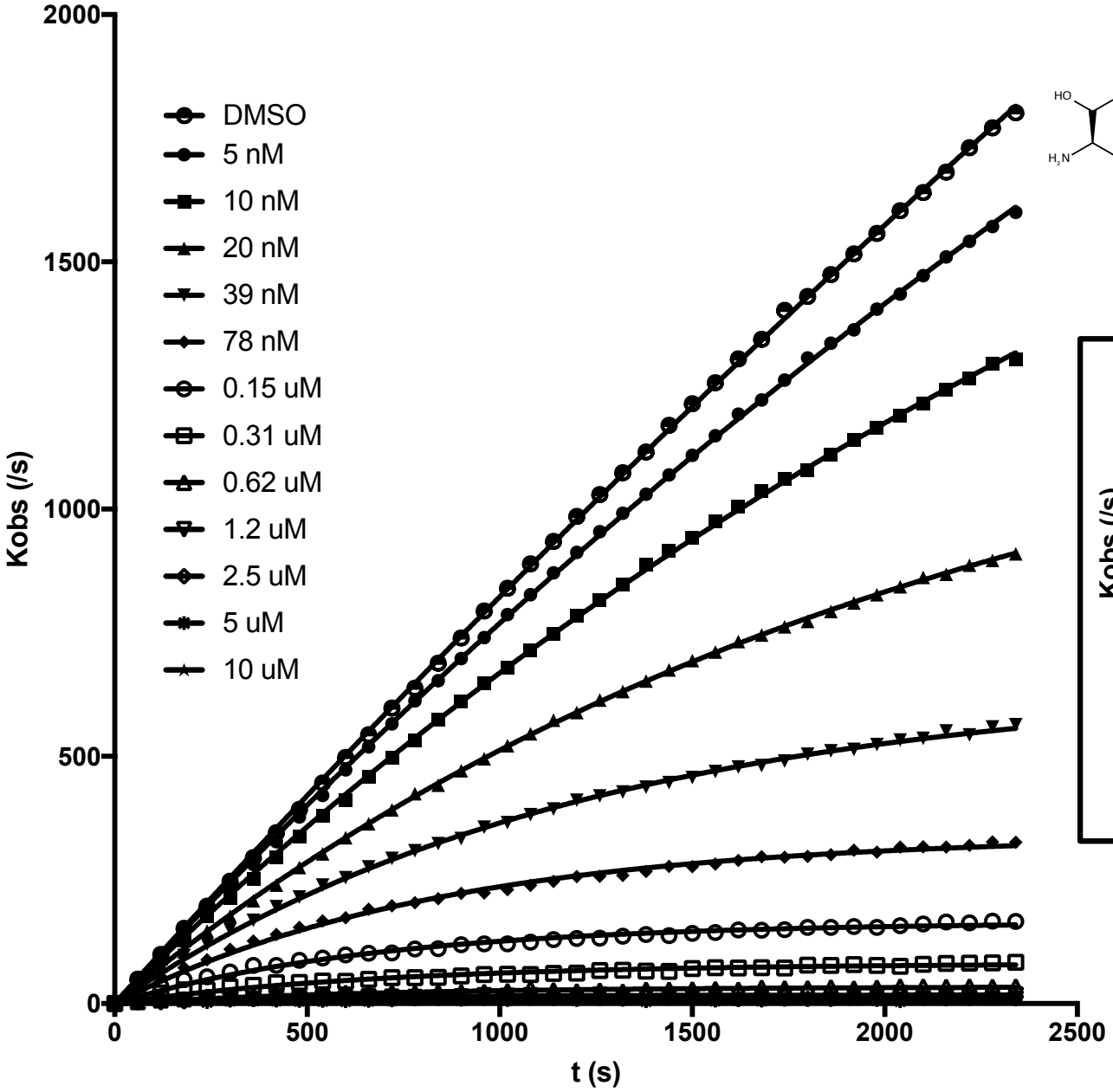


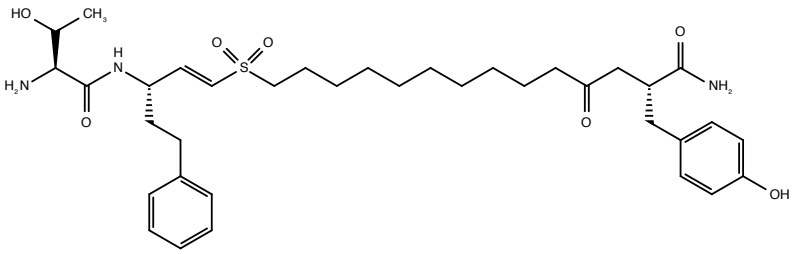
k_{inact} = 0.0025 ± 0.0001 s^{-1}

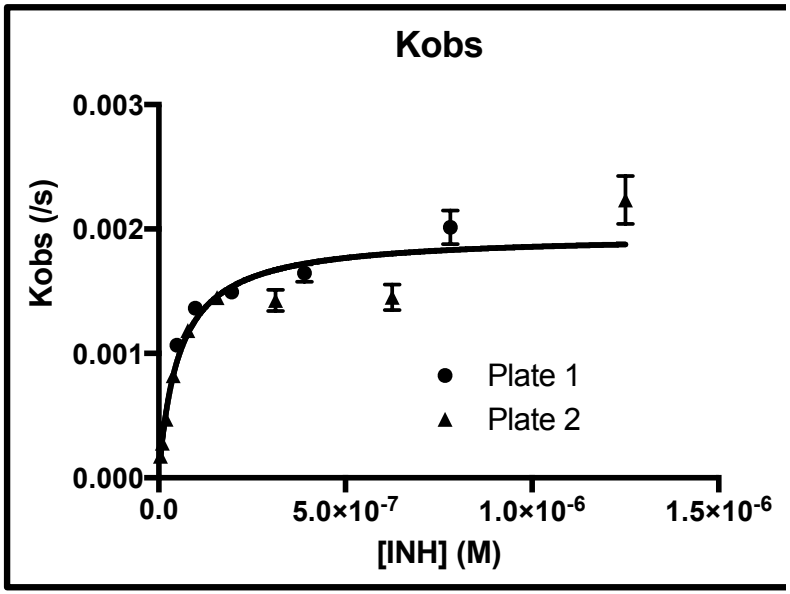
K_i = 0.42 ± 0.03 μM

K_{inact}/K_i = 17200 ± 2500 $M^{-1}s^{-1}$

P2: Thr





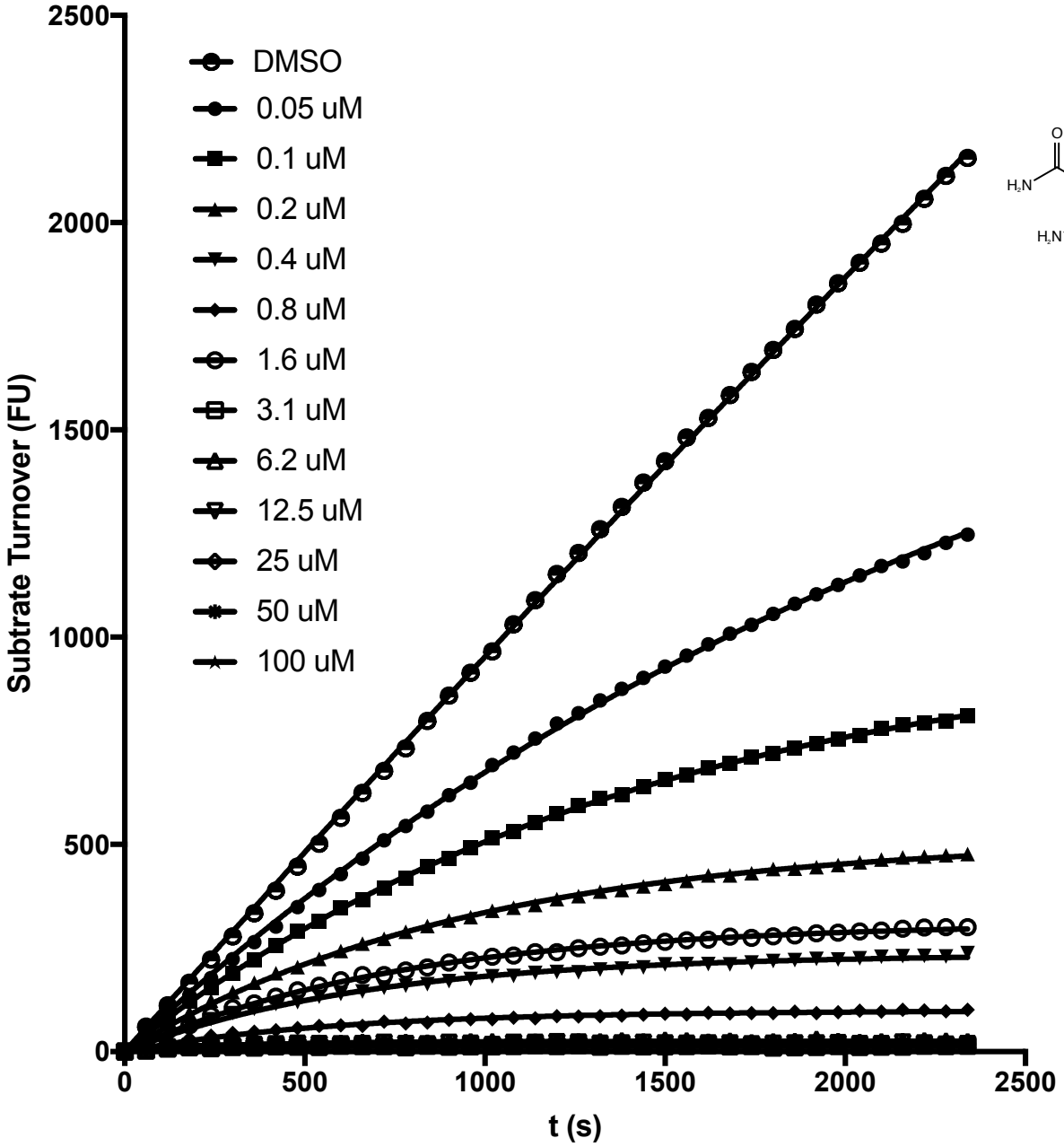


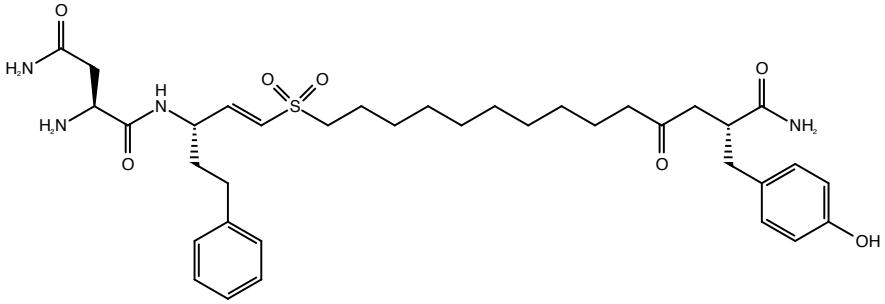
k_{inact} = 0.0020 ± 0.0001 s^{-1}

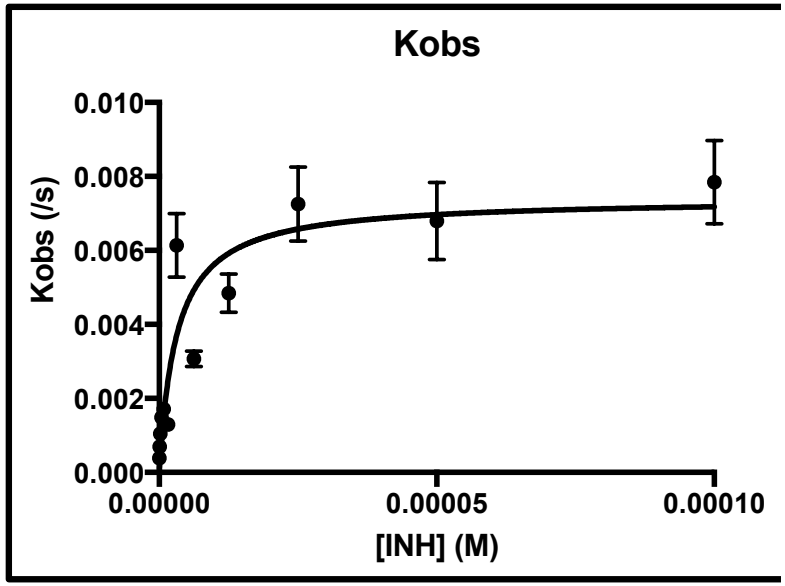
K_i = 36 ± 8 nM

K_{inact}/K_i = 55000 ± 10000 $M^{-1}s^{-1}$

P2: Asn





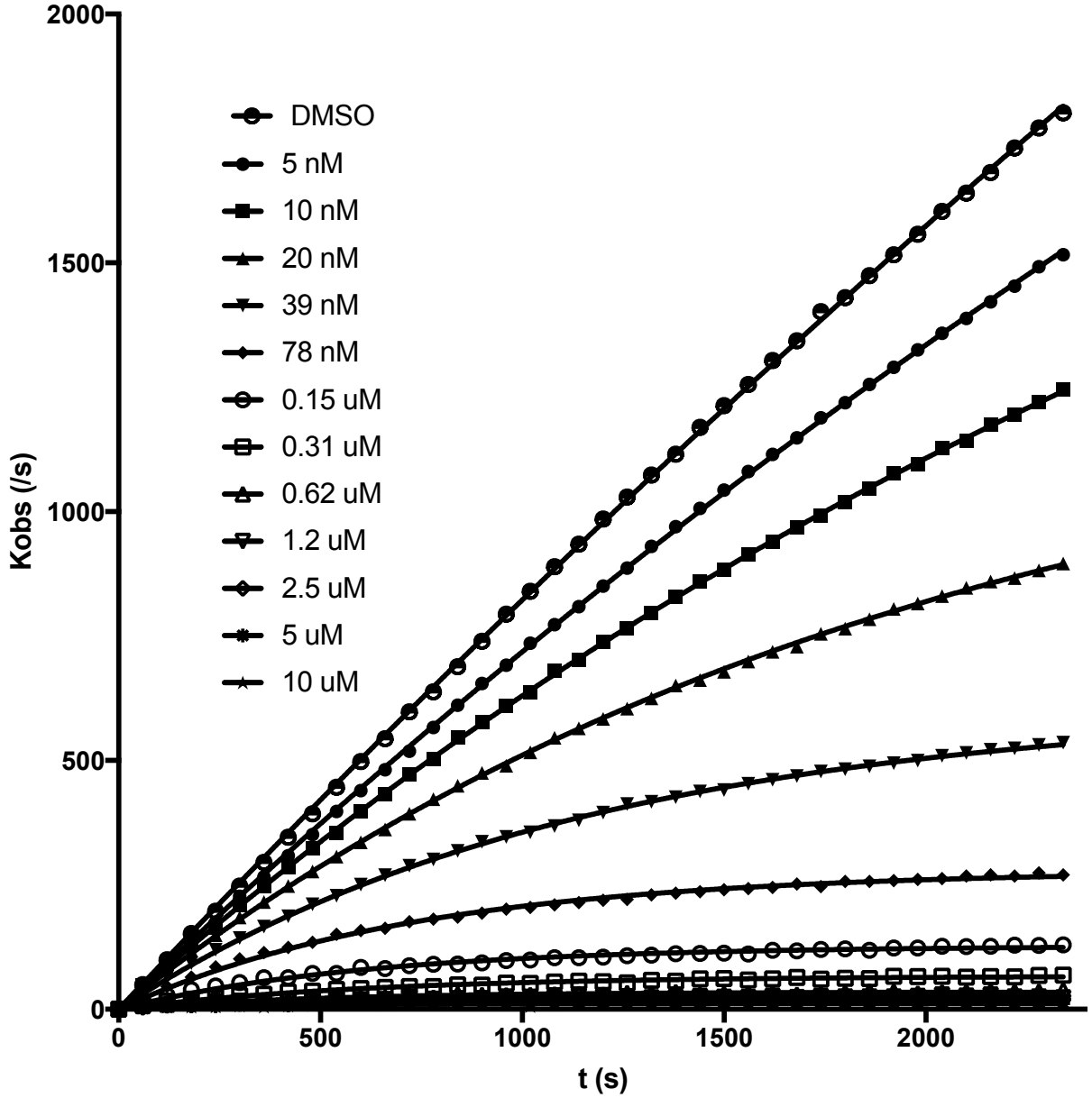


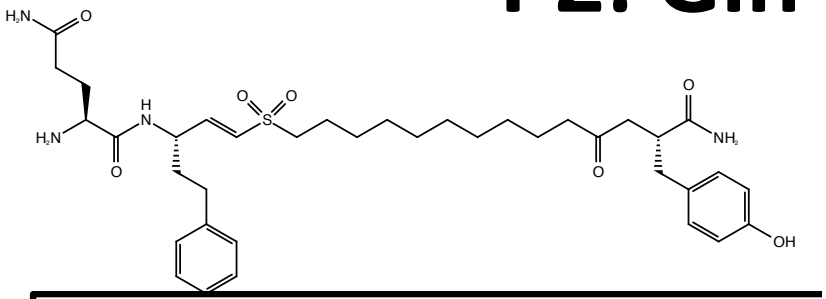
k_{inact} = 0.0074 ± 0.0008 s^{-1}

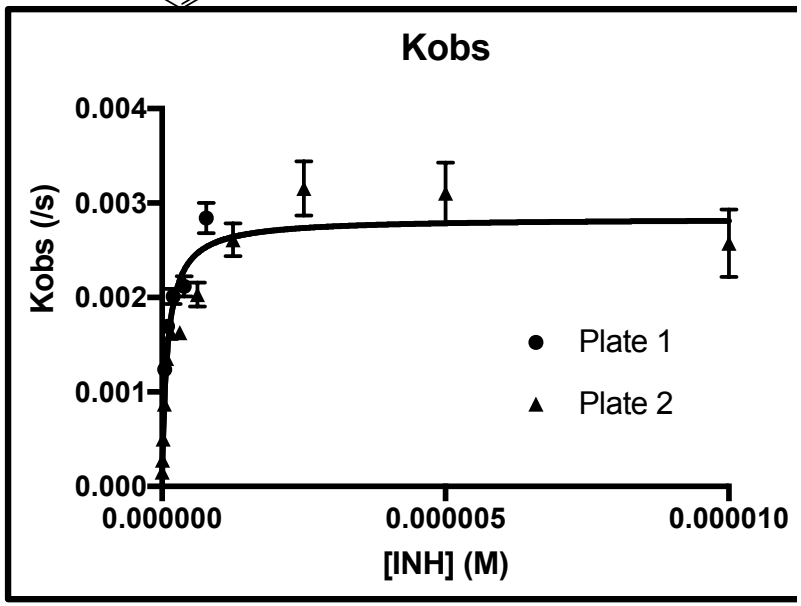
K_i = 2.1 ± 1 μM

K_{inact}/K_i = 3500 ± 1400 $M^{-1}s^{-1}$

P2: Gln





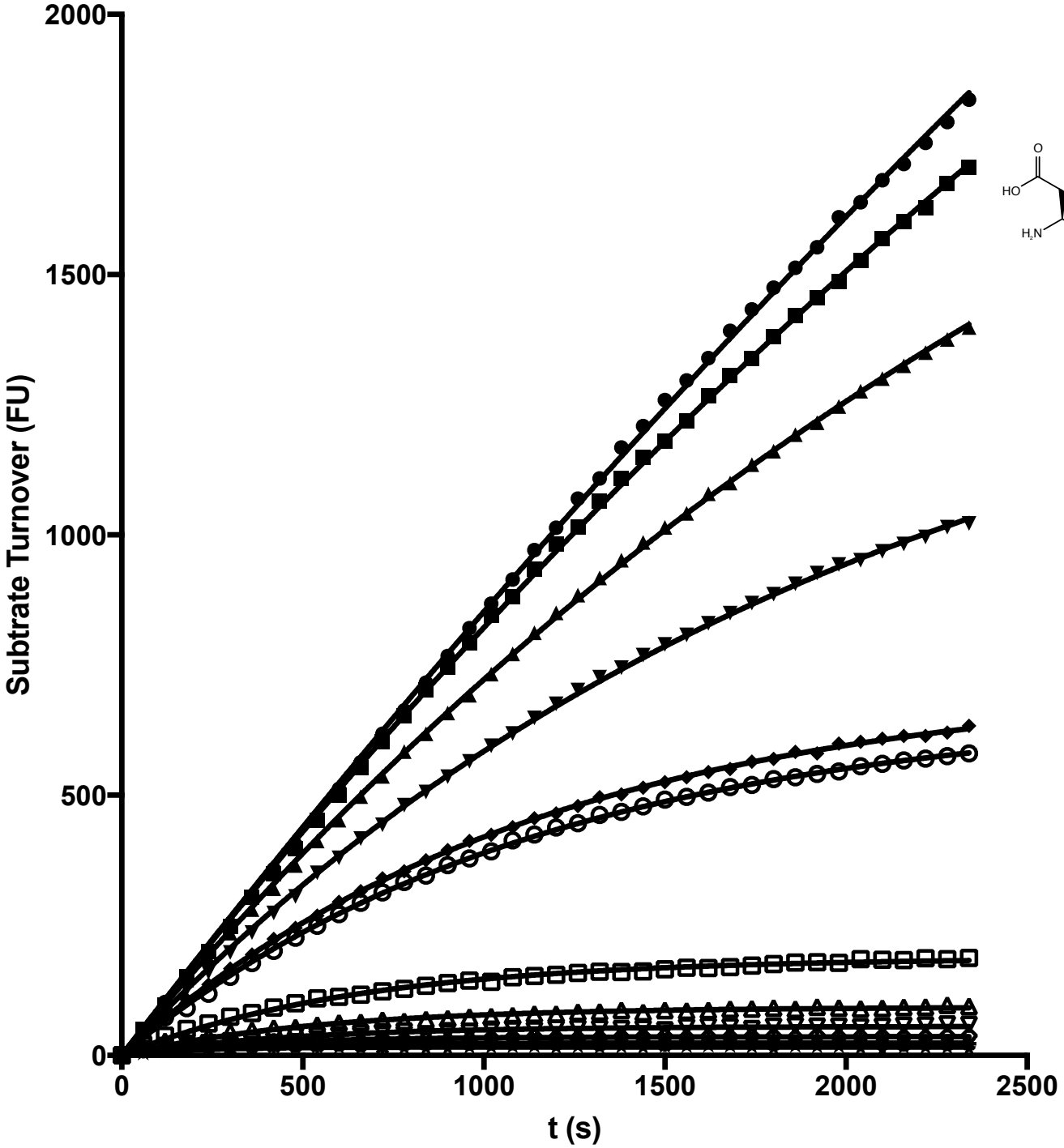


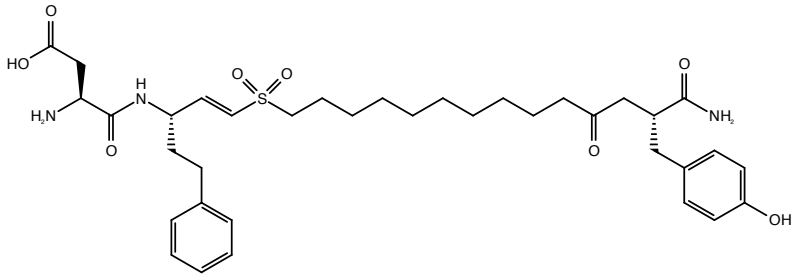
k_{inact} = 0.0028 ± 0.0001 s^{-1}

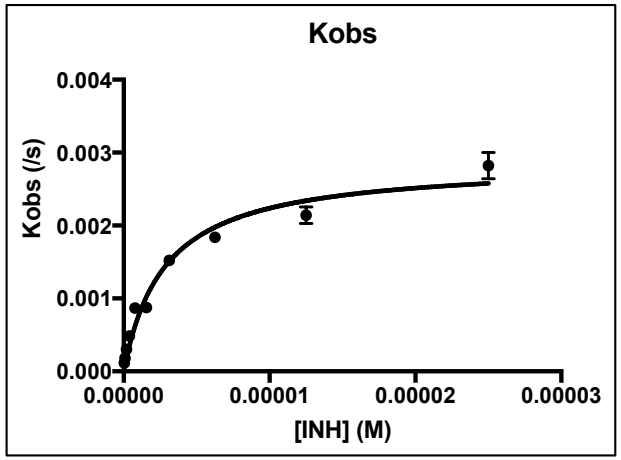
K_i = 62 ± 13 nM

K_{inact}/K_i = 45600 ± 7800 $M^{-1}s^{-1}$

P2: Asp





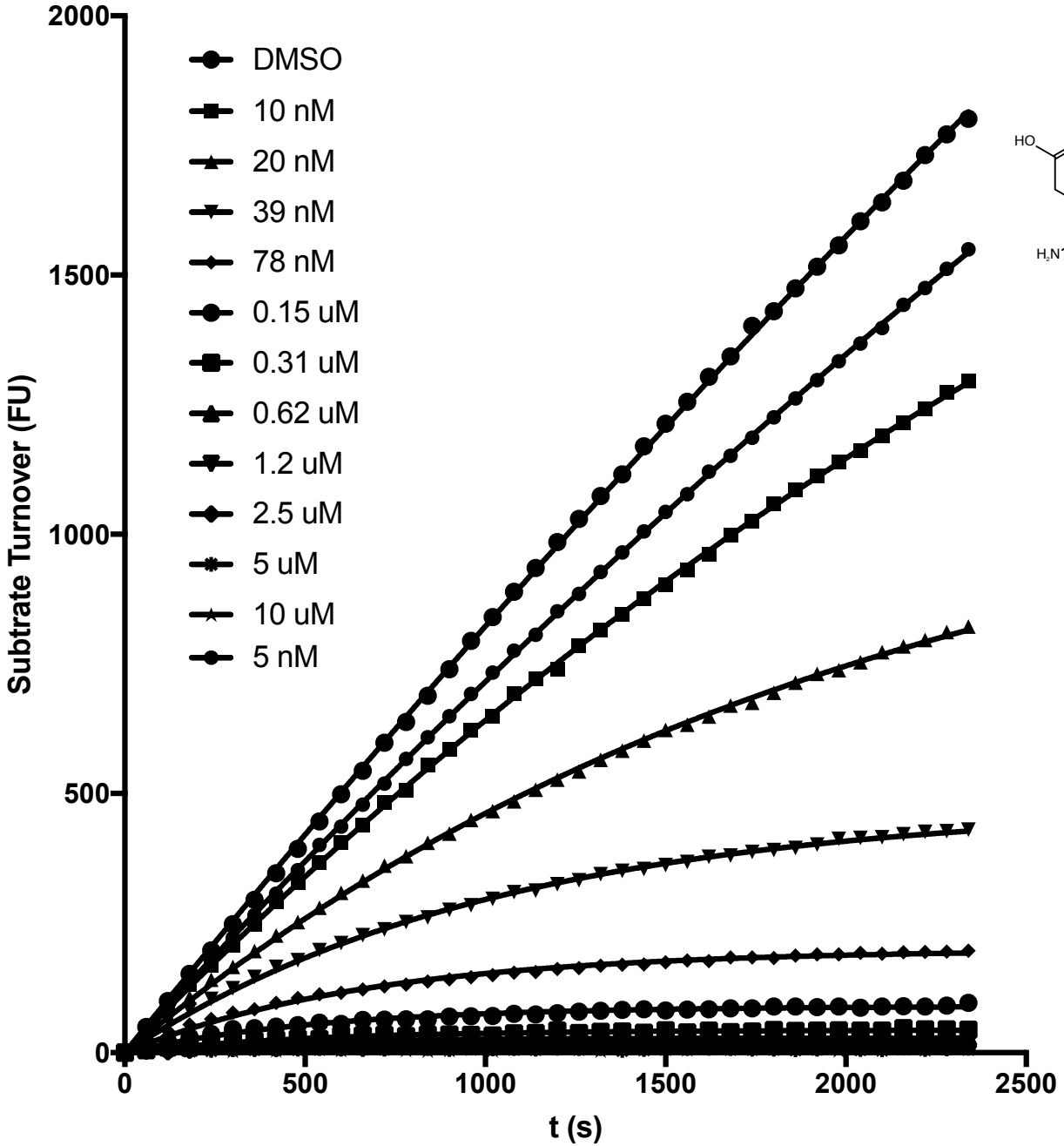


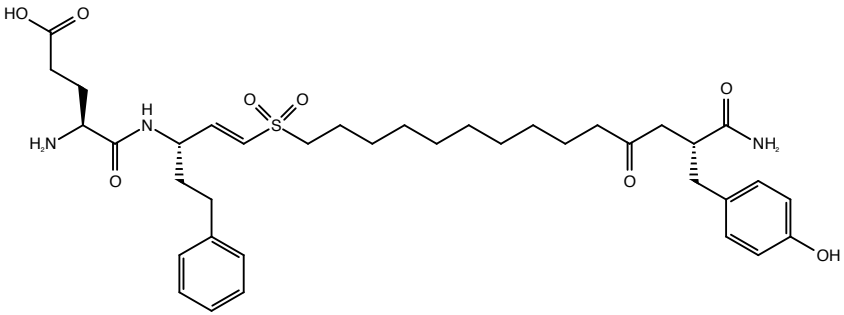
k_{inact} = 0.0029 ± 0.0002 s^{-1}

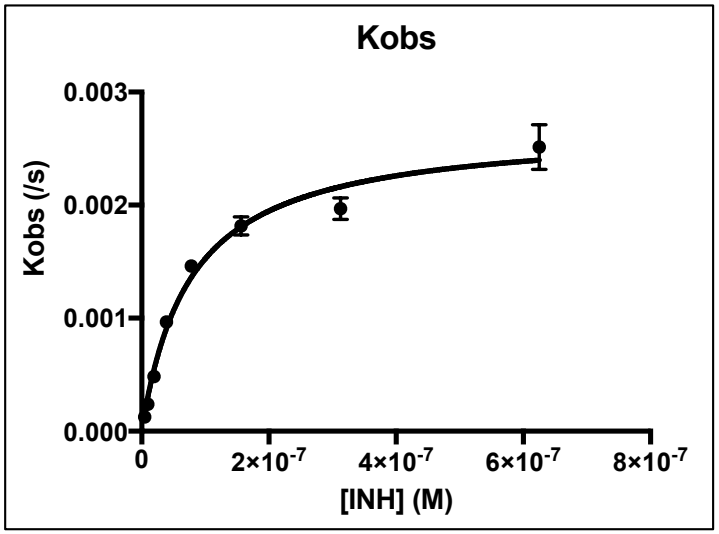
K_i = 1.9 ± 0.4 μM

K_{inact}/K_i = 1530 ± 250 $M^{-1}s^{-1}$

P2: Glu





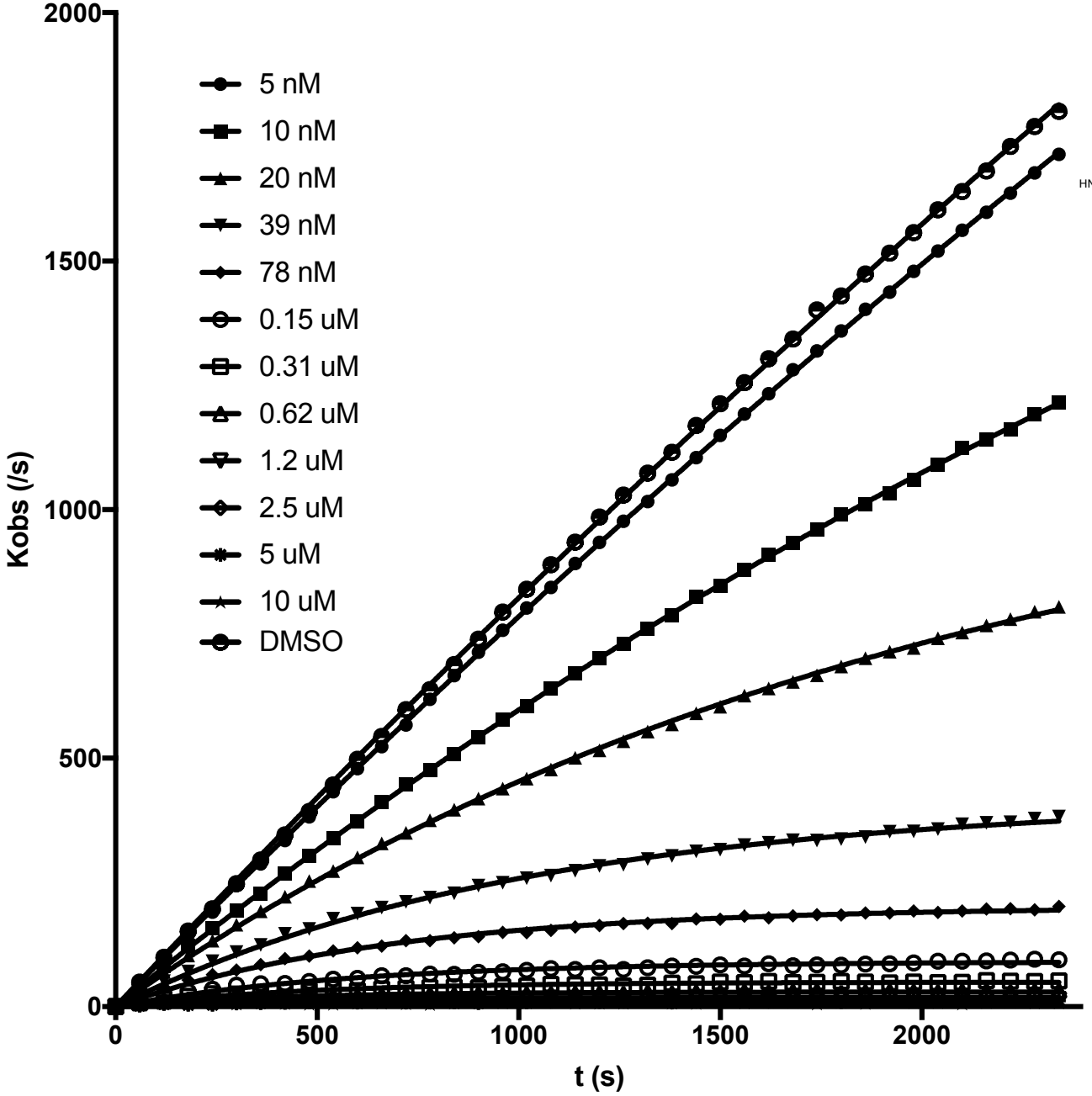


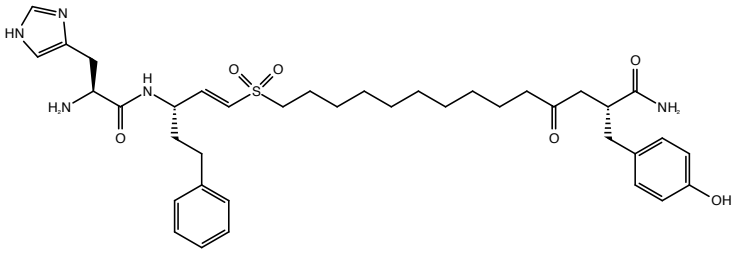
k_{inact} = 0.0027 ± 0.0001 s^{-1}

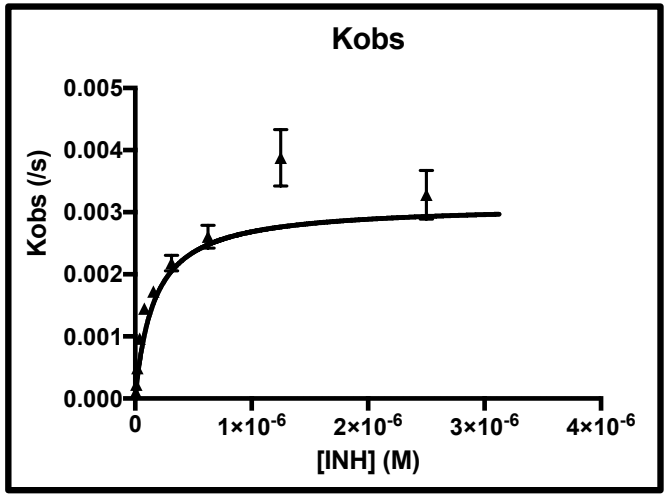
K_i = 51 ± 8 nM

K_{inact}/K_i = 53000 ± 6000 $M^{-1}s^{-1}$

P2: His





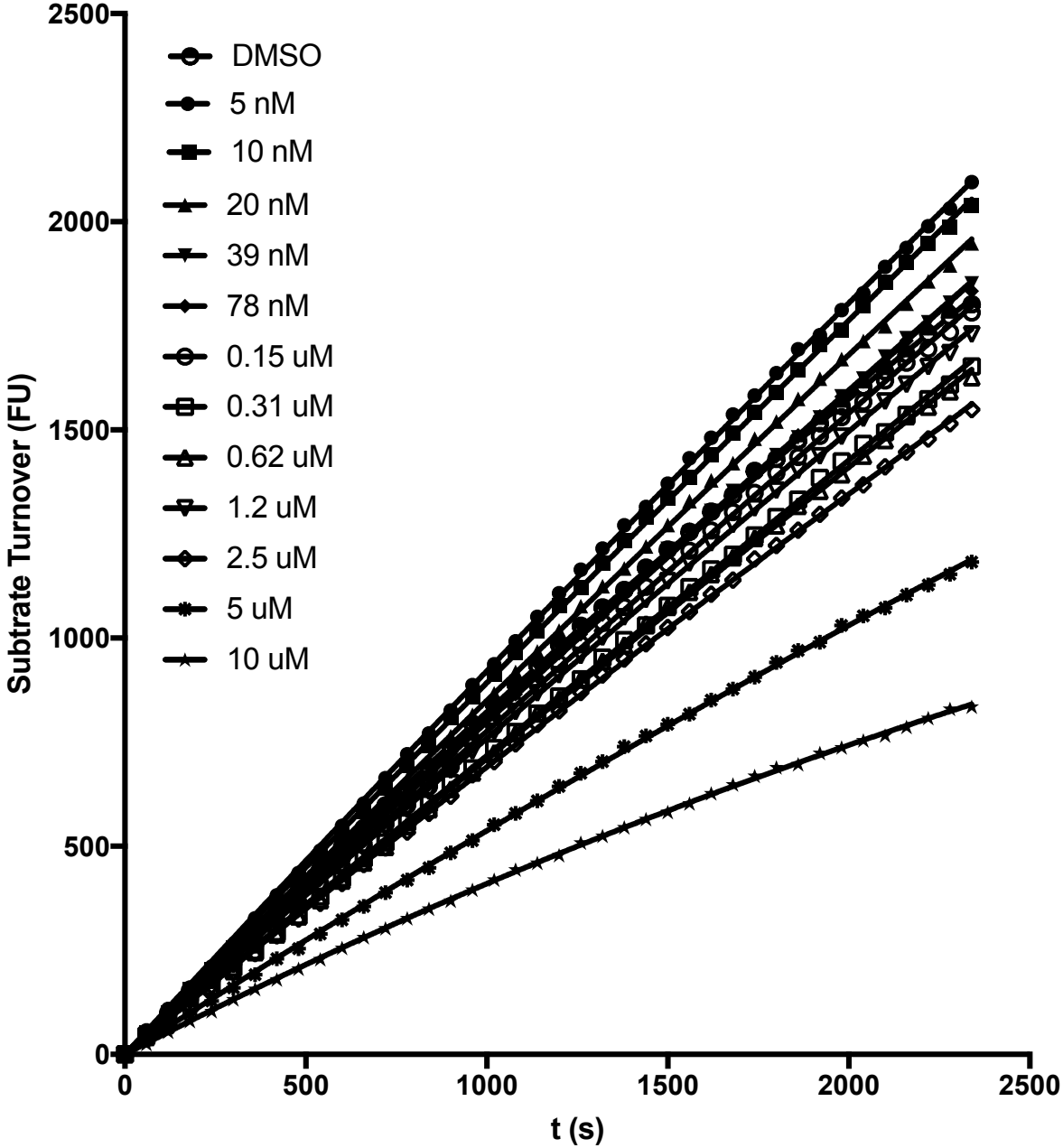


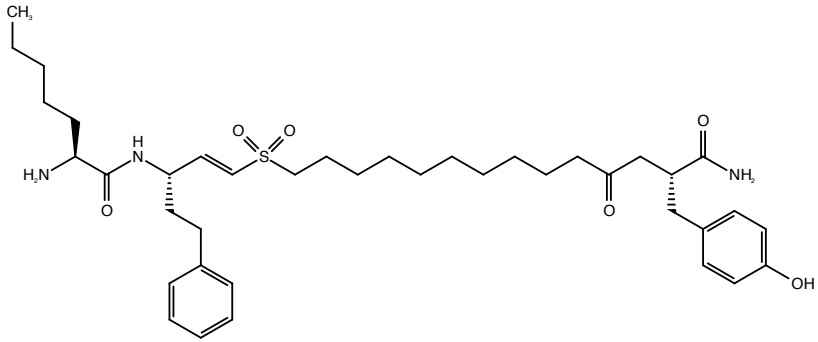
k_{inact} = 0.0031 ± 0.0003 s^{-1}

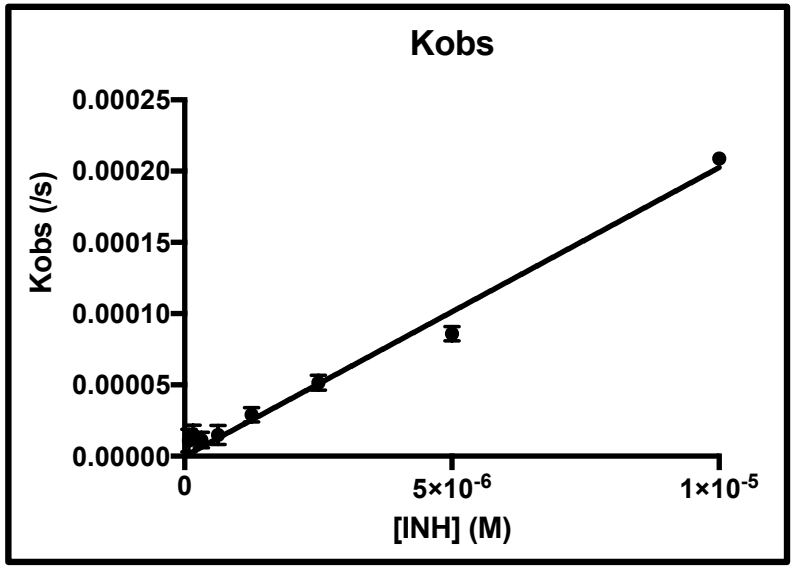
K_i = 110 ± 35 nM

K_{inact}/K_i = 28700 ± 7600 $M^{-1}s^{-1}$

P2: Lys

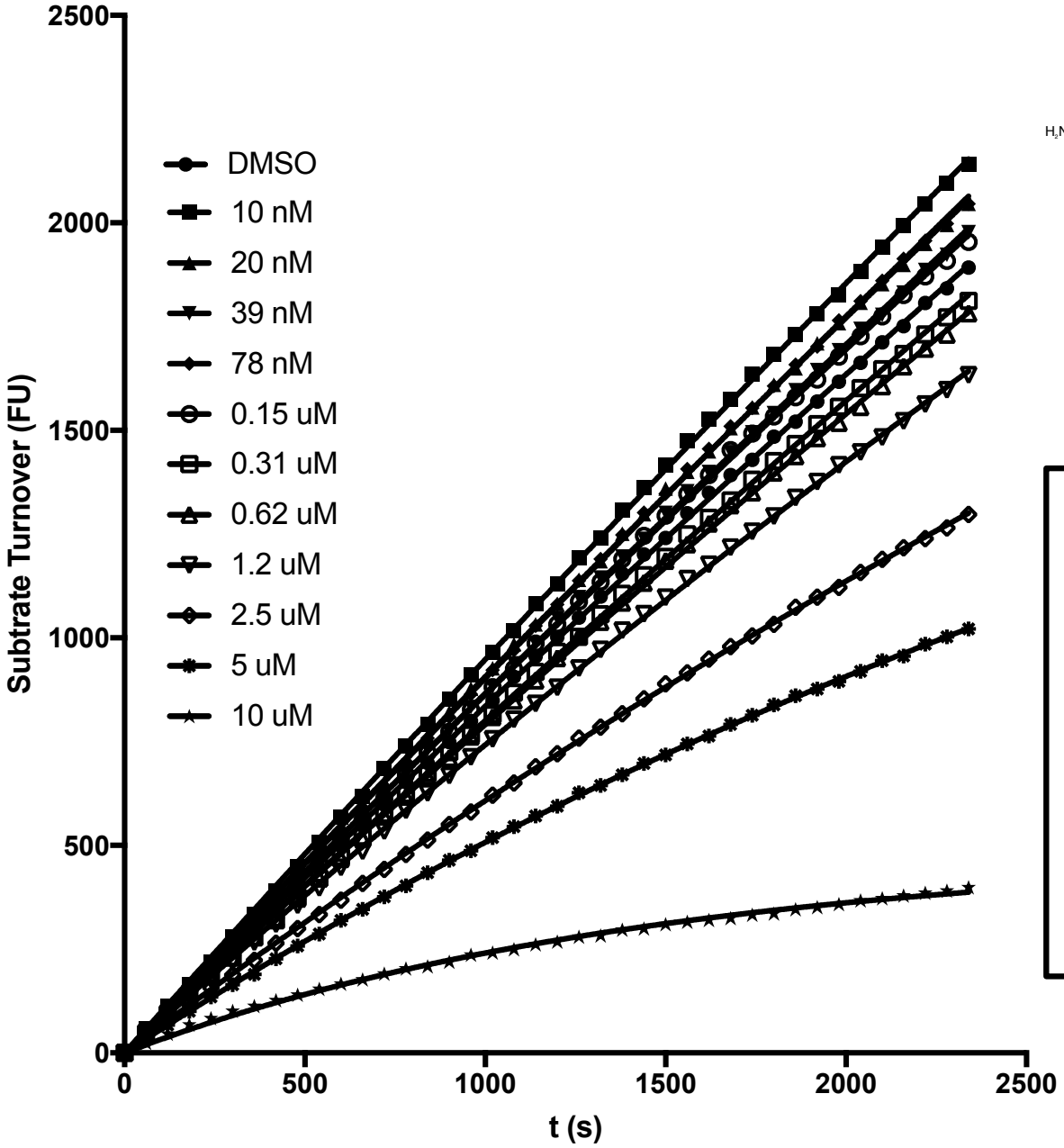


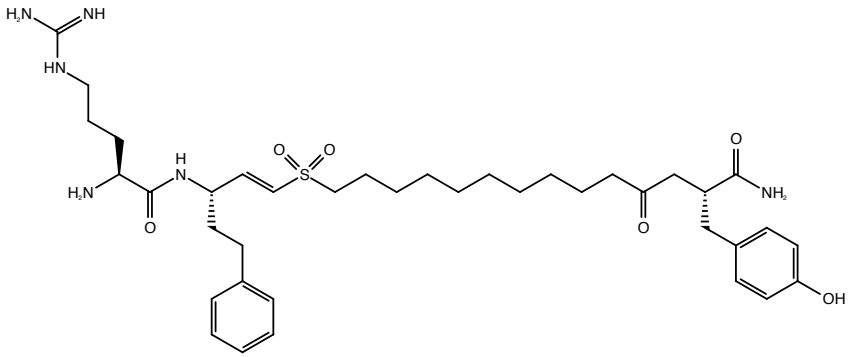


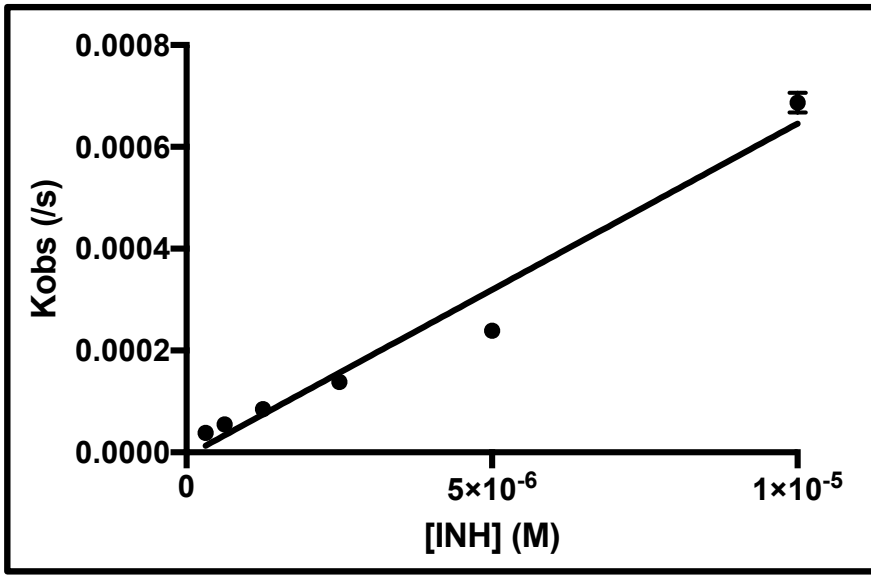


K_{inact}/K_i = 20 ± 1 $M^{-1}s^{-1}$

P2: Arg

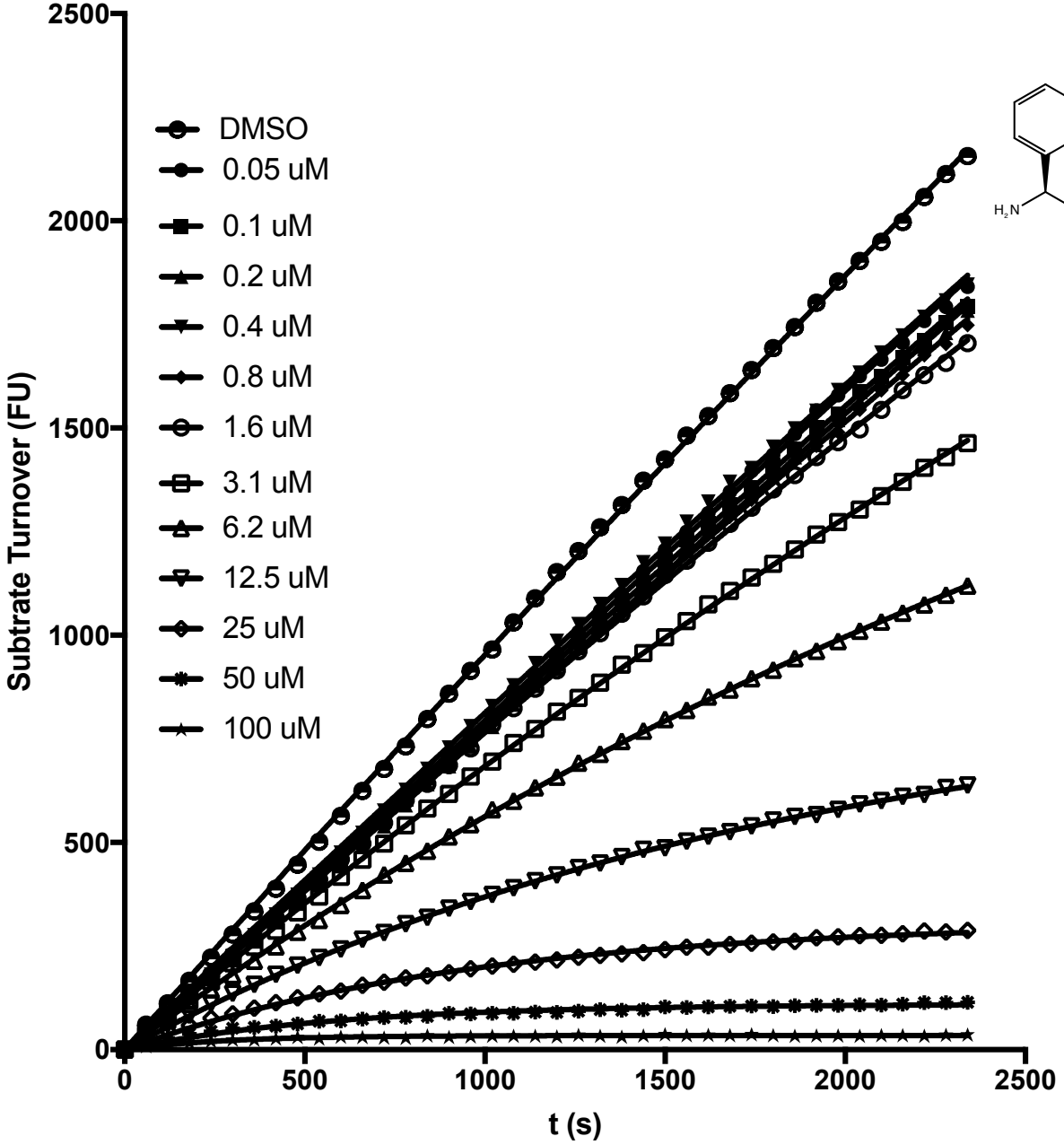


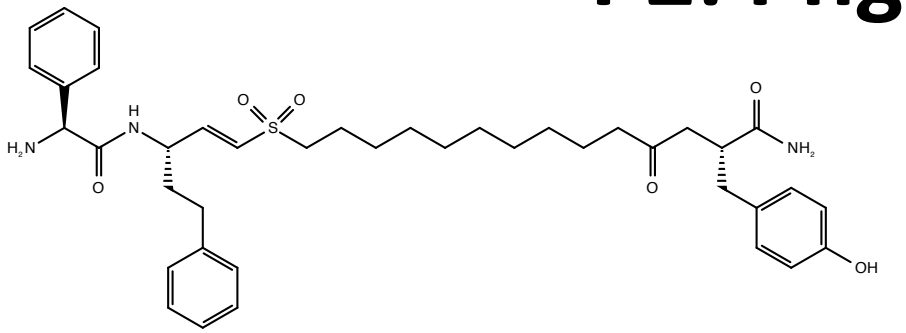


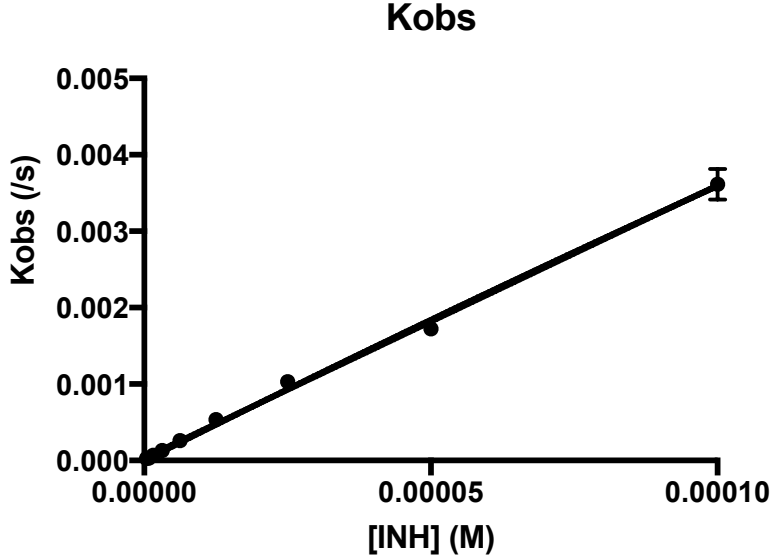


K_{inact}/K_i = 65 ± 6 $M^{-1}s^{-1}$

P2: Phg

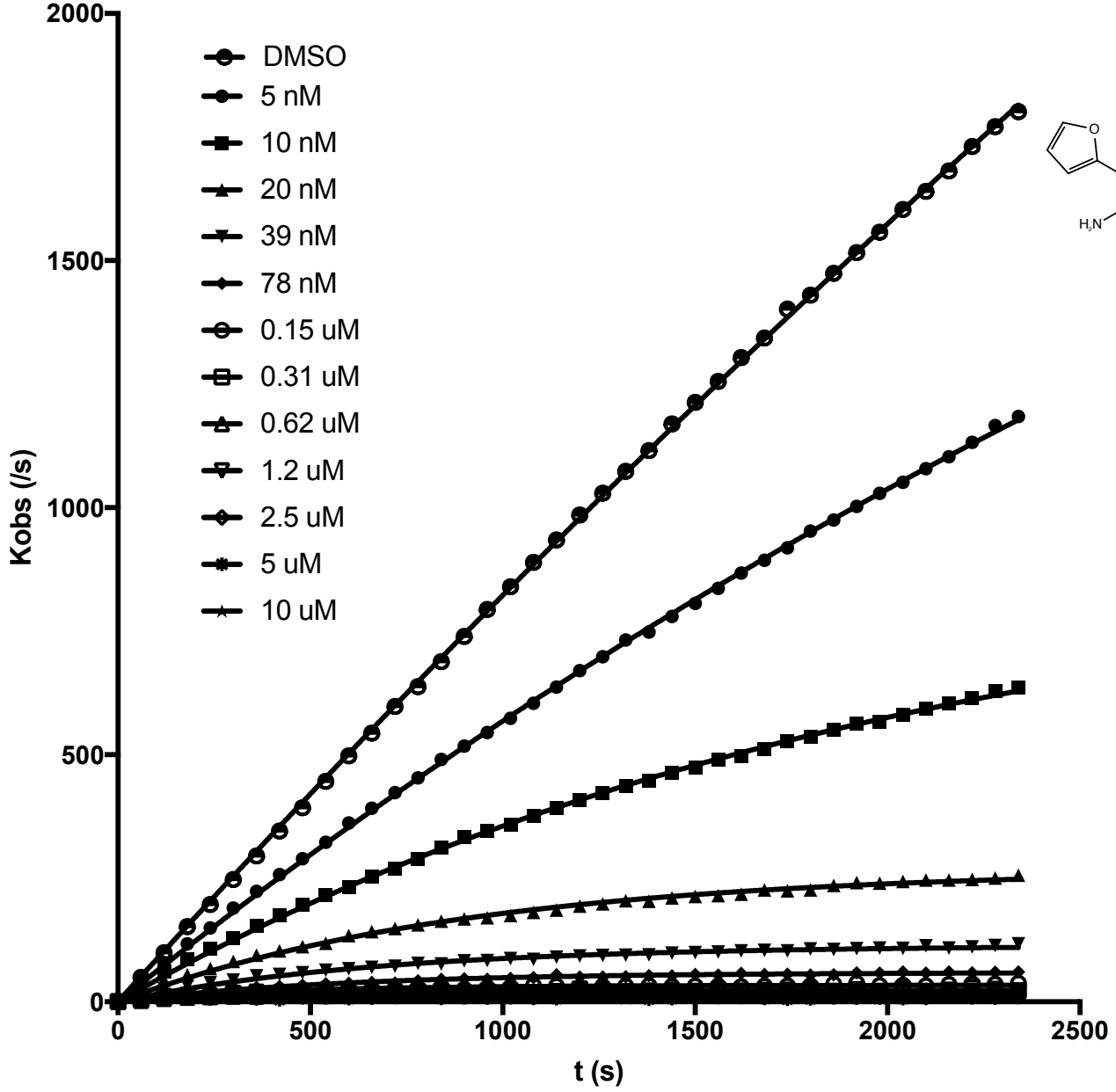


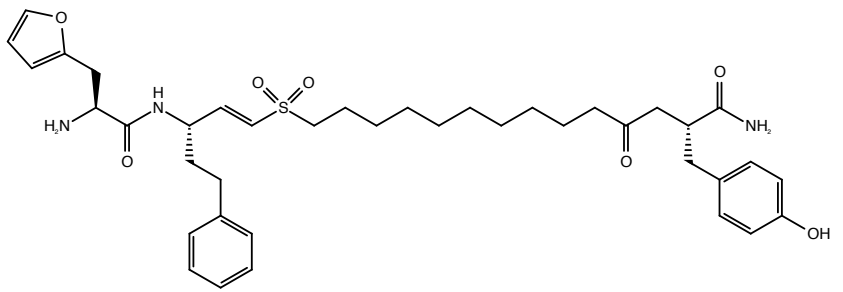


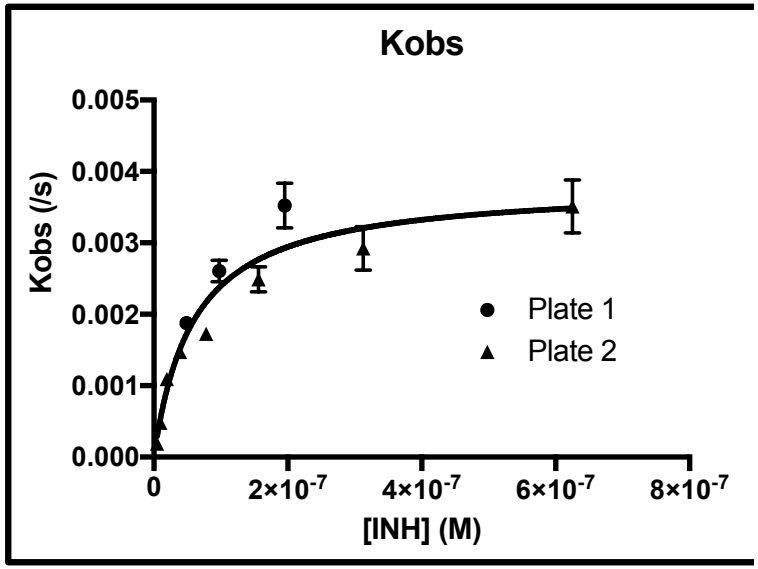


K_{inact}/K_i = 35.7 ± 0.7 $M^{-1}s^{-1}$

P2: 2fa





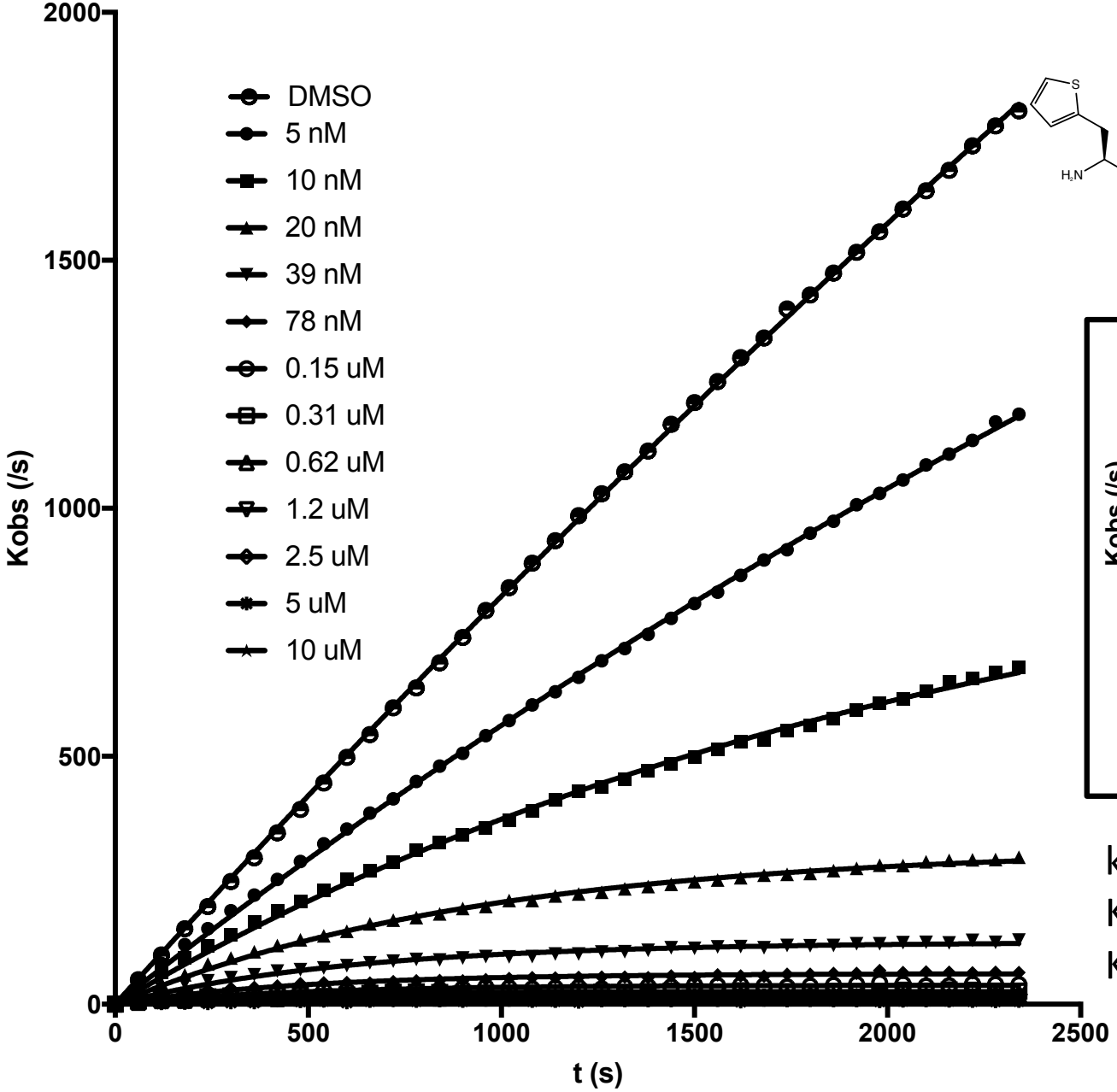


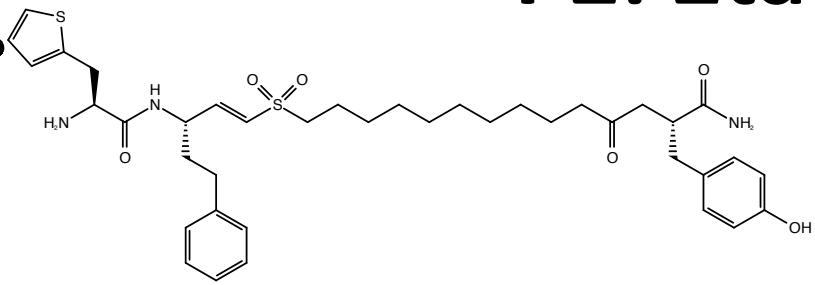
k_{inact} = 0.00378 ± 0.0003 s^{-1}

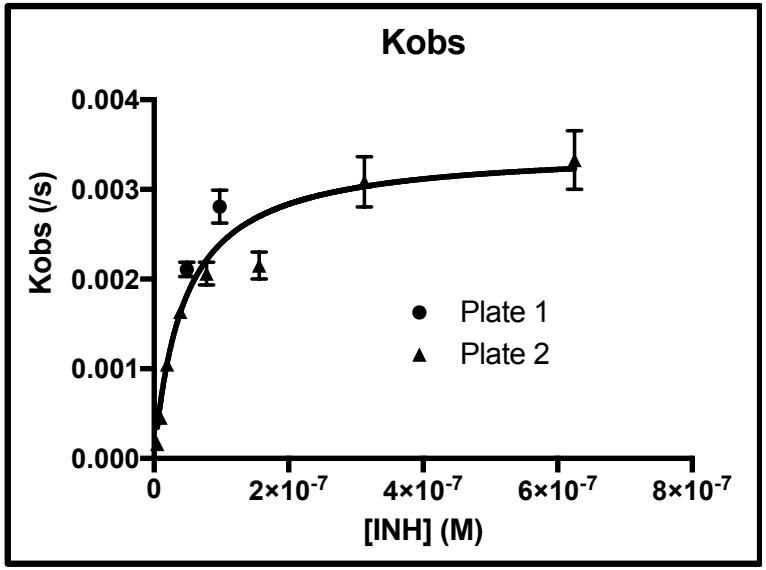
K_i = 39 ± 10 nM

K_{inact}/K_i = 96700 ± 17500 $M^{-1}s^{-1}$

P2: 2ta





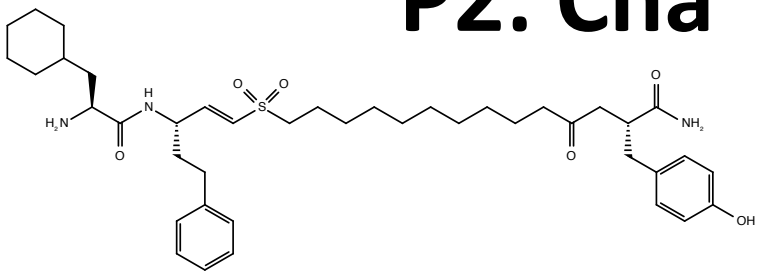


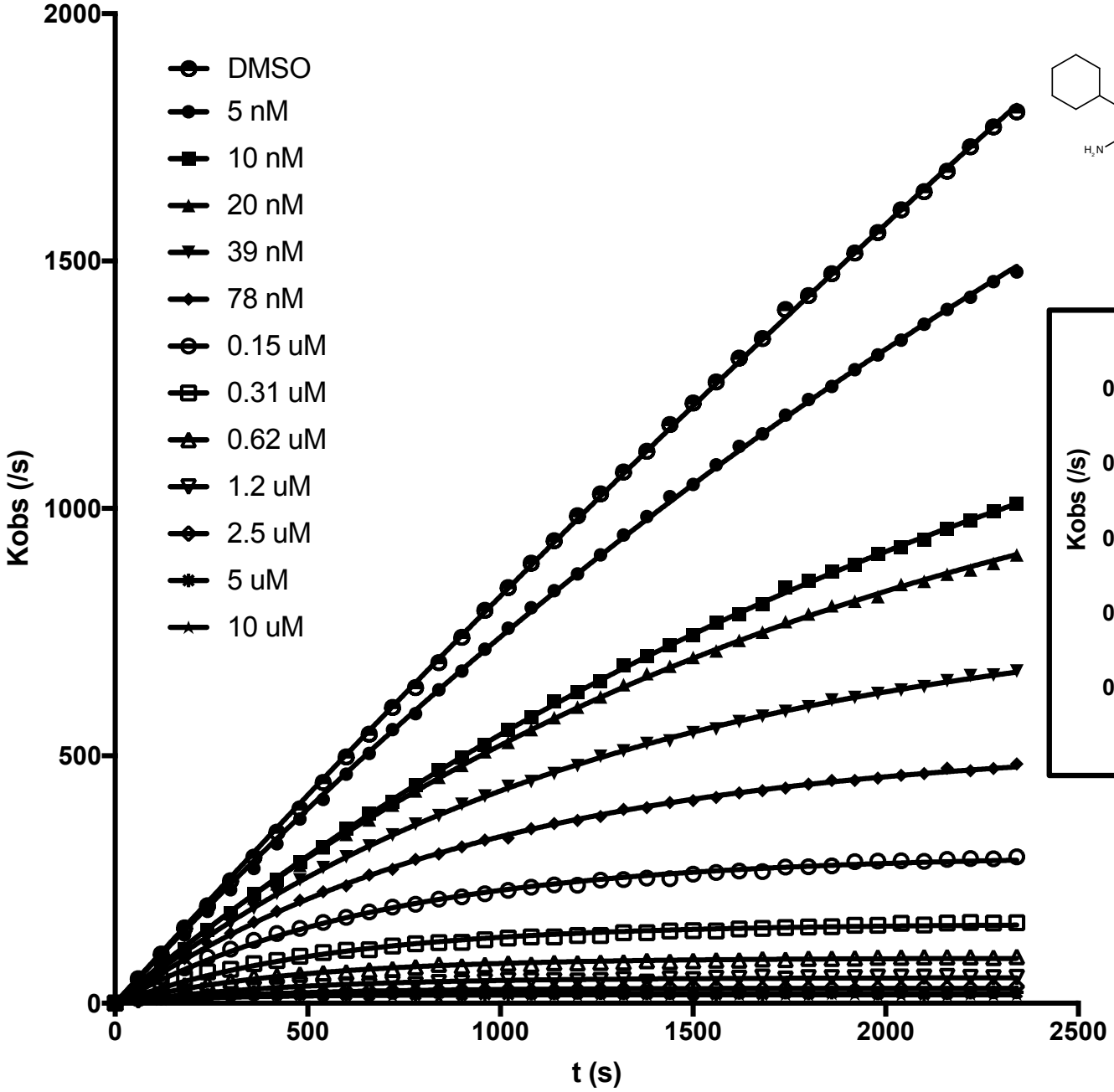
k_{inact} = 0.0034 ± 0.0003 s^{-1}

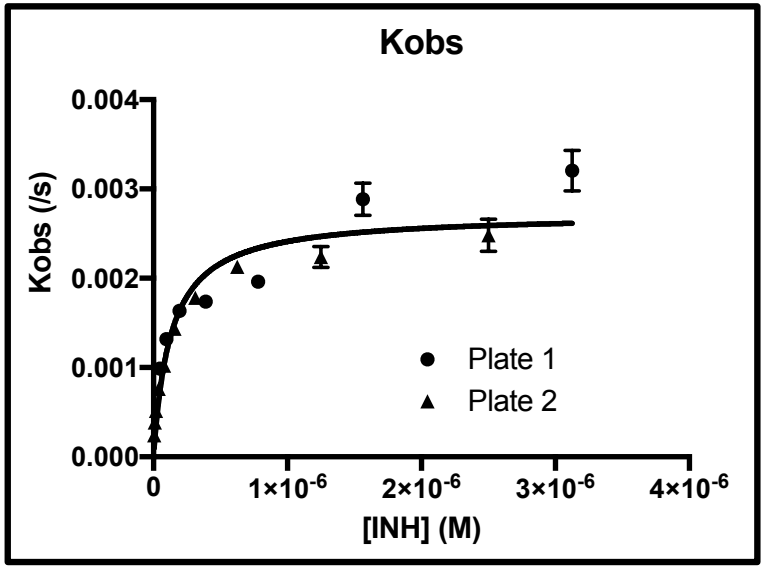
K_i = 29 ± 7 nM

K_{inact}/K_i = 118000 ± 22500 $M^{-1}s^{-1}$

P2: Cha





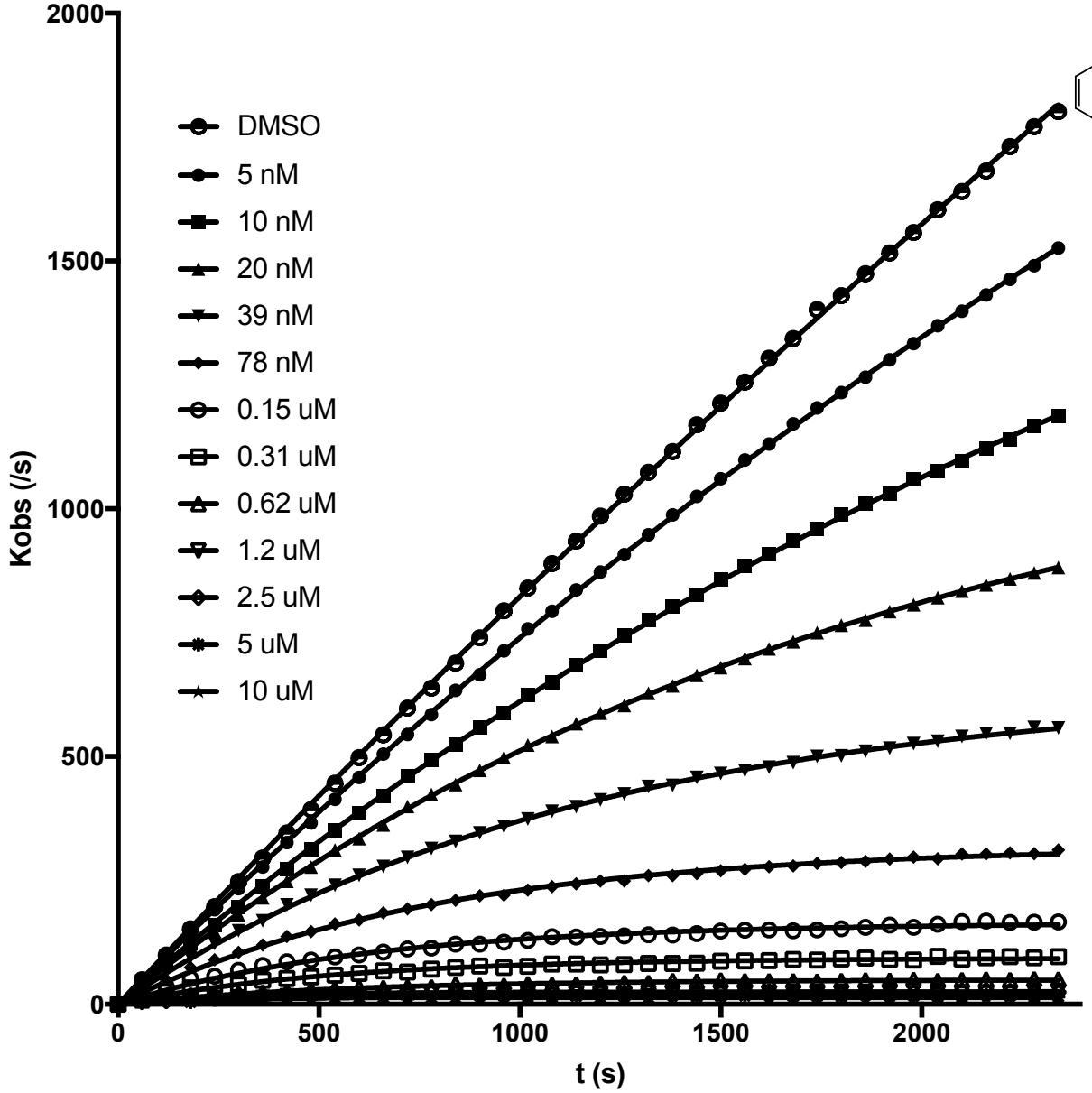


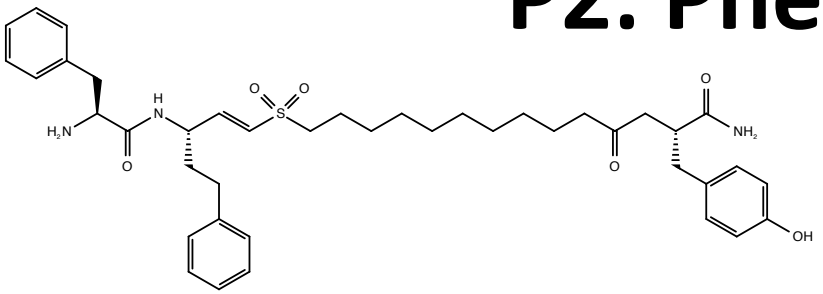
k_{inact} = 0.0027 ± 0.0002 s^{-1}

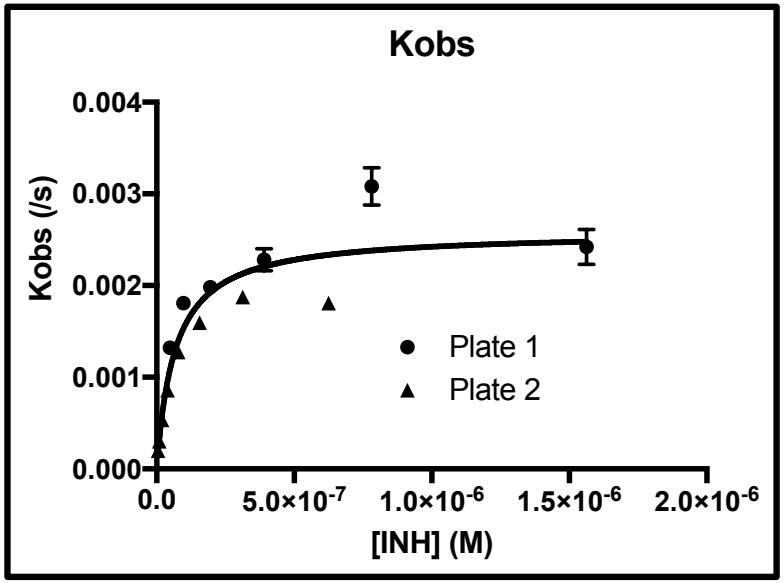
K_i = 86 ± 18 nM

K_{inact}/K_i = 31600 ± 5500 $M^{-1}s^{-1}$

P2: Phe





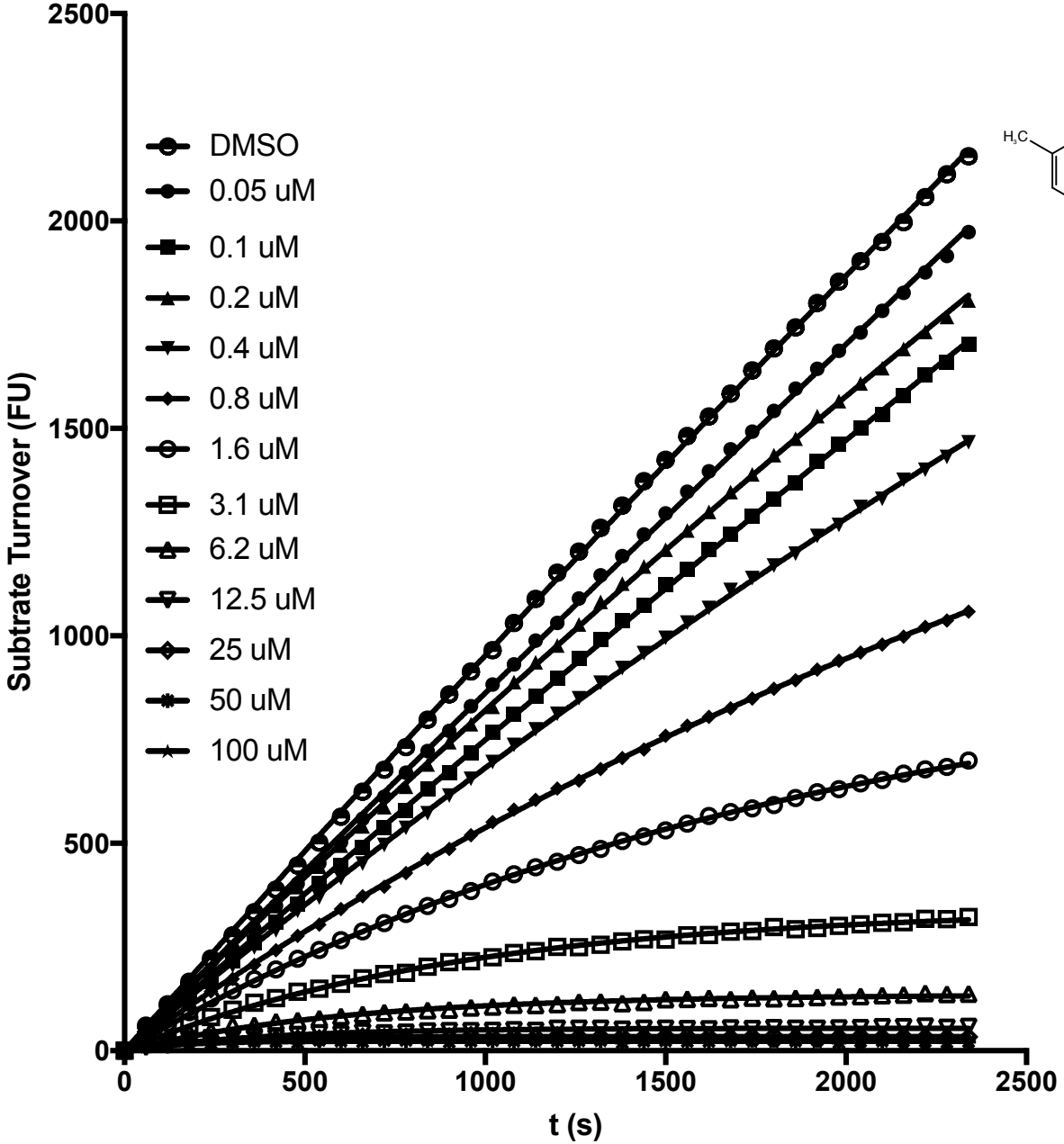


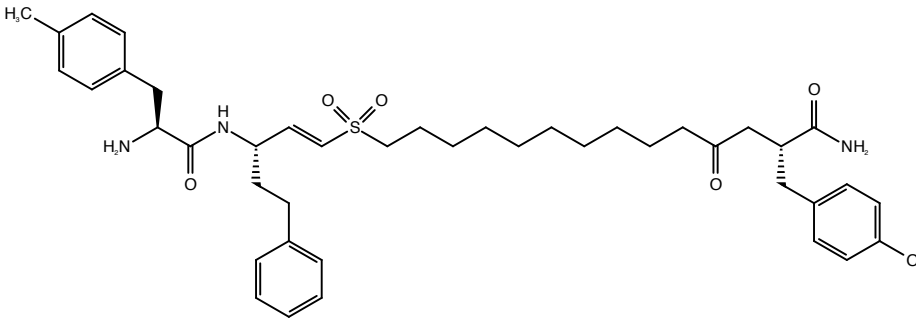
k_{inact} = 0.00207 ± 0.00007 s^{-1}

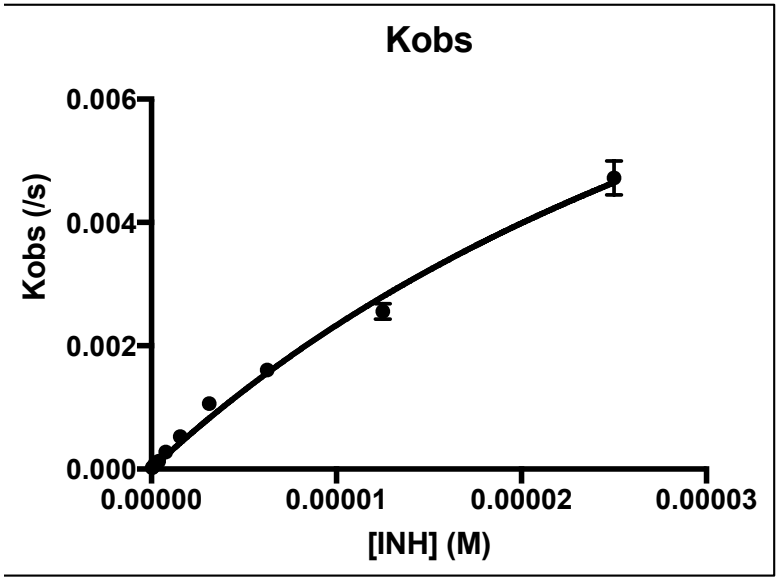
K_i = 37 ± 4 nM

K_{inact}/K_i = 60000 ± 5000 $M^{-1}s^{-1}$

P2: 4mPhe





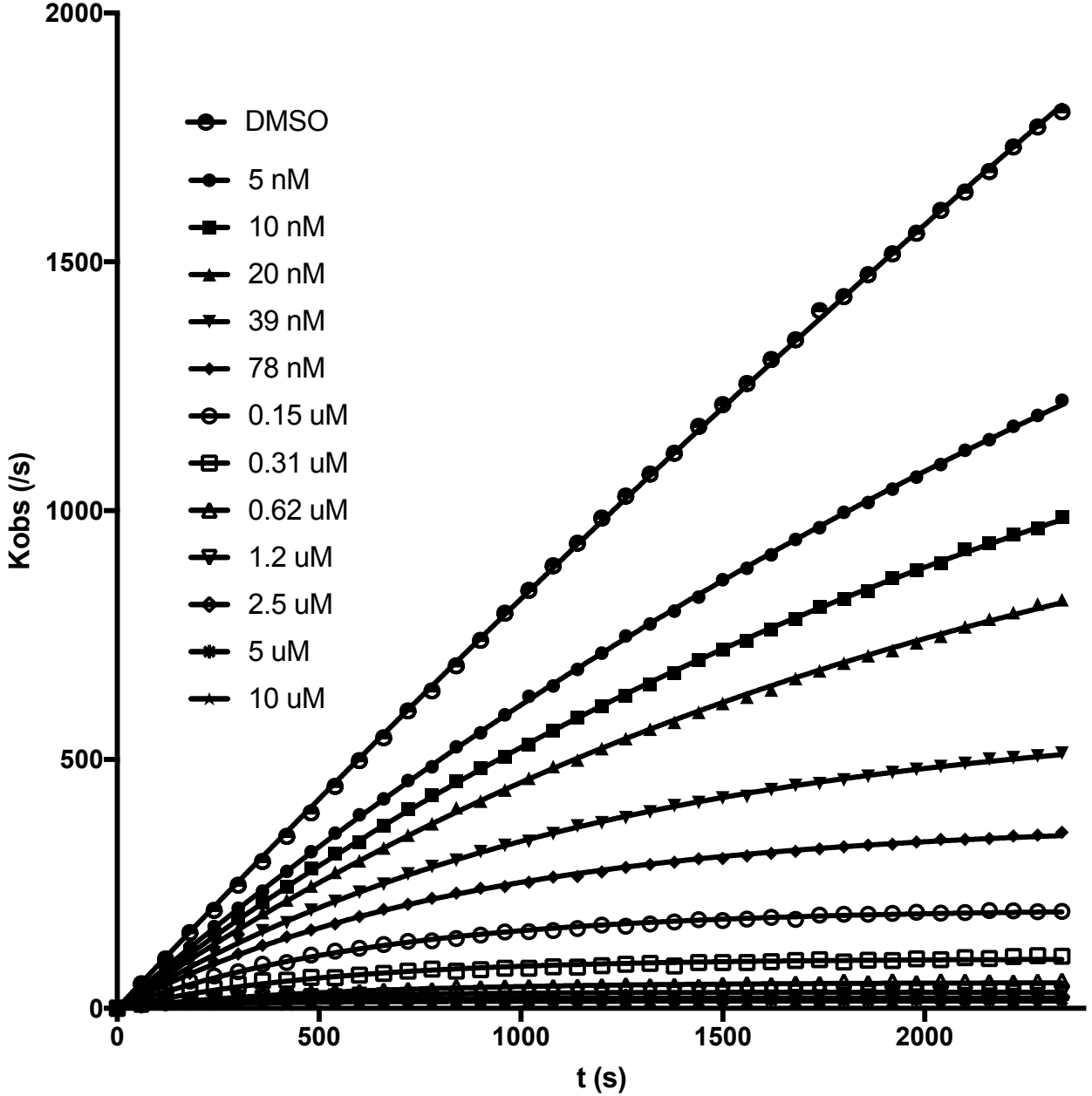


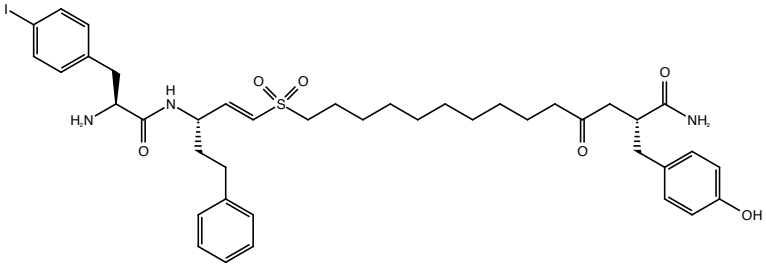
k_{inact} = 0.014 ± 0.003 s^{-1}

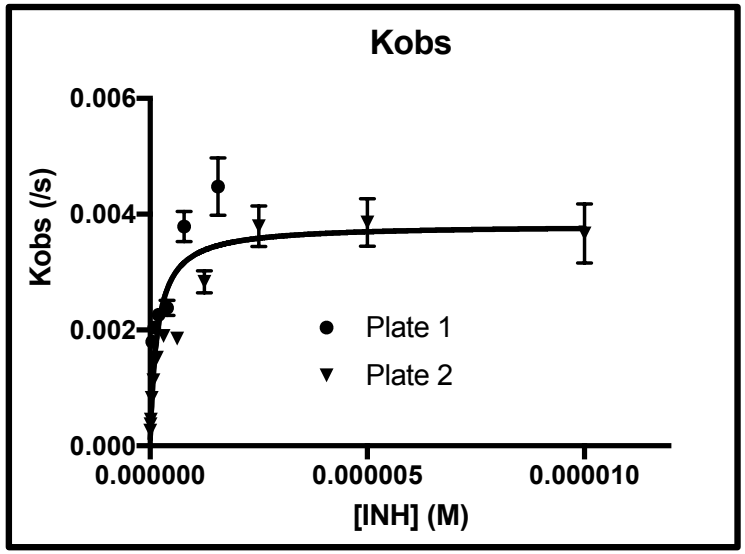
K_i = 33 ± 9 uM

K_{inact}/K_i = 420 ± 30 $M^{-1}s^{-1}$

P2: 4iPhe





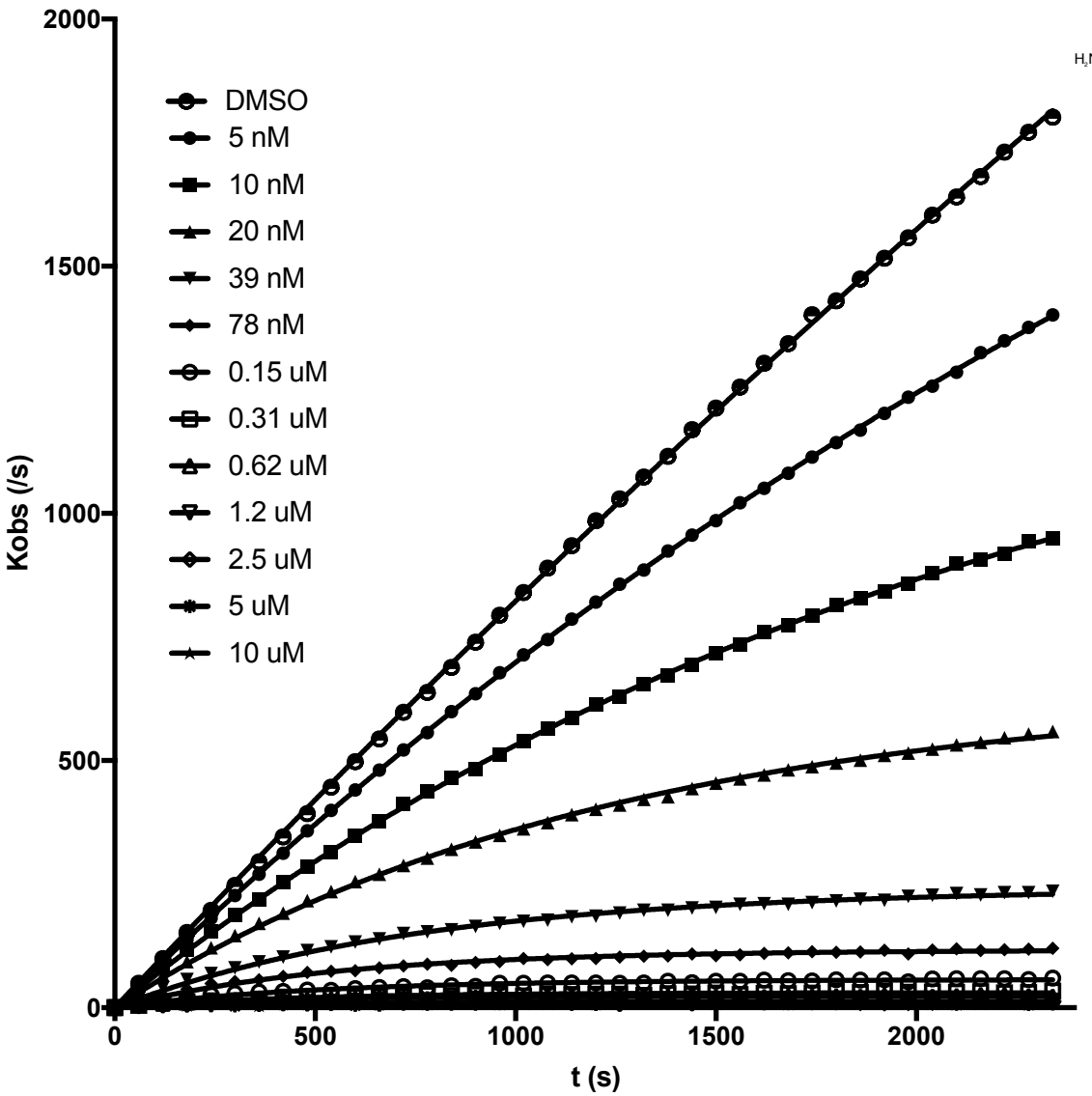


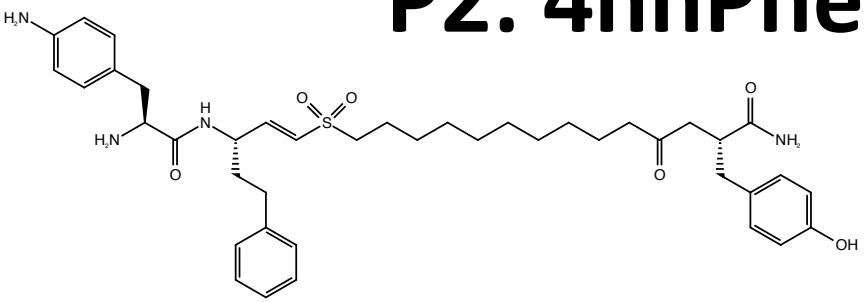
k_{inact} = 0.0038 ± 0.0003 s^{-1}

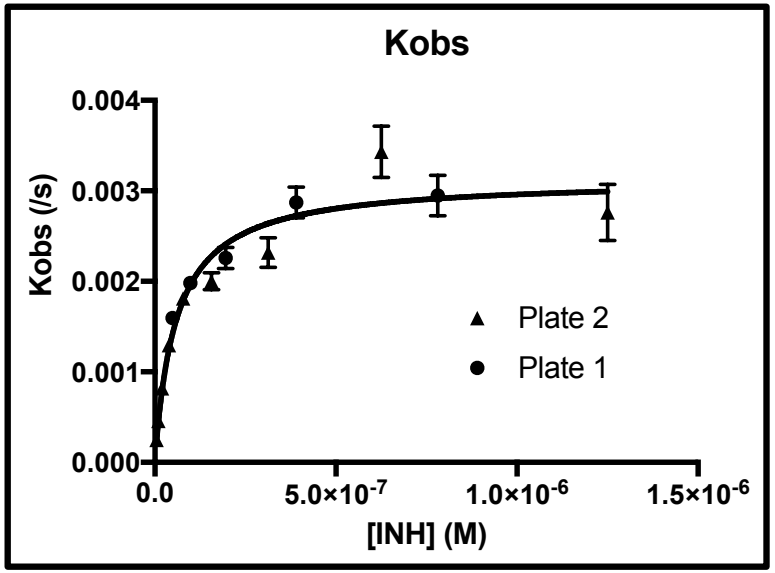
K_i = 0.11 ± 0.03 μM

K_{inact}/K_i = 35500 ± 9300 $M^{-1}s^{-1}$

P2: 4nhPhe





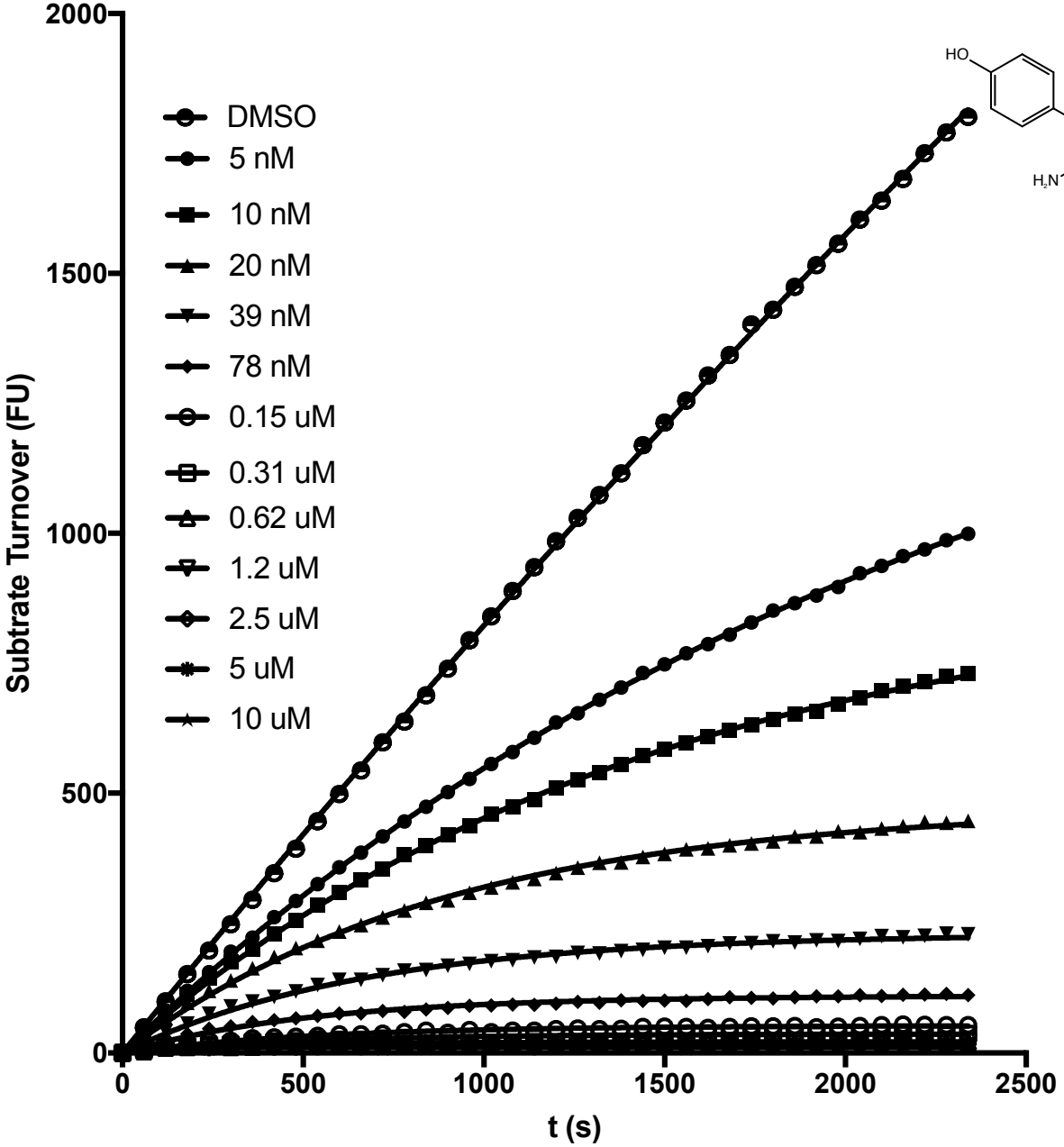


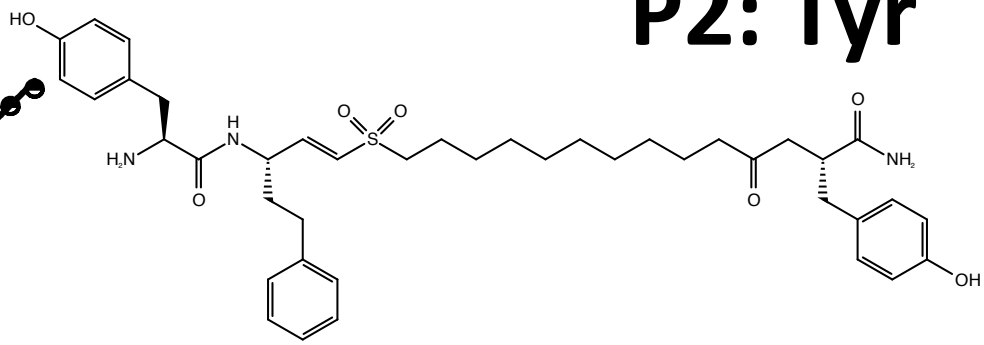
k_{inact} = 0.0031 ± 0.0002 s^{-1}

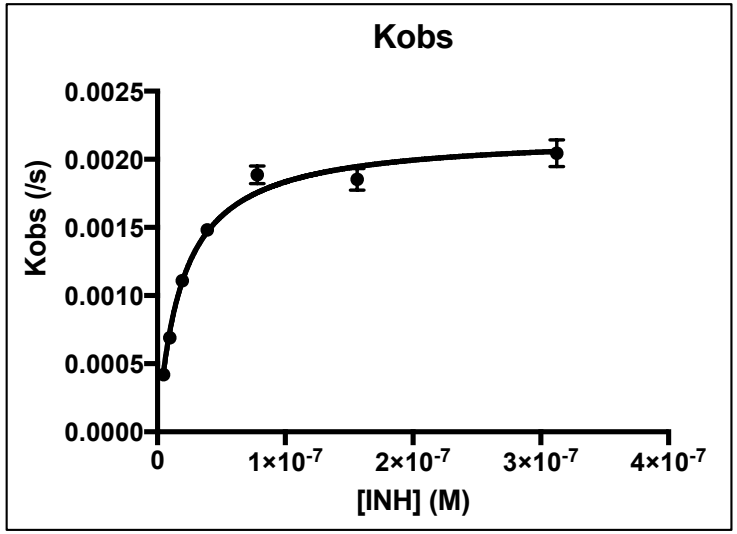
K_i = 39 ± 7 nM

K_{inact}/K_i = 80000 ± 12000 $M^{-1}s^{-1}$

P2: Tyr





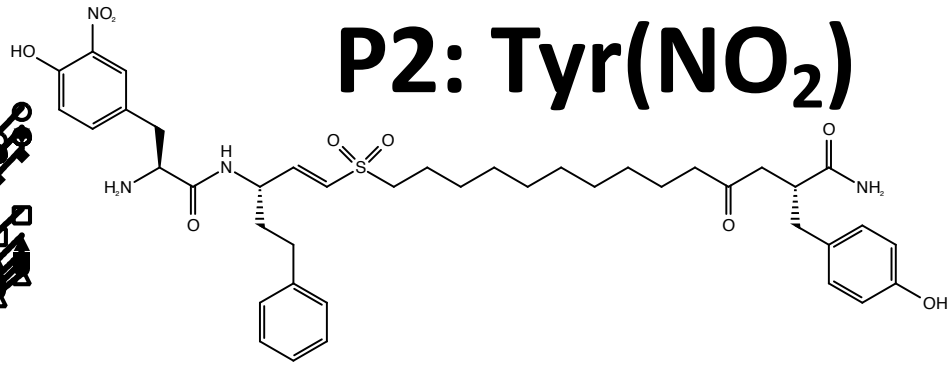


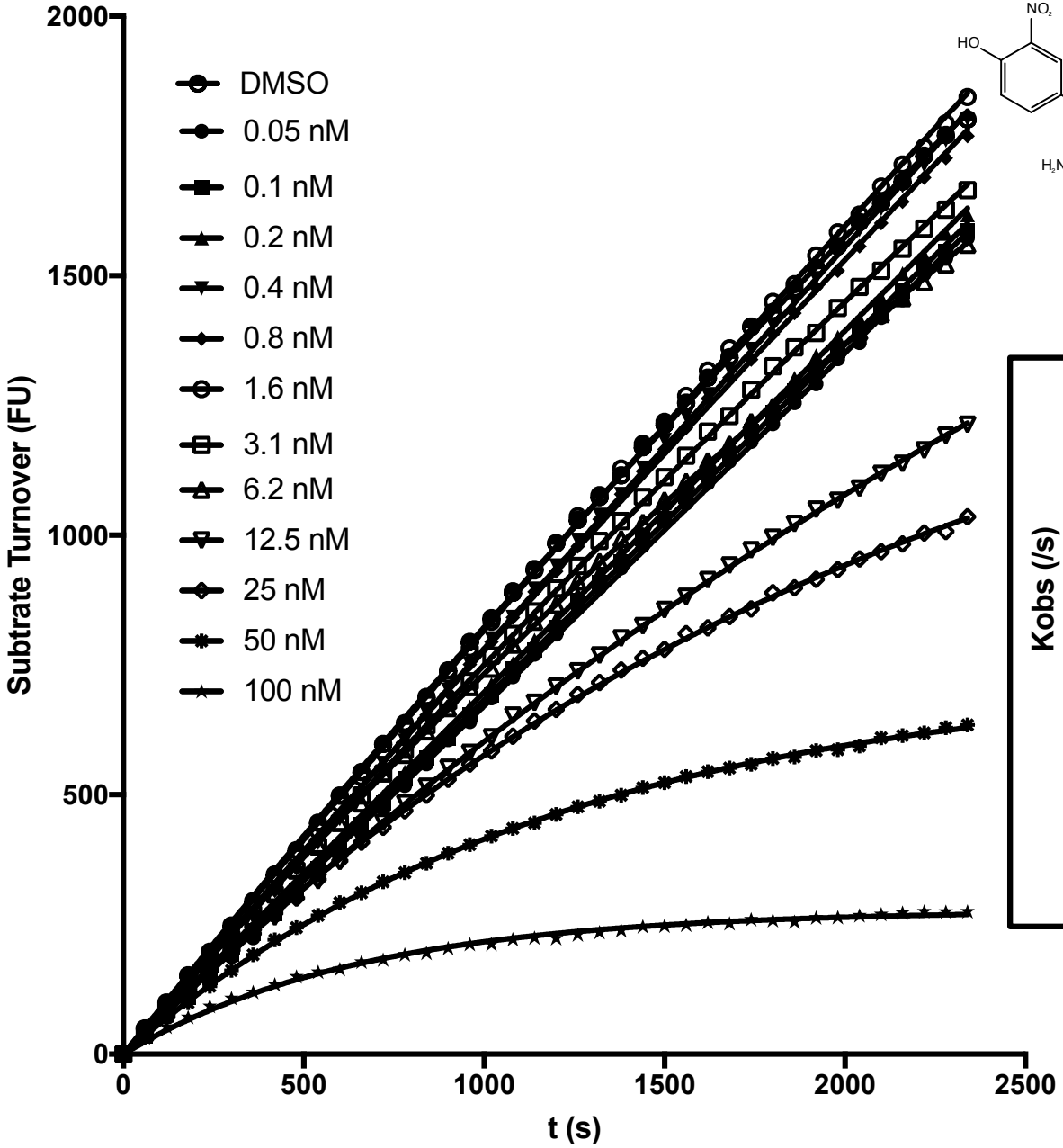
k_{inact} = 0.00218 ± 0.00007 s^{-1}

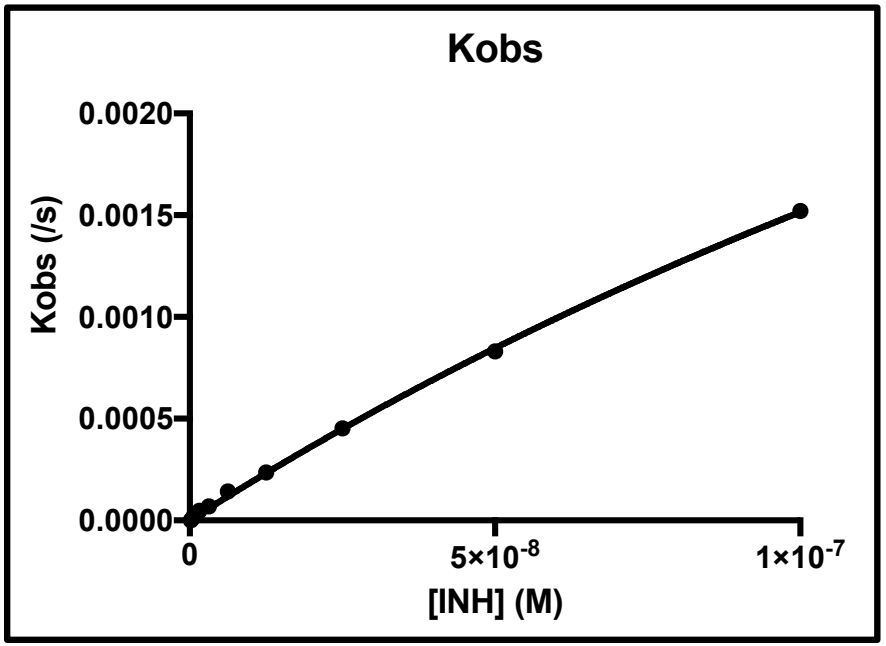
K_i = 12.7 ± 1.5 nM

K_{inact}/K_i = 172000 ± 16000 $M^{-1}s^{-1}$

P2: Tyr(NO_2)





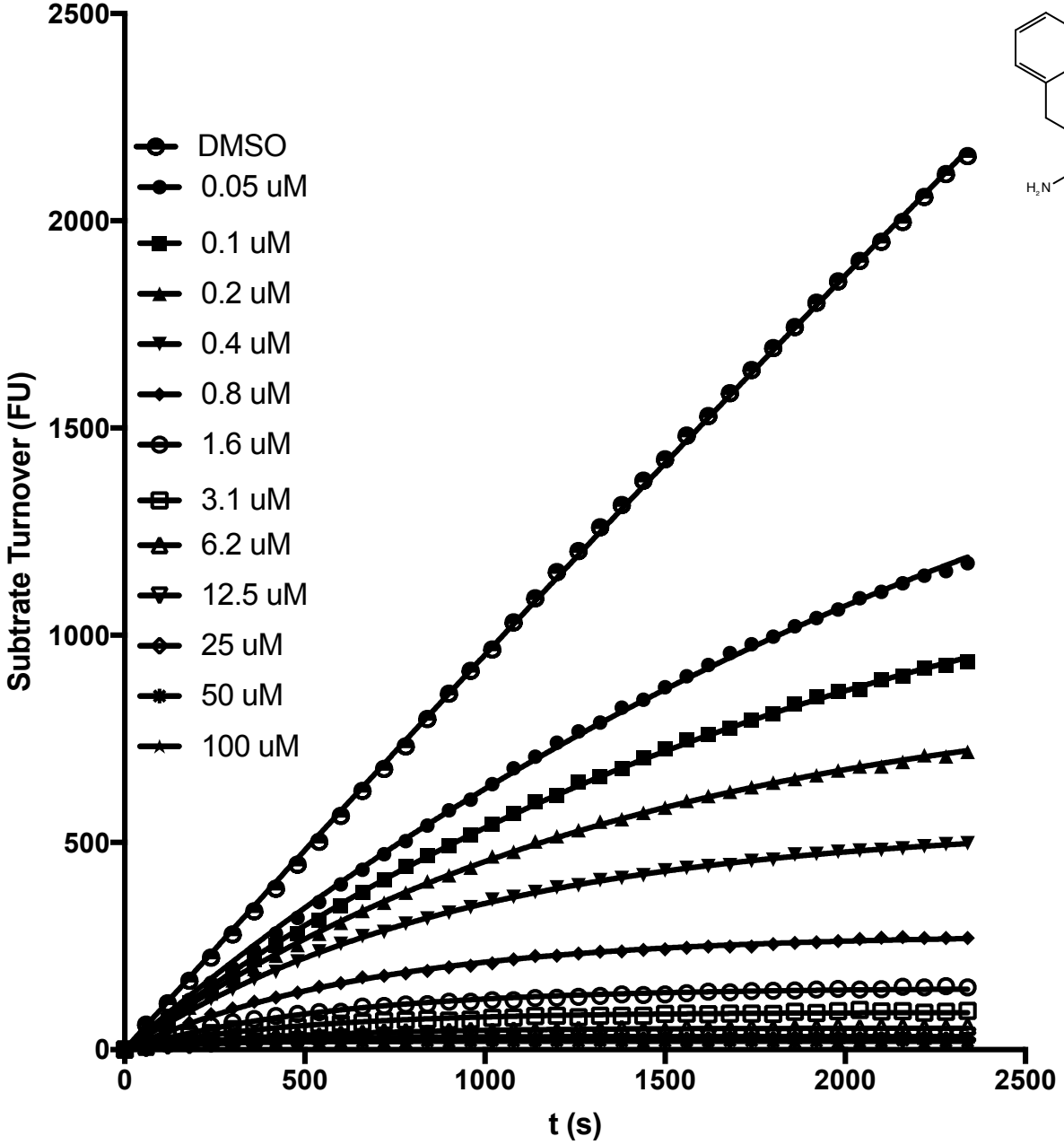


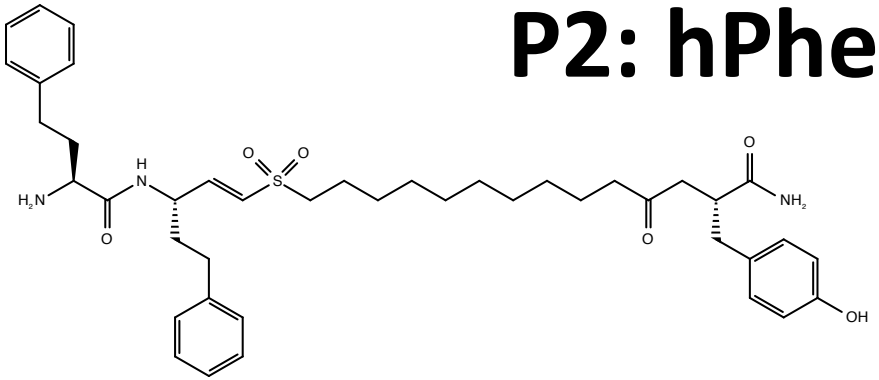
k_{inact} = 0.0072 ± 0.0009 s^{-1}

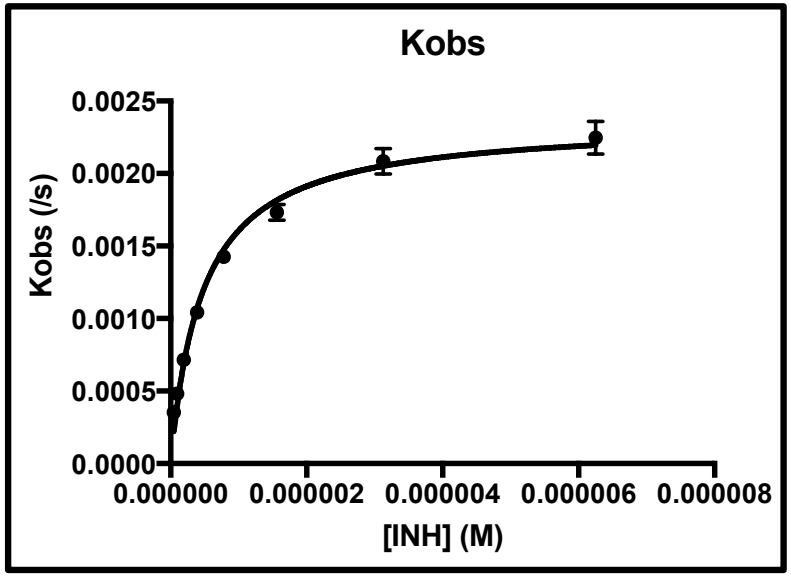
K_i = 251 ± 37 nM

K_{inact}/K_i = 28800 ± 800 $M^{-1}s^{-1}$

P2: hPhe





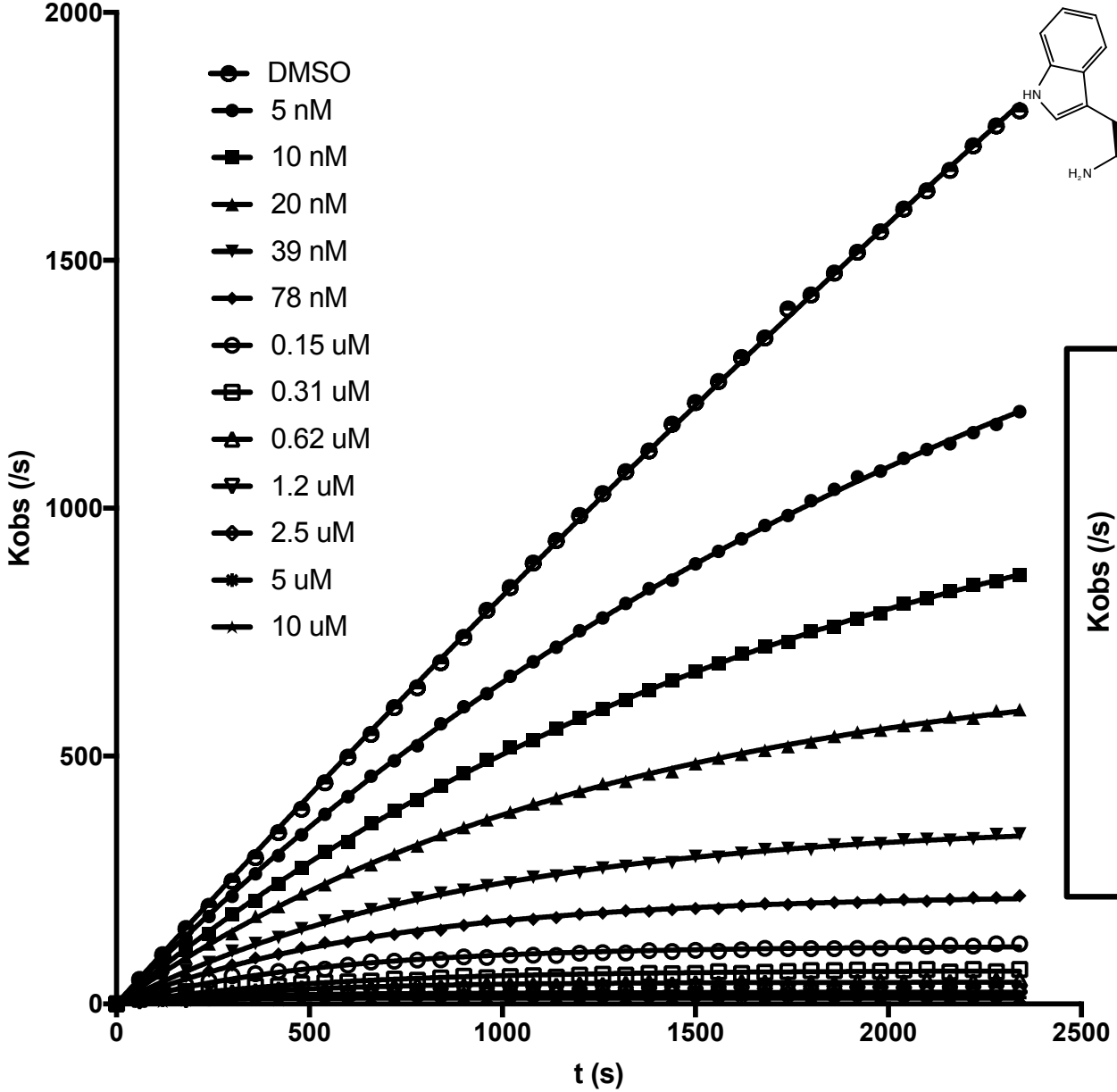


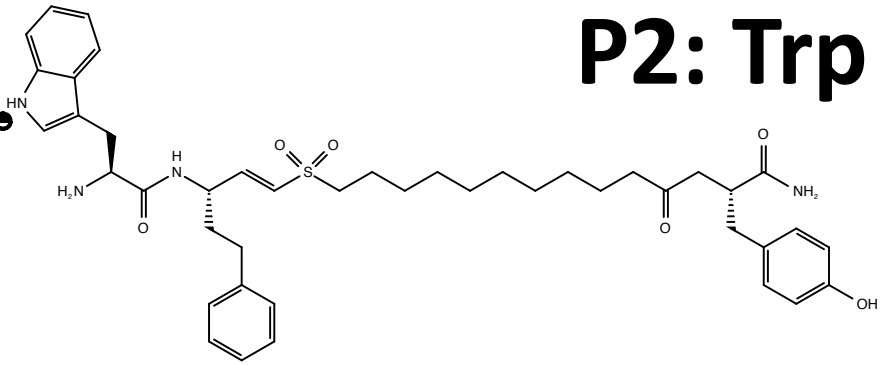
k_{inact} = 0.00236 ± 0.00008 s^{-1}

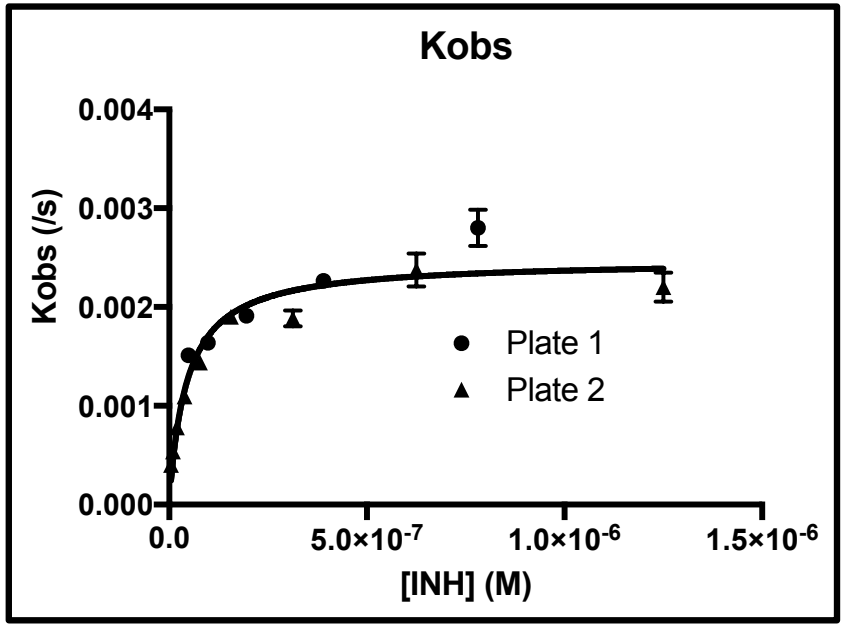
K_i = 312 ± 34 nM

K_{inact}/K_i = 7550 ± 660 $M^{-1}s^{-1}$

P2: Trp





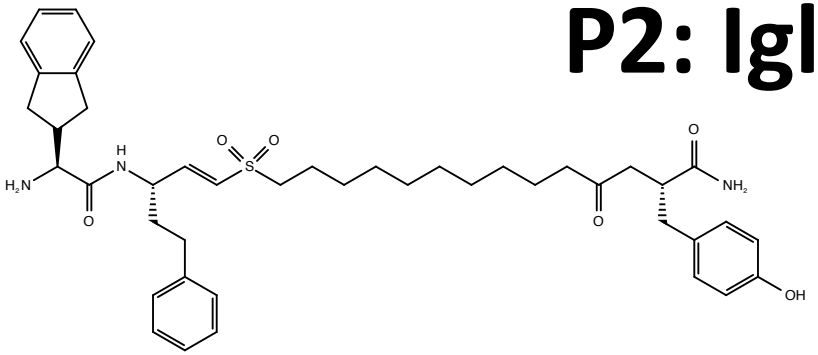


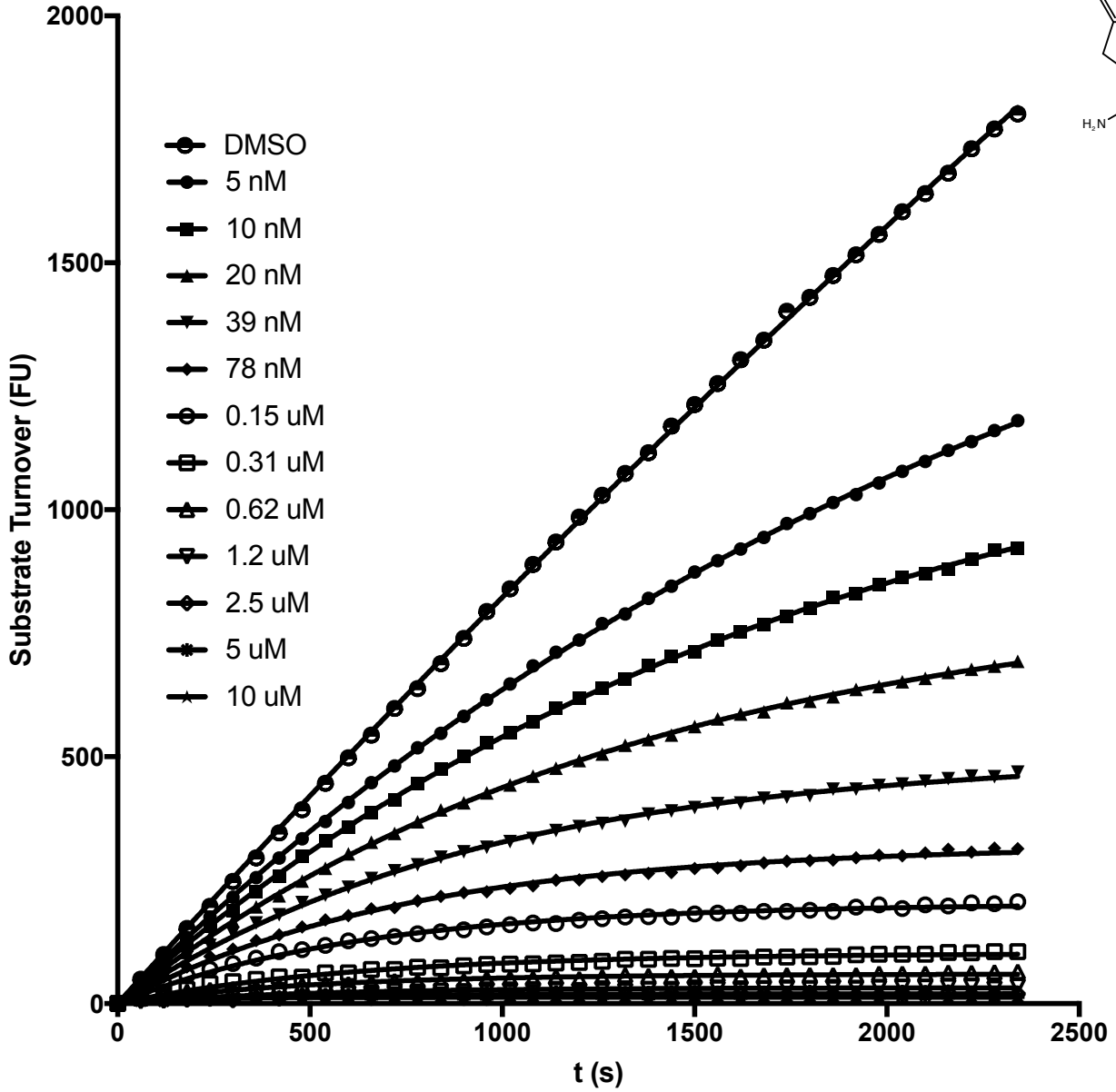
k_{inact} = 0.0025 ± 0.0001 s^{-1}

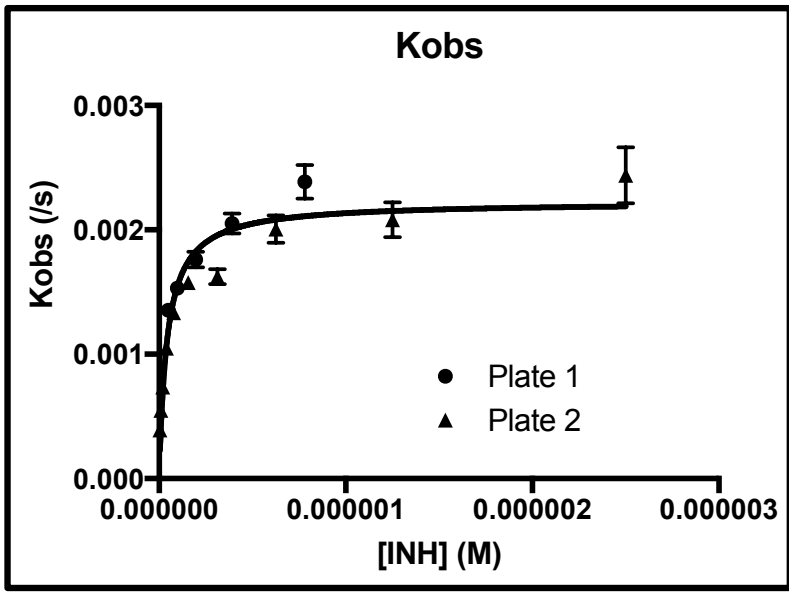
K_i = 30 ± 6 nM

K_{inact}/K_i = 83600 ± 13400 $M^{-1}s^{-1}$

P2: Igl





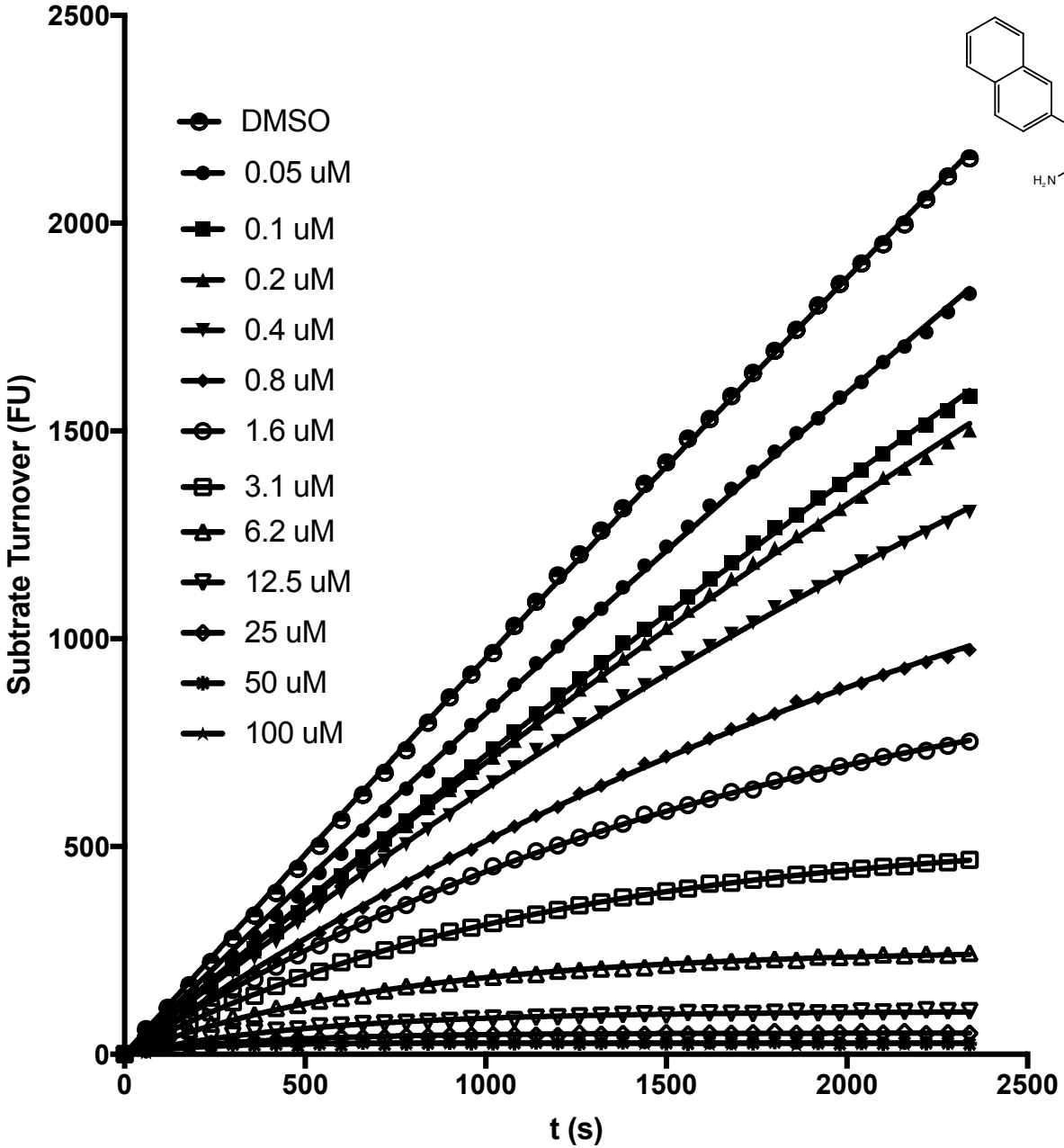


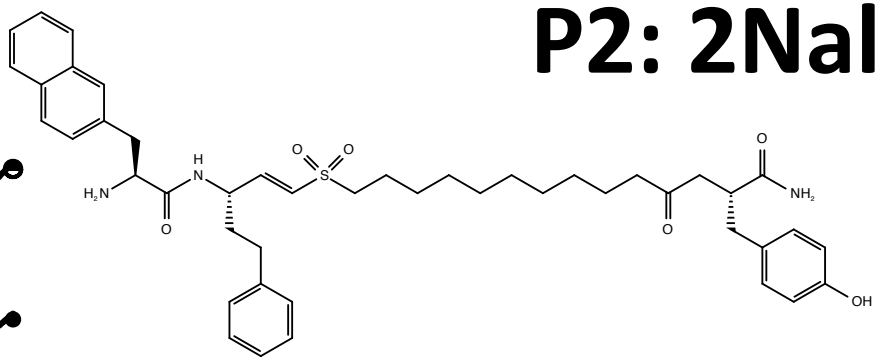
k_{inact} = 0.0022 ± 0.0001 s^{-1}

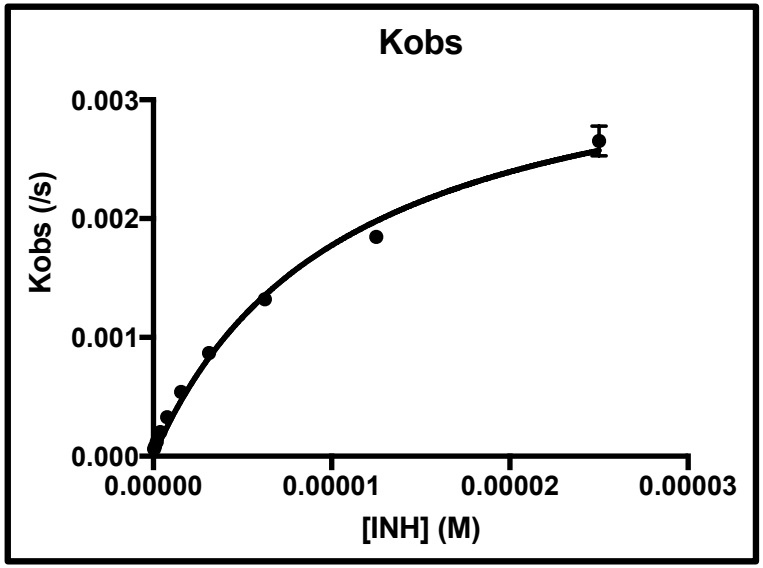
K_i = 29 ± 5 nM

K_{inact}/K_i = 77700 ± 11800 $M^{-1}s^{-1}$

P2: 2Nal





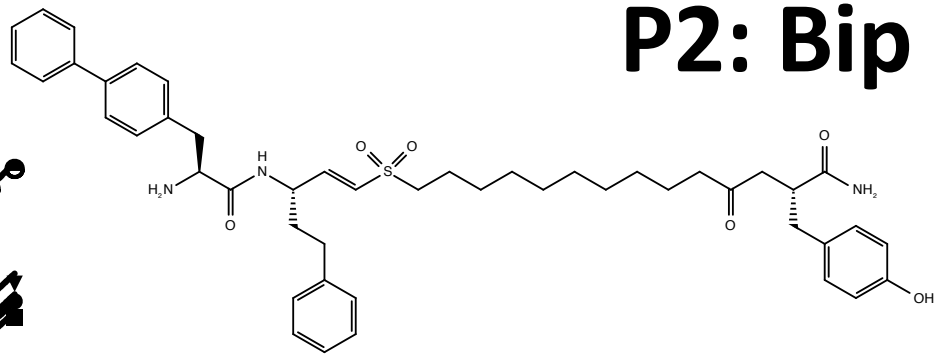


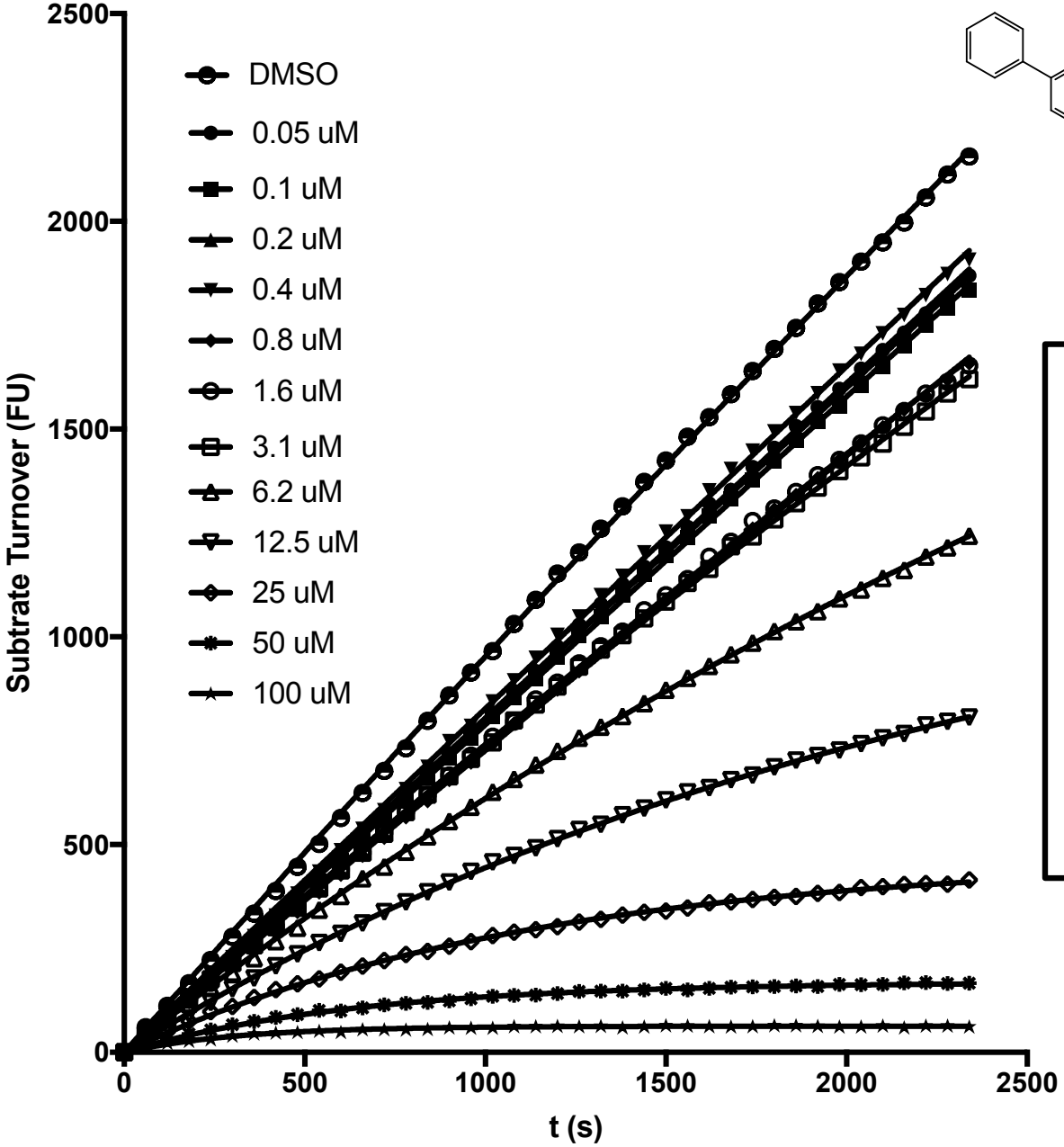
k_{inact} = 0.0037 ± 0.0003 s^{-1}

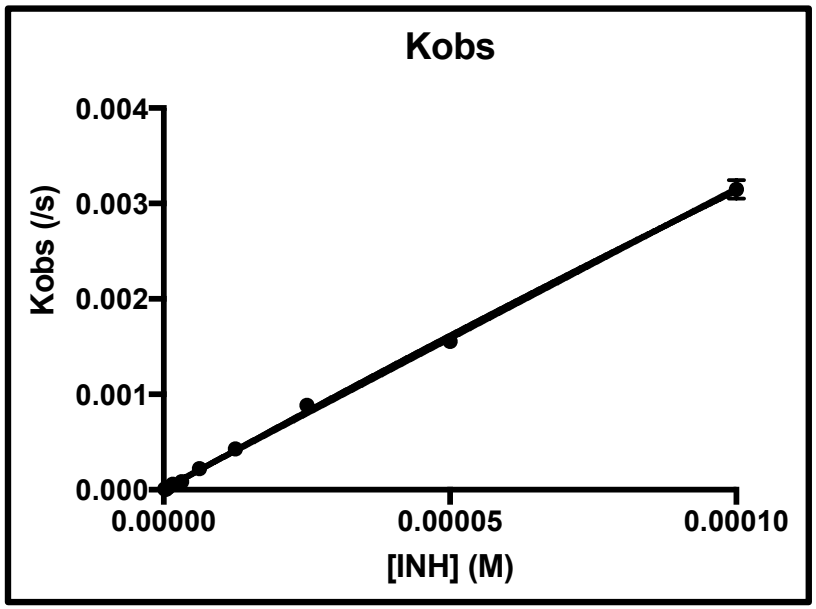
K_i = 7.1 ± 1 uM

K_{inact}/K_i = 514 ± 42 $M^{-1}s^{-1}$

P2: Bip





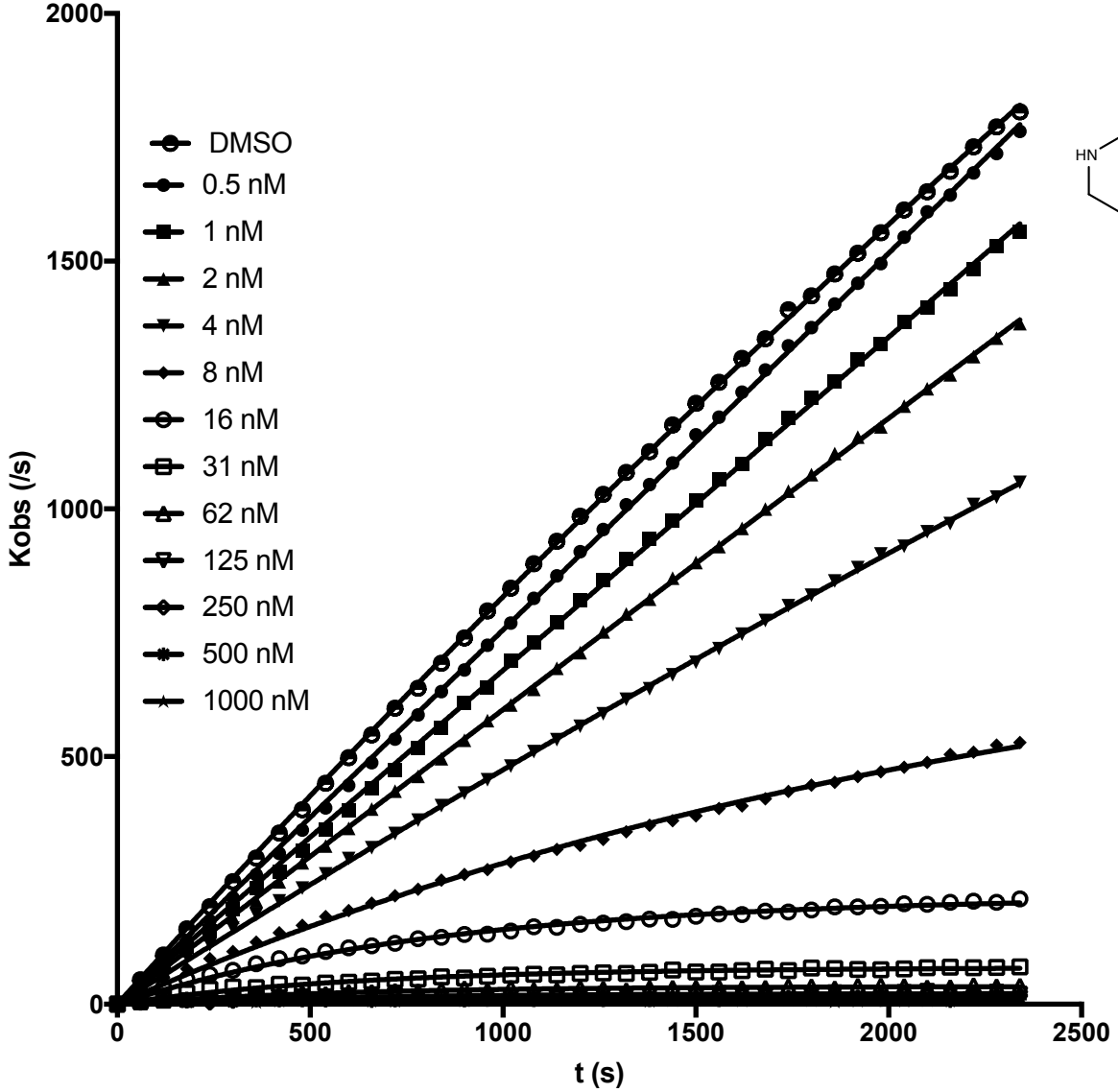


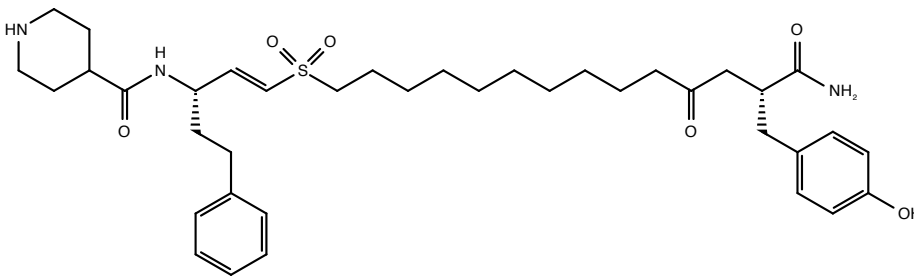
k_{inact} = N/A

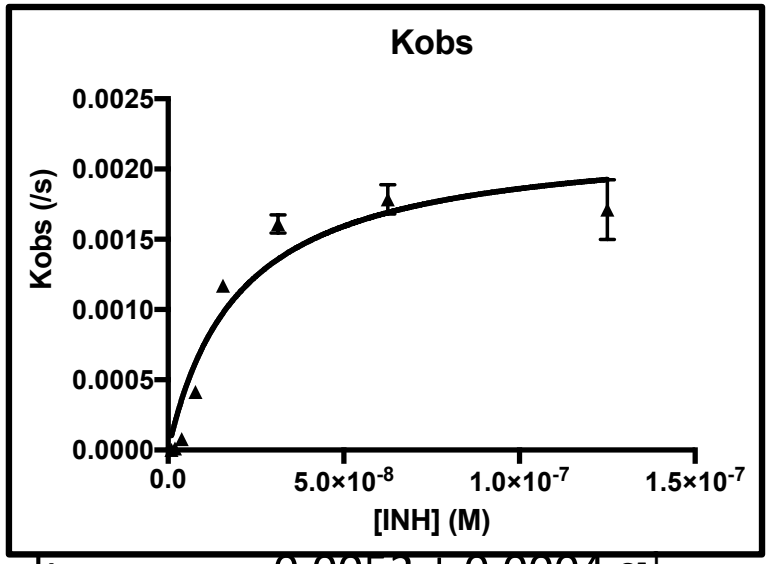
K_i = N/A

K_{inact}/K_i = 31.4 ± 0.4 $M^{-1}s^{-1}$

P2: Inp





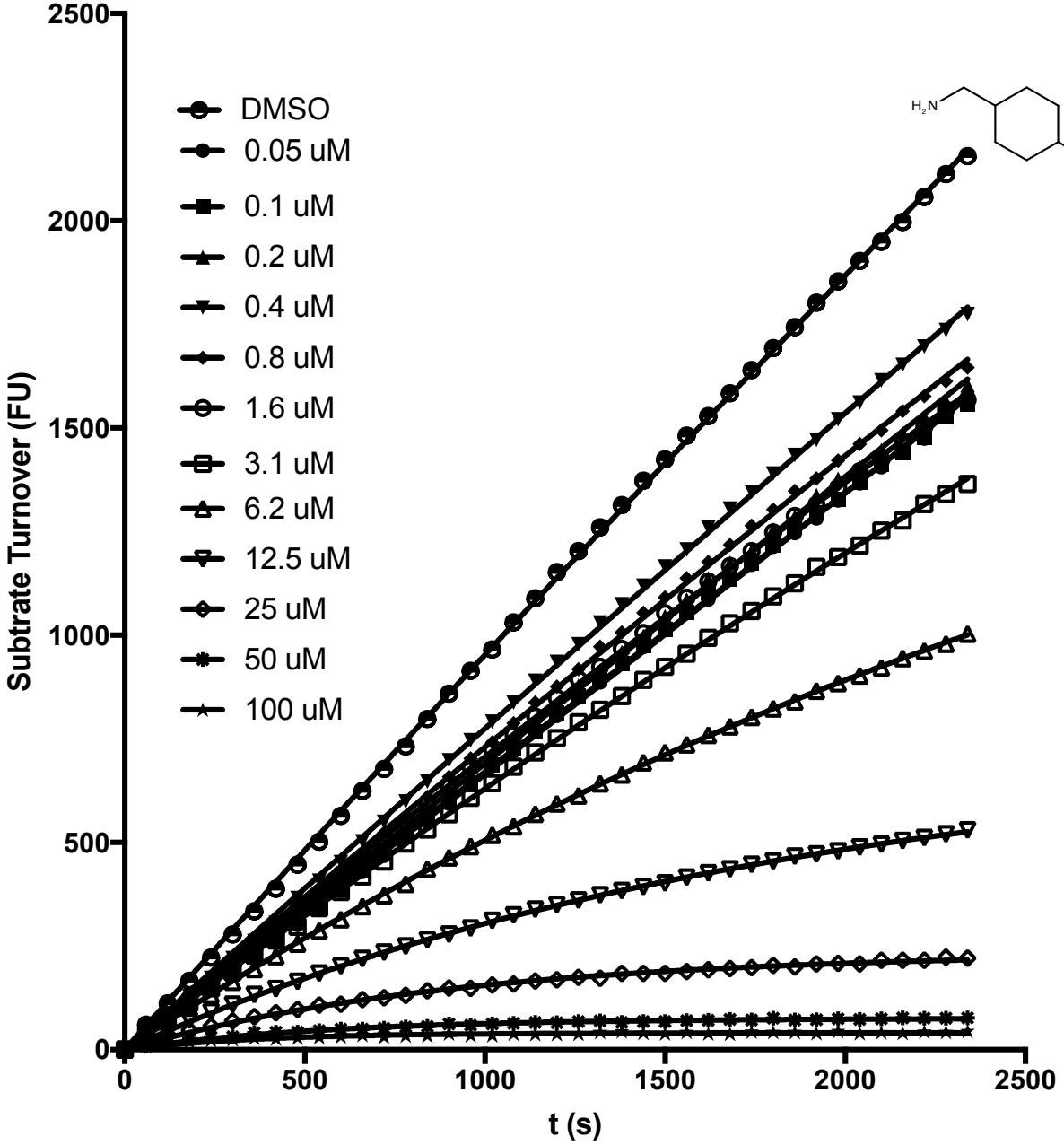


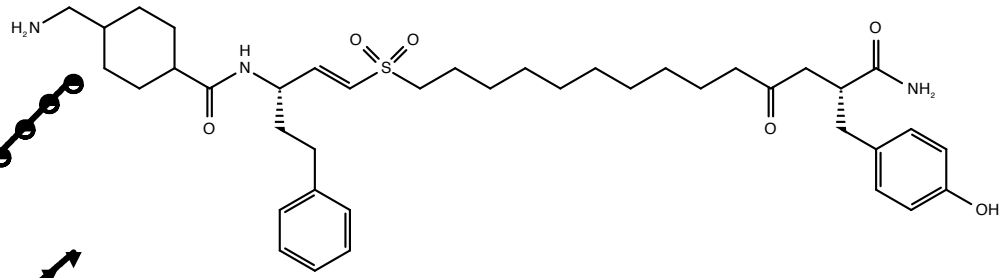
K_{inact} = 0.0052 ± 0.0004 s^{-1}

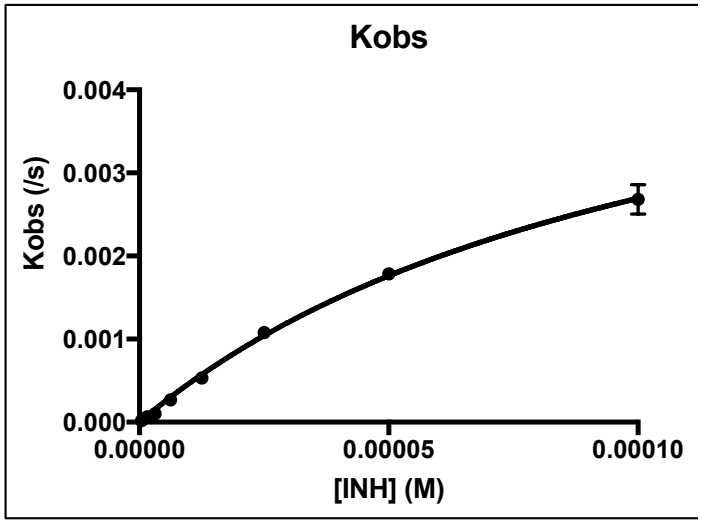
K_i = 65 ± 8 μM

K_{inact}/K_i = 79 ± 4 $M^{-1}s^{-1}$

P2: Amc





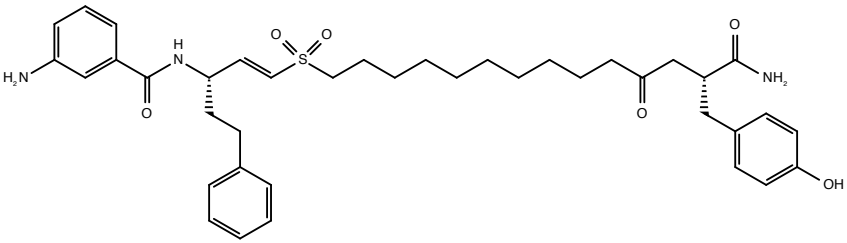


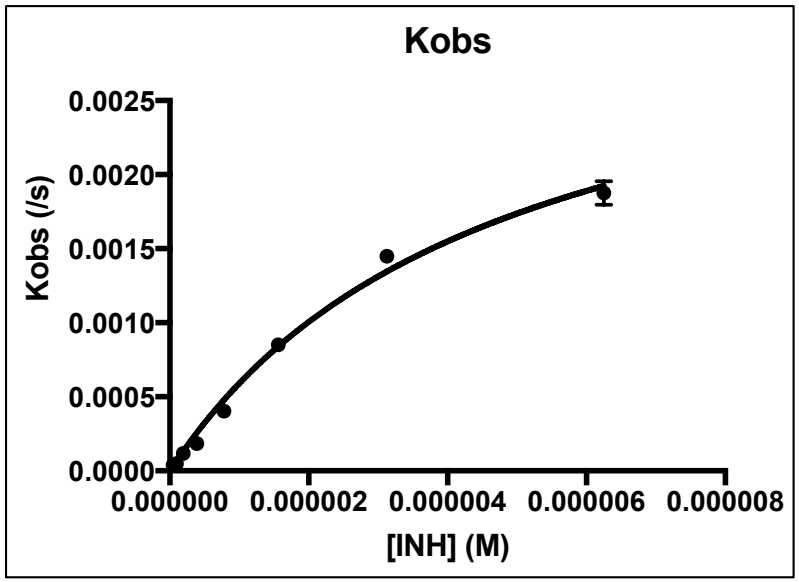
k_{inact} = 0.0057 ± 0.0003 s^{-1}

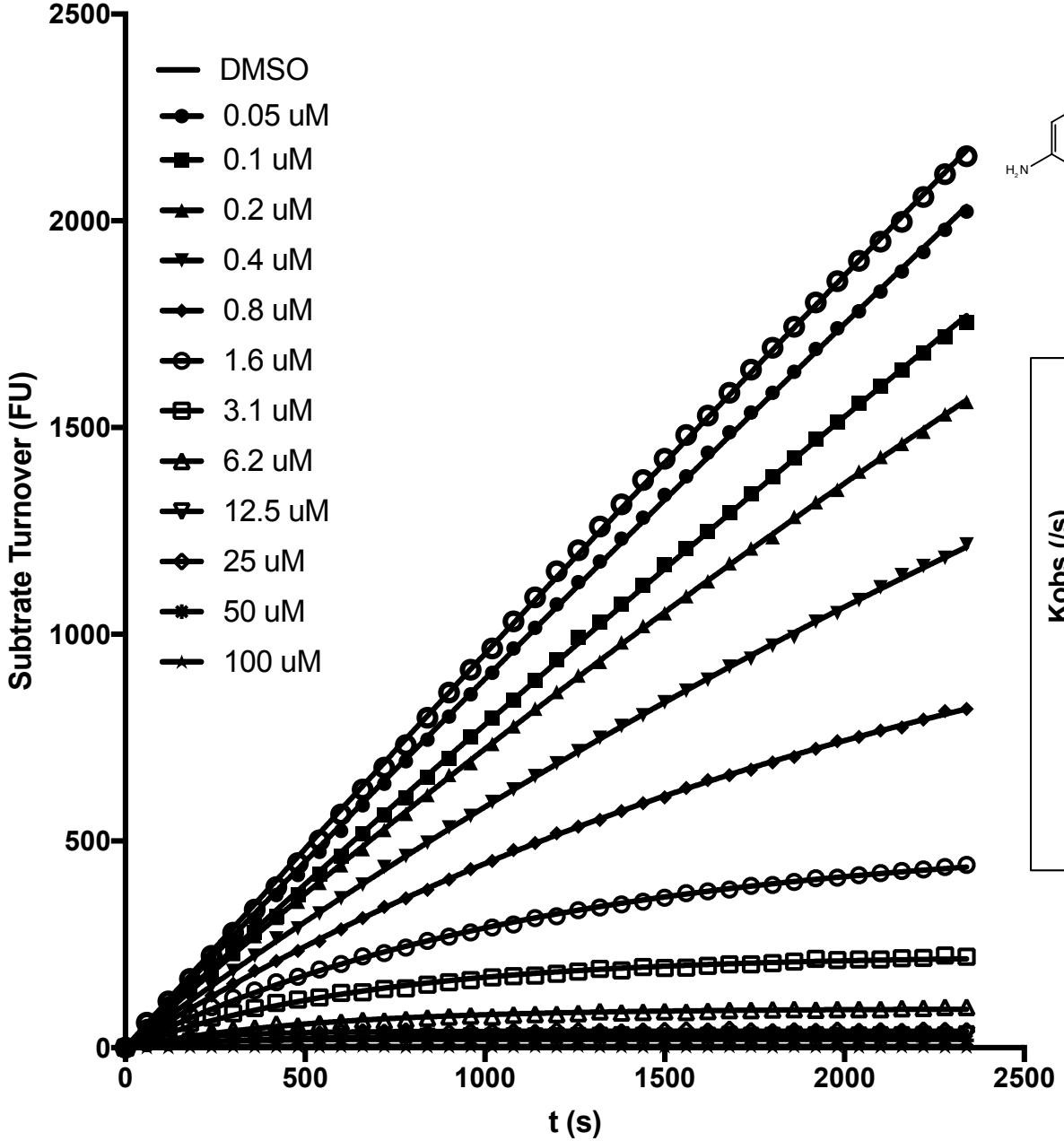
K_i = 75 ± 7 uM

K_{inact}/K_i = 76 ± 3 $M^{-1}s^{-1}$

P2: 3Abz



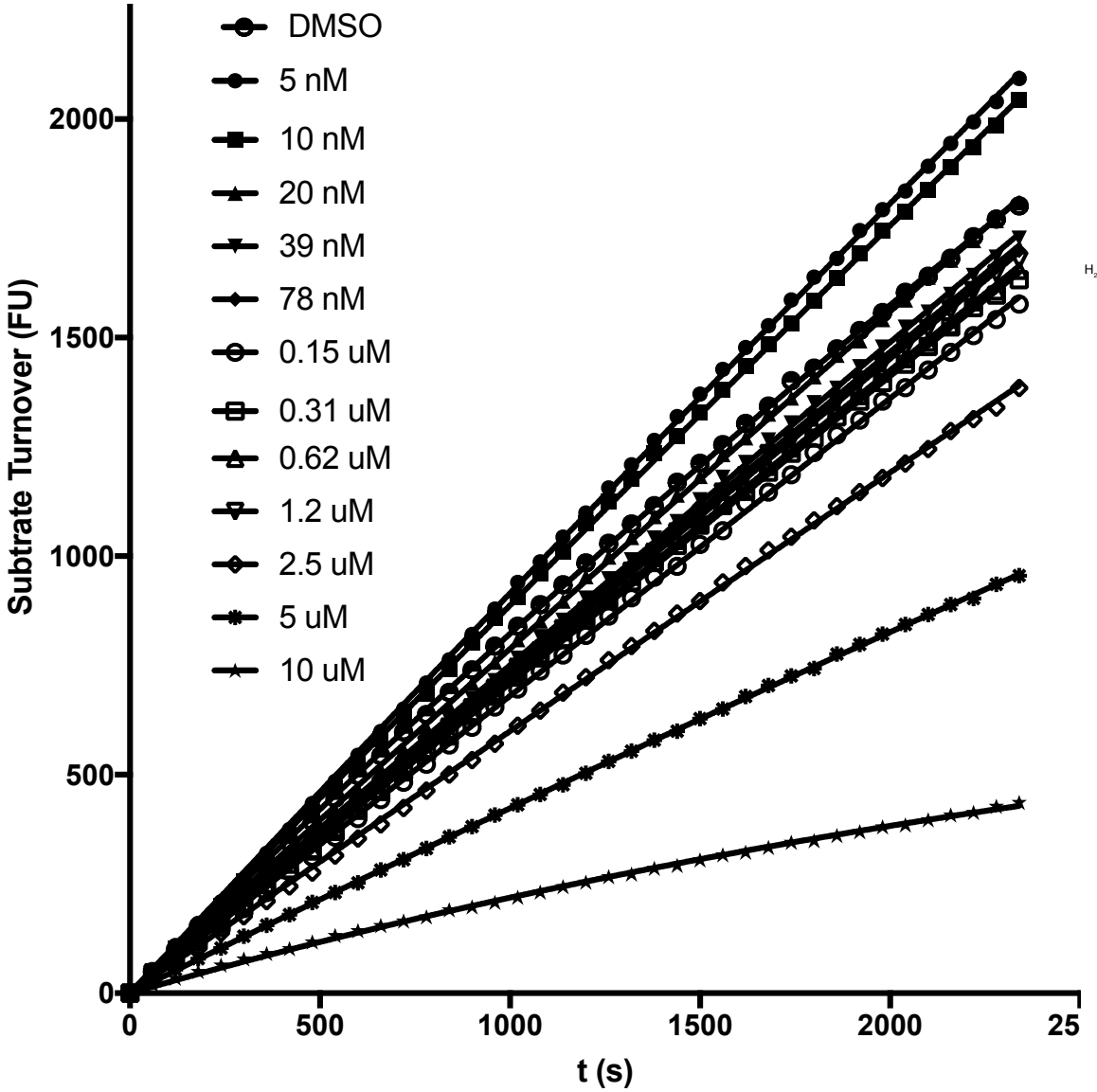




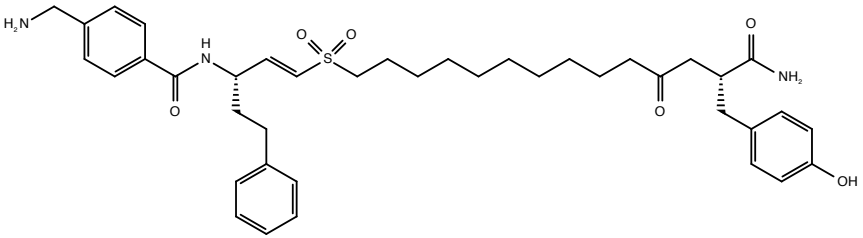
k_{inact} = 0.0034 ± 0.0004 s^{-1}

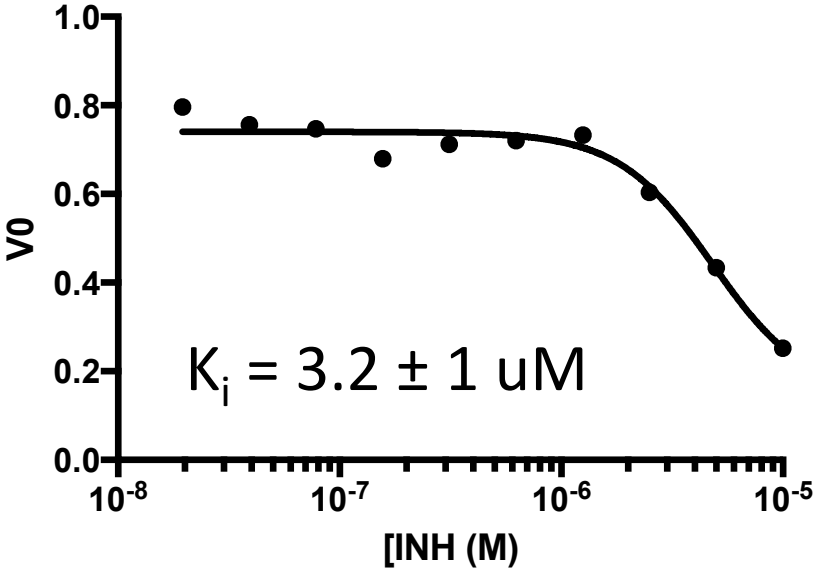
K_i = 3.1 ± 0.6 uM

K_{inact}/K_i = 1074 ± 95 $M^{-1}s^{-1}$

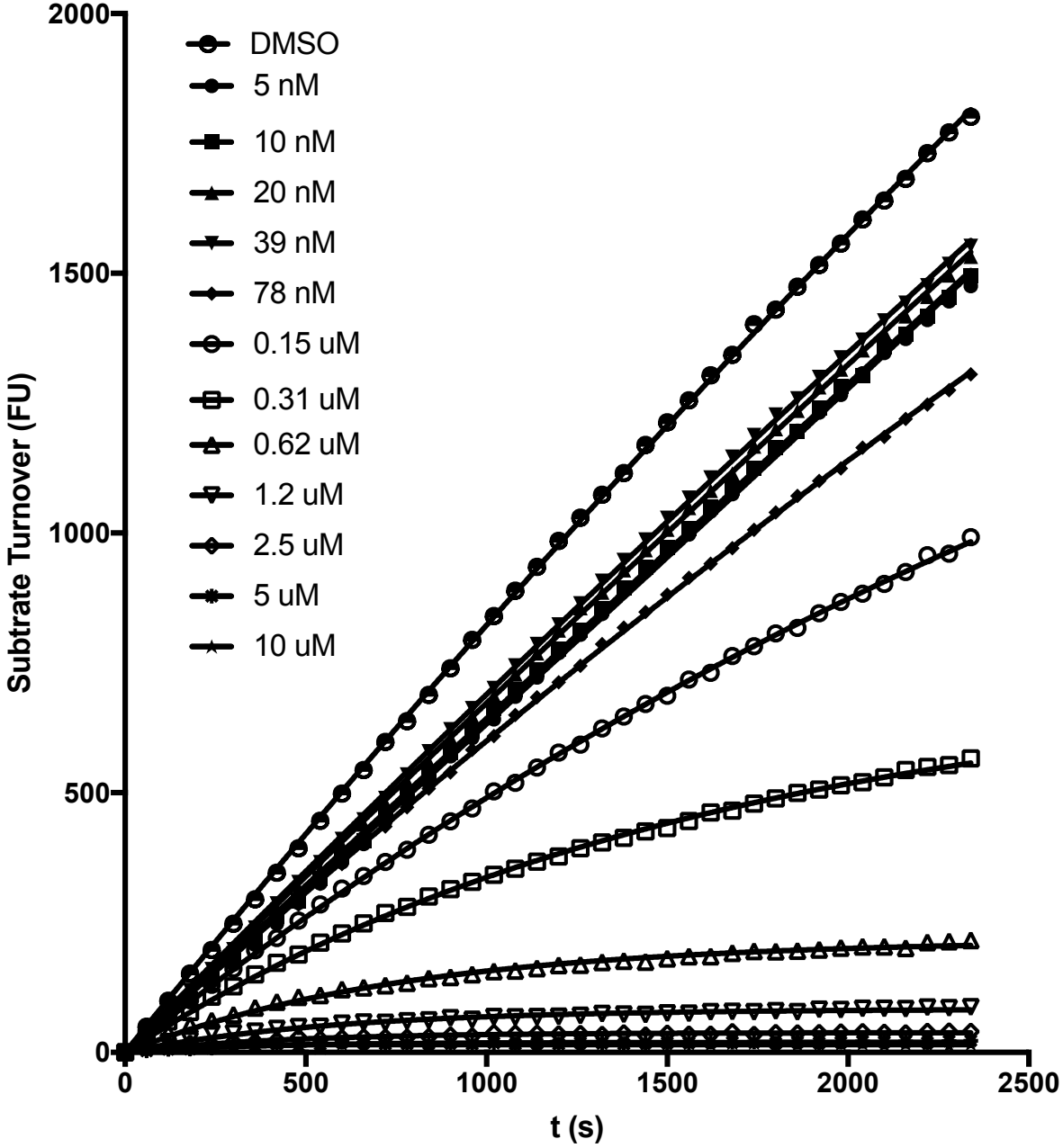

DMSO
5 nM
10 nM
20 nM
39 nM
78 nM
0.15 uM
0.31 uM
0.62 uM
1.2 uM
2.5 uM
5 uM
10 uM
Subtrate Turnover (FU)
2000
1500
1000
500
0
0
500
1000
1500
2000
25
t (s)

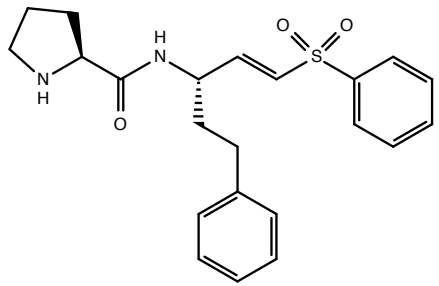
P2: Amb

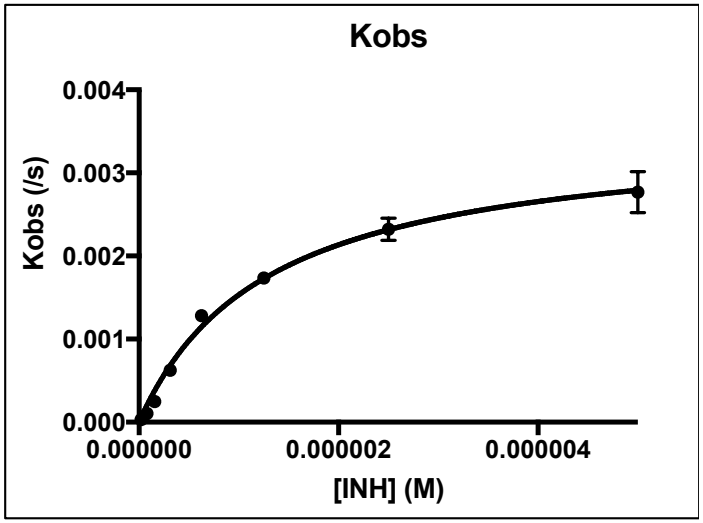



1.0
0.8
0.6
0.4
0.2
0.0
V0
10^{-8}
10^{-7}
10^{-6}
10^{-5}
[INH (M)
K_i = 3.2 ± 1 uM

P2: Pro





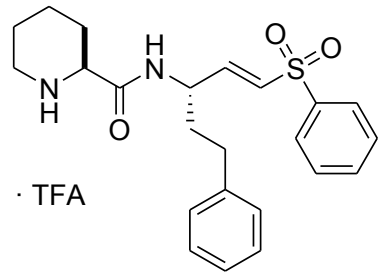


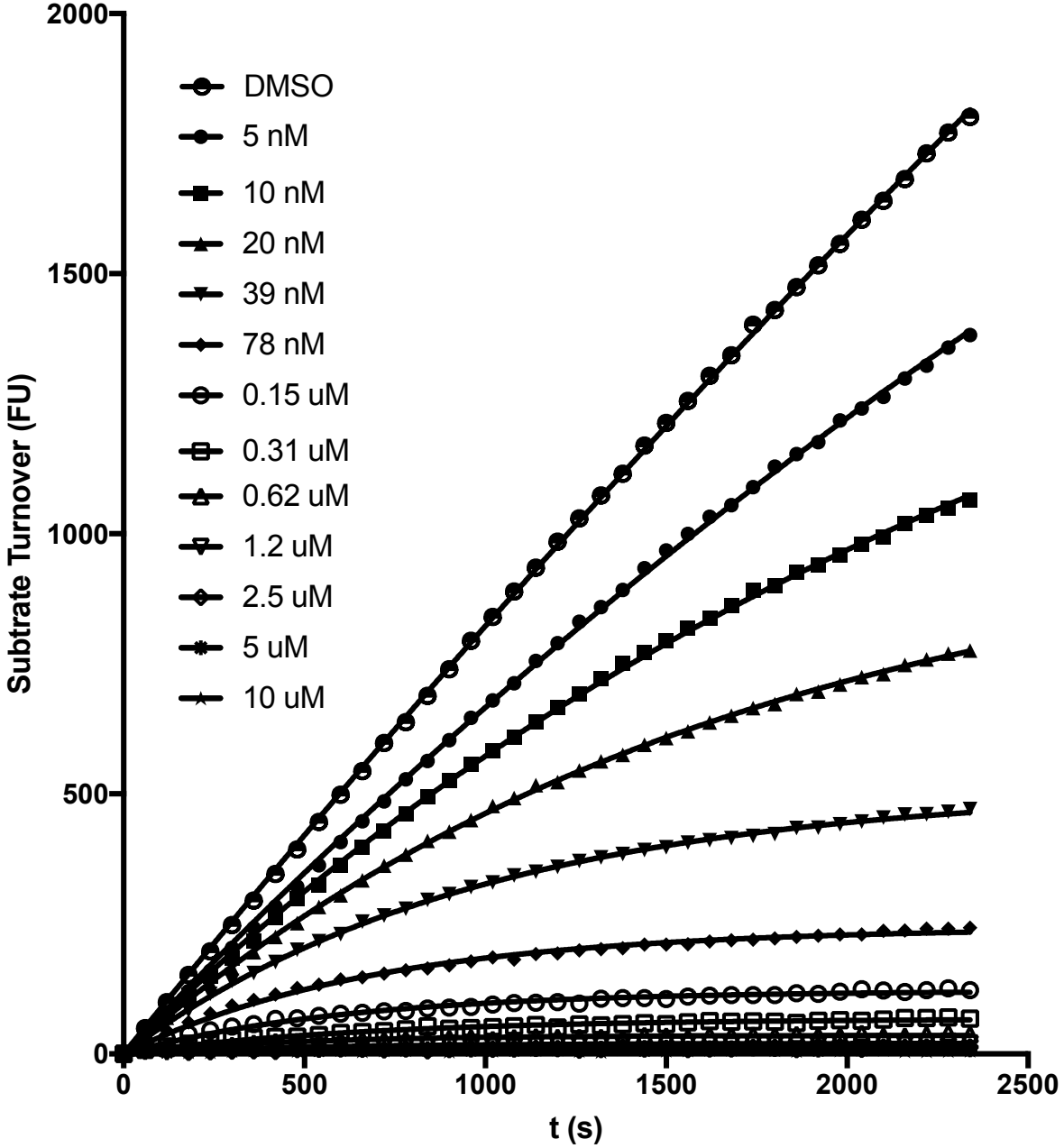
k_{inact} = 0.0035 ± 0.0002 s^{-1}

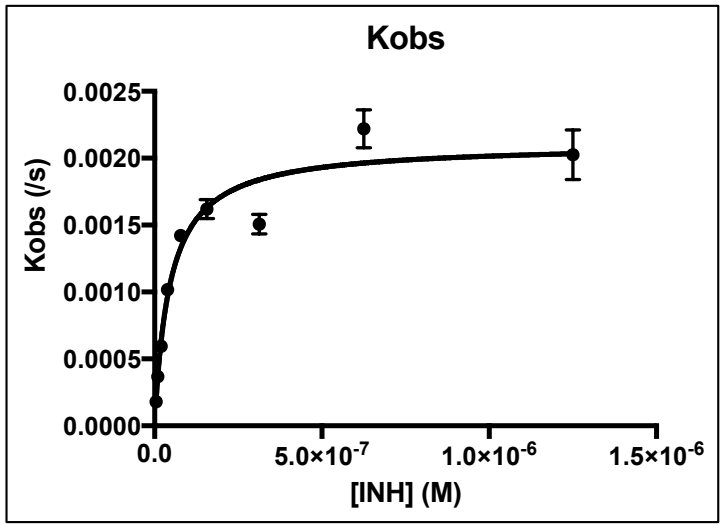
K_i = 860 ± 100 nM

K_{inact}/K_i = 4080 ± 320 $M^{-1}s^{-1}$

P2: hPro





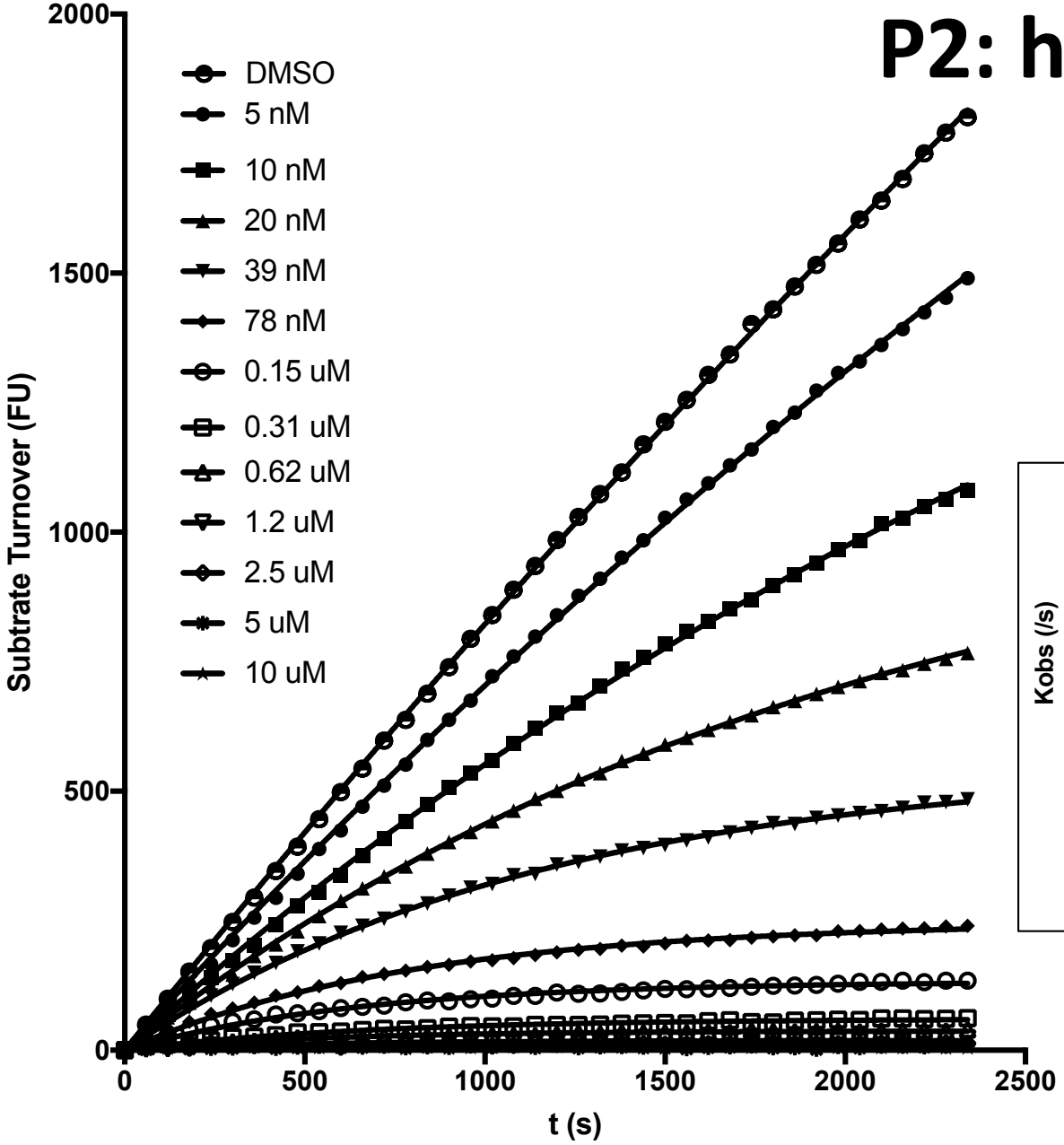


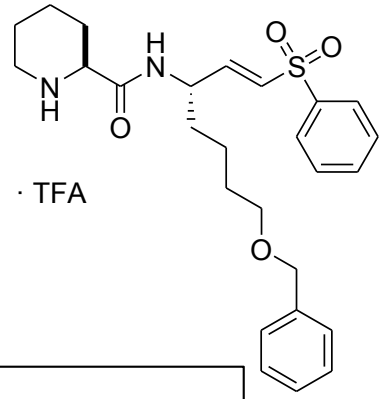
k_{inact} = 0.0021 ± 0.0001 s^{-1}

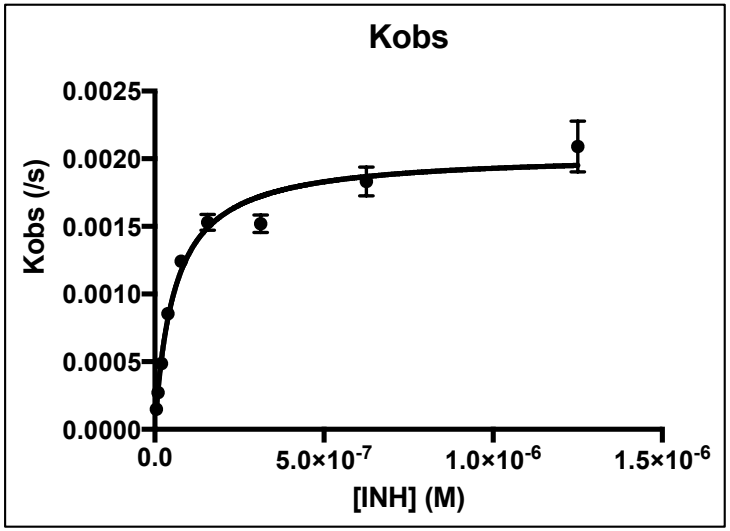
K_i = 30 ± 7 nM

K_{inact}/K_i = 69200 ± 13200 $M^{-1}s^{-1}$

P2: hPro, P1:nLeu(O-Bzl)





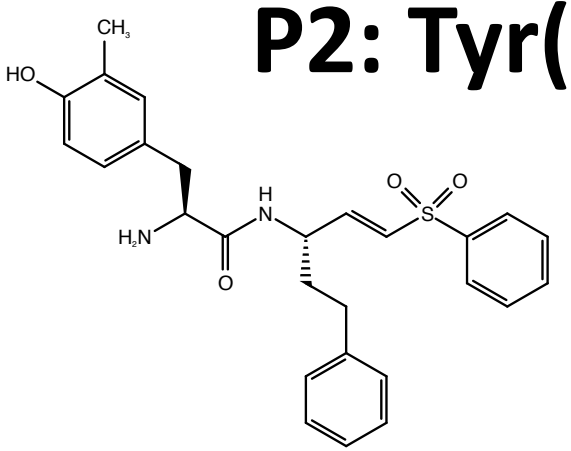


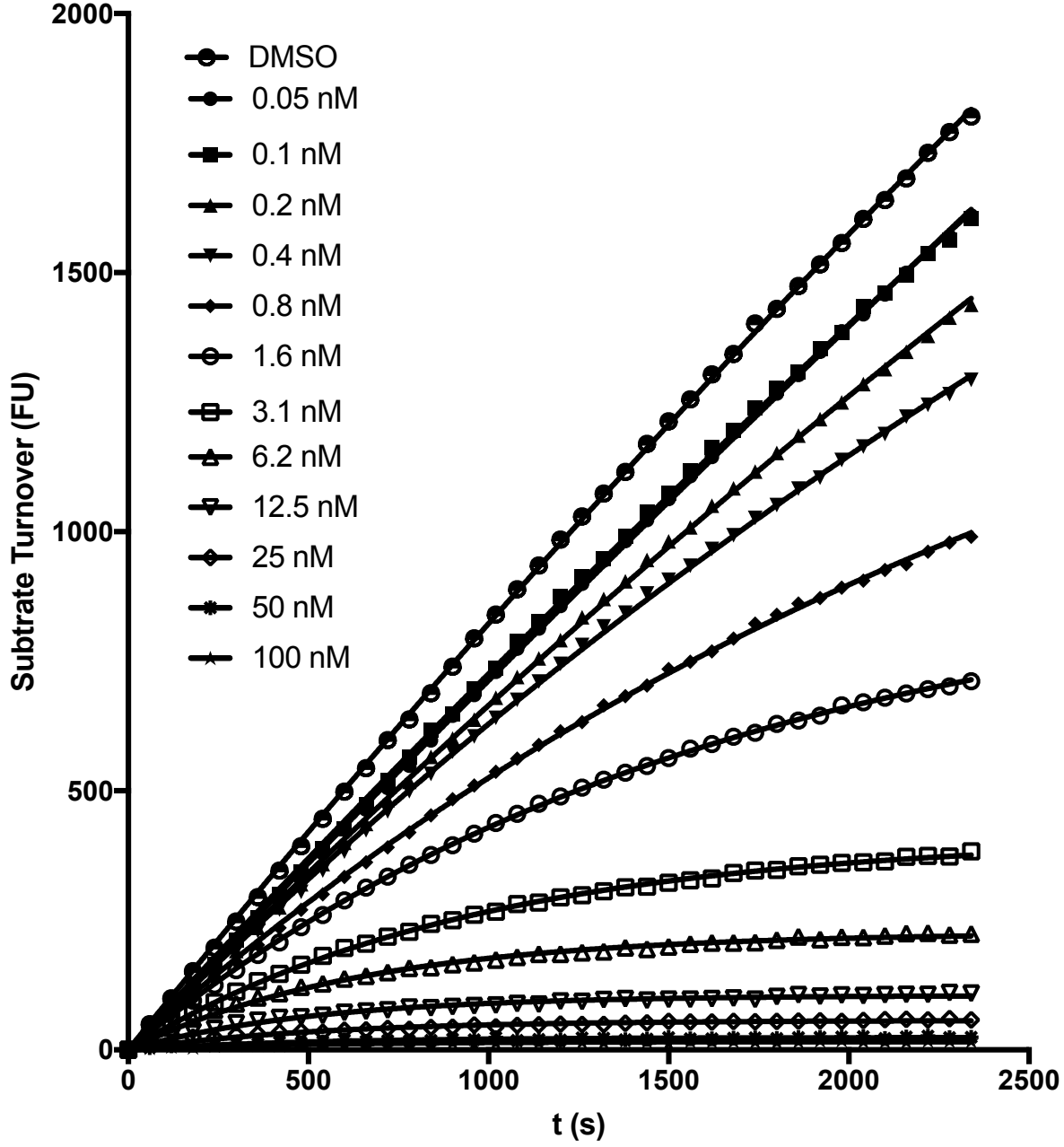
k_{inact} = 0.0020 ± 0.00008 s^{-1}

K_i = 39 ± 6 nM

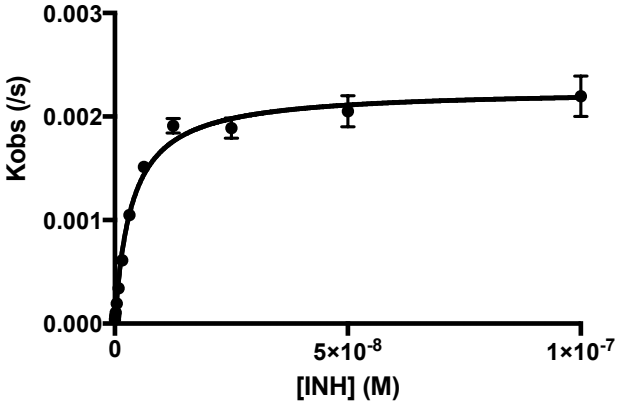
K_{inact}/K_i = 53000 ± 6500 $M^{-1}s^{-1}$

P2: Tyr(NO_2)





Kobs

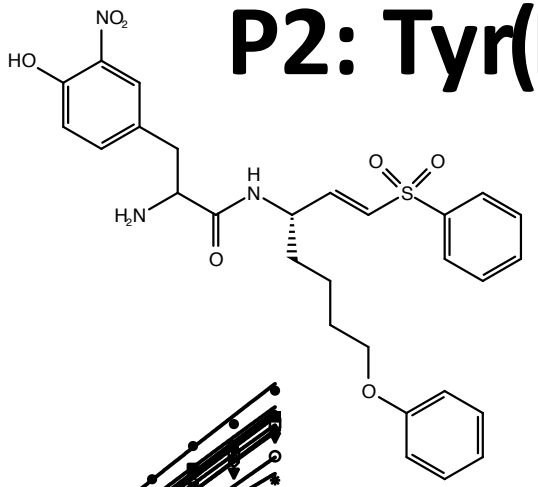


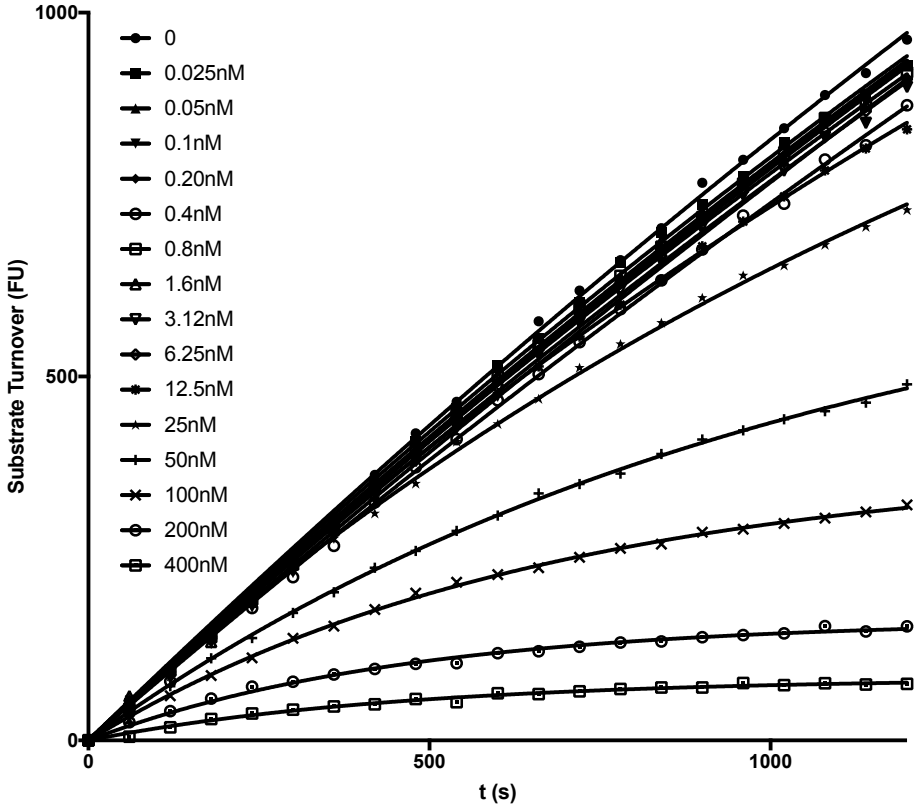
k_{inact} = 0.00226 ± 0.00005 s^{-1}

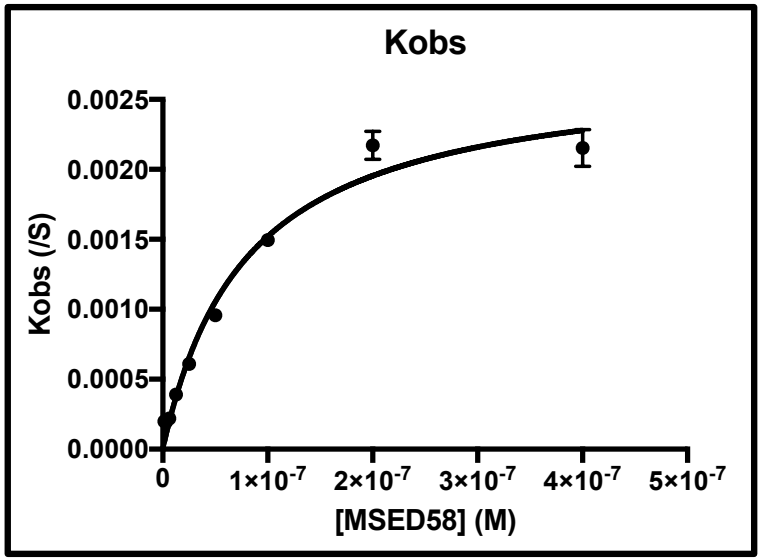
K_i = 2.4 ± 0.2 nM

K_{inact}/K_i = 949600 ± 73500 $M^{-1}s^{-1}$

P2: Tyr(NO_2), P1:nLeu(O-Bzl)



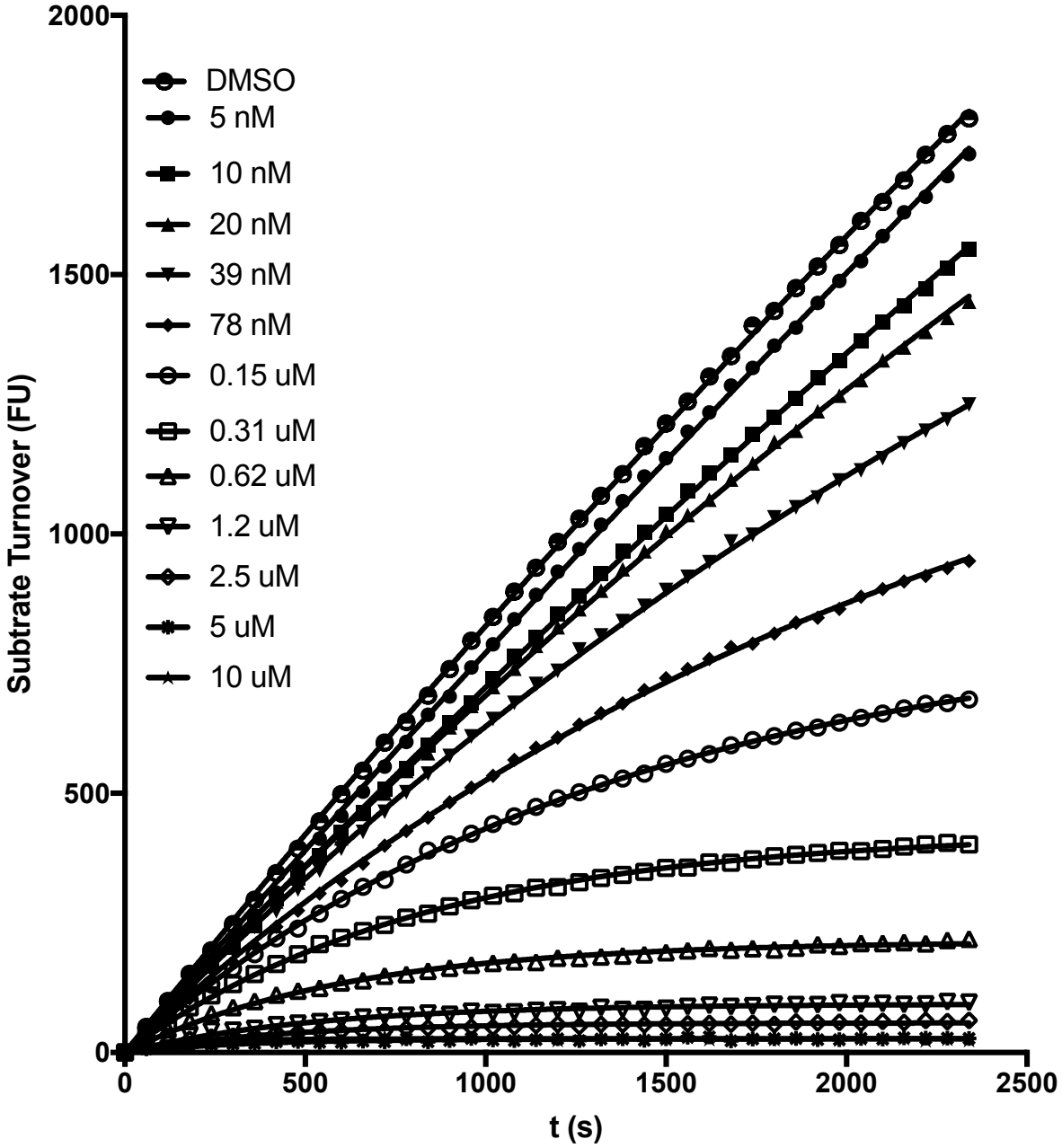




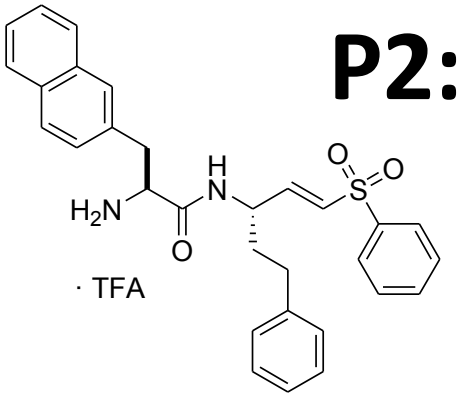
k_{inact} = 0.0027 ± 0.0002 s^{-1}

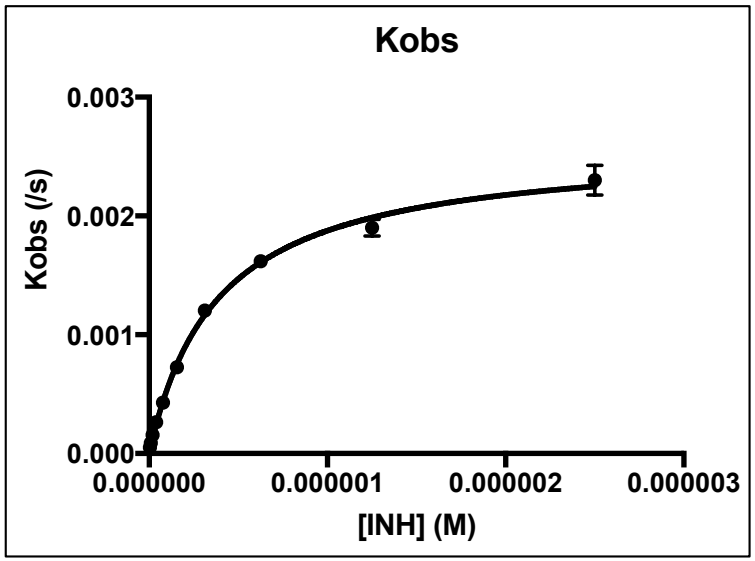
K_i = 80 ± 15 nM

K_{inact}/K_i = 34250 ± 4700 $M^{-1}s^{-1}$



P2: 2Nal



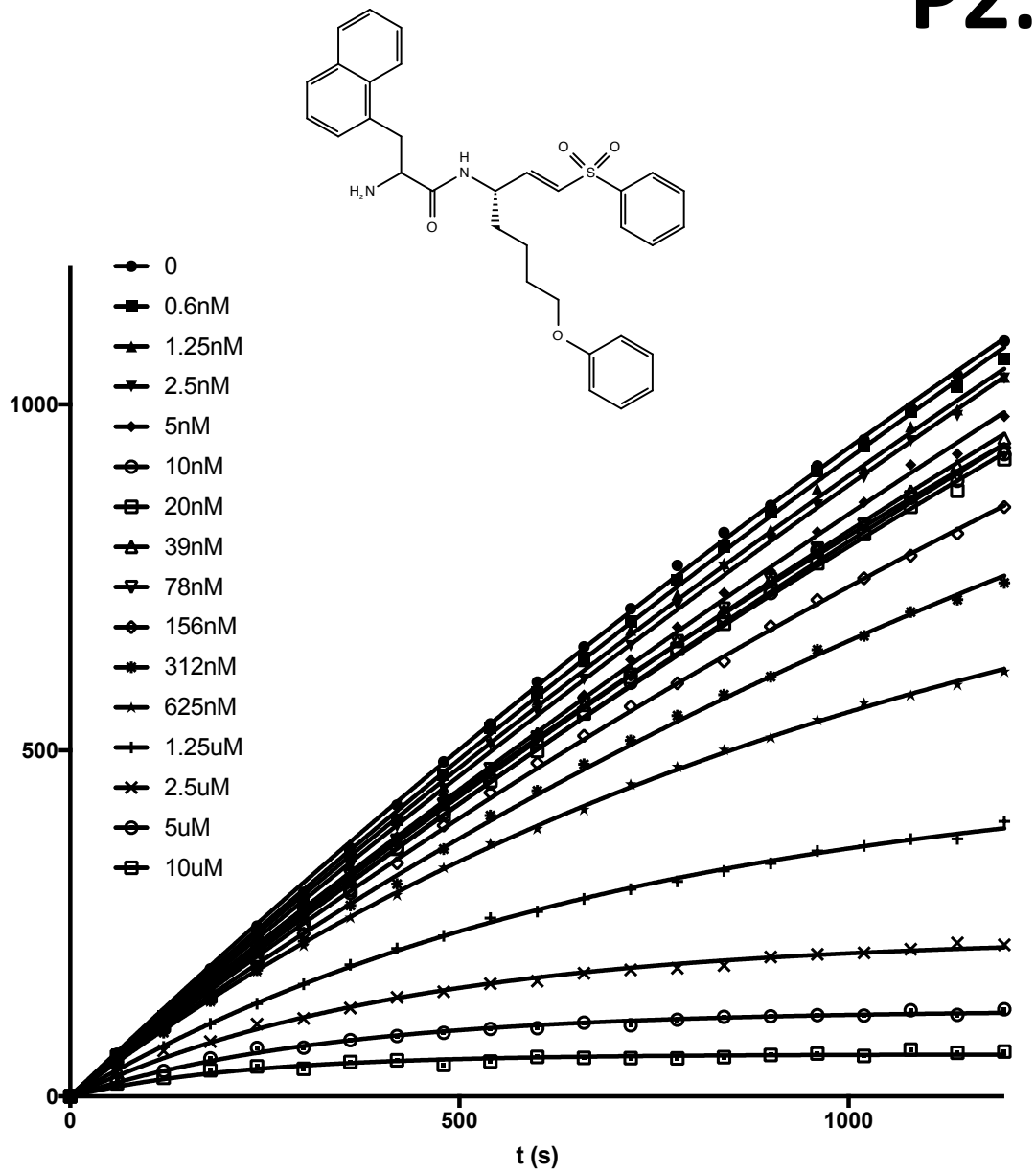


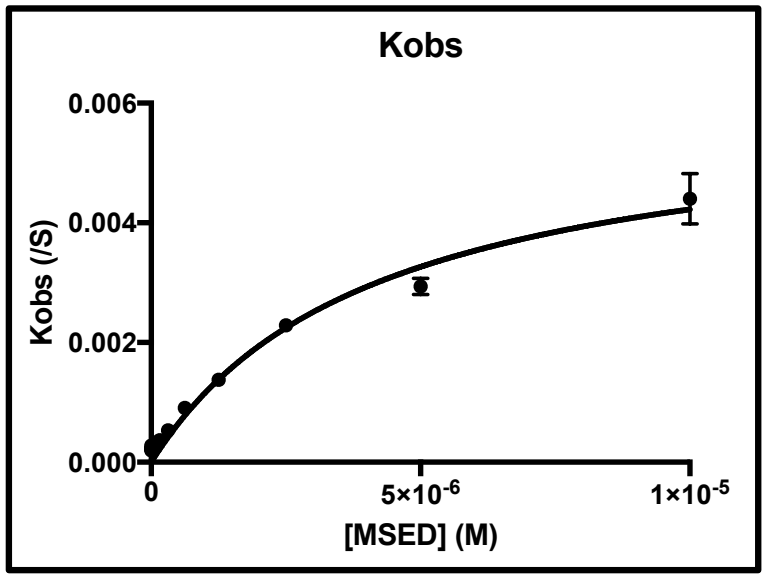
k_{inact} = 0.00259 ± 0.00007 s^{-1}

K_i = 254 ± 16 nM

K_{inact}/K_i = 10200 ± 500 $M^{-1}s^{-1}$

P2: 2Nal, P1:nLeu(O-Bzl)



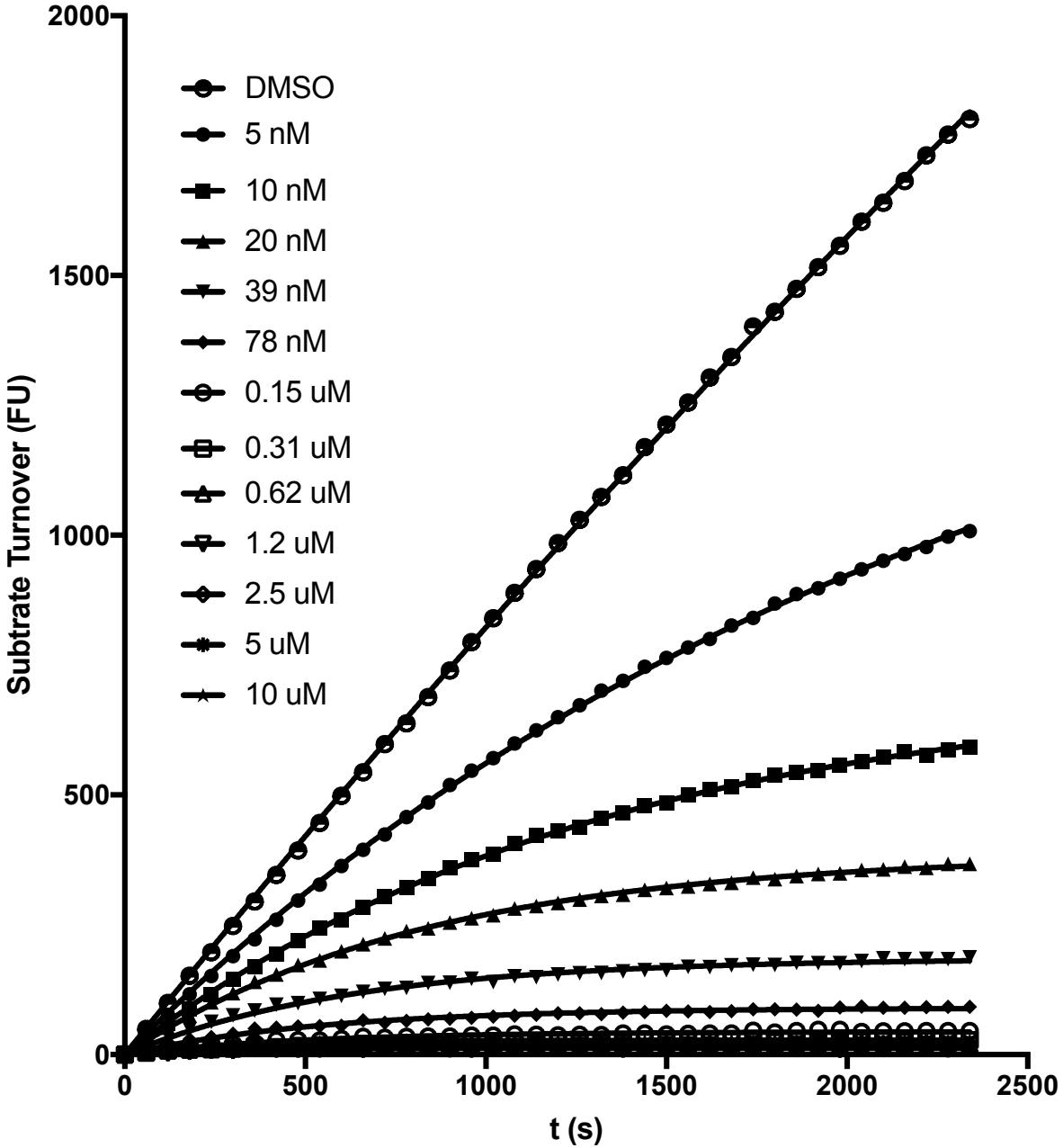


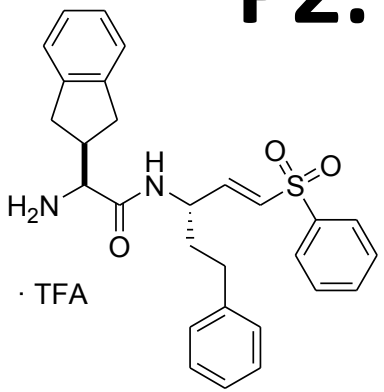
k_{inact} = 0.006 ± 0.0007 s^{-1}

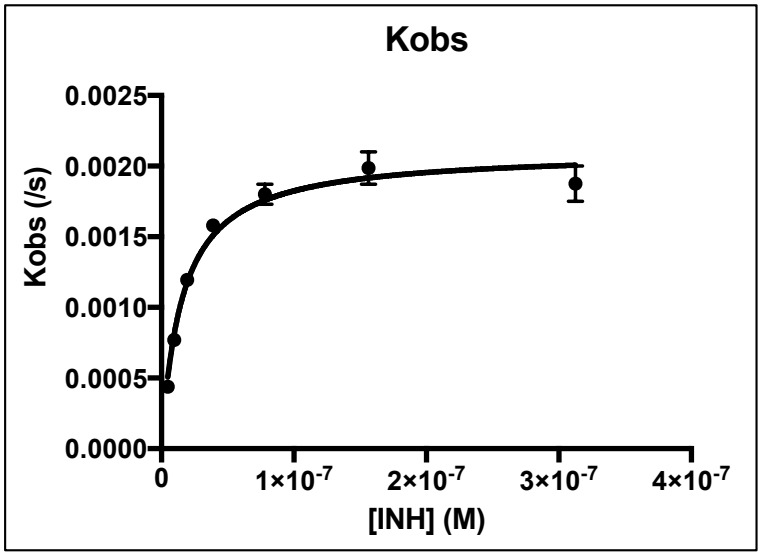
K_i = 4200 ± 100 nM

K_{inact}/K_i = 1450 ± 200 $M^{-1}s^{-1}$

P2: Igl





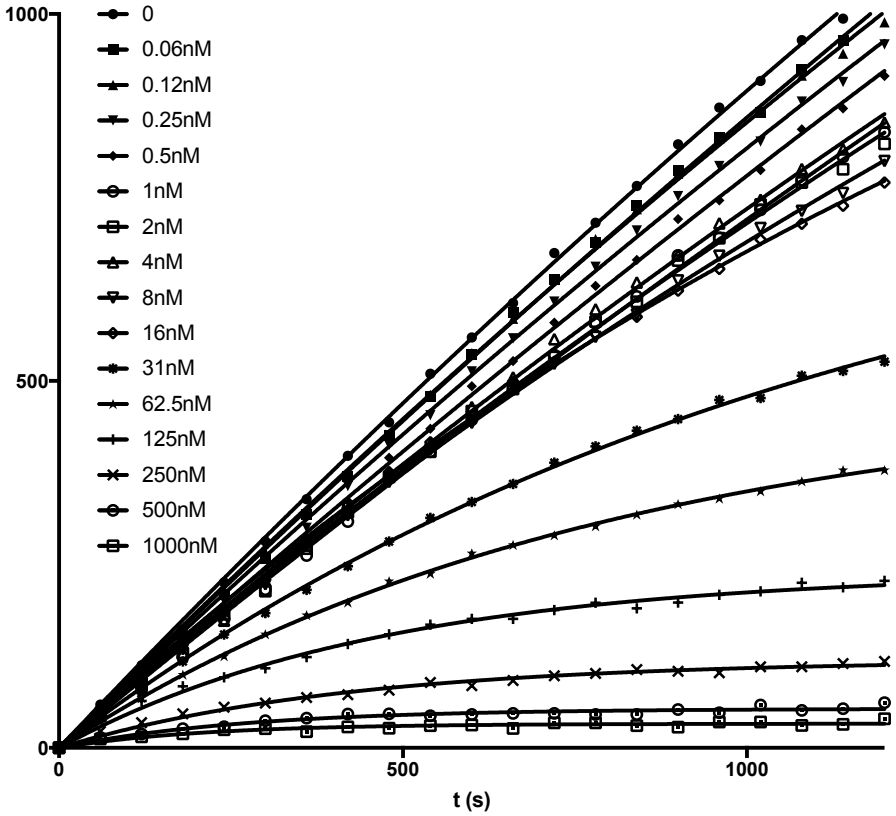


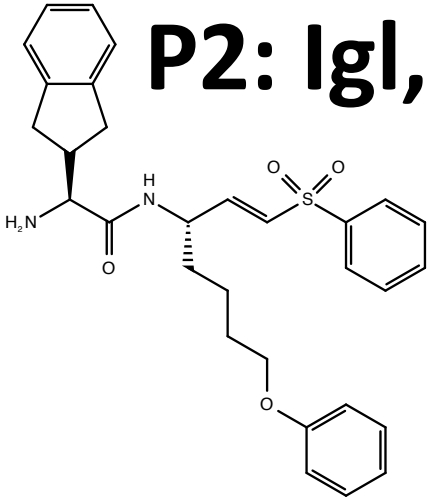
k_{inact} = 0.0021 ± 0.00007 s^{-1}

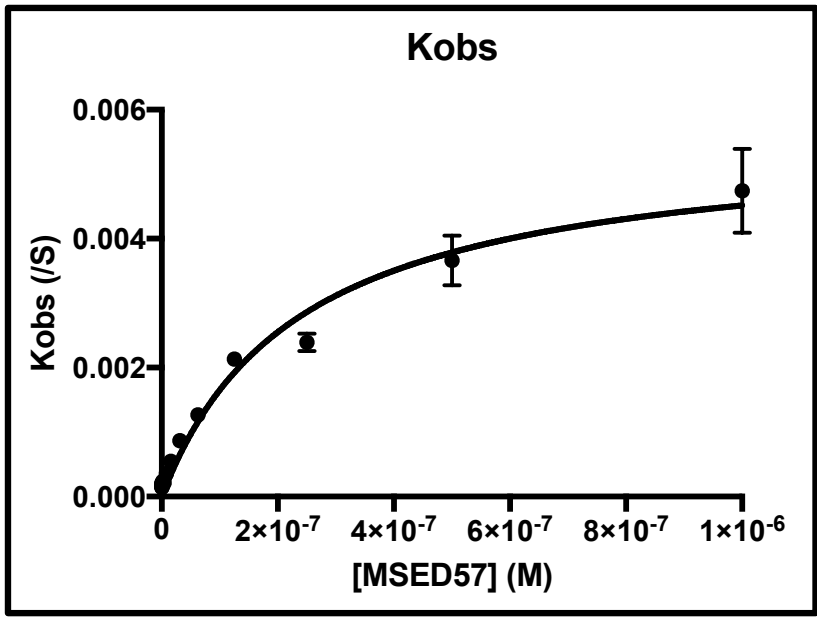
K_i = 10 ± 1 nM

K_{inact}/K_i = 205400 ± 22000 $M^{-1}s^{-1}$

P2: Igl, P1:nLeu(O-Bzl)





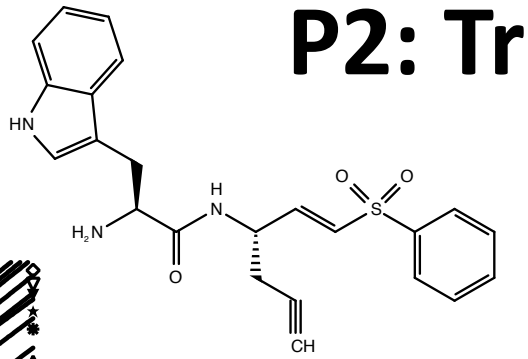


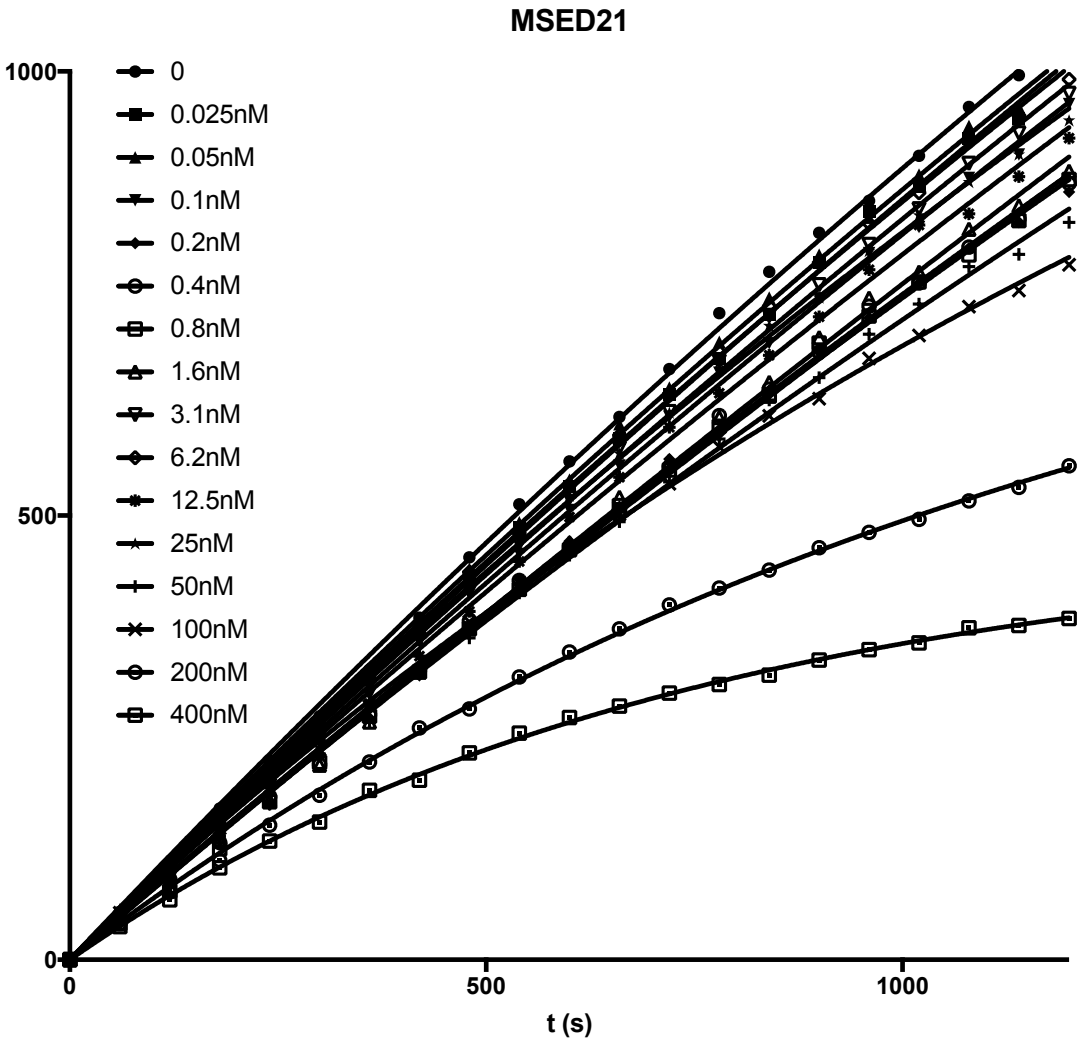
k_{inact} = 0.0056 ± 0.0004 s^{-1}

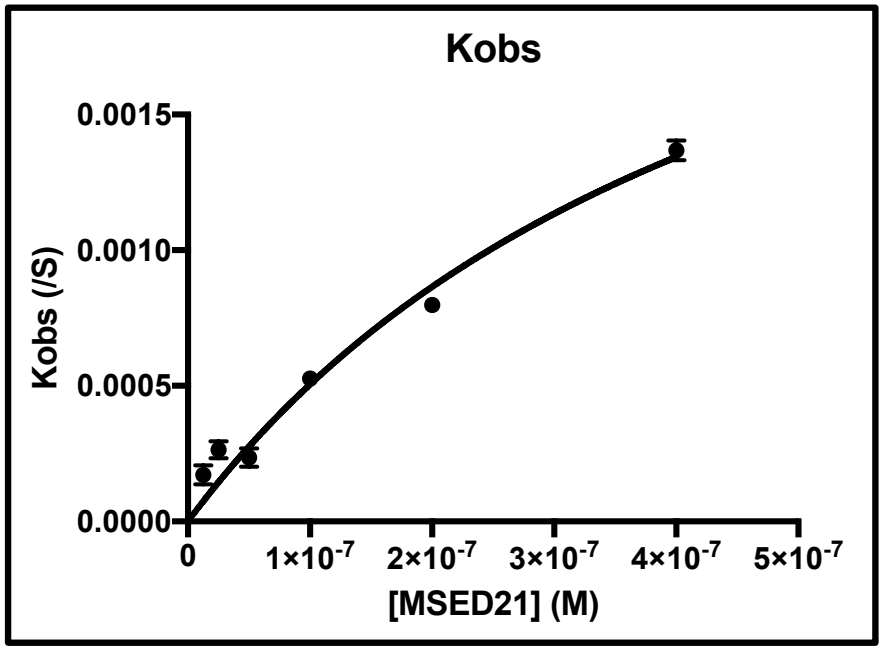
K_i = 240 ± 40 nM

K_{inact}/K_i = 23500 ± 3000 $M^{-1}s^{-1}$

P2: Trp, P1:hPG





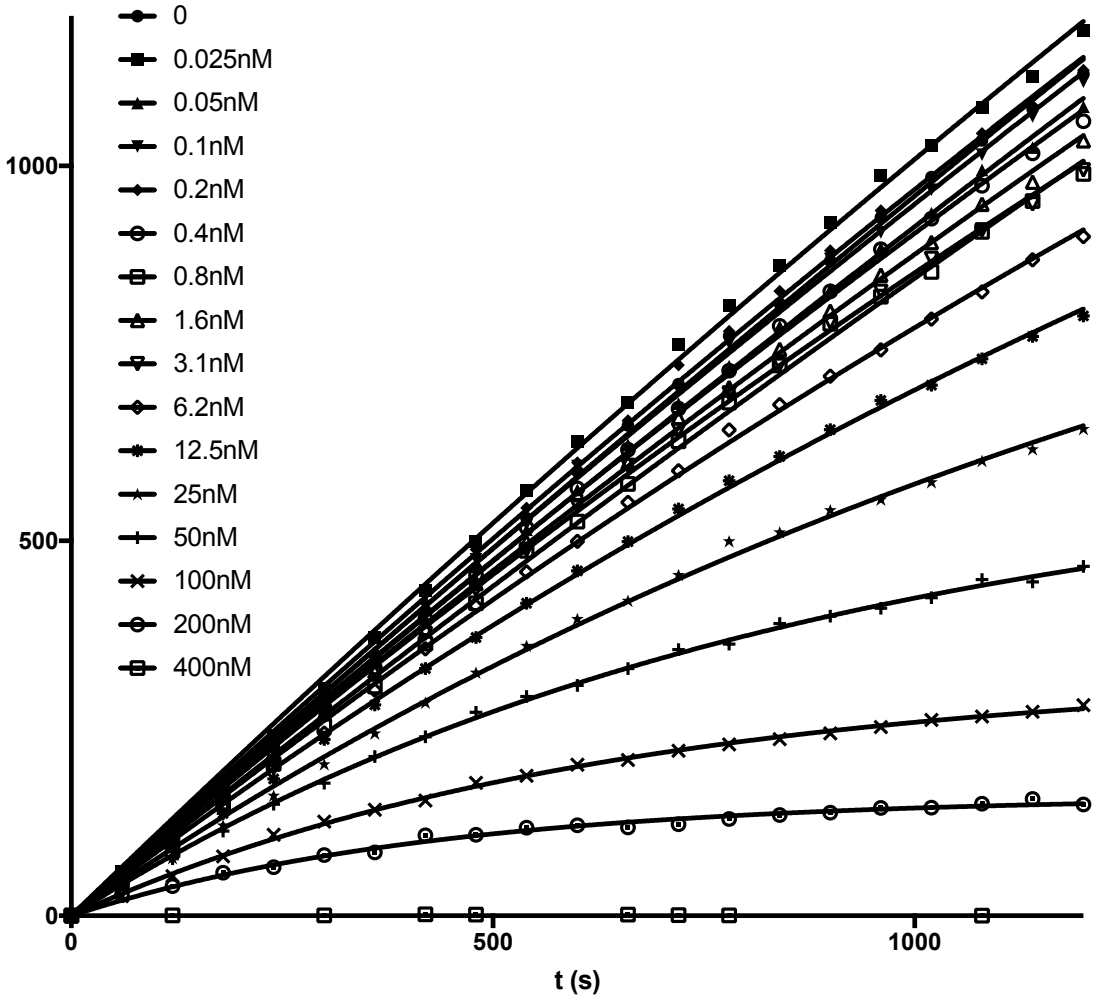


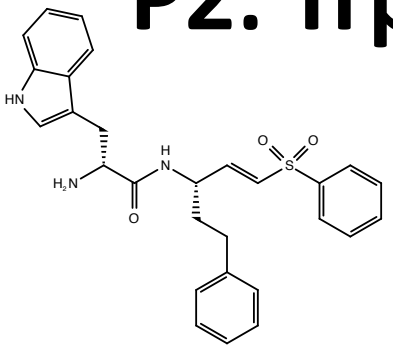
k_{inact} = 0.003 ± 0.001 s^{-1}

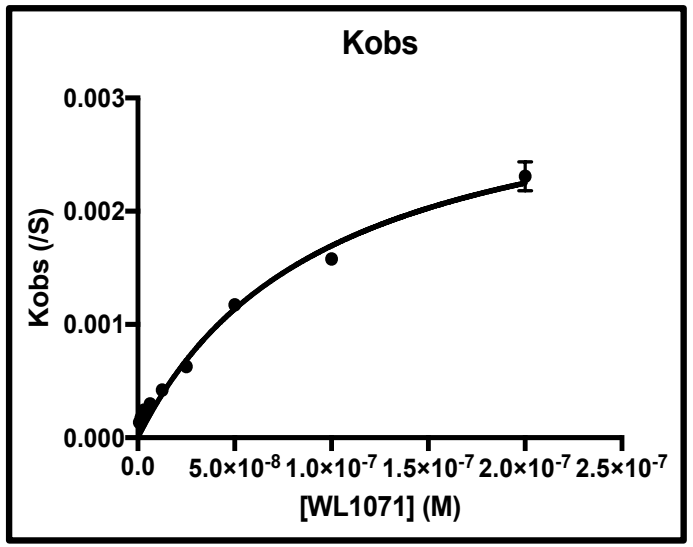
K_i = 500 ± 240 nM

K_{inact}/K_i = 6070 ± 1100 $M^{-1}s^{-1}$

P2: Trp, P1:hPhe





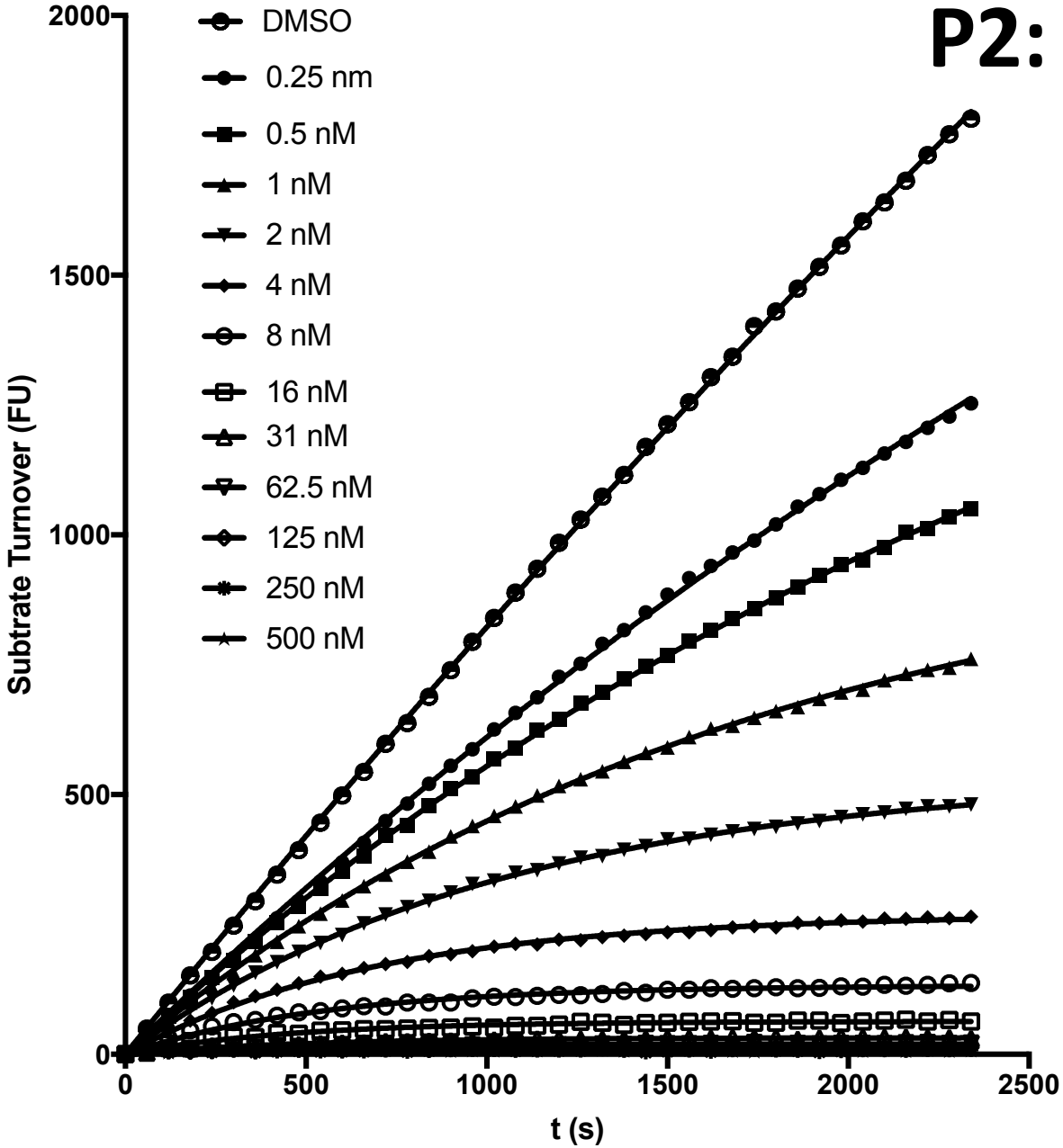


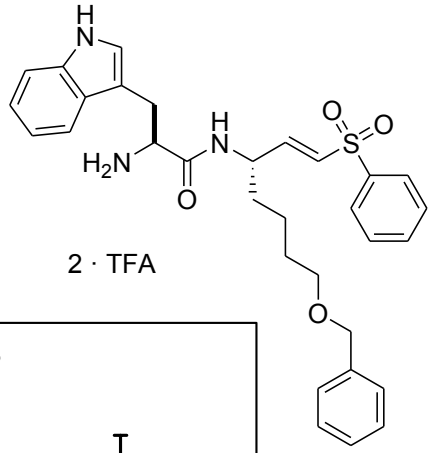
k_{inact} = 0.0033 ± 0.0004 s^{-1}

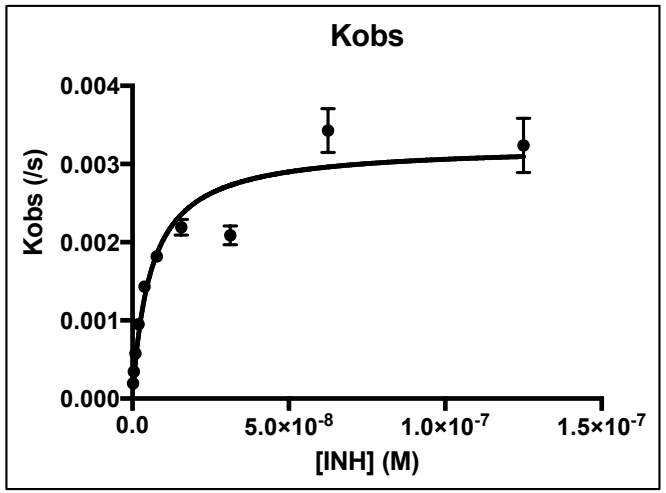
K_i = 100 ± 20 nM

K_{inact}/K_i = 34400 ± 4300 $M^{-1}s^{-1}$

P2: Trp, P1:nLeu(O-Bzl)





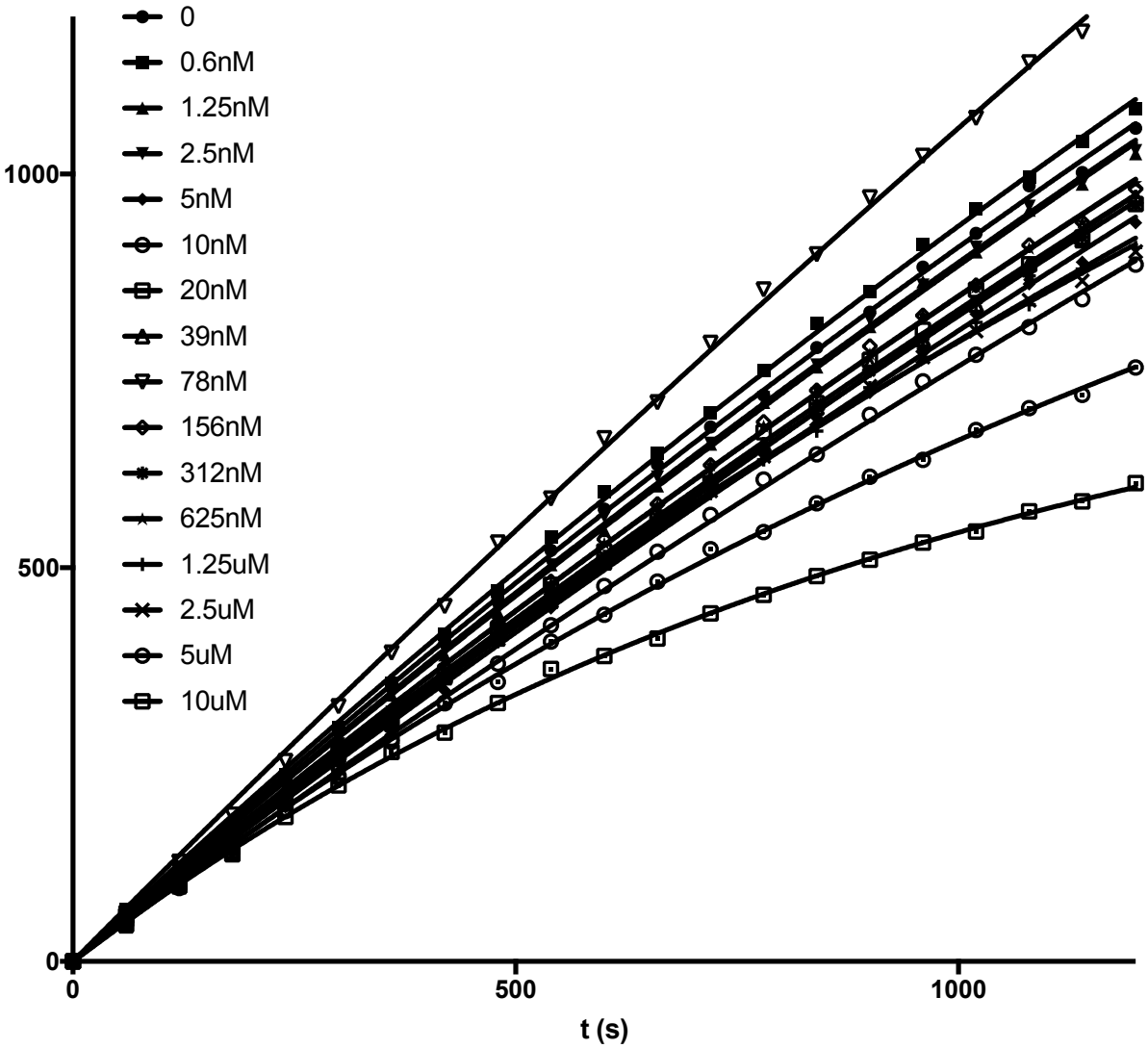


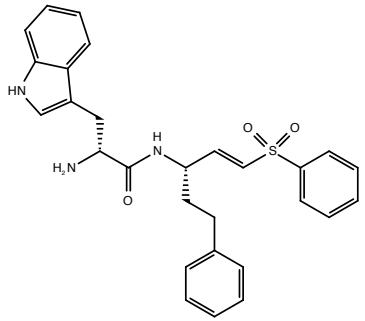
k_{inact} = 0.0032 ± 0.0003 s^{-1}

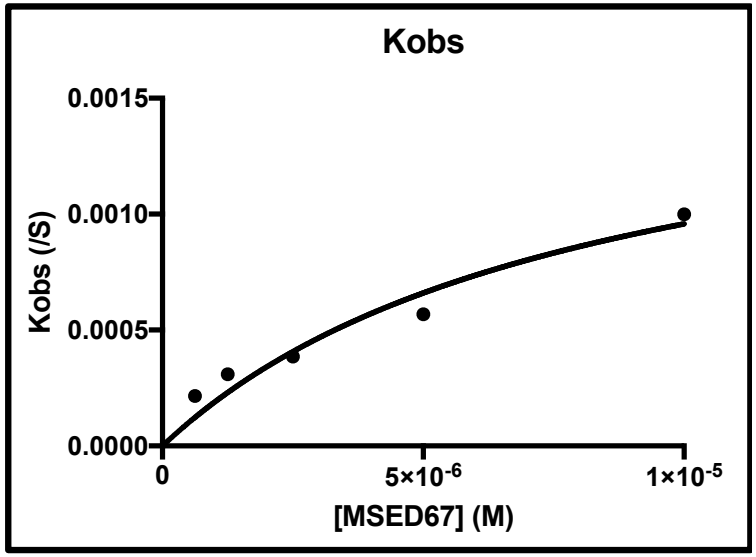
K_i = 3.9 ± 1 nM

K_{inact}/K_i = 826400 ± 193000 $M^{-1}s^{-1}$

P2: D-Trp, P1:hPhe





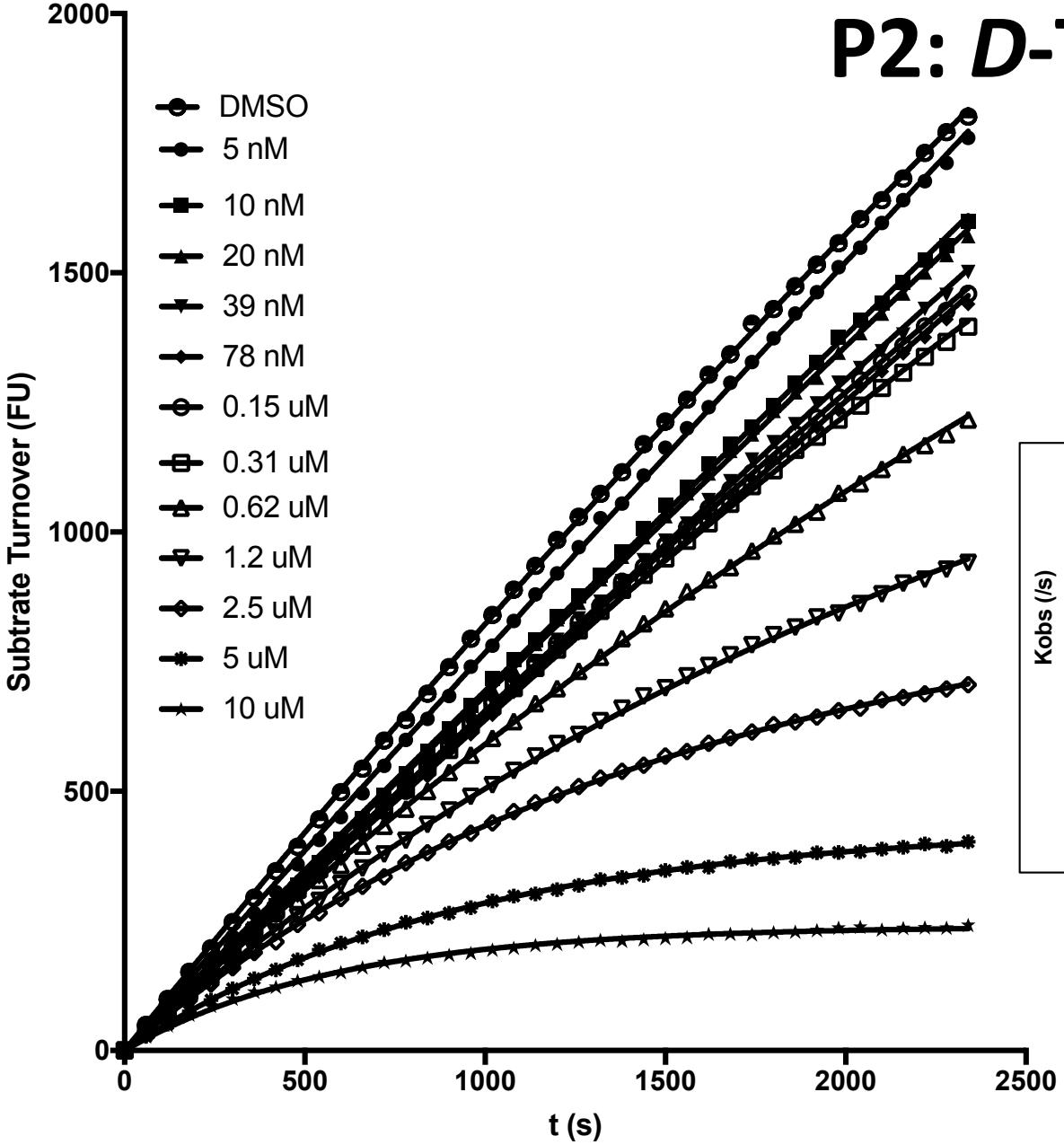


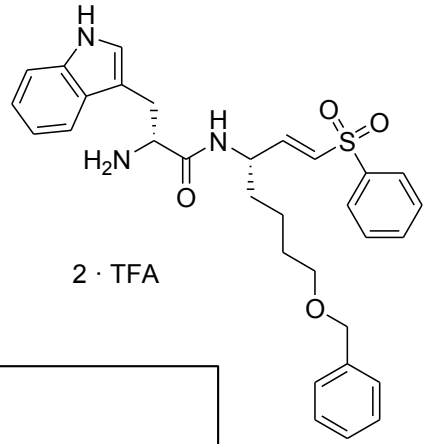
k_{inact} = 0.0017 ± 0.0006 s^{-1}

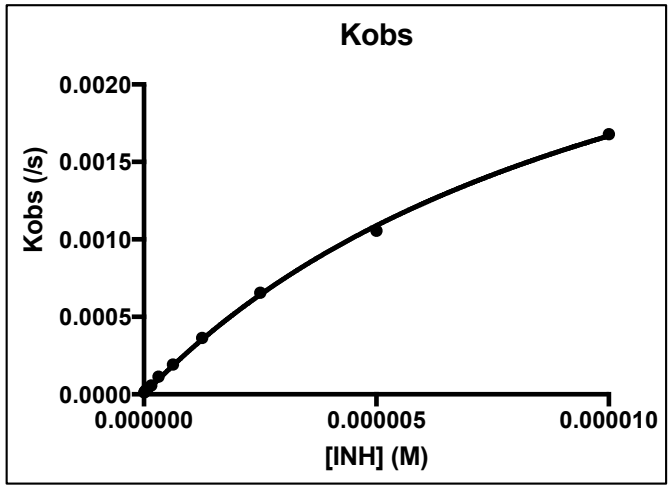
K_i = 8200 ± 4500 nM

K_{inact}/K_i = 210 ± 60 $M^{-1}s^{-1}$

P2: *D*-Trp, P1:nLeu(O-Bzl)





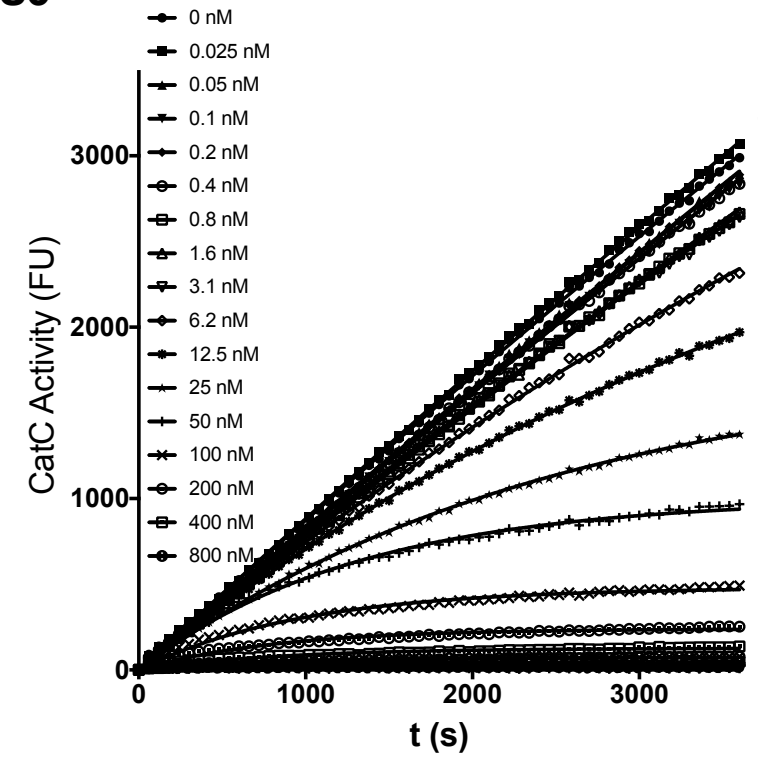


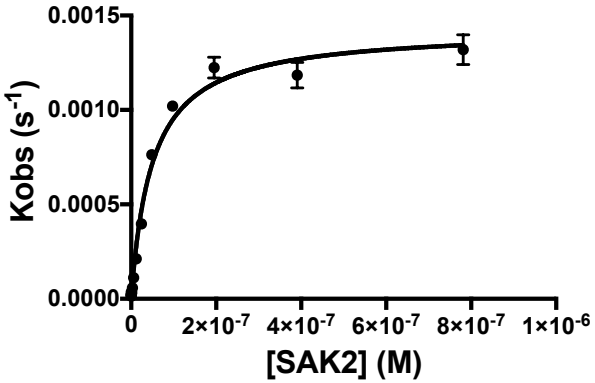
k_{inact} = 0.0036 ± 0.0002 s^{-1}

K_i = 7.6 ± 0.6 uM

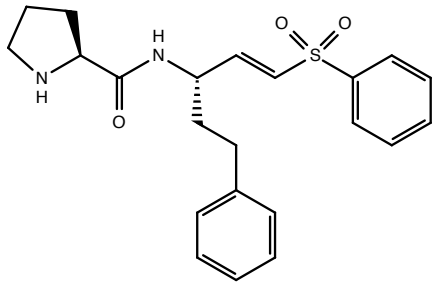
K_{inact}/K_i = 470 ± 13 $M^{-1}s^{-1}$

Fig. S3





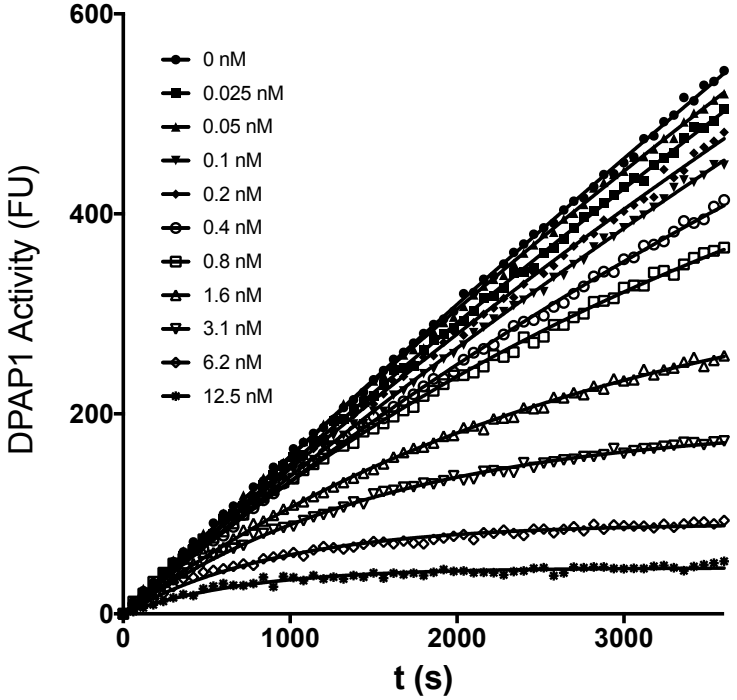
P2: Pro,
P1: hPhe

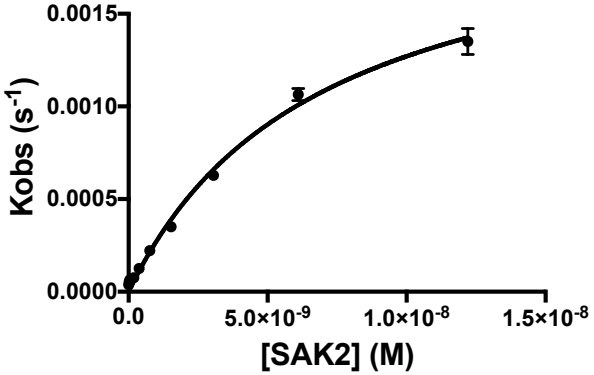


k_{inact} = 0.00145 ± 0.00007 s^{-1}

K_i = 0.034 ± 0.005 uM

K_{inact}/K_i = 42000 ± 5000 $M^{-1}s^{-1}$



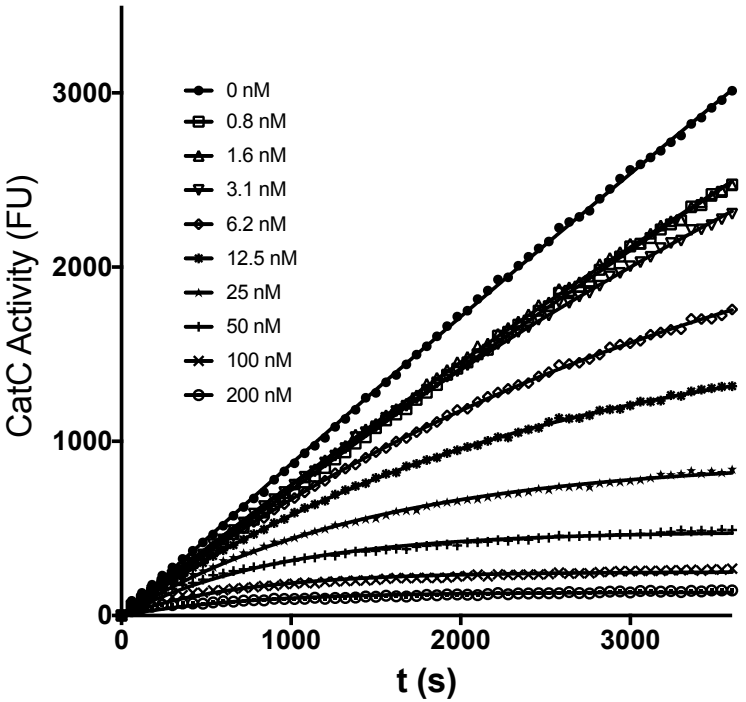


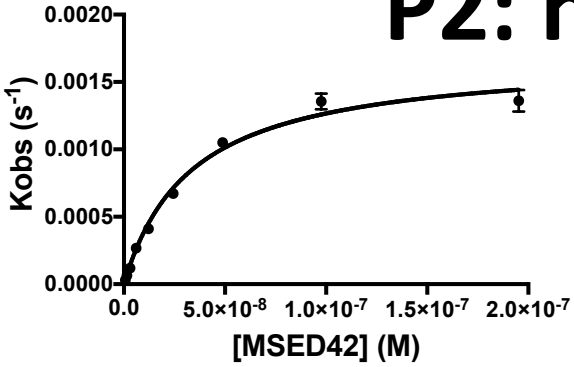
k_{inact} = 0.0021 ± 0.0002 s^{-1}

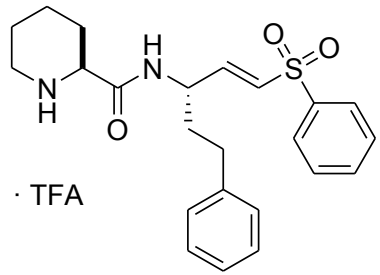
K_i = 0.0046 ± 0.0007 uM

K_{inact}/K_i = 470000 ± 35000 $M^{-1}s^{-1}$

P2: hPro, P1: hPhe



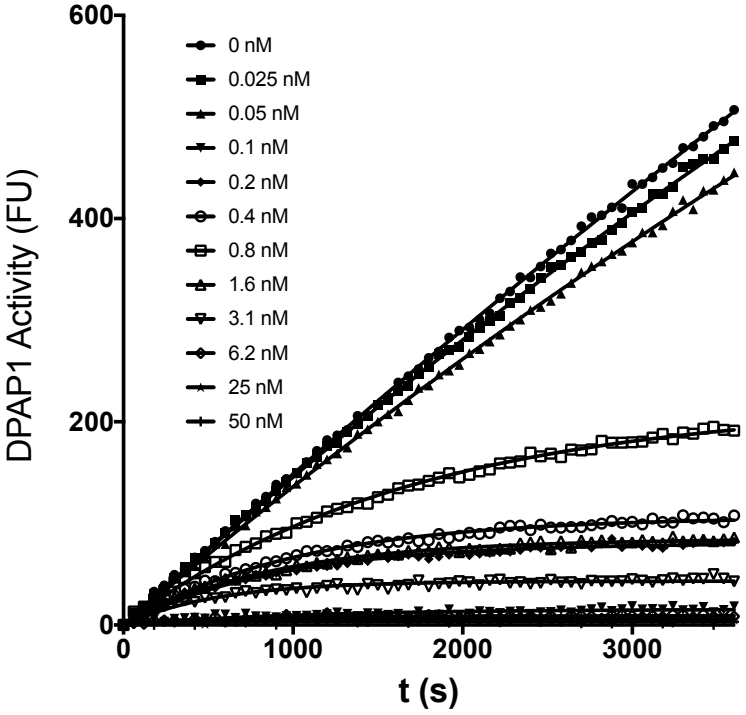


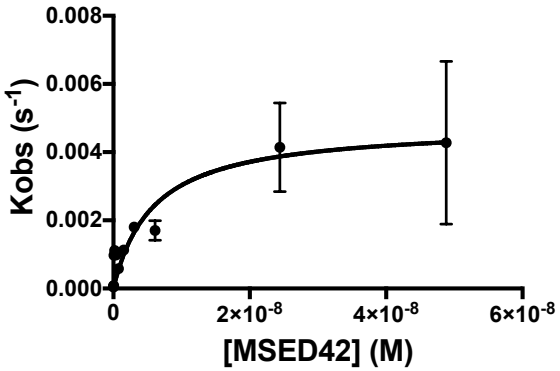


k_{inact} = 0.00167 ± 0.00008 s^{-1}

K_i = 0.022 ± 0.003 uM

K_{inact}/K_i = 76000 ± 800 $M^{-1}s^{-1}$



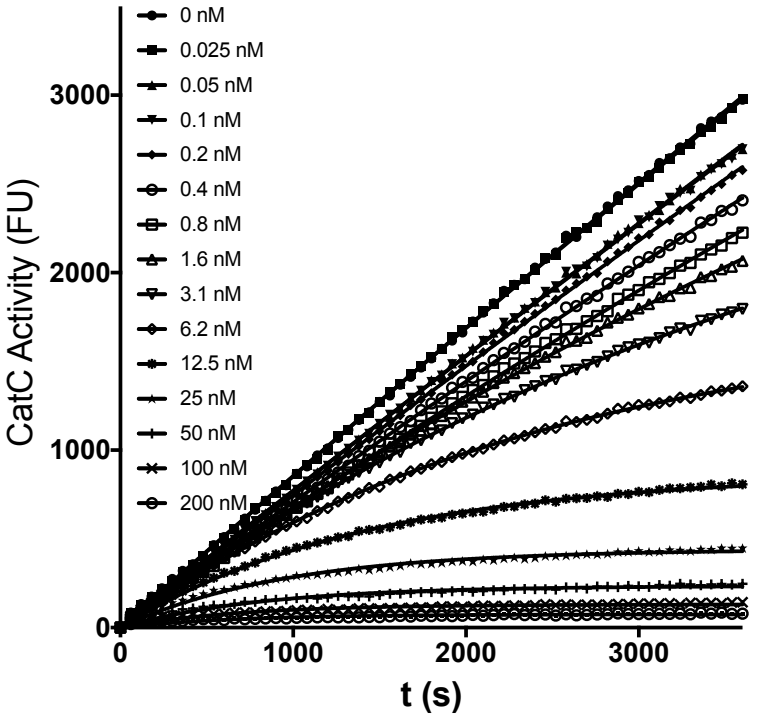


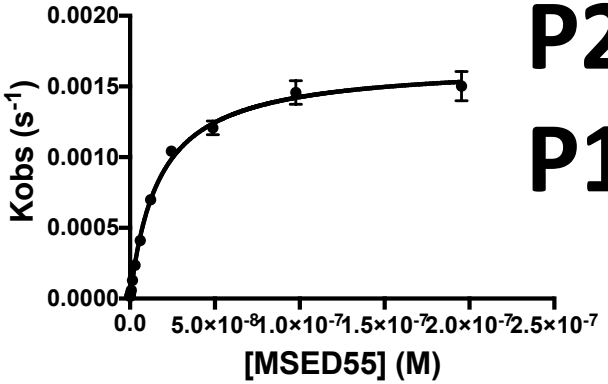
k_{inact} = 0.0048 ± 0.0006 s^{-1}

K_i = 0.0039 ± 0.0015 uM

K_{inact}/K_i = 1230000 ± 400000$M^{-1}s^{-1}$

P2: hPro,
P1: nLeu(o-Bzl)

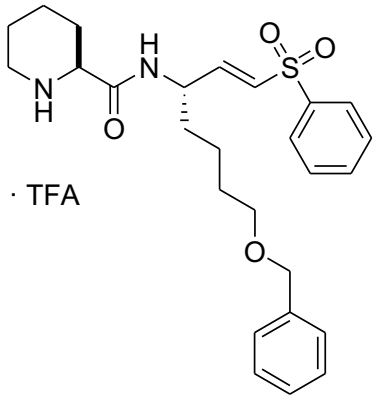


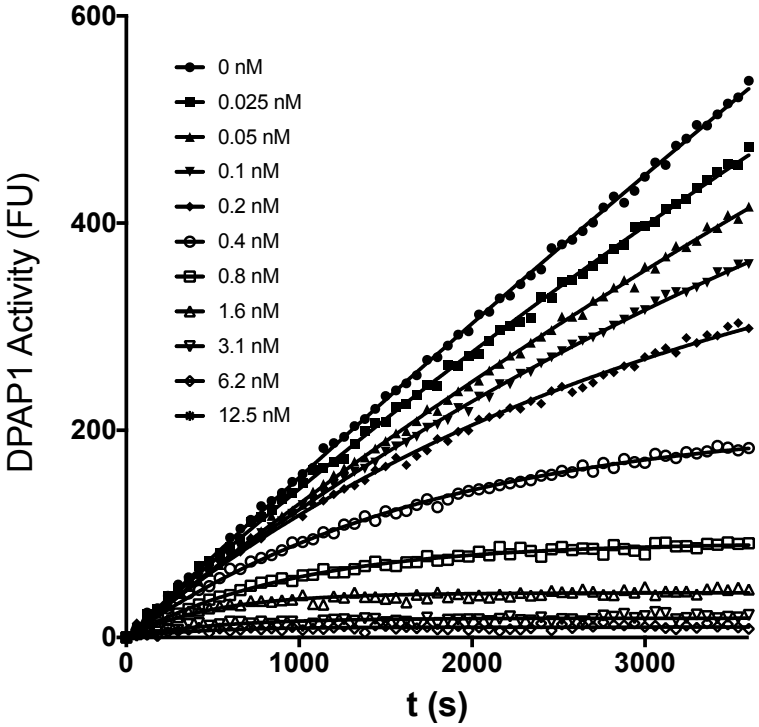


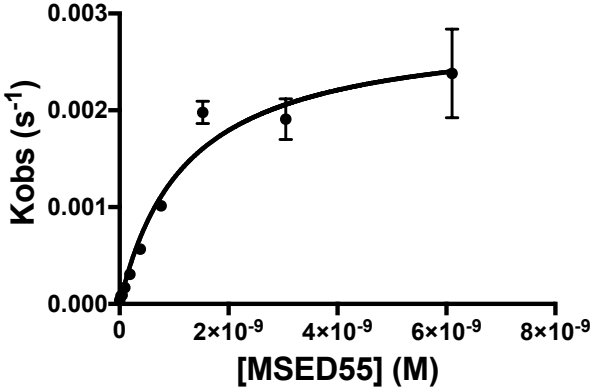
k_{inact} = 0.00167 ± 0.00003 s^{-1}

K_i = 0.0112 ± 0.0006 uM

K_{inact}/K_i = 150000 ± 6000 $M^{-1}s^{-1}$





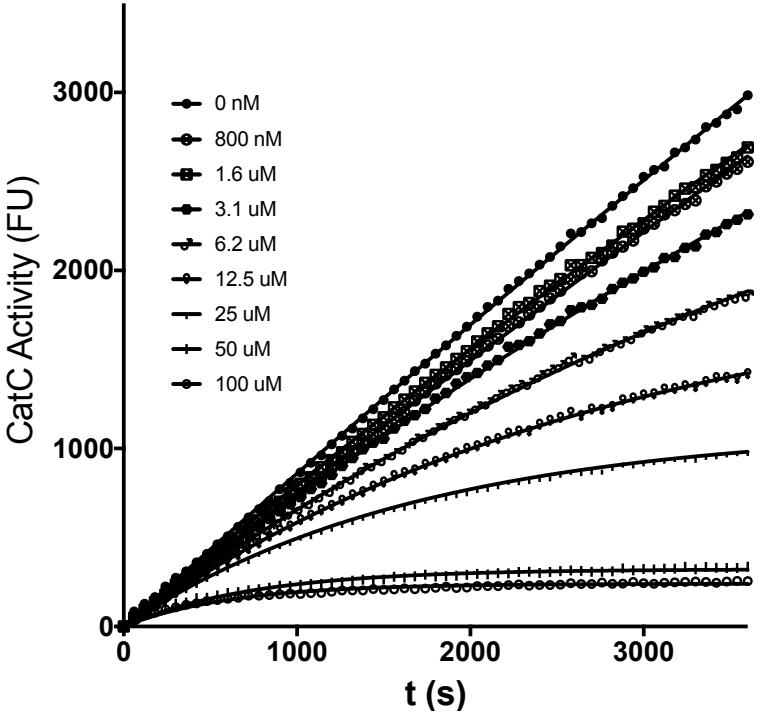


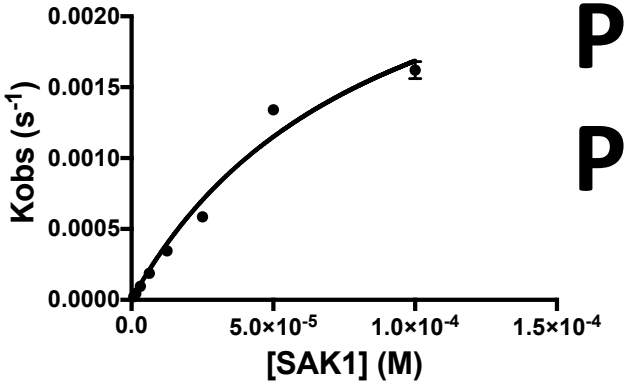
k_{inact} = 0.0029 ± 0.0003 s^{-1}

K_i = 0.0008 ± 0.0002 uM

K_{inact}/K_i = 3560000 ± 150000 $M^{-1}s^{-1}$

P2: Tyr(NO_2), P1: hPhe

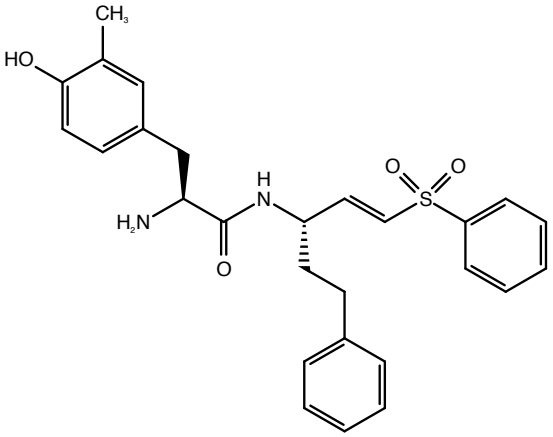


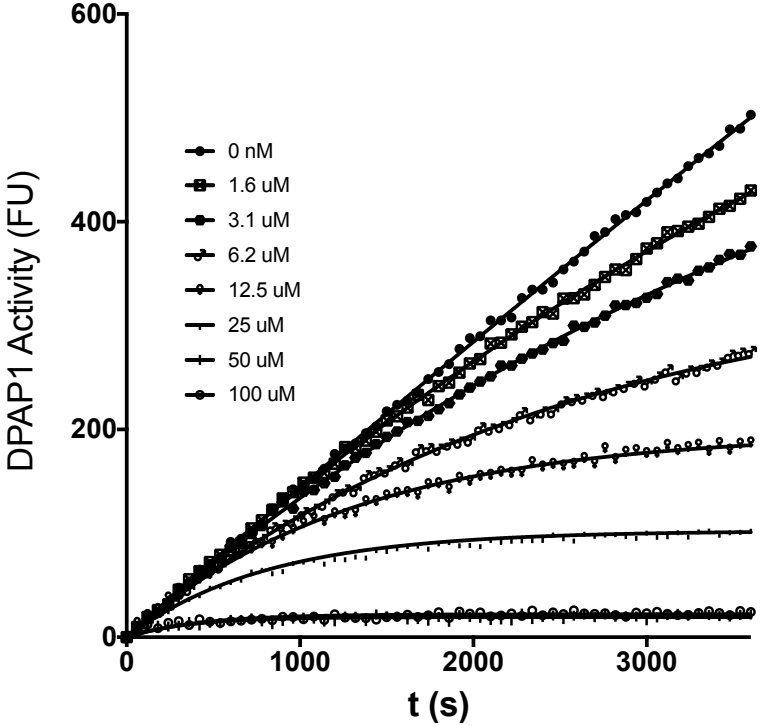


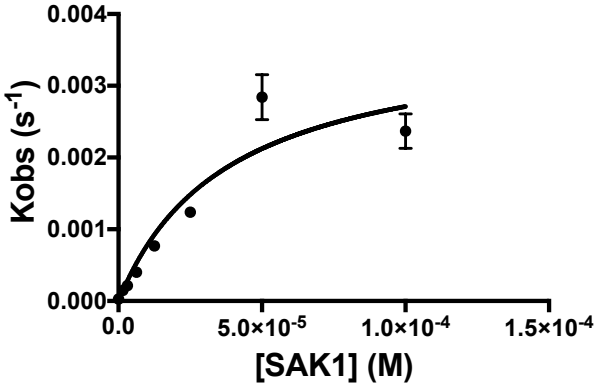
k_{inact} = 0.0032 ± 0.0006 s^{-1}

K_i = 58 ± 20 uM

K_{inact}/K_i = 54 ± 8 $M^{-1}s^{-1}$





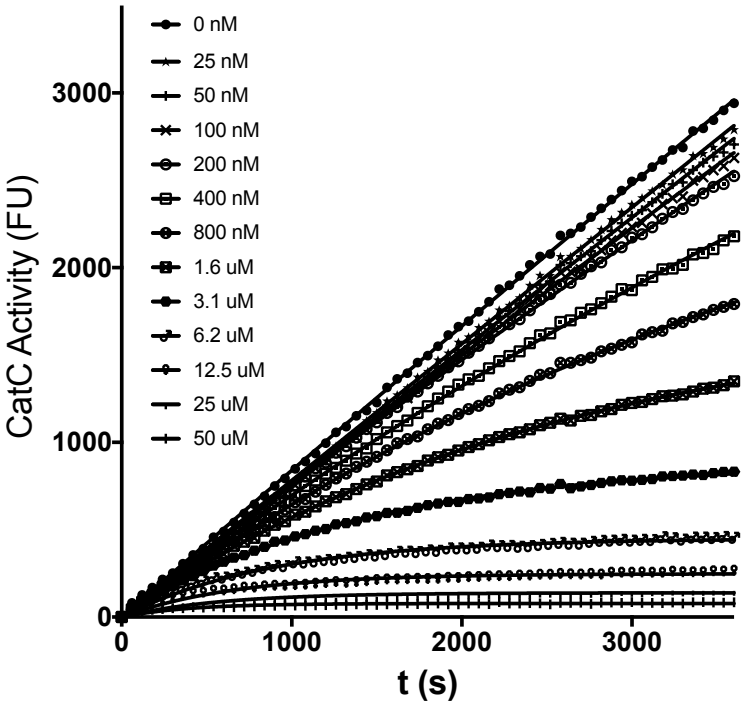


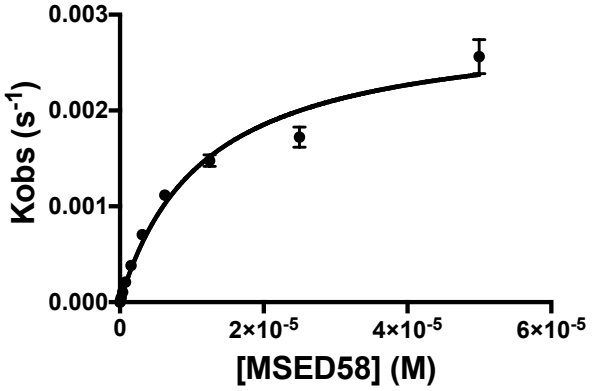
k_{inact} = 0.0038 ± 0.001 s^{-1}

K_i = 25 ± 14 uM

K_{inact}/K_i = 150 ± 50 $M^{-1}s^{-1}$

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

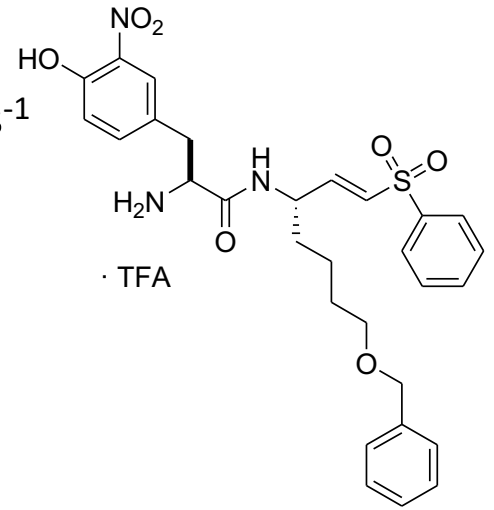


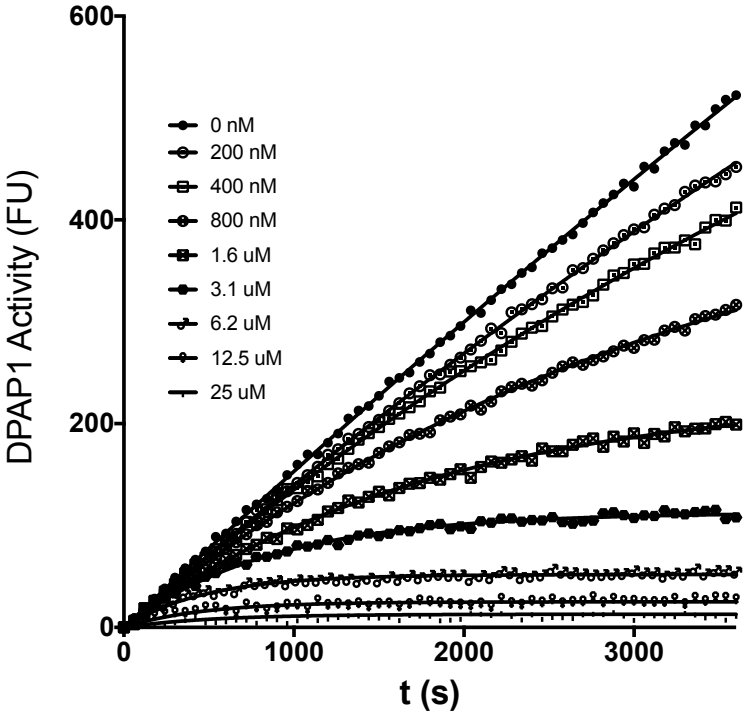


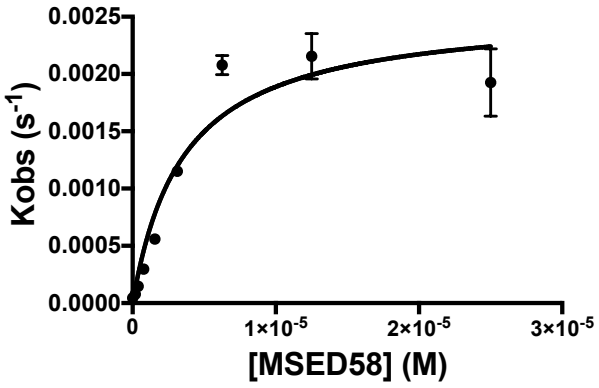
k_{inact} = 0.0029 ± 0.0002 s^{-1}

K_i = 7.7 ± 1.5 uM

K_{inact}/K_i = 380 ± 50 $M^{-1}s^{-1}$





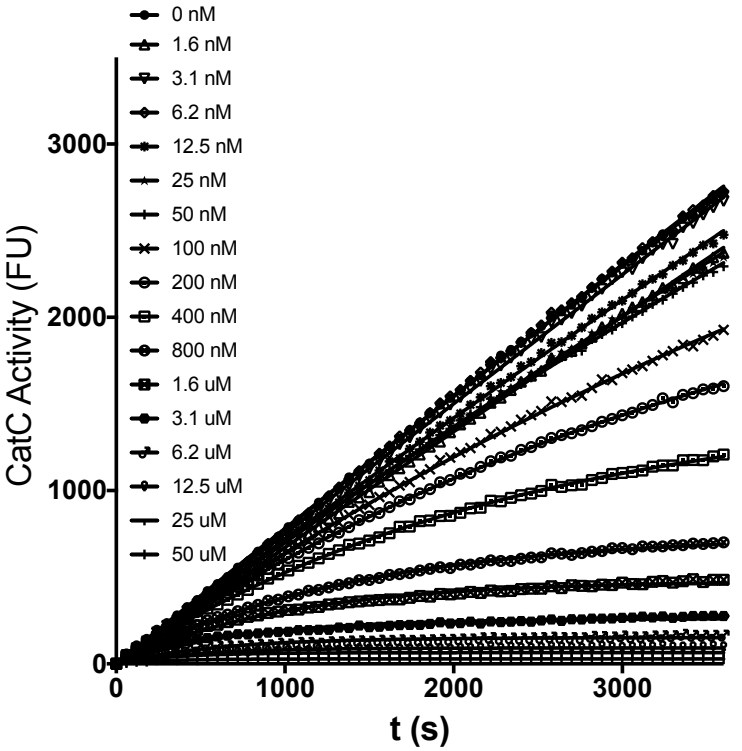


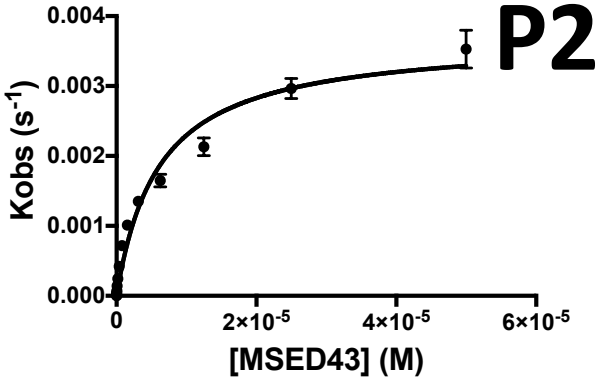
k_{inact} = 0.0026 ± 0.0003 s^{-1}

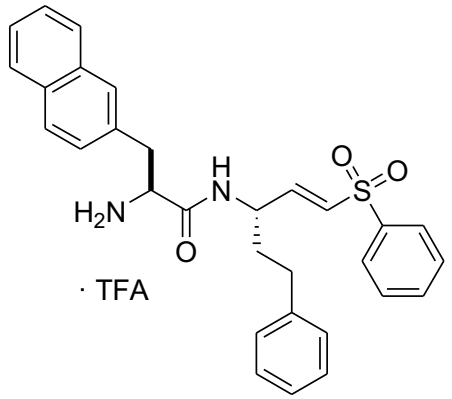
K_i = 2.4 ± 0.9 uM

K_{inact}/K_i = 1100 ± 300 $M^{-1}s^{-1}$

P2: Nal, P1: hPhe



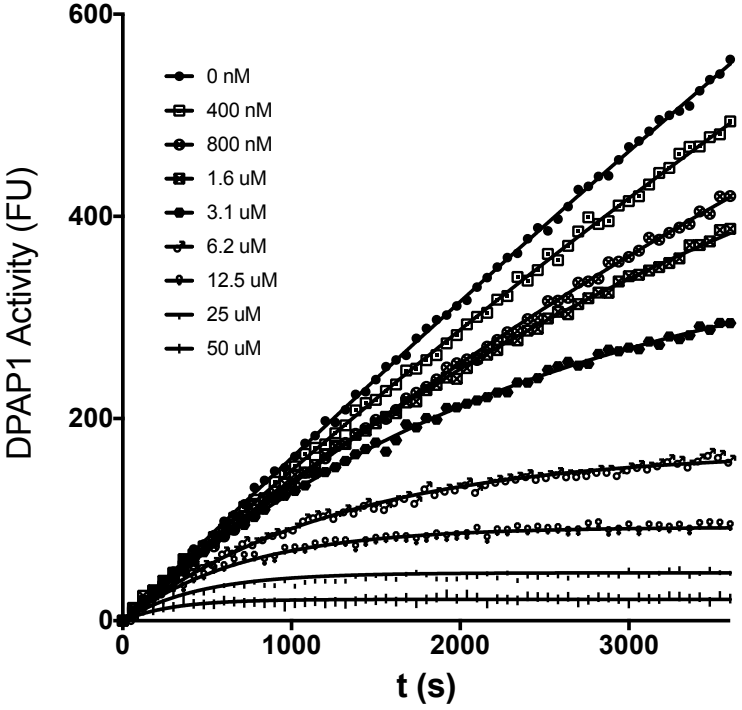


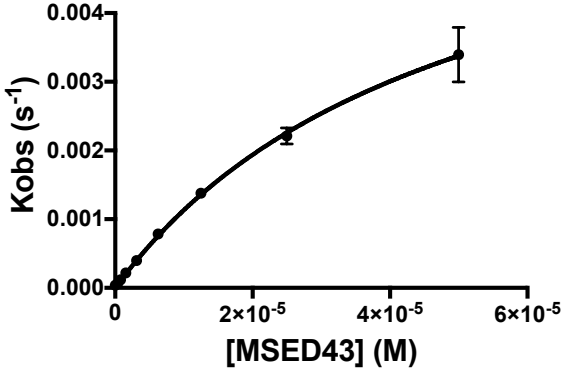


k_{inact} = 0.0037 ± 0.0002 s^{-1}

K_i = 4.0 ± 0.7 uM

K_{inact}/K_i = 900 ± 100 $M^{-1}s^{-1}$



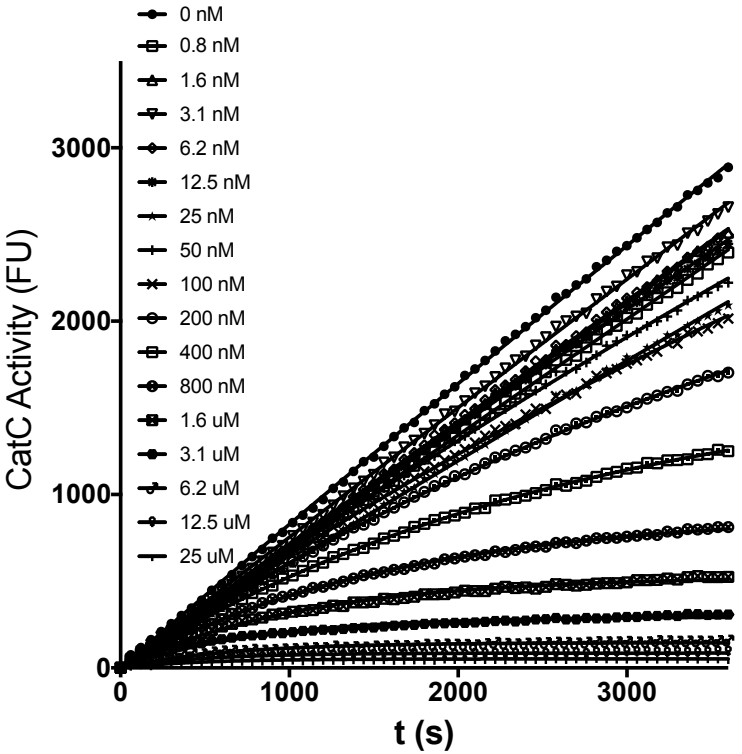


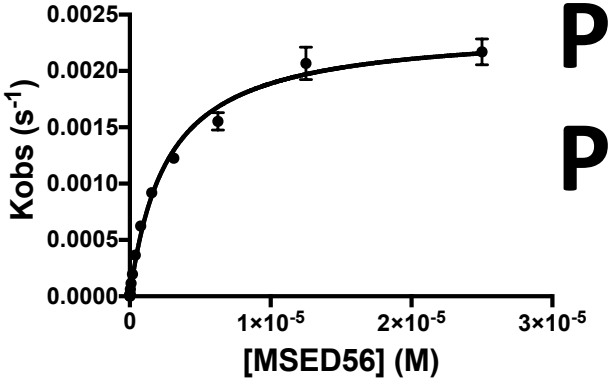
k_{inact} = 0.0067 ± 0.0002 s^{-1}

K_i = 33 ± 2 uM

K_{inact}/K_i = 200 ± 5 $M^{-1}s^{-1}$

P2: Nal,
P1: nLeu(o-Bzl)

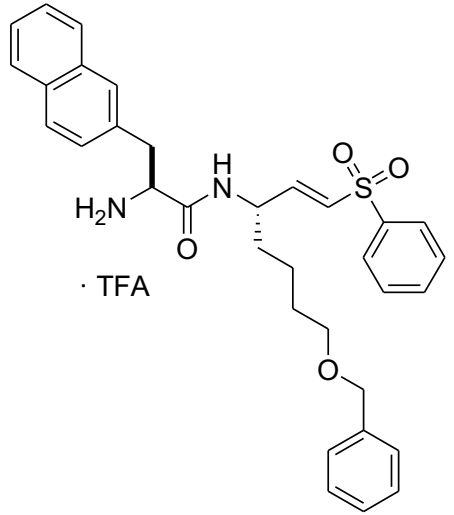


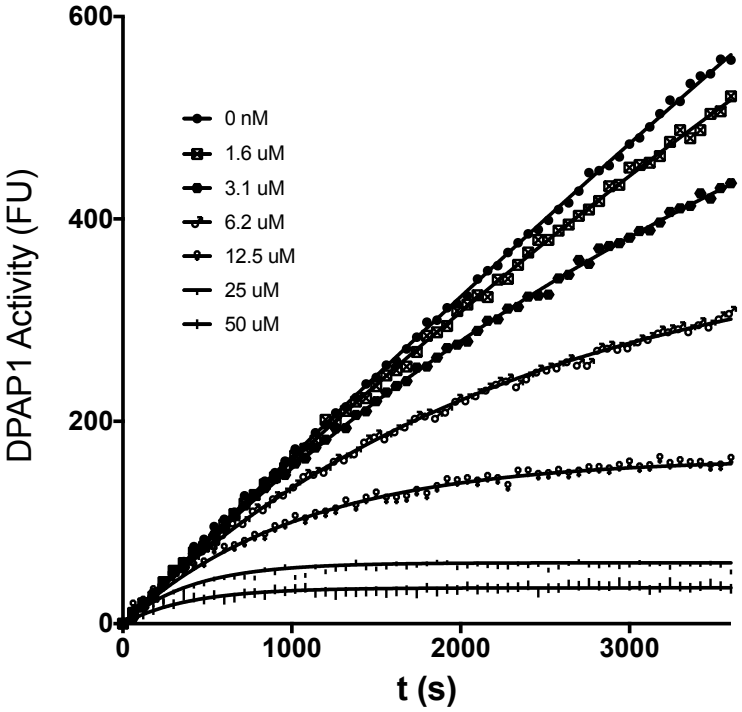


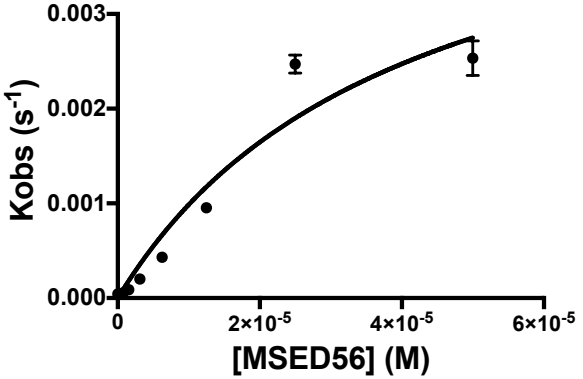
k_{inact} = 0.0024 ± 0.0006 s^{-1}

K_i = 1.74 ± 0.15 uM

K_{inact}/K_i = 1370 ± 90 $M^{-1}s^{-1}$





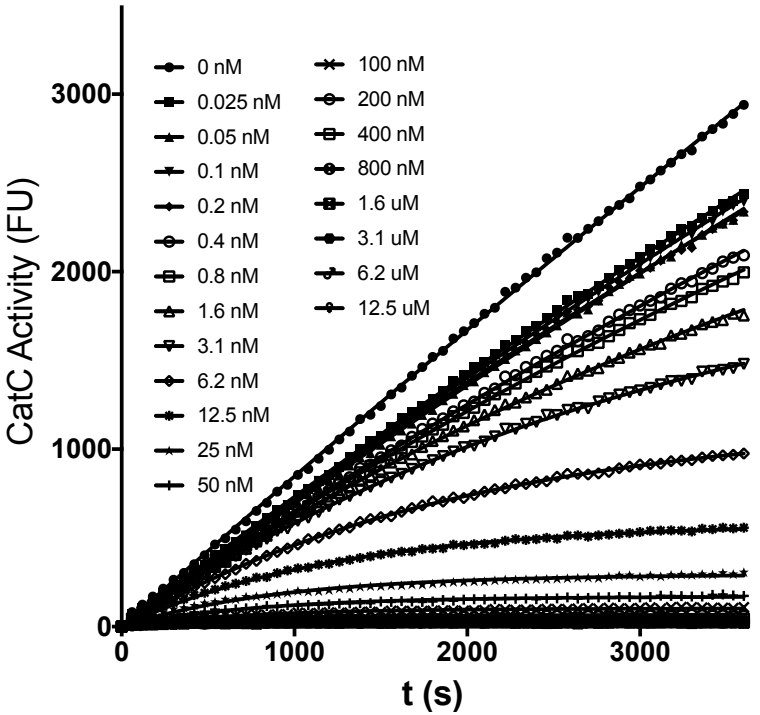


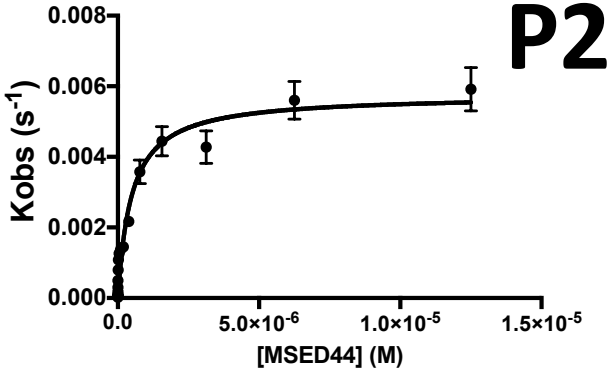
k_{inact} = 0.005 ± 0.002 s^{-1}

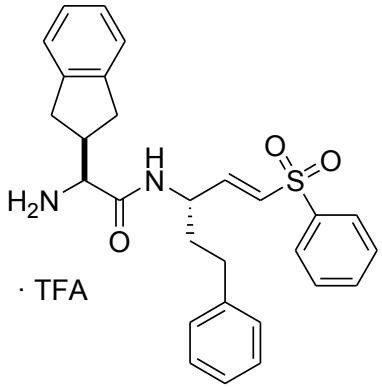
K_i = 27 ± 16 uM

K_{inact}/K_i = 190 ± 50 $M^{-1}s^{-1}$

P2: Igl, P1: hPhe



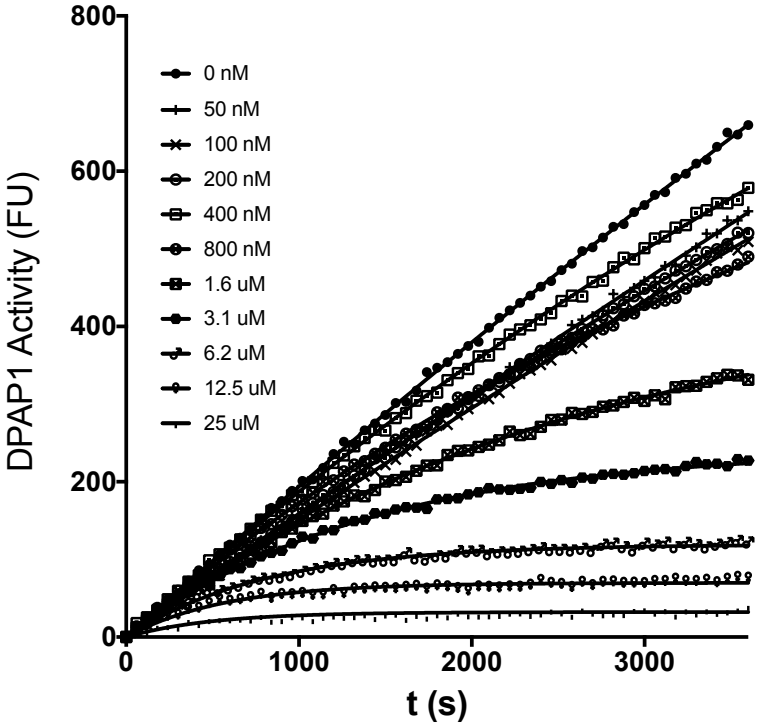


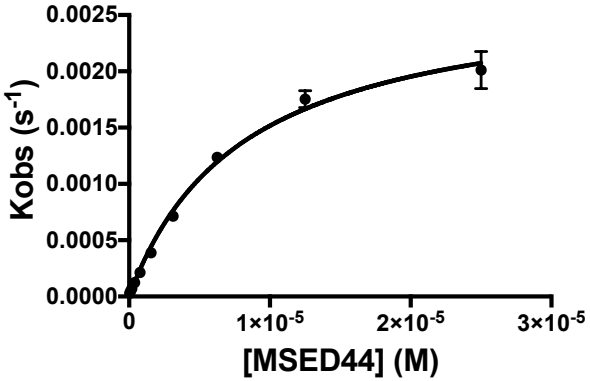


k_{inact} = 0.0058 ± 0.0003 s^{-1}

K_i = 0.32 ± 0.07 uM

K_{inact}/K_i = 18000 ± 300 $M^{-1}s^{-1}$



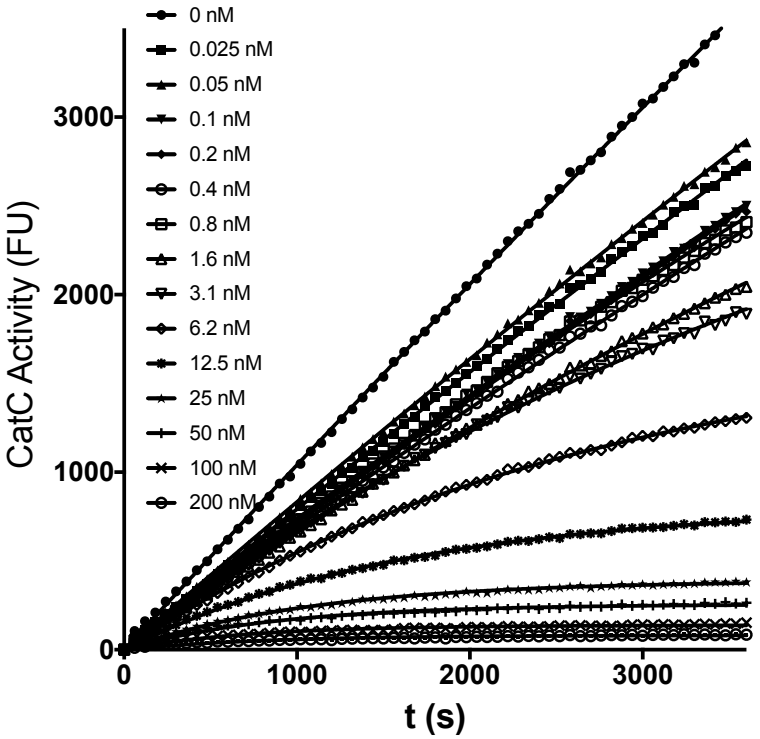


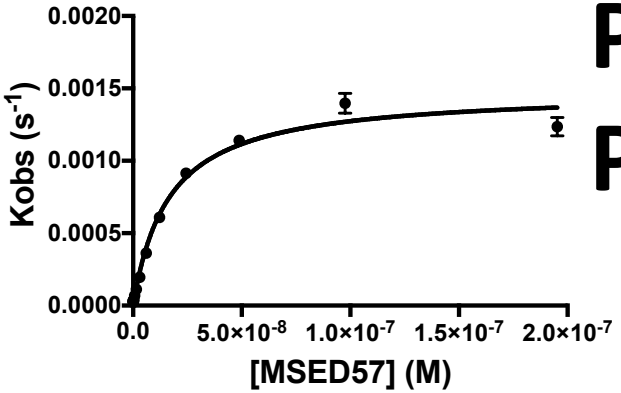
k_{inact} = 0.0027 ± 0.0001 s^{-1}

K_i = 5.4 ± 0.5 uM

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

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

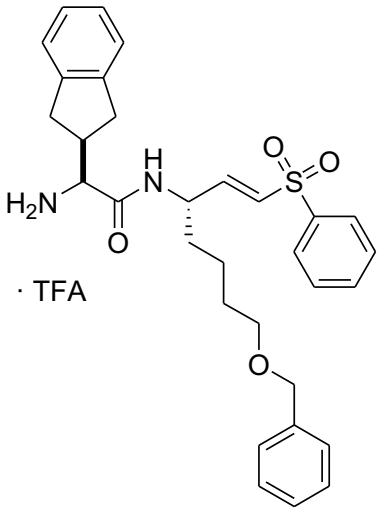


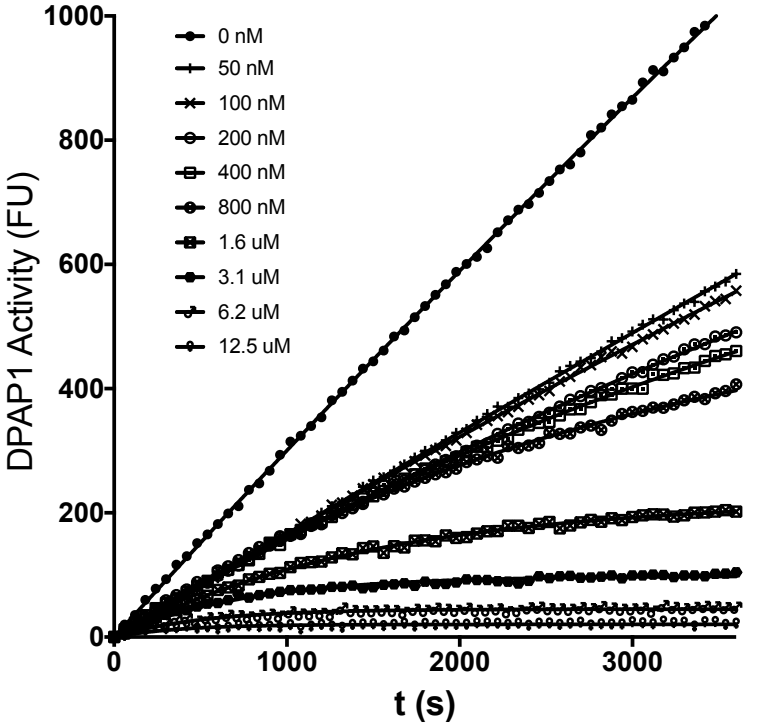


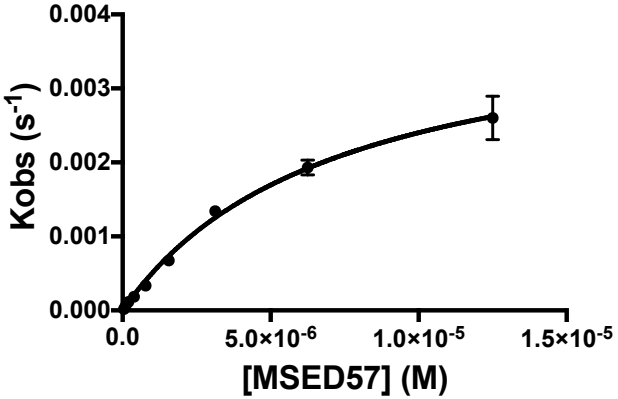
k_{inact} = 0.00148 ± 0.00006 s^{-1}

K_i = 0.011 ± 0.002 uM

K_{inact}/K_i = 135000 ± 14000 $M^{-1}s^{-1}$





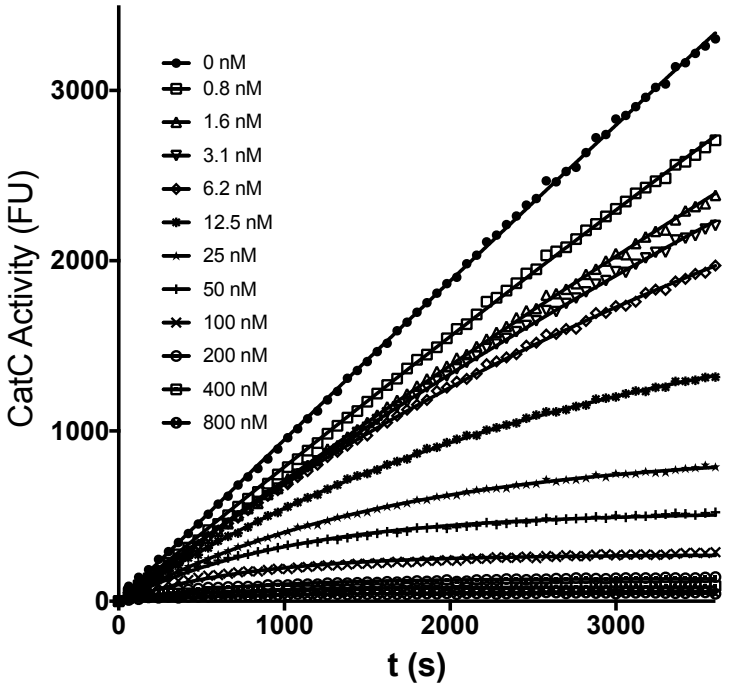


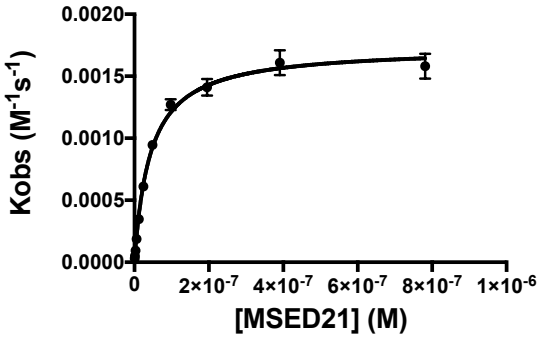
k_{inact} = 0.0041 ± 0.0002 s^{-1}

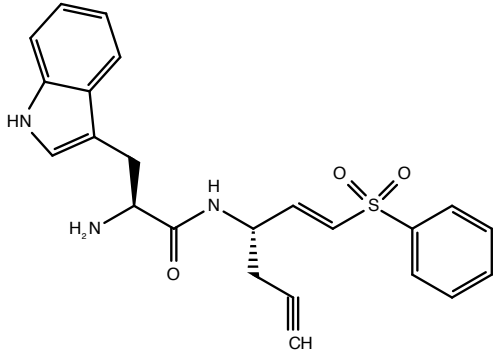
K_i = 4.8 ± 0.5 uM

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

P2: Trp, P1:hPG



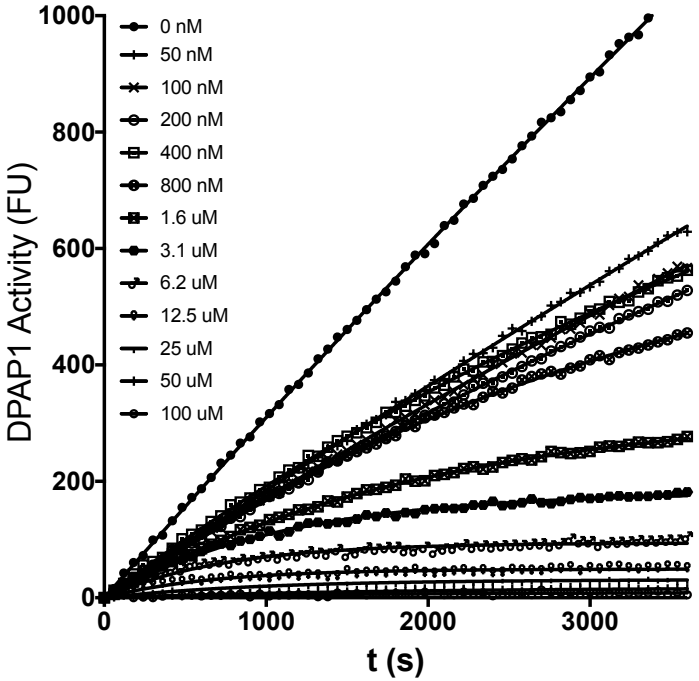


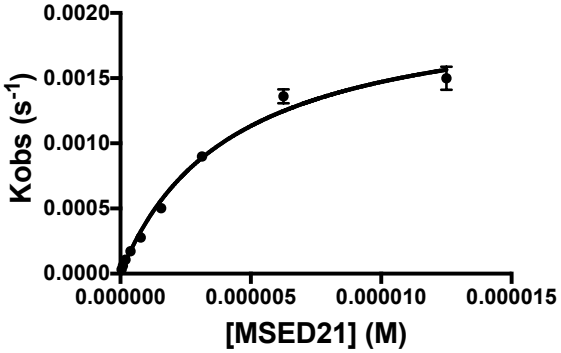


k_{inact} = 0.00173 ± 0.00003 s^{-1}

K_i = 0.028 ± 0.002 uM

K_{inact}/K_i = 61000 ± 4000 $M^{-1}s^{-1}$



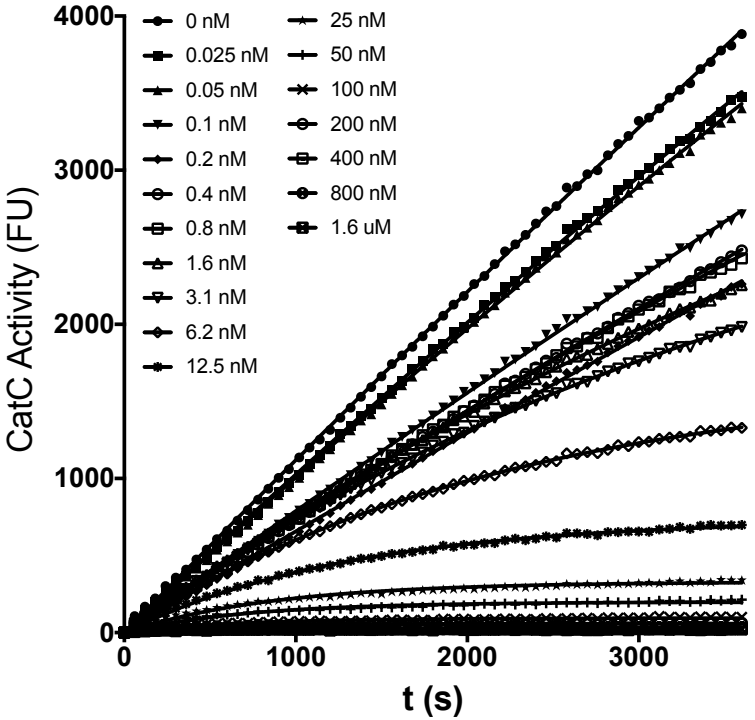


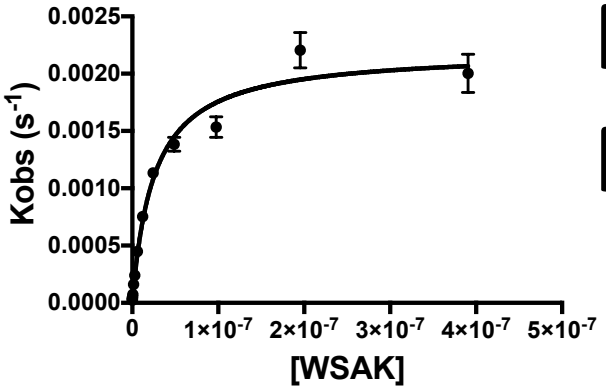
k_{inact} = 0.0021 ± 0.0002 s^{-1}

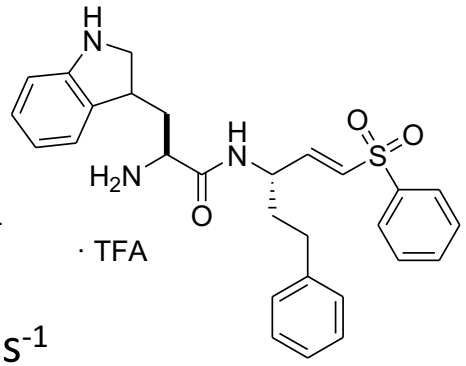
K_i = 2.8 ± 0.45 uM

K_{inact}/K_i = 740 ± 70 $M^{-1}s^{-1}$

P2: *L*-Trp,
P1: hPhe



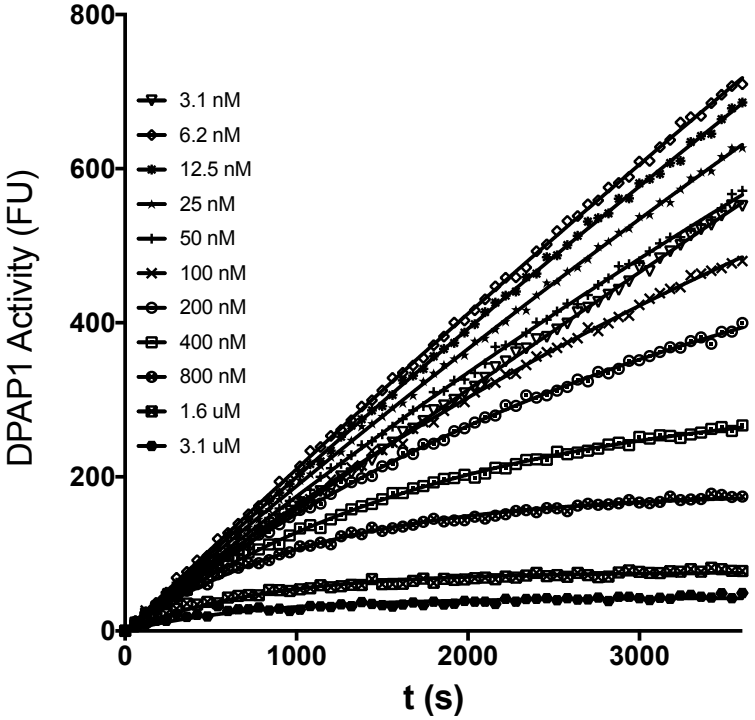


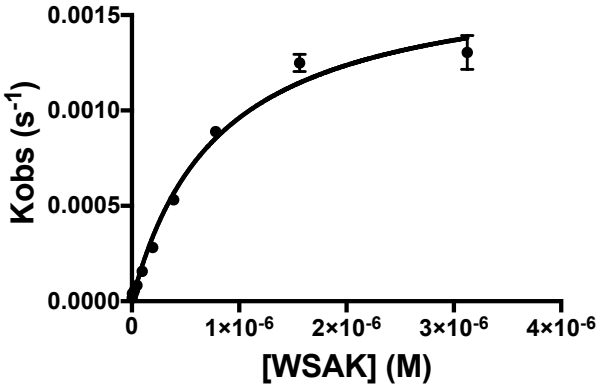


k_{inact} = 0.0022 ± 0.00009 s^{-1}

K_i = 0.016 ± 0.003 uM

K_{inact}/K_i = 131000 ± 15000 $M^{-1}s^{-1}$



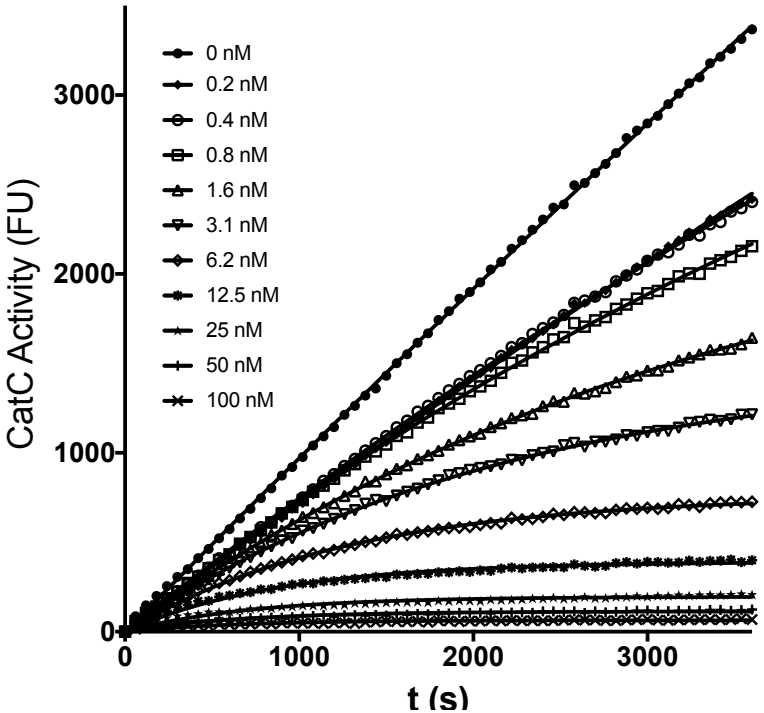


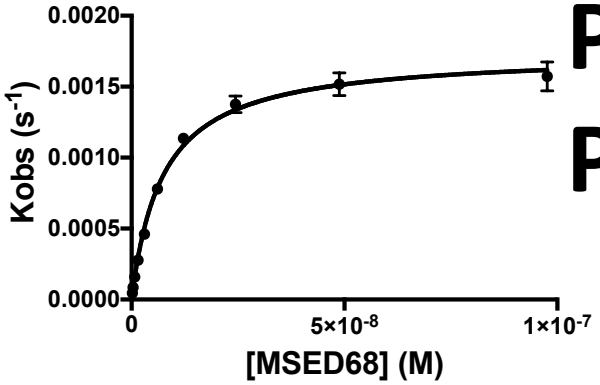
k_{inact} = 0.0017 ± 0.0001 s^{-1}

K_i = 0.54 ± 0.08 uM

K_{inact}/K_i = 3200 ± 300 $M^{-1}s^{-1}$

P2: *L*-Trp,
P1: nLeu(o-Bzl)

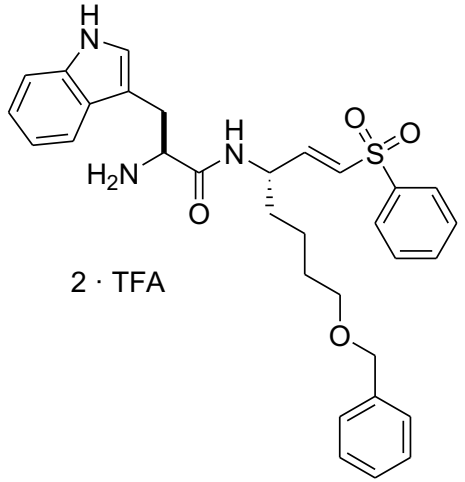


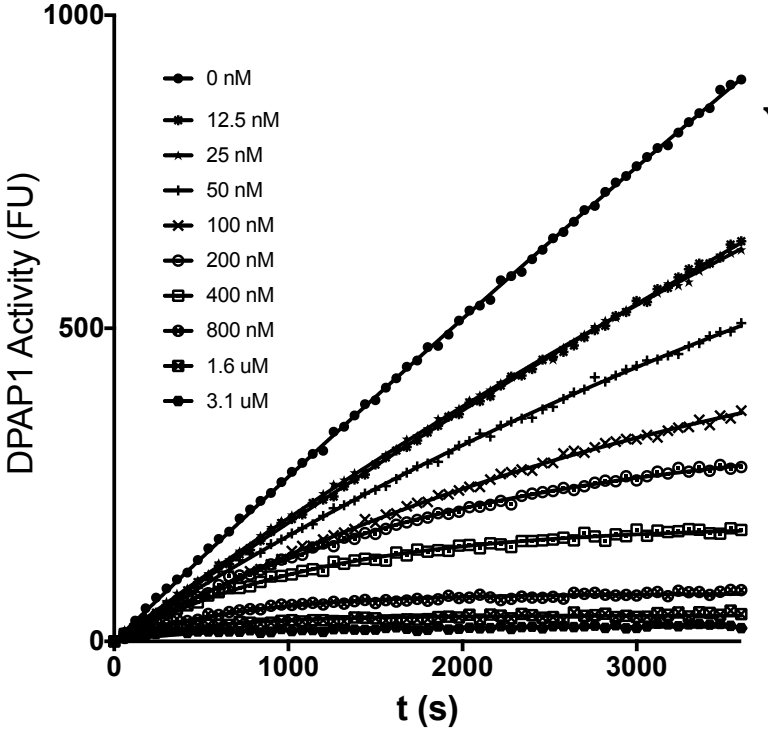


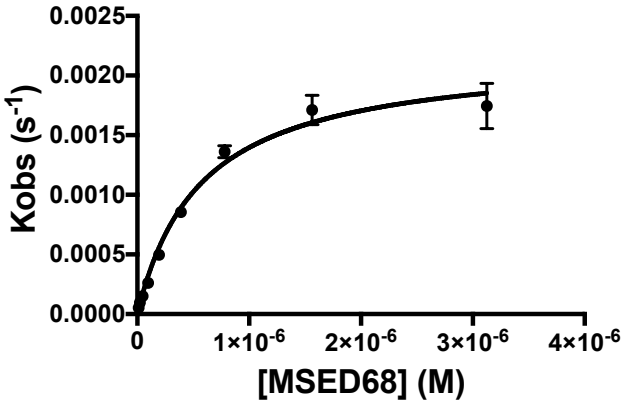
k_{inact} = 0.0022 ± 0.00009 s^{-1}

K_i = 0.016 ± 0.003 uM

K_{inact}/K_i = 131000 ± 15000 $M^{-1}s^{-1}$





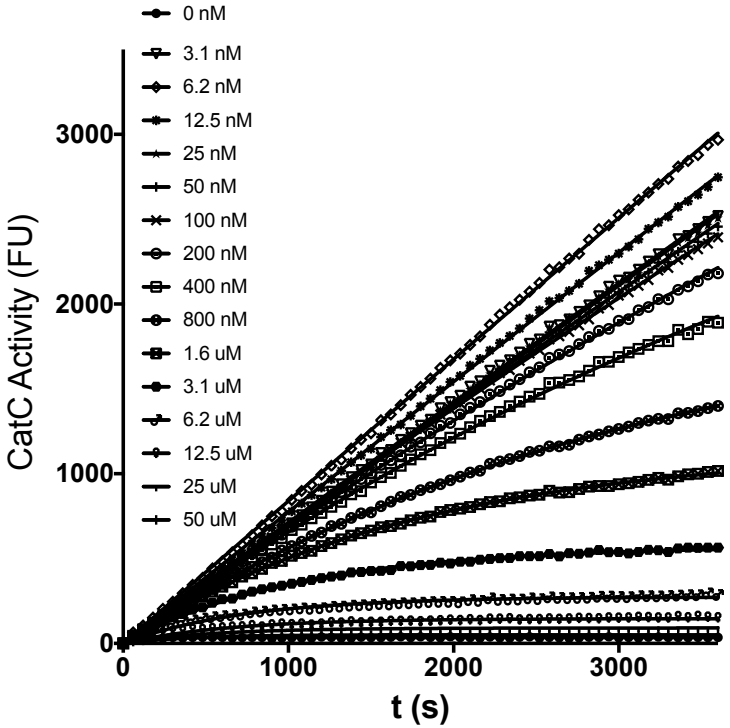


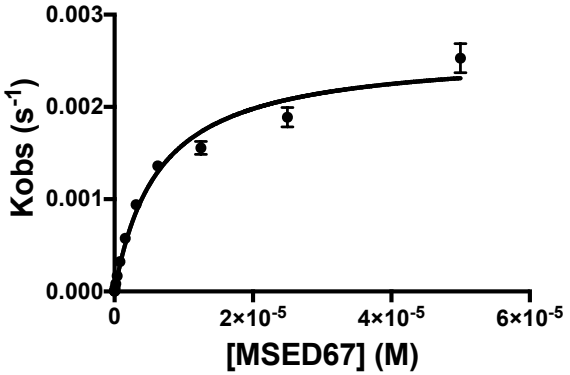
k_{inact} = 0.0017 ± 0.0001 s^{-1}

K_i = 0.54 ± 0.08 uM

K_{inact}/K_i = 3200 ± 300 $M^{-1}s^{-1}$

P2: D-Trp, P1: hPhe

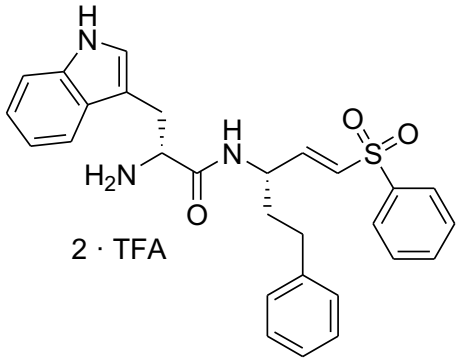


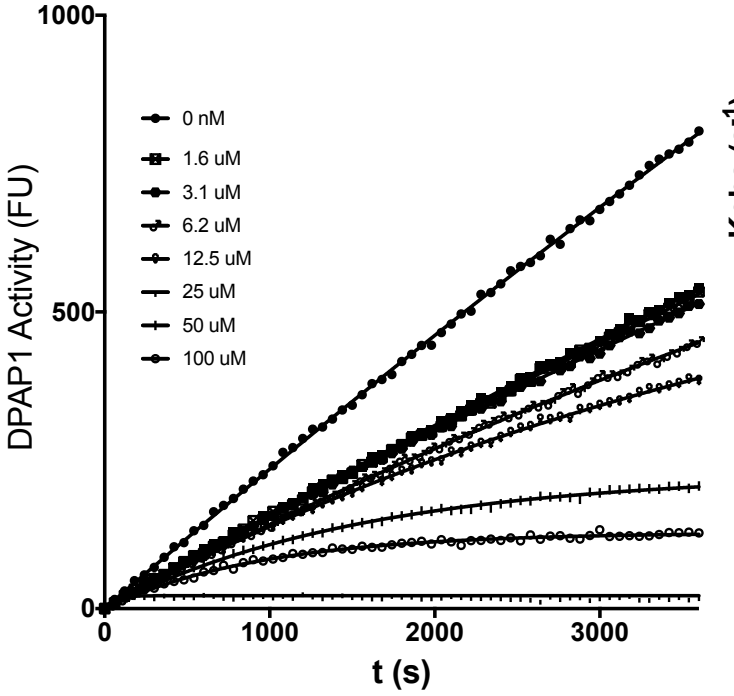


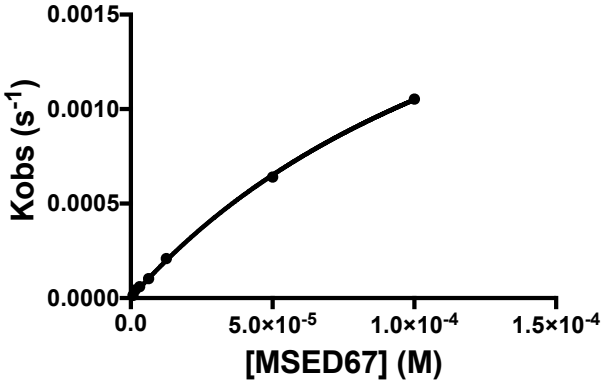
k_{inact} = 0.0026 ± 0.0001 s^{-1}

K_i = 4.2 ± 0.6 uM

K_{inact}/K_i = 620 ± 70 $M^{-1}s^{-1}$





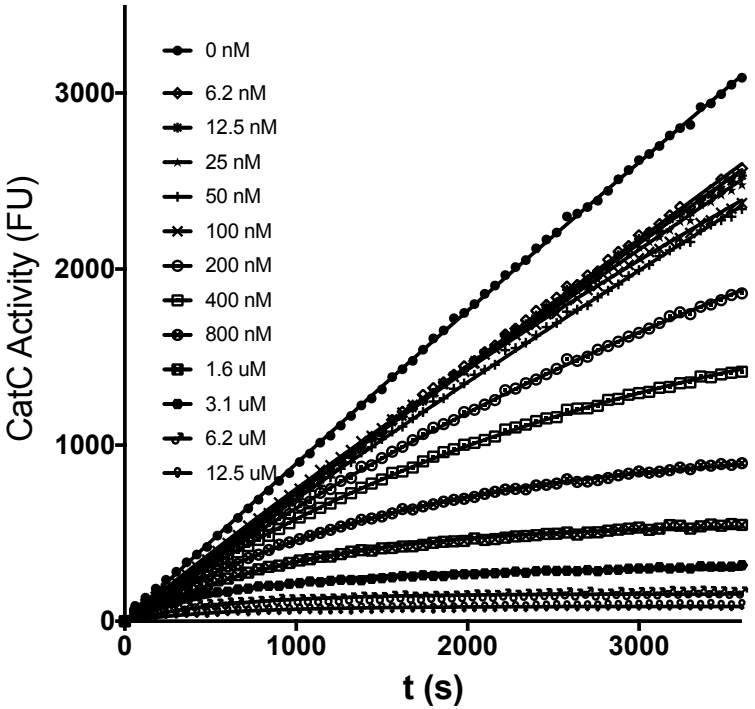


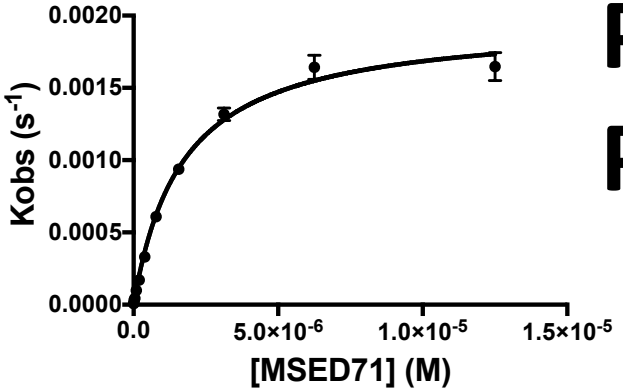
k_{inact} = 0.0027 ± 0.0002 s^{-1}

K_i = 105 ± 10 uM

K_{inact}/K_i = 26 ± 1 $M^{-1}s^{-1}$

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

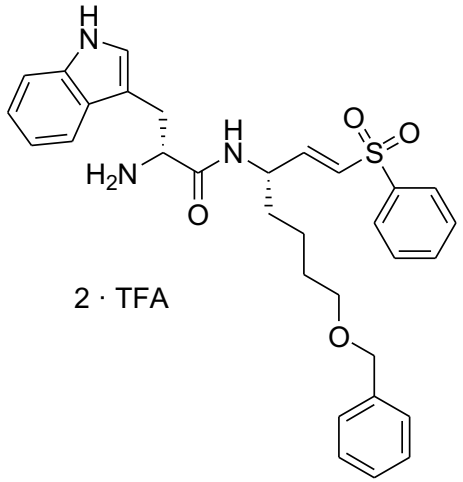


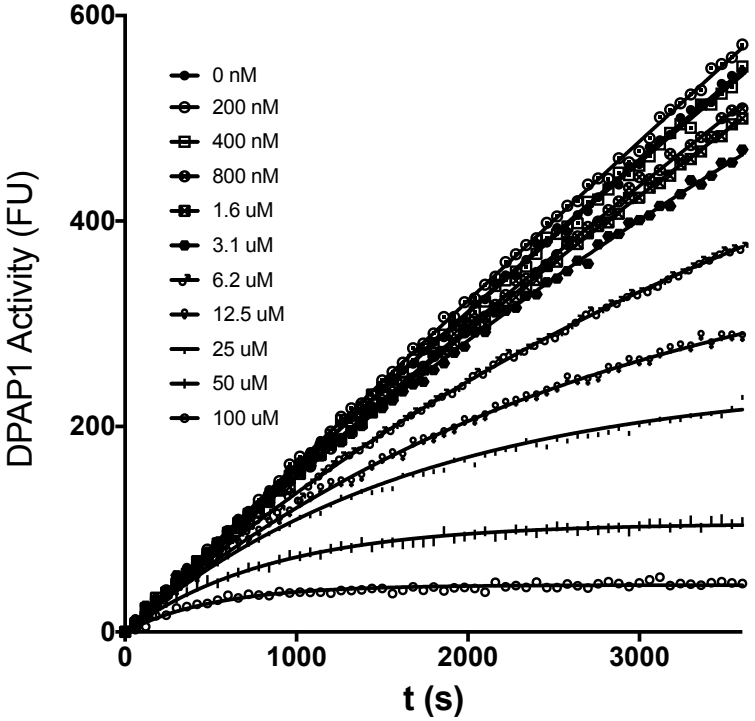


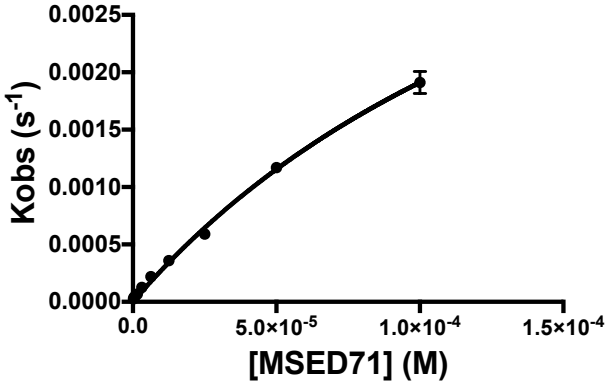
k_{inact} = 0.00197 ± 0.00006 s^{-1}

K_i = 1.1 ± 0.1 uM

K_{inact}/K_i = 1700 ± 100 $M^{-1}s^{-1}$





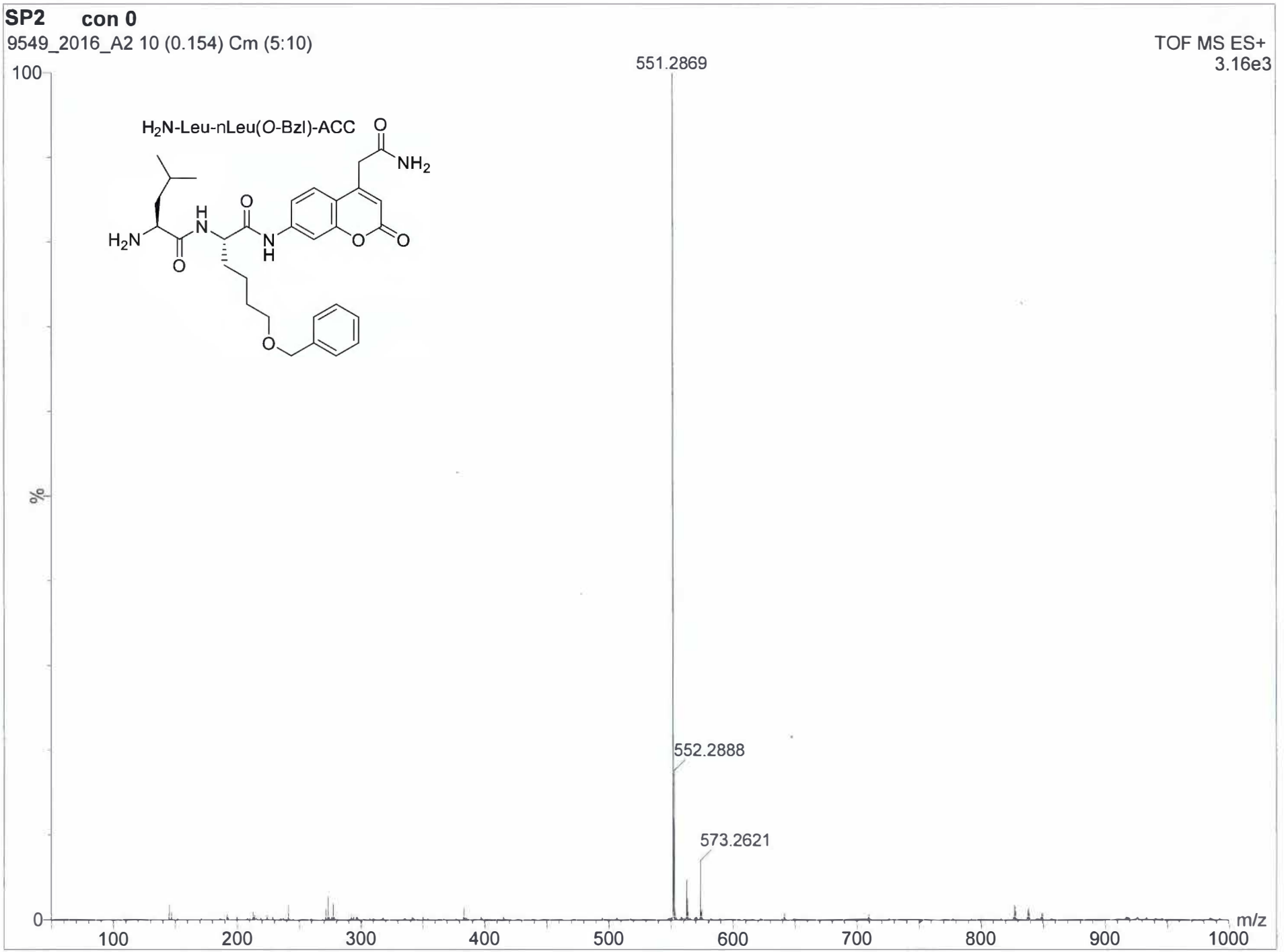


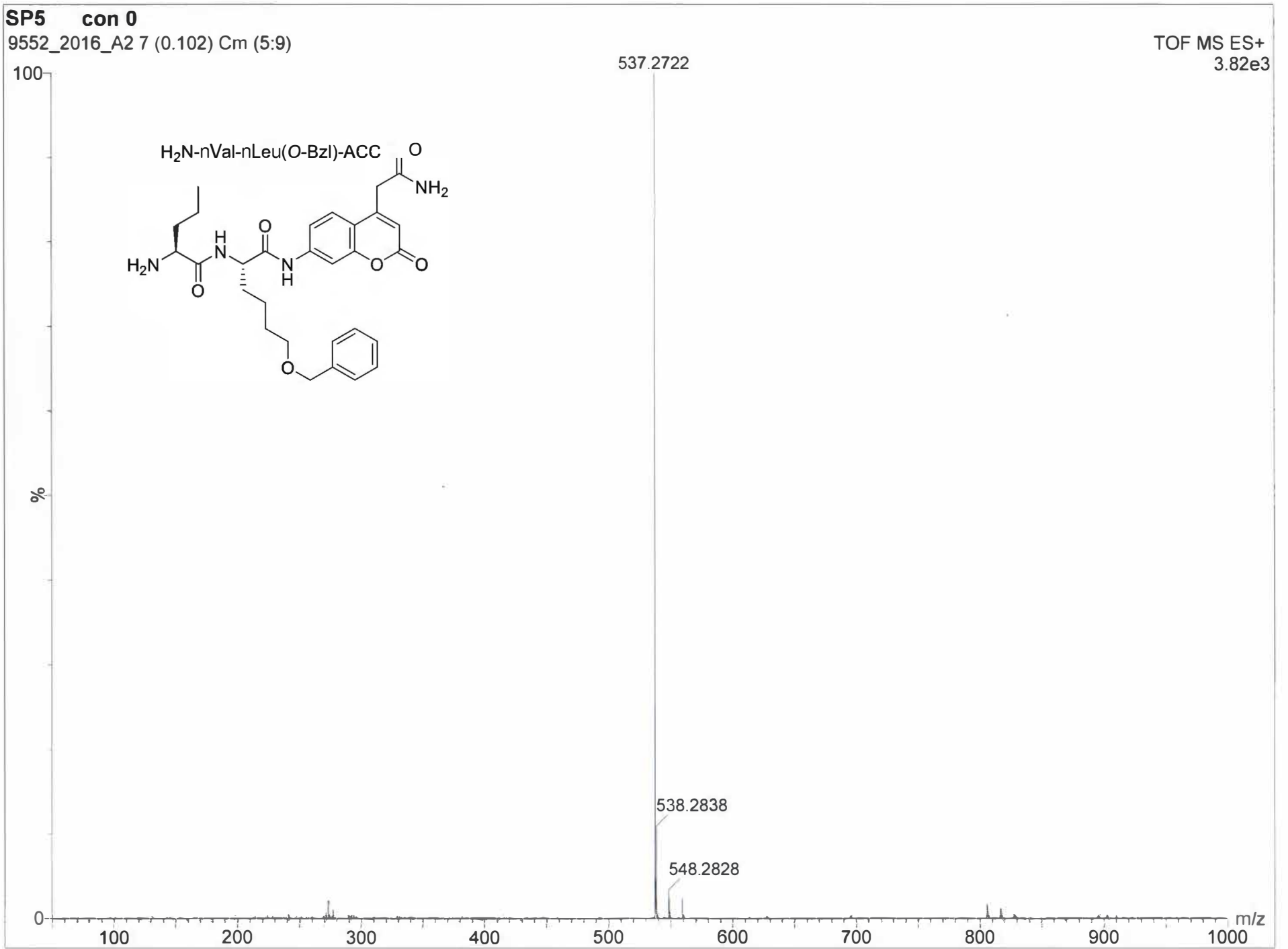
k_{inact} = 0.0055 ± 0.0007 s^{-1}

K_i = 127 ± 20 uM

K_{inact}/K_i = 44 ± 2 $M^{-1}s^{-1}$

Fig. S4

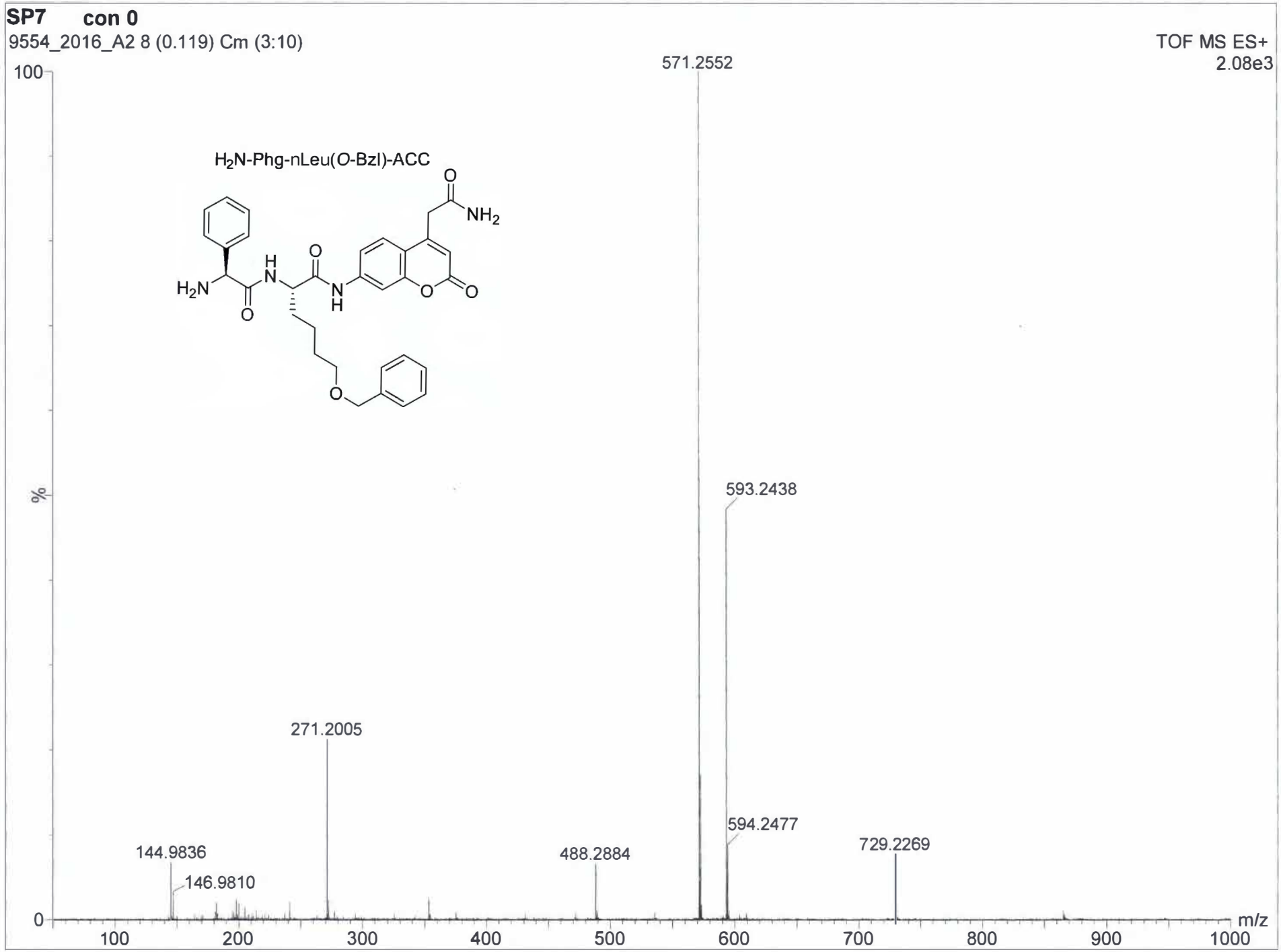

SP2 con 0
9549_2016_A2 10 (0.154) Cm (5:10)
TOF MS ES+
3.16e3
551.2869
552.2888
573.2621
H2N-Leu-nLeu(O-Bzl)-ACC
100
%
0
100
200
300
400
500
600
700
800
900
1000
m/z


SP5 con 0
9552_2016_A2 7 (0.102) Cm (5:9)
TOF MS ES+
3.82e3
537.2722
100
H2N-nVal-nLeu(O-Bzl)-ACC
O
NH2
H2N
H
N
O
N
H
O
O
O
%
538.2838
548.2828
0
m/z
100
200
300
400
500
600
700
800
900
1000

SP7 con 0

9554_2016_A2 8 (0.119) Cm (3:10)

TOF MS ES+
2.08e3



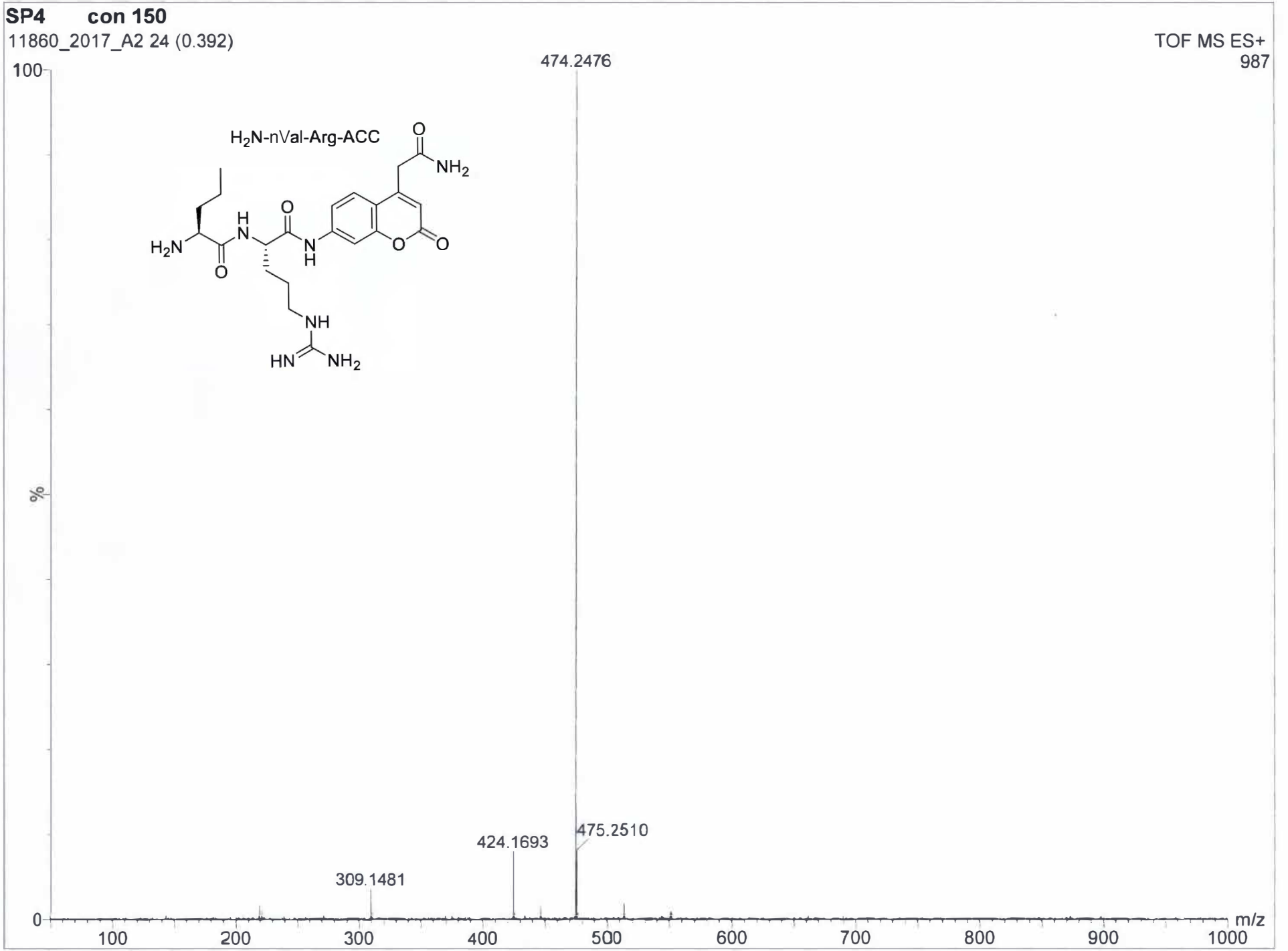
H_2N-Phg-nLeu(*O*-Bzl)-ACC

SP4 con 150

11860_2017_A2 24 (0.392)

TOF MS ES+

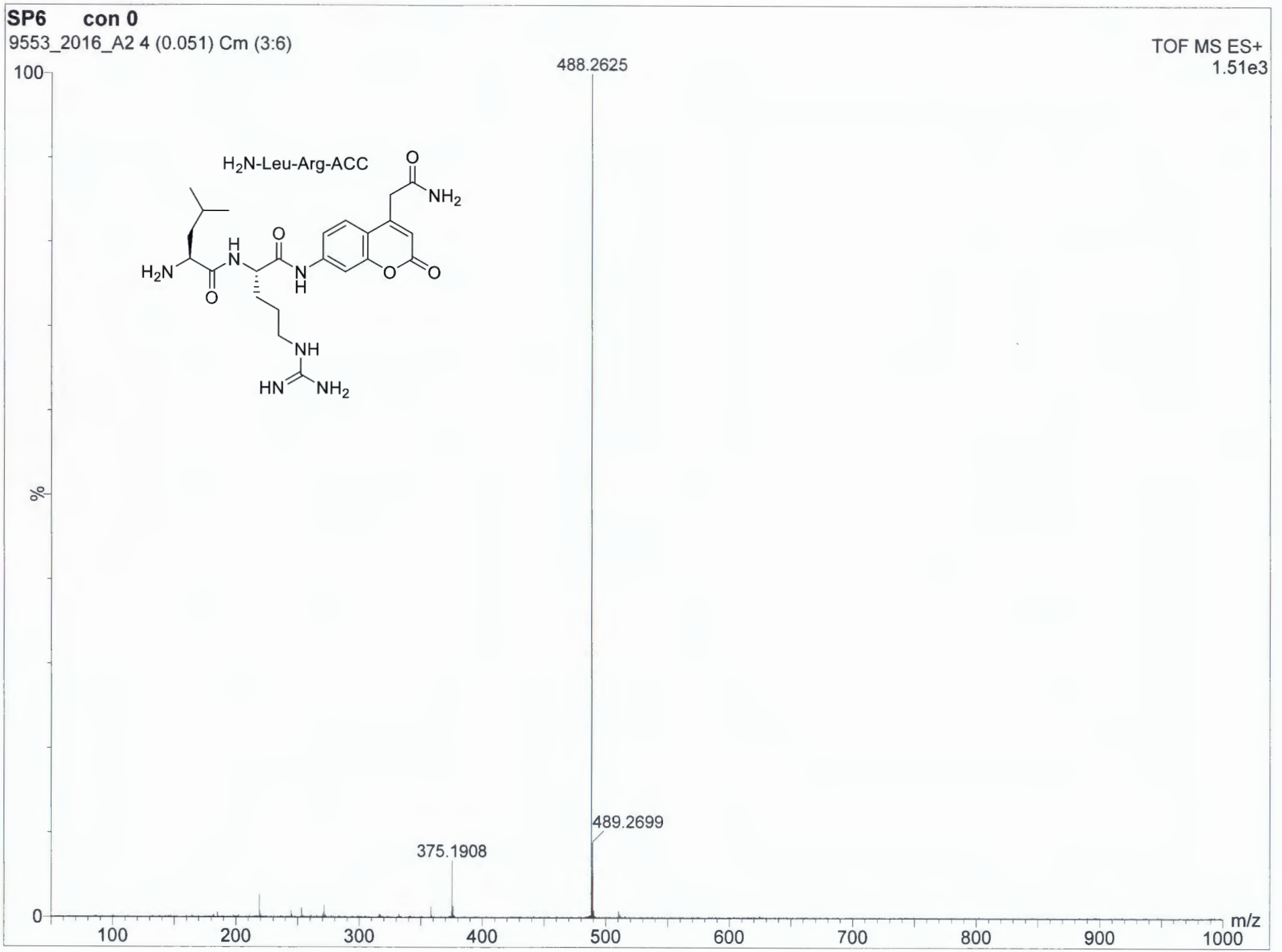
987



SP6 con 0

9553_2016_A2 4 (0.051) Cm (3:6)

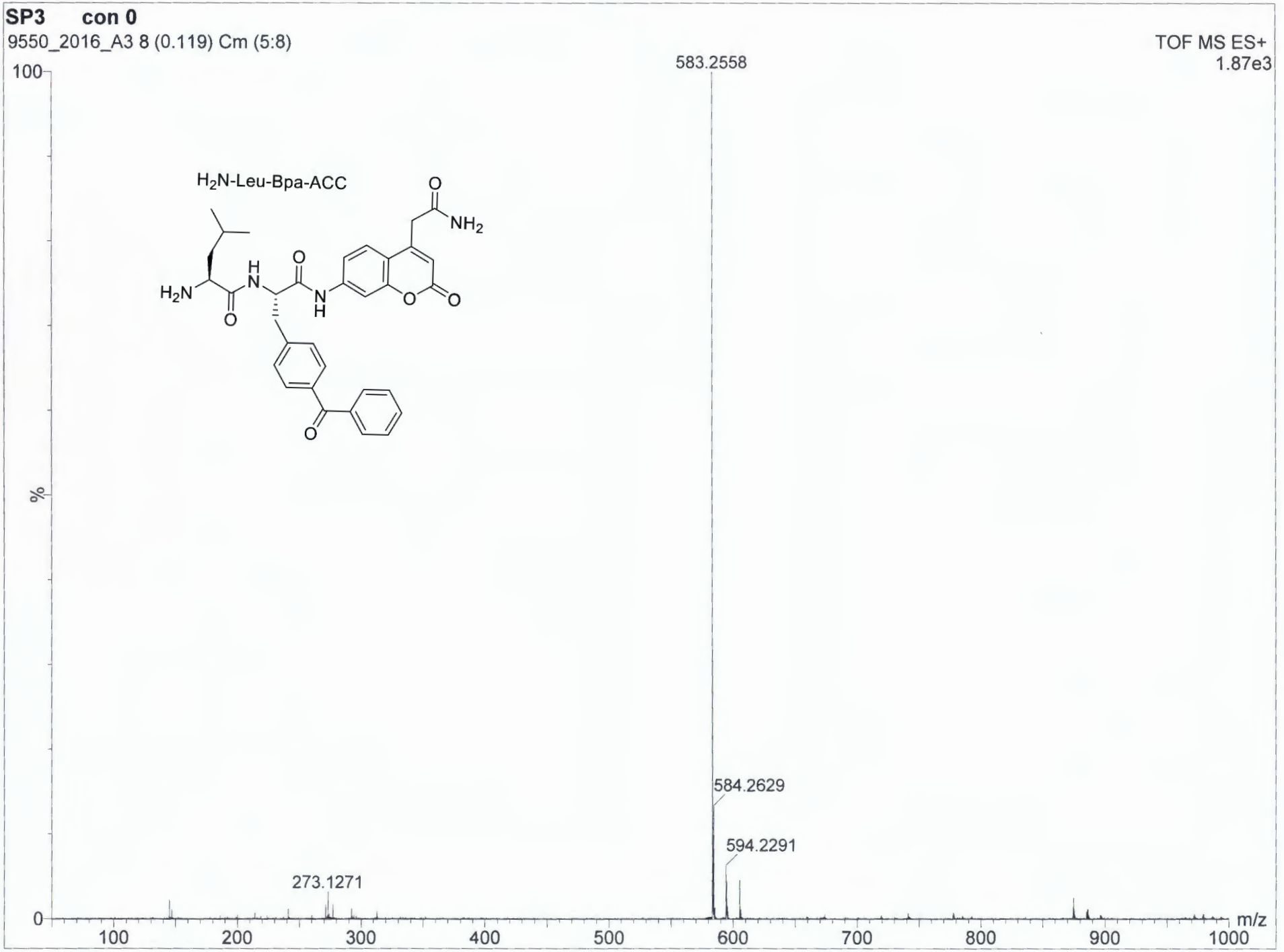
TOF MS ES+
1.51e3



SP3 con 0

9550_2016_A3 8 (0.119) Cm (5:8)

TOF MS ES+
1.87e3



H_2N-Leu-Bpa-ACC