Structure Activity Relationship Towards Design of Cryptosporidium Specific Thymidylate Synthase Inhibitor

Czyzyk, D.J.[a,‡], Valhondo, M.[b,‡], Deiana, L.[b] Jorgensen, W.L.[b,*], and Anderson, K.S.[a,*]

[a]Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

[b]Department of Chemistry, Yale University, 225 Prospect Street, PO Box 208107, New Haven, CT 06520, USA

*Corresponding author. Tel.: +1 203 432 6278; fax: +1 203 432 6299 (W.L.J.); Tel.: +1 203 785 7670; fax: +1 203 785 7670 (K.S.A.).

E-mail address: william.jorgensen@yale.edu (W.L. Jorgensen), karen.anderson@yale.edu (K.S. Anderson)

‡Authors have contributed equally to this work.

Supplementary Data

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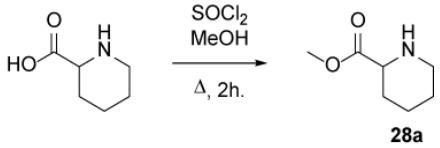
1.1. Procedure for the synthesis of non-commercial amine intermediate 28a



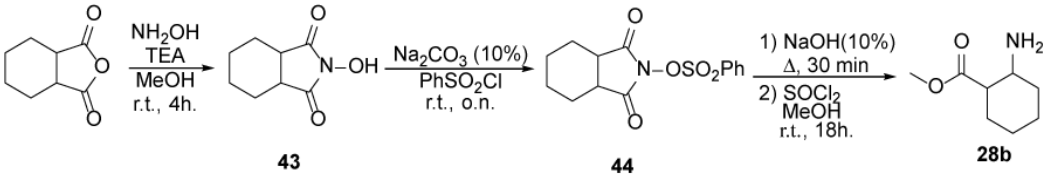
Scheme S1. Synthesis of amine intermediate **28a**

A solution of piperidine-2-carboxylic acid (1 g, 5.98 mmol) in methanol (15,5 mL) was cooled to 0 ºC followed by dropwise addition of thionyl chloride (1.4 mL). The mixture was stirred at reflux for 2 h. After evaporation of the solvent and neutralization by addition of a saturated aqueous $NaHCO_3$ solution, the mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were then washed with saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product, a yellow oil, was used in subsequent reaction without purification ***methyl piperidine-2-carboxylate*** (**28a**).

methyl piperidine-2-carboxylate (**28a**)

(565 mg, 3.95 mmol, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.35 (dd, J = 9.9, 3.2 Hz, 1H), 3.11 – 3.03 (m, 1H), 2.69 – 2.61 (m, 1H), 1.99 – 1.91 (m, 2H), 1.83 – 1.74 (m, 1H), 1.61 – 1.38 (m, 4H). LC-MS (ESI) m/z 144.1 $[M+H]^+$.

1.2. Procedure for the synthesis of non-commercial amine intermediate 28b



Scheme S2. Synthesis of amine intermediate **28b**

A solution of hexahydrophthalic anhydride (5 g, 32.4 mmol) in methanol (32.5 mL) was added to a mixture of hydroxylamine hydrochloride (2.25 g, 32.4 mmol) and triethylamine (4.5 mL) in methanol (6.5 mL). After the reaction mixture was stirred for 4 h at room temperature, the solvent was removed under reduced pressure. The crude product was used in the subsequent reaction without purification as **N-Hydroxyimide** (**43**) (white solid). **43** (5.48 g, 32.4 mmol) in 40 mL of aqueous 10% Na_2CO_3 solution with benzenesulfonyl chloride (4 mL) was stirred at room temperature overnight. Methanol was added to the mixture and cooled to -20 ^oC and the resulting precipitate was filtered to provide **N-phenyl-sulfonyloxyhexahydrophthalimide** (**44**) as a white solid, which was used in the subsequent reaction without purification. **44** (5.93 g. 19.17 mmol) was heated with 38 mL of aqueous 10% NaOH solution for 30 min. The clear solution was acidified with HCl and evaporated the solvent to dryness in vacuo.[1] The residue was dissolved in methanol (68.5 mL) and thionyl chloride (7.5 g, 63.12 mmol, 4.6 mL) was added dropwise at 0 ^oC. The mixture was allowed to warm to room temperature and stirred for 18 h. The clear solution was then evaporated to dryness, and the residual volatiles were removed on high vacuum. To the remaining crude material was added a saturated aqueous $NaHCO_3$ (20 mL) and the resulting mixture was stirred at 25 ^oC for 30 min. The reaction mixture was extracted with ethyl acetate (3 x 40 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide ***methyl 2-aminocyclohexane-1-carboxylate*** (**28b**) as a brown oil, which was used in subsequent reaction without purification.

N-Hydroxyimide (**43**)

(5.48 g, 32.40 mmol, 99% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 5.43 (bs, 1H), 2.89 – 2.80 (m, 2H), 1.90 – 1.71 (m, 4H), 1.49 – 1.39 (m, 4H).7.36 (d, J = 3.1 Hz, 1H), 6.96 (dd, J = 8.9, 3.1 Hz, 1H), 6.64 (d, J = 8.9 Hz, 1H), 3.89 (s, 3H), 3.77 (s, 3H). LC-MS (ESI) m/z 170.1 $[M+H]^+$.

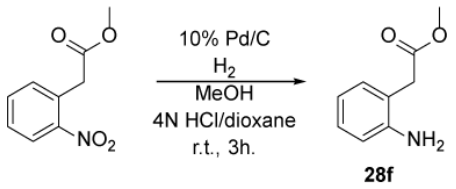
N-phenyl-sulfonyloxyhexahydrophthalimide (**44**)

(5.93 g, 19.17 mmol, 59% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 – 7.99 (m, 2H), 7.93 – 7.87 (m, 1H), 7.76 – 7.69 (m, 2H), 3.08 (td, *J* = 4.5, 2.2 Hz, 2H), 1.79 – 1.68 (m, 2H), 1.64 – 1.54 (m, 2H), 1.45 – 1.24 (m, 4H). LC-MS (ESI) m/z 310.1 $[M+H]^+$.

methyl 2-aminocyclohexane-1-carboxylate (**28b**)

(677 mg, 4.3 mmol, 22% yield). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 3.68 (s, 3H), 3.32 – 3.26 (m, 1H), 2.59 – 2.53 (m, 1H), 1.89 – 1.51 (m, 6H), 1.47 – 1.36 (m, 1H), 1.36 – 1.24 (m, 1H). LC-MS (ESI) m/z 158.1 $[M+H]^+$.

1.3. Procedure for the synthesis of non-commercial amine intermediate 28f



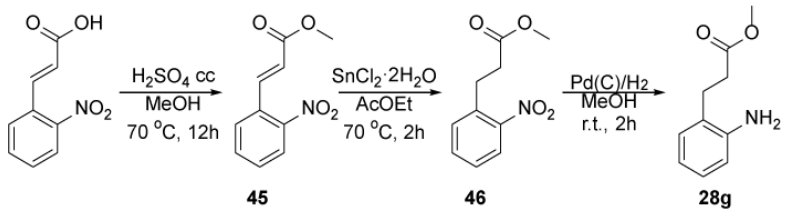
Scheme S3. Synthesis of amine intermediate **28f**

The 2-nitrophenyl acid methyl ester (1 g, 4.87 mmol, 95%) was dissolved in ethyl acetate (30 mL) and 0.26 g of Pd(C) 10% was suspended into the solution, the reaction mixture was maintained under hydrogen atmosphere at room temperature for 4 h.[2] The palladium was removed by filtration and concentrated under vacuo to yield ***methyl 2-(2-aminophenyl)acetate*** (**28f**) as an orange-red oil.

methyl 2-(2-aminophenyl)acetate (**28f**)

(0.62 g, 3.75 mmol, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.15 – 7.04 (m, 2H), 6.80 – 6.67 (m, 2H), 4.07 (bs, 2H), 3.69 (s, 3H), 3.57 (s, 2H). LC-MS (ESI) m/z 166.1 $[M+H]^+$.

1.4. Procedure for the synthesis of non-commercial amine intermediate 28g



Scheme S4. Synthesis of amine intermediate **28g**

To a solution of 2-nitrocinnamic acid (5 g, 25.88 mmol) in methanol (96 mL) was dropped a small amount of concentrated sulfuric acid at room temperature and the reaction mixture was stirred at 70 ºC for 12 h. After the reaction was complete, the resulting solution was basified with saturated aqueous solution of $NaHCO_3$ to weak basicity and extracted with dichloromethane (3 x 100 mL). Then, the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuo[3]. **Methyl 2-nitrocinnamate** (**45**) was obtained as a yellow solid and used in the subsequent reaction without purification. To a solution of **45** (4.477 g, 21.61 mmol) in ethyl acetate (130 mL) was added $SnCl_2 \cdot H_2O$ (60.06 g, 0.27 mol) at room temperature, and the resulting solution was stirred at 70 °C for 2 h. After the reaction was complete, the solution was poured into 57 mL of cool water and cooled in an ice bath. This was followed by careful addition of saturated aqueous solution of $NaHCO_3$ to weak basicity (800 mL), and filtration over Celite. The aqueous phase was extracted with ethyl acetate three times, and the combined organic phases were dried over anhydrous Na_2SO_4 followed by solvent evaporation under reduced pressure to give **methyl 2-aminocinnamate** (**46**)[4] as a yellow solid which was used in the subsequent reaction without purification. A mixture of methyl 2-aminocinnamate (**46**) (3.59 g, 20.27 mmol) and 360 mg of Pd(C) 10% in 185 mL of methanol were pressurized with a balloon of hydrogen (5 bar) for 2 h at room temperature. The reaction mixture was filtered over Celite and methanol was removed by distillation in vacuo to give ***methyl 3-(2-aminophenyl)propanoate*** (**28g**)[4] as a brown oil.

Methyl 2-nitrocinnamate (**45**)

(5.25 g, 25.36 mmol, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.11 (d, *J* = 15.8 Hz, 1H), 8.04 (d, *J* = 8.1 Hz, 1H), 7.70 – 7.59 (m, 2H), 7.54 (td, *J* = 7.5, 6.7, 2.2 Hz, 1H), 6.36 (d, *J* = 15.8 Hz, 1H), 3.82 (s, 3H). LC-MS (ESI) m/z 208.1 $[M+H]^+$.

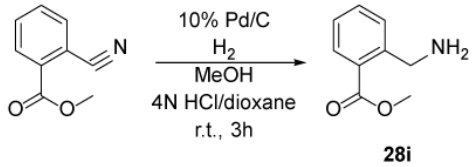
methyl 2-aminocinnamate (**46**)

(3.59 g, 20.27 mmol, 94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.83 (d, *J* = 15.8 Hz, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 6.77 (t, *J* = 7.5 Hz, 1H), 6.70 (d, *J* = 8.1 Hz, 1H), 6.36 (d, *J* = 15.8 Hz, 1H), 3.96 (bs, 2H), 3.80 (s, 3H). LC-MS (ESI) m/z 178.1 $[M+H]^+$.

methyl 3-(2-aminophenyl)propanoate (**28g**)

(3.59 g, 19.15 mmol, 95% yield)l. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.04 (t, *J* = 7.9 Hz, 2H), 6.77 – 6.71 (m, 1H), 6.68 (d, *J* = 7.8 Hz, 1H), 3.79 (s, 2H), 3.68 (s, 3H), 2.84 (t, *J* = 7.6 Hz, 2H), 2.66 (t, *J* = 7.5 Hz, 2H). LC-MS (ESI) m/z 180.1 $[M+H]^+$.

1.5. Procedure for the synthesis of non-commercial amine intermediate 28i



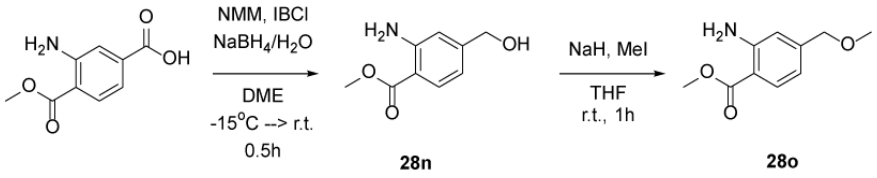
Scheme S5. Synthesis of amines intermediate **28i**

A mixture of methyl 2-cyanobenzoate (0.65 g, 4.05 mmol) and PtO_2 (65 mg) in 13 mL ethanol and 0.65 mL of chloroform was hydrogenated under hydrogen balloon for 2 h. The reaction mixture was then filtered through Celite and concentrated on a rotary evaporator to give **methyl 2-(aminomethyl)benzoate** (**28i**)[2].

methyl 2-(aminomethyl)benzoate (**28i**)

(0.296 g, 1.79 mmol, 41% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.03 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.62 (td, *J* = 7.6, 1.5 Hz, 1H), 7.49 (t, *J* = 7.7 Hz, 1H), 5.30 (s, 2H), 3.91 (s, 3H). LC-MS (ESI) m/z 201.1 $[M+H]^+$.

1.6. Procedure for the synthesis of non-commercial amine intermediates 28n-o



Scheme S6. Synthesis of amines intermediate **28n** and **28o**

To a solution of 1-methyl 2-aminoterephthalate (1 g, 5.12 mmol) and NMM (0.58 mL, 1 eq) in DME (9 mL) at -15 °C was added dropwise IBCl (0.67 mL, 1 eq). The mixture was then stirred at this temperature for 15 min. The salt was removed by filtration and the solution was cooled down to -15 °C. A solution of $NaBH_4$ (306 mg, 1.5 eq) in water (3 mL) was carefully added dropwise . At the end of the addition, water (10 mL) was added and the mixture was stirred at r.t. for 15 min. Aqueous NaOH solution (2N, 5 mL) was added and the aqueous phase was extracted twice with ethyl acetate. The organic phases were combined and dried over anhydrous Na_2SO_4. The solution was concentrated in vacuo to afford ***methyl 2-amino-4-(hydroxymethyl)benzoate*** (**28n**)[3] as a white solid. To NaH (243 mg, 3.3 mmol, 60% in oil) in THF (18 mL) was added successively (**28n**) (1 g, 5.52 mmol) and MeI (784 mg, 5.52 mmol). After 1 h, the reaction was quenched by addition of NaCl(aq), extracted with diethyl ether, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude residue was purified by flash column chromatography (0-40 % EtOAc in dichloromethane) to provide ***methyl 2-amino-4-(methoxymethyl)benzoate*** (**28o**) as a yellow oil.

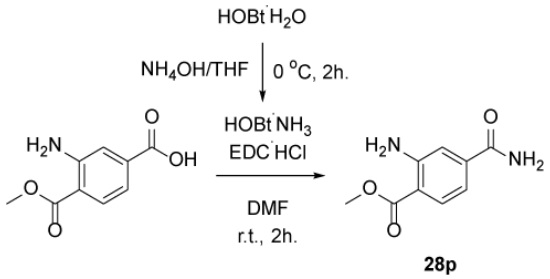
methyl 2-amino-4-(hydroxymethyl)benzoate (**28n**)

(800 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 8.2 Hz, 1H), 6.68 (s, 1H), 6.60 (dd, J = 8.3, 1.6 Hz, 1H), 4.62 (s, 2H), 3.86 (s, 3H). LC-MS (ESI) m/z 182.1 $[M+H]^+$.

methyl 2-amino-4-(methoxymethyl)benzoate (**28o**)

(197 mg, 1.01 mmol, 18% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 8.2 Hz, 1H), 6.65 (d, J = 1.7 Hz, 1H), 6.58 (dd, J = 8.2, 1.6 Hz, 1H), 5.73 (bs, 2H), 4.38 (s, 2H), 3.86 (s, 3H), 3.39 (s, 3H). LC-MS (ESI) m/z 196.1 $[M+H]^+$.

1.7. Procedure for the synthesis of non-commercial amine intermediate 28p



Scheme S7. Synthesis of amines intermediate **28p**

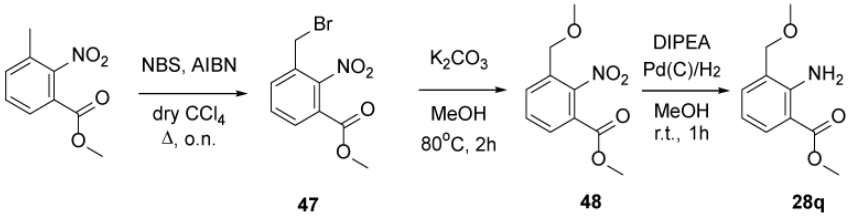
To a solution of 1-methyl-2-aminoterephthalate (1g, 5.12 mmol) in DMF (5 mL) was added 1-hydroxy-1H-benzotriazole ammonium salt (858 mg, 5.64 mmol) and 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.08g, 5.64 mmol) under ice-cooling, and the mixture was stirred for 1.5 h at the same temperature and then for 30 min at room temperature. The reaction mixture was combined with icewater, and the precipitated crystals were collected by filtration, and washed with water and diethyl ether. The filtrate was neutralized with $NaHCO_3$ and extracted (4 x 25 mL) with a mixed solution of tetrahydrofuran and ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in

vacuo. The crude residue was purified by flash column chromatography (0-10 % methanol in dichloromethane) to provide ***methyl 2-amino-4-carbamoylbenzoate*** (**28p**) as a white solid.

methyl 2-amino-4-carbamoylbenzoate (**28p**)

(880 mg, 4.53 mmol, 88% yield) ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (s, 1H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.37 (bs, 1H), 7.24 (d, *J* = 1.6 Hz, 1H), 6.94 (dd, *J* = 8.3, 1.7 Hz, 1H), 6.74 (bs, 2H), 3.80 (s, 3H). LC-MS (ESI) m/z 194.1 [M+H] .

1.8. Procedure for the synthesis of non-commercial amine intermediate 28q



Scheme S8. Synthesis of amine intermediate **28q**

2-nitro-3-methylbenzoic acid methyl ester (5g, 25.62 mmol), N-bromosuccinimide (5.34 g, 30 mmol) and azodiisobutyronitrile (30 mg, 0.183 mmol) were dissolved in 50 mL dry CCl_4 and the reaction mixture was refluxed overnight. After completion of the reaction, the residue was poured into water, and then was extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with saturated NaCl solution, dried over anhydrous $NaSO_4$, filtered and concentrated in vacuo. The crude residue was purified by flash column chromatography (0-20 % ethyl acetate in hexane) to provide **methyl 3-(bromomethyl)-2-nitrobenzoate** (**47**) as an orange oil. Methyl 3-(bromomethyl)-2-nitrobenzoate (**47**) (700 mg, 2.55 mmol) was dissolved in methanol (35 mL) and K_2CO_3 (354 mg, 2.55 mmol) was added. The mixture was stirred 2 h at 80 °C and was concentrated in vacuo to provide **methyl 3-(methoxymethyl)-2-nitrobenzoate** (**48**) as an orange oil. The crude product was used in the subsequent reaction without

purification. A mixture of methyl 3-(methoxymethyl)-2-nitrobenzoate (**48**) (434 mg, 1.93 mmol), DIPEA (64 mL) and 206 mg of palladium on charcoal (10%) in 10 mL of methanol were pressurized with a balloon of hydrogen (5 bar) 50 min at room temperature. After the reaction was completed, filtered through celite, dried over anhydrous Na_2SO_4 and concentrated in vacuo to provide ***methyl 2-(2-amino-4-methoxyphenyl)acetate*** (**28q**) as an yellow oil. The crude product was used in subsequent reaction without purification.

methyl 3-(bromomethyl)-2-nitrobenzoate (**47**)

(1.41 g, 5.16 mmol, 20% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, J = 7.8, 1.4 Hz, 1H), 7.74 (dd, J = 7.8, 1.4 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 4.45 (s, 2H), 3.90 (s, 3H). LC-MS (ESI) m/z 275.1 $[M+H]^+$.

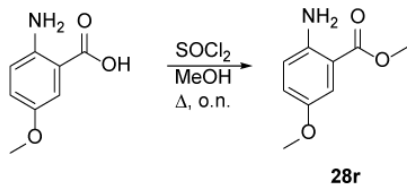
methyl 3-(methoxymethyl)-2-nitrobenzoate (**48**)

(434 mg, 1.93 mmol, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, J = 7.8, 1.4 Hz, 1H), 7.78 (dd, J = 7.8, 1.5 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 4.50 (s, 2H), 3.90 (s, 3H), 3.40 (s, 3H). LC-MS (ESI) m/z 226.1 $[M+H]^+$.

methyl 2-(2-amino-4-methoxyphenyl)acetate (**28q**)

(350 mg, 1.79 mmol, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (dd, J = 8.1, 1.7 Hz, 1H), 7.20 (dd, J = 7.2, 1.7 Hz, 1H), 6.60 (t, J = 7.4 Hz, 1H), 6.42 – 6.34 (s, 2H), 4.49 (s, 2H), 3.87 (s, 3H), 3.33 (s, 3H).LC-MS (ESI) m/z 196.1 $[M+H]^+$.

1.9. Procedure for the synthesis of non-commercial amine intermediate 28r



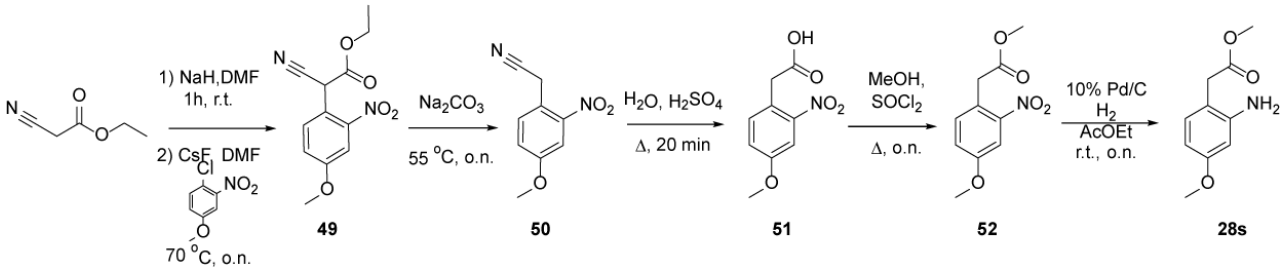
Scheme S9. Synthesis of amine intermediate **28r**

A solution of 2-amino-5-methoxybenzoic acid (1 g, 5.98 mmol) was dissolved in methanol (12 mL), cooled to 0 °C, and followed by dropwise addition of thionyl chloride (1.1 mL). The mixture was stirred overnight at reflux. After evaporation of the solvent, and neutralized by addition of a saturated aqueous $NaHCO_3$ solution, mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with saturated brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo, providing ***methyl 2-amino-5-methoxybenzoate*** (**28r**) as a brown oil. The crude product was used in subsequent reaction without purification.

methyl 2-amino-5-methoxybenzoate (**28r**)

(98 mg, 3.3 mmol, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 3.1 Hz, 1H), 6.96 (dd, J = 8.9, 3.1 Hz, 1H), 6.64 (d, J = 8.9 Hz, 1H), 3.89 (s, 3H), 3.77 (s, 3H). LC-MS (ESI) m/z 182.1 $[M+H]^+$.

1.10. Procedure for the synthesis of non-commercial amine intermediate 28s



Scheme S10. Synthesis of amine intermediate **28s**

Ethyl cyanoacetate (2.56 mL, 24.0 mmol) was added dropwise to a suspension of 959 mg of NaH (60%, 24.0 mmol) in 10 mL of DMF. The mixture was stirred for 1 h at room temperature. CsF (61 mg, 0.4 mmol) and a solution of 4-chloro-3-nitro-anisole (1.5 g, 8.0 mmol) in 2 mL of DMF were added and the mixture was stirred overnight at 70 °C. The reaction mixture was cooled to room temperature and was quenched by the addition of 5 ml of water. Aqueous 1N HCl (5 mL) was added to adjust the pH to 3-4 and the mixture was diluted with dichloromethane. The organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography (0-40 % EtOAc in hexane) to provide **cyano-(4-methoxy-2-nitro-phenyl)-acetic acid ethyl ester** (**49**)[5] as an orange oil. To cyano-(4-methoxy-2-nitro-phenyl)-acetic acid ethyl ester (**49**) (1.43 g, 5.43 mmol) was added 46 mL of a saturated solution of aqueous sodium carbonate. The mixture was stirred overnight at 55 °C. After cooling to room temperature, the mixture was diluted with ethyl acetate. The organic layer was washed with saturated brine, dried over anhydrous Na_2SO4 and concentrated in vacuo to provide **(4-methoxy-2-nitro-phenyl)-acetonitrile** (**50**)[5] as an orange oil. The crude product was used in the subsequent reaction without purification. A suspension of (4-methoxy-2-nitro-phenyl)-acetonitrile (**50**) (906 mg, 4.71 mmol) in a mixture of 10.6 mL of water and 7 mL of concentrated sulfuric acid was heated at reflux. After 20 min, the reaction mixture was treated with ice resulting in the precipitation of brown solids. After filtration **(4-methoxy-2-nitro-phenyl)-acetic acid** (**51**) was obtained as a brown solid. The crude product was used in subsequent reaction without purification. (4-methoxy-2-nitro-phenyl)-acetic acid (**51**) (657 mg, 3.11 mmol) was dissolved in methanol (8.7 mL) and thionyl chloride (0.7 mL, 9.33 mmol) was added. The mixture was stirred overnight at reflux and was concentrated in vacuo. The residue was partitioned between 200 mL of ethyl acetate and 40 mL of saturated aqueous $NaHCO_3$. The organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (0-40 % EtOAc in hexanes) to provide **(4-**

methoxy-2-nitro-phenyl)-acetic acid methyl ester (**52**) as an orange oil. A mixture of (4-methoxy-2-nitro-phenyl)-acetic acid methyl ester (**52**) (607 mg, 2.69 mmol) and 144 mg of palladium on charcoal (10%) in 11 mL of ethyl acetate were pressurized with a balloon of hydrogen (5 bar) overnight at room temperature. After the reaction was completed, the solution was filtered through celite, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (0-40 % EtOAc in dichloromethane) to provide ***methyl 2-(2-amino-4-methoxyphenyl)acetate*** (**28s**) as a yellow oil.

cyano-(4-methoxy-2-nitro-phenyl)-acetic acid ethyl ester (**49**)

(1.44 g, 5.43 mmol, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 2.8 Hz, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.27 – 7.23 (m, 1H), 5.55 (s, 1H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.92 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H). LC-MS (ESI) m/z 265.1 $[M+H]^+$.

(4-methoxy-2-nitro-phenyl)-acetonitrile (**50**)

(908 mg, 4.72 mmol, 87% yield). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.70 (d, *J* = 2.7 Hz, 1H), 7.60 (d, *J* = 8.6 Hz, 1H), 7.22 (dd, *J* = 8.6, 2.7 Hz, 1H), 4.12 (s, 2H), 3.90 (s, 3H). LC-MS (ESI) m/z 192.1 $[M+H]^+$.

(4-methoxy-2-nitro-phenyl)-acetic acid (**51**)

(660 mg, 3.13 mmol, 66% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.66 (d, *J* = 2.7 Hz, 1H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.40 (dd, *J* = 8.6, 2.8 Hz, 1H), 4.22 (s, 2H), 3.87 (s, 3H). LC-MS (ESI) m/z 212.1 $[M+H]^+$.

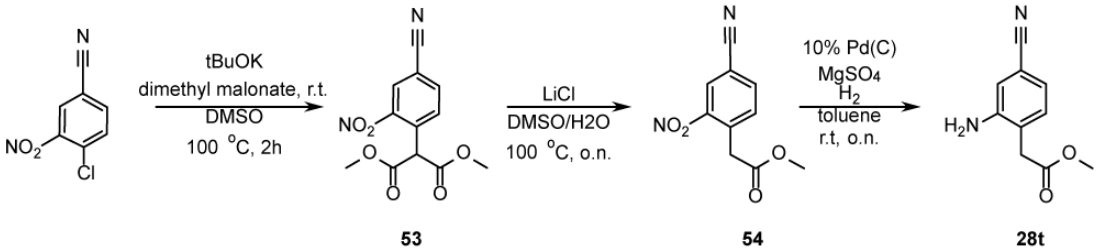
(4-methoxy-2-nitro-phenyl)-acetic acid methyl ester (**52**)

(607 mg, 2.7 mmol, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 2.7 Hz, 1H), 7.25 (d, J = 8.6 Hz, 2H), 7.13 (dd, J = 8.5, 2.7 Hz, 1H), 3.95 (s, 2H), 3.88 (s, 3H), 3.71 (s, 3H). LC-MS (ESI) m/z 226.1 $[M+H]^+$.

methyl 2-(2-amino-4-methoxyphenyl)acetate (**28s**)

(393 mg, 2.012 mmol, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, J = 8.3 Hz, 1H), 6.32 (dd, J = 8.3, 2.6 Hz, 1H), 6.27 (d, J = 2.5 Hz, 1H), 4.07 (bs, 2H), 3.75 (s, 3H), 3.68 (s, 3H), 3.50 (s, 2H). LC-MS (ESI) m/z 196.1 $[M+H]^+$.

1.11. Procedure for the synthesis of non-commercial amine intermediate 28t



Scheme S11. Synthesis of amine intermediate **28t**

Potassium tert-butoxide (4.39 g, 37.2 mmol) was dissolved in dimethylsulfoxide (13.5 mL) at 20 °C, and dimethyl malonate (4.96 g, 37.2 mmol, 4.3 mL) was slowly added. Stirring was continued for 1 h at room temperature. After that time, 4-chloro-3-nitro-benzonitrile (2 g, 10.6 mmol) was slowly added and the mixture was stirred at 100 °C for 1 h. After being cooled, the mixture was poured into water (100 mL) and neutralized with 1N HCl (pH 7). The precipitate was filtered off and washed with water. The residue was taken up in DCM (100 mL), extracted with water (100 mL), and dried over anhydrous Na_2SO_4 to give **dimethyl 2-(4-cyano-2-nitro-phenyl)propanedioate** (**53**) as a white solid, which was used in the next reaction without purification. Dimethyl 2-(4-cyano-2-nitro-phenyl)propanedioate (**53**) (3.40 g, 12.2 mmol) was

dissolved in dimethylsufoxide (60 mL), and LiCl (1.05 g, 24.4 mmol) and water (0.22 mL) were added. The mixture was stirred overnight at 100 ^{o}C. After being cooled, the mixture was poured into ice water (250 mL) and stirred for 10 min. The mixture was extracted with dichloromethane (3 x 125 mL) and the organic phase was washed with saturated aqueous solution NaCl (3 x 200 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The crude was purified by flash column chromatography (hexanes to hexanes in ethyl acetate 6:4). **Methyl 2-(4-cyano-2-nitro-phenyl)acetate** (**54**) as a yellow solid. To a solution of methyl 2-(4-cyano-2-nitro-phenyl)acetate (**54**) (1.63 g, 7.40 mmol) in toluene (23 mL) was added $MgSO_4$ (446 mg, 3.70 mmol) and 10 % Pd/C (50% water) (156 mg) and the mixture was subjected to catalytic reaction at room temperature overnight under hydrogen atmosphere. The catalyst and magnesium sulfate were separated by filtration over celite and the filtrate was concentrated under reduced pressure to give the ***methyl 2-(2-amino-4-cyano-phenyl)acetate*** (**28t**) as a yellow solid.

dimethyl 2-(4-cyano-2-nitro-phenyl)propanedioate (**53**)

(2.85 g, 10.2 mmol, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 1.7 Hz, 1H), 7.92 (dd, J = 8.2, 1.7 Hz, 1H), 7.73 (d, J = 8.1 Hz, 1H), 5.37 (s, 1H), 3.82 (s, 6H). LC-MS (ESI) m/z 277.1 $[M+H]^+$.

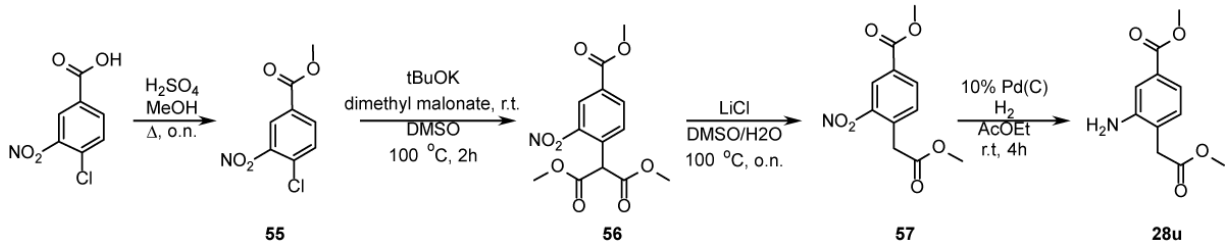
Methyl 2-(4-cyano-2-nitro-phenyl)acetate (**54**)

(1.64 g, 7.43 mmol, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 1.7 Hz, 1H), 7.88 (dd, J = 8.0, 1.7 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 4.11 (s, 2H), 3.73 (s, 3H). LC-MS (ESI) m/z 219.0 $[M+H]^+$.

methyl 2-(2-amino-4-cyano-phenyl)acetate (**28t**)

(1.32 g, 6.95 mmol, 94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (d, *J* = 7.7 Hz, 1H), 7.01 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.95 (d, *J* = 1.6 Hz, 1H), 4.28 (s, 2H), 3.71 (s, 3H), 3.59 (s, 2H). LC-MS (ESI) m/z 191.1 $[M+H]^+$.

1.12. Procedure for the synthesis of non-commercial amine intermediate 28u



Scheme S12. Synthesis of amine intermediate **28u**

To a solution of 4-chloro-3-nitrobenzoic acid (4.00 g, 19.8 mmol) in methanol (30 mL) was added concentrated sulfuric acid (98%, 8.00 mL, 194 mmol) and the reaction mixture was refluxed for 16 h. The solvent was evaporated, water (100 mL) was added and the mixture extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to obtain **methyl 4-chloro-3-nitro-benzoate** (**55**) as a yellow solid. Potassium tert-butoxide (7.28 g, 64.9 mmol) was dissolved in dimethyl sulfoxide (23 mL) at 20 ºC, and dimethyl malonate (7.42 mL, 64.9 mmol) was slowly added, stirring was continued for 1 h at room temperature. After, methyl 4-chloro-3-nitrobenzoate (**55**) (4.00 g, 18.5 mmol) was slowly added and the mixture was stirred at 100 ºC for 1 h. After being cooled, the mixture was poured into water (100 mL) and neutralized with 1N HCl (2.5 mL). Dichloromethane (100 mL) was added and the phases were separated. The organic layer was washed with water (100 mL) and dried over anhydrous Na_2SO_4 to provide **dimethyl 2-(4-methoxycarbonyl-2-nitro-phenyl)propanedioate** (**56**) as a yellow solid. Dimethyl 2-(4-

methoxycarbonyl-2-nitro-phenyl)propanedioate (**56**) (1 g, 3.21 mmol) was dissolved in dimethylsufoxide (15.8 mL), and LiCl (272 mg, 6.43 mmol) and water (0.6 mL) were added. The mixture was stirred overnight at 100 ^{o}C. After being cooled, the mixture was poured into ice water (50 mL) and stirred for 10 min. The mixture was extracted with dichloromethane (3 x 25 mL) and the organic phase was washed with saturated aqueous solution NaCl (3 x 40 mL). The rganic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The crude was purified by flash column chromatography (hexanes to hexanes in ethyl acetate 6:4) to provide **methyl 4-(2-methoxy-2-oxo-ethyl)-3-nitro-benzoate** (**57**) as a yellow solid. To a solution of methyl 4-(2-methoxy-2-oxo-ethyl)-3-nitro-benzoate (**57**) (3.64 g, 14.40 mmol) in ethyl acetate (59 mL), 0.76 g of Pd(C) 10% was suspended into the solution, the reaction mixture was under hydrogen atmosphere at room temperature for 4 h. The palladium was removed by filtration and concentrated under vacuo to yield ***methyl 3-amino-4-(2-methoxy-2-oxoethyl)benzoate*** (**28u**) as a yelow oil.

methyl 4-chloro-3-nitro-benzoate (**55**)

(4.00 g, 18.5 mmol, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, J = 2.0 Hz, 1H), 8.16 (dd, J = 8.4, 2.0 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 3.97 (s, 3H). LC-MS (ESI) m/z 216.1 $[M+H]^+$.

dimethyl 2-(4-methoxycarbonyl-2-nitro-phenyl)propanedioate (**56**)

(5.47 g, 17.58 mmol, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, J = 1.8 Hz, 1H), 8.27 (dd, J = 8.2, 1.8 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 5.35 (s, 1H), 3.97 (s, 3H), 3.80 (s, 6H). LC-MS (ESI) m/z 312.1 $[M+H]^+$.

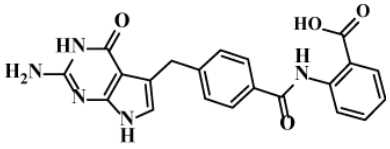
methyl 4-(2-methoxy-2-oxo-ethyl)-3-nitro-benzoate (**57**)

(1.64 g, 7.43 mmol, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 1.7 Hz, 1H), 7.88 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 4.11 (s, 2H), 3.73 (s, 3H). LC-MS (ESI) m/z 219.0 $[M+H]^+$.

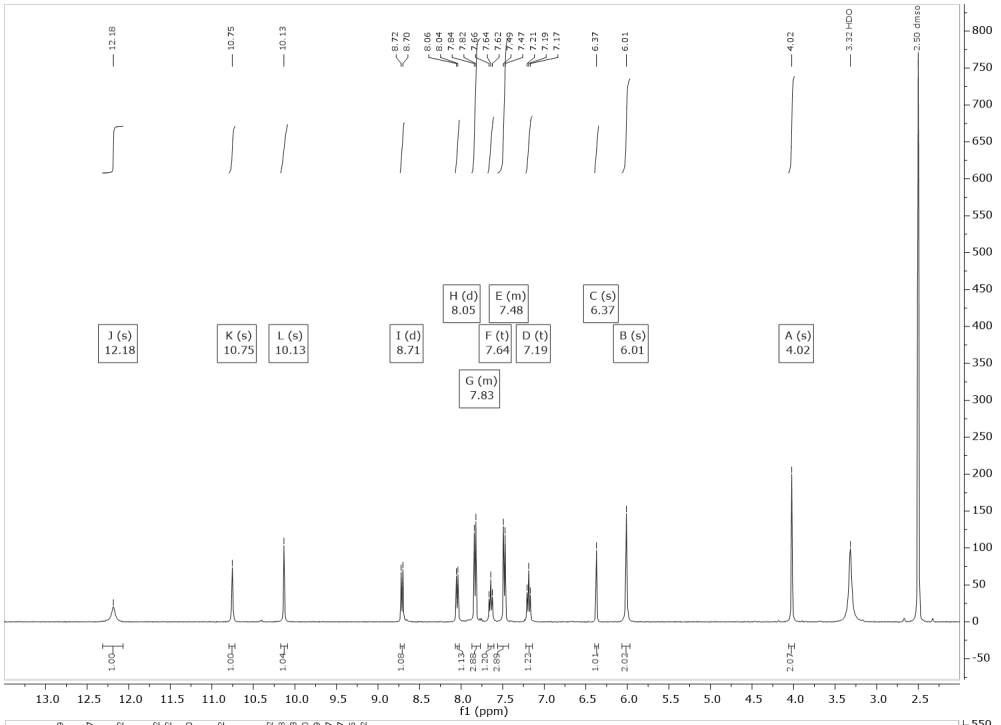
methyl 3-amino-4-(2-methoxy-2-oxoethyl)benzoate (**28u**)

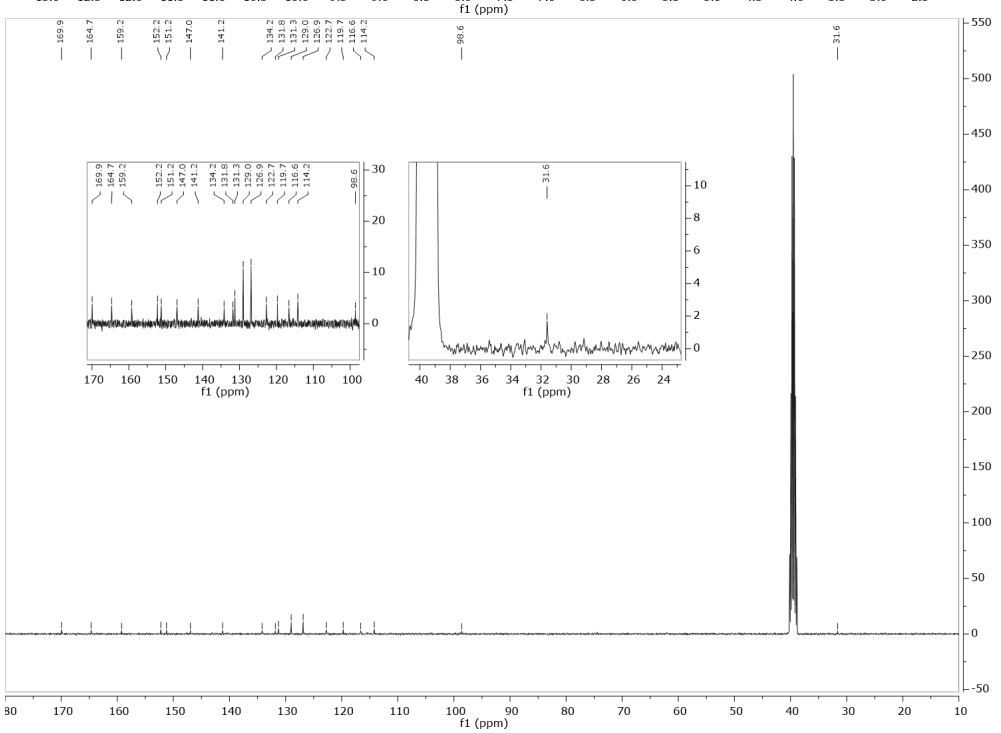
(2.89 g, 12.9 mmol, 90% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.38 (m, 2H), 7.14 (d, *J* = 7.7 Hz, 1H), 4.18 (s, 2H), 3.88 (s, 3H), 3.69 (s, 3H), 3.60 (s, 2H). LC-MS (ESI) m/z 224,1 $[M+H]^+$

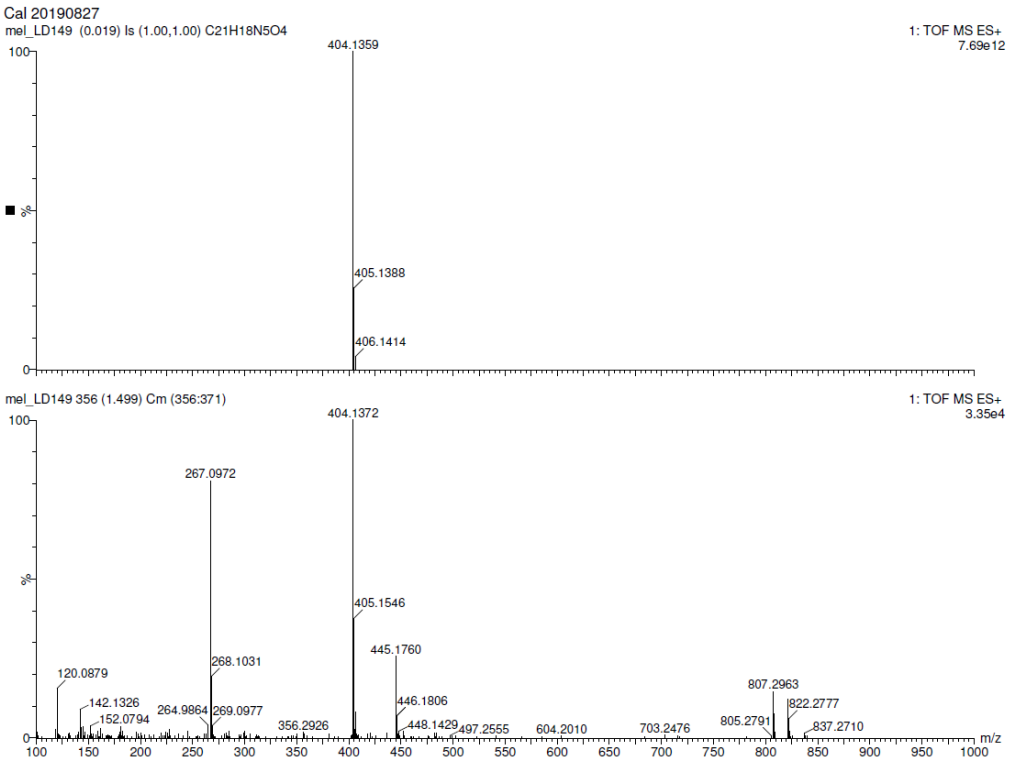
2. ^{1}H, ^{13}C and HRMS spectra of representative compounds



2



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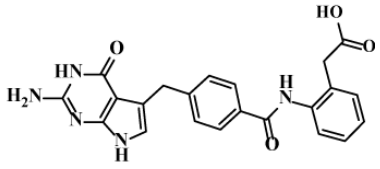
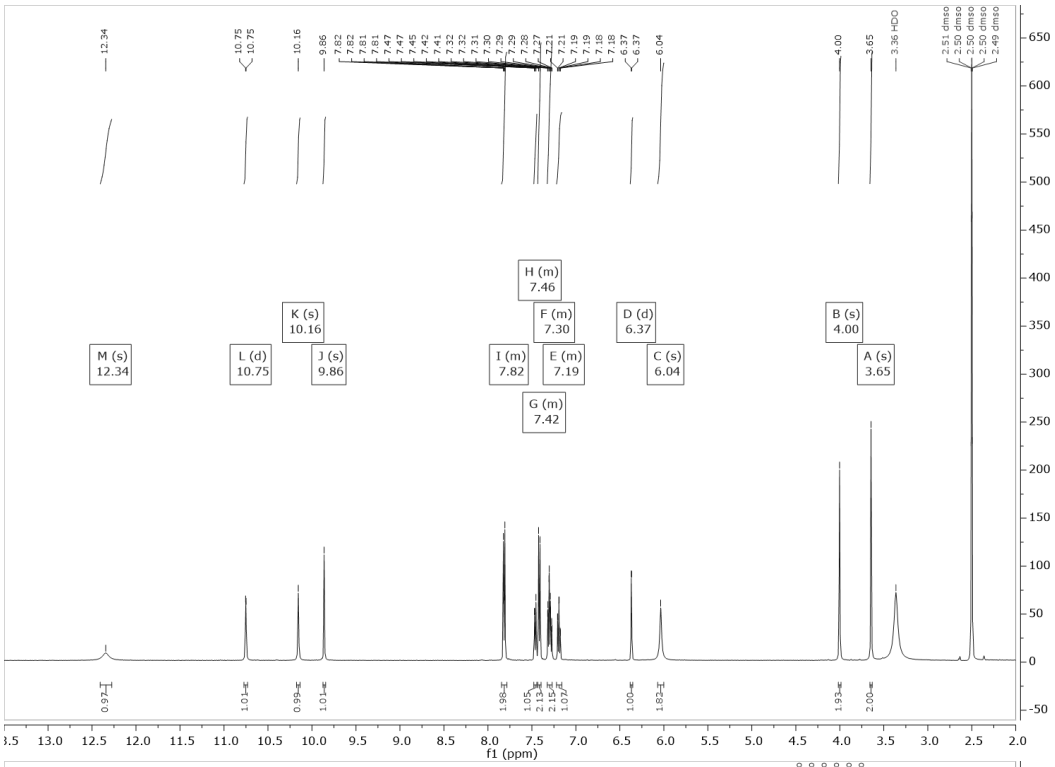
404.1359
405.1388
406.1414
mel_LD149 356 (1.499) Cm (356:371)
1: TOF MS ES+
3.35e4
404.1372
267.0972
405.1546
445.1760
268.1031
120.0879
142.1326
152.0794
264.9864
269.0977
356.2926
446.1806
448.1429
497.2555
604.2010
703.2476
807.2963
805.2791
822.2777
837.2710
%
m/z


HO
O
HN
O
H_2N
N
H
N
H
N
O

5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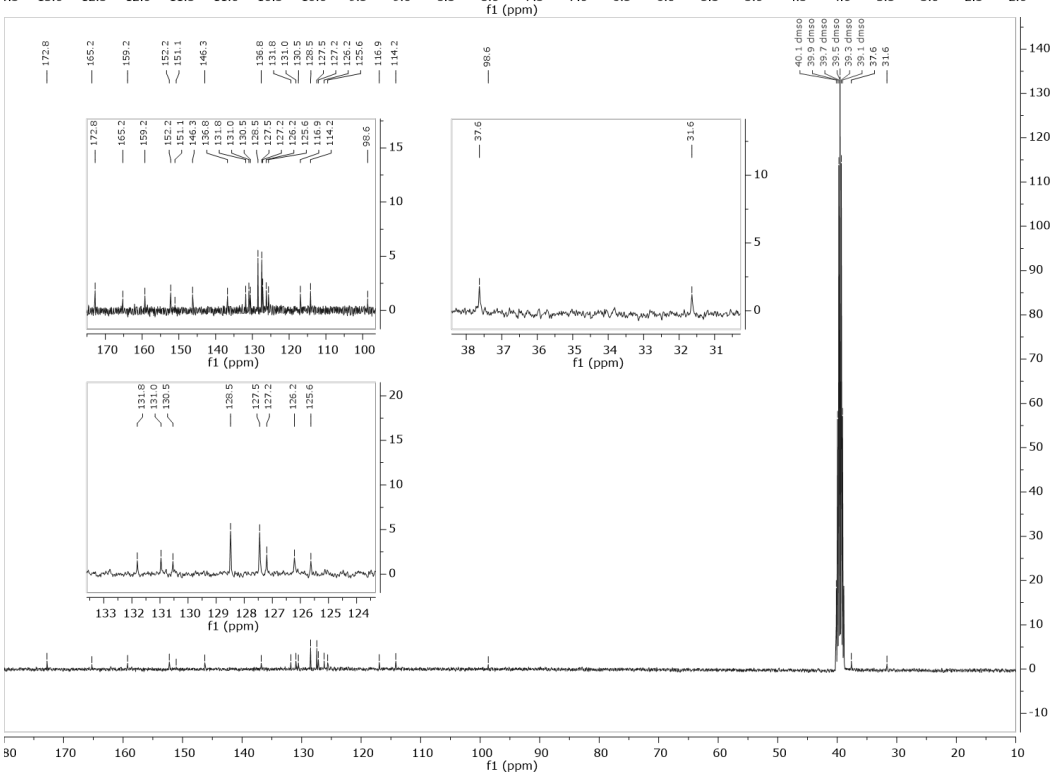
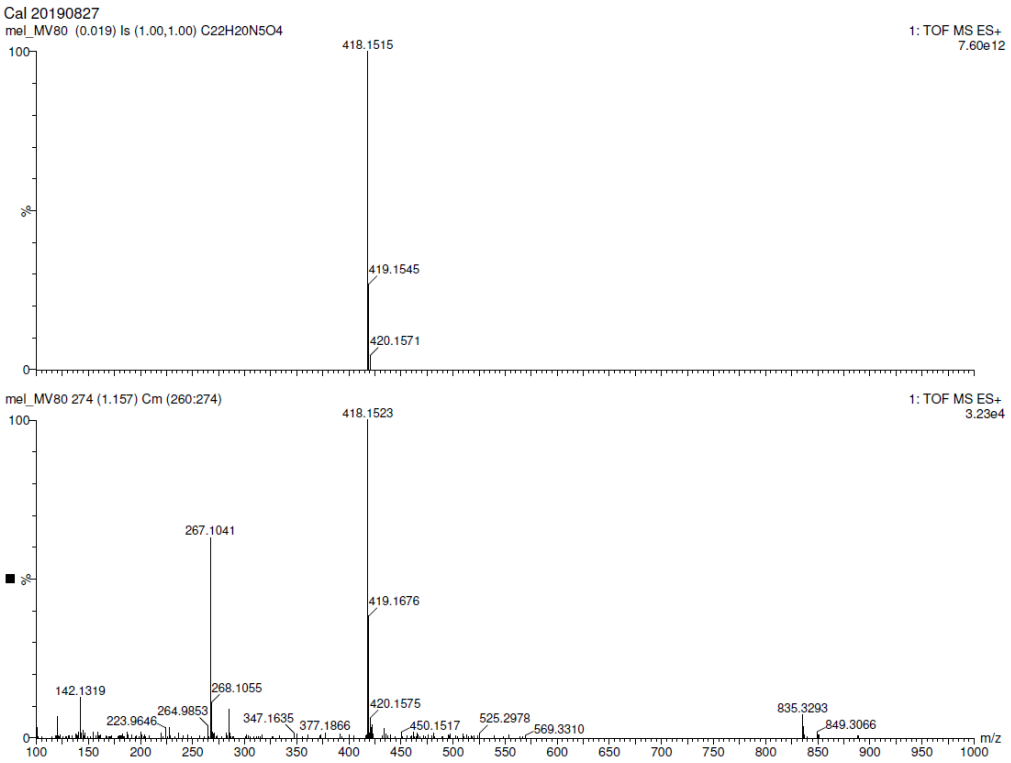
K (s) 10.16
J (s) 9.86
I (m) 7.82
E (m) 7.19
H (m) 7.46
F (m) 7.30
G (m) 7.42
D (d) 6.37
C (s) 6.04
B (s) 4.00
A (s) 3.65
f1 (ppm)


f1 (ppm)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418.1515
419.1545
420.1571
mel_MV80 274 (1.157) Cm (260:274)
1: TOF MS ES+
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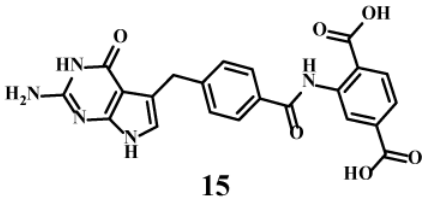
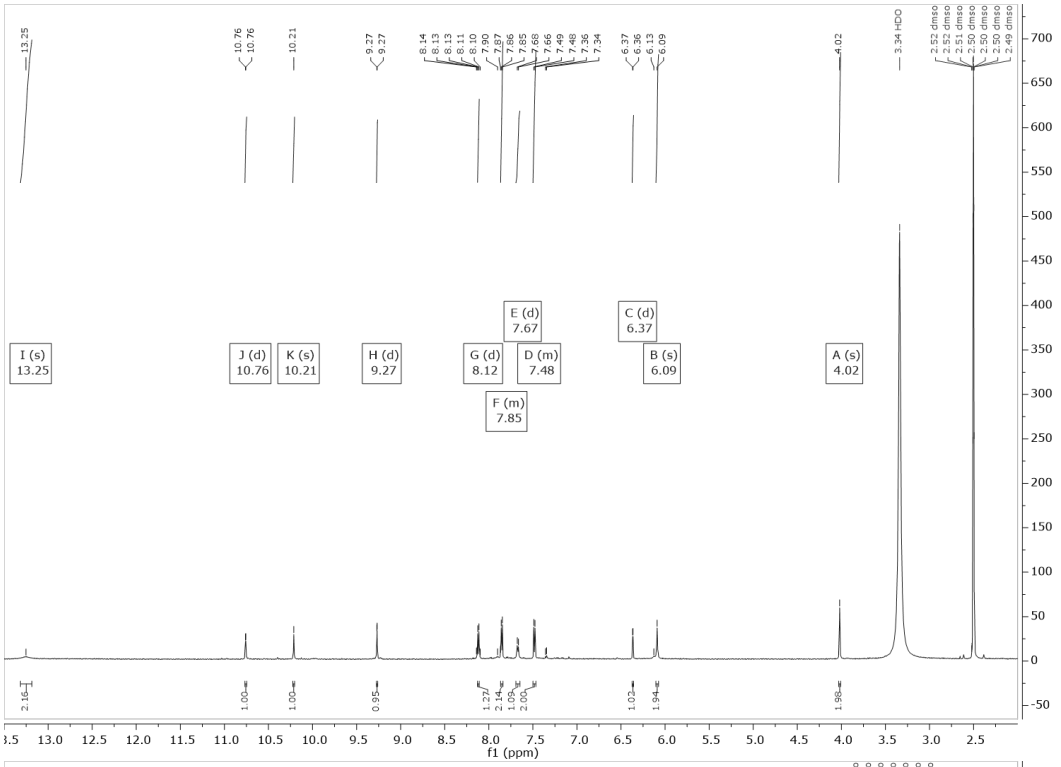
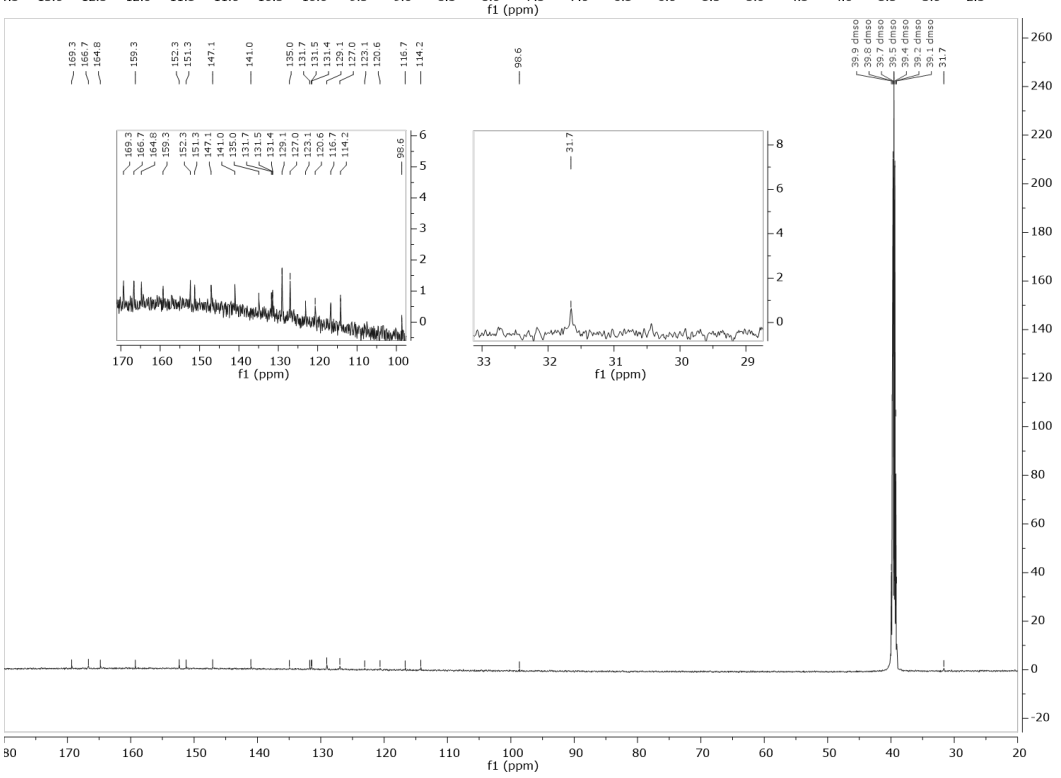
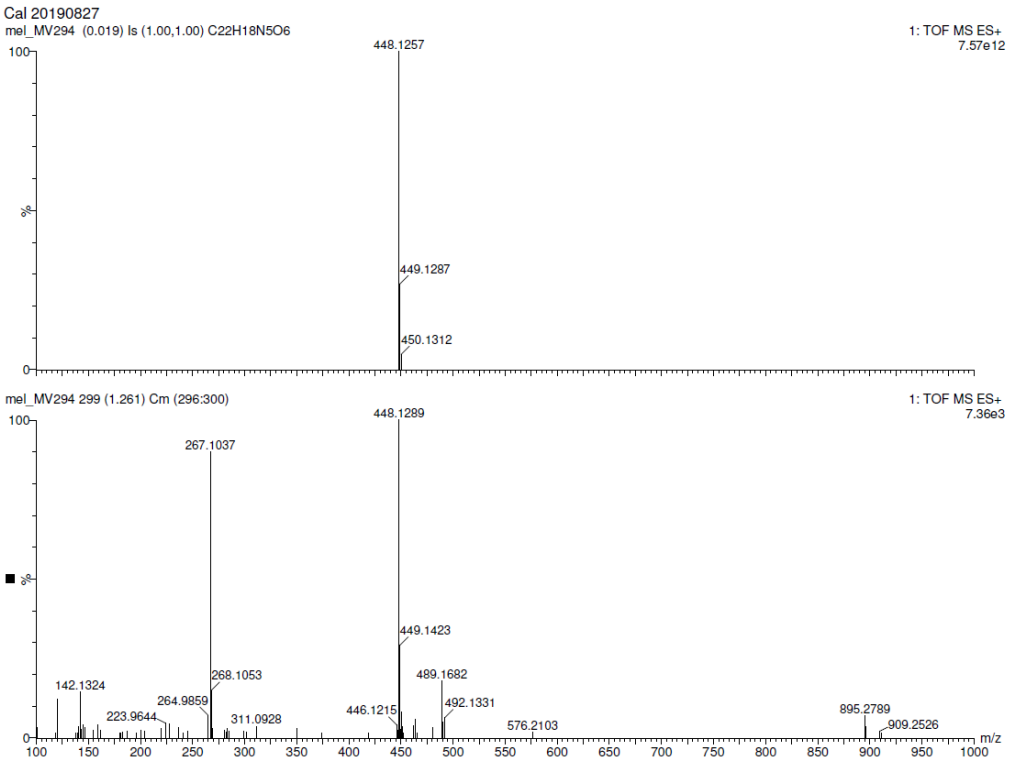
418.1523
267.1041
419.1676
142.1319
268.1055
420.1575
223.9646
264.9853
347.1635
377.1866
450.1517
525.2978
569.3310
835.3293
849.3066
%
m/z



15


I (s) 13.25
J (d) 10.76
K (s) 10.21
H (d) 9.27
G (d) 8.12
F (m) 7.85
E (d) 7.67
D (m) 7.48
C (d) 6.37
B (s) 6.09
A (s) 4.02
f1 (ppm)


f1 (ppm)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449.1287
450.1312
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267.1037
449.1423
268.1053
489.1682
142.1324
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446.1215
576.2103
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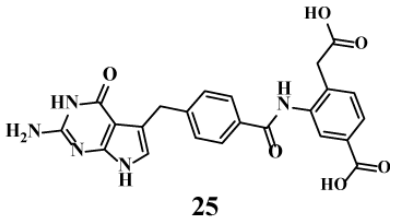
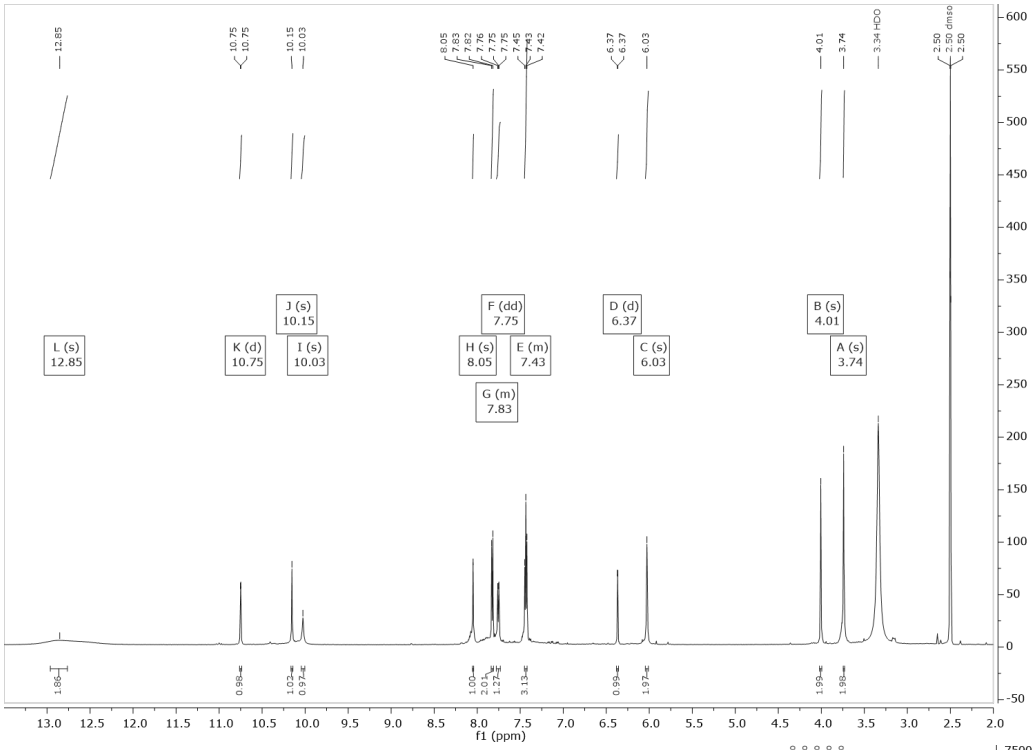
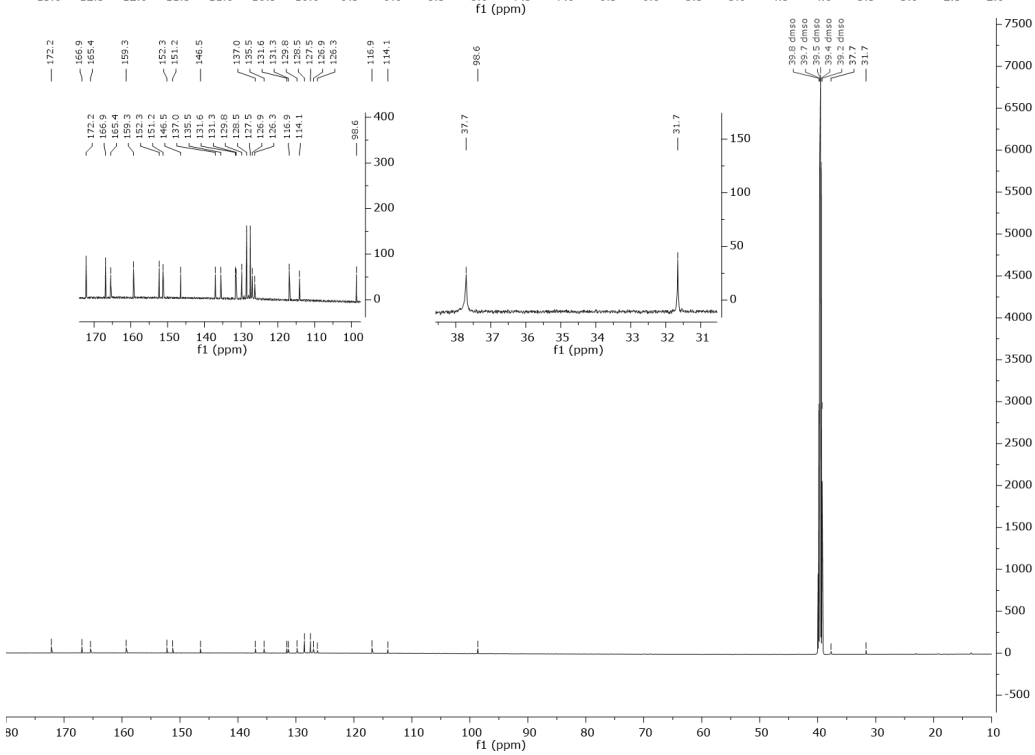
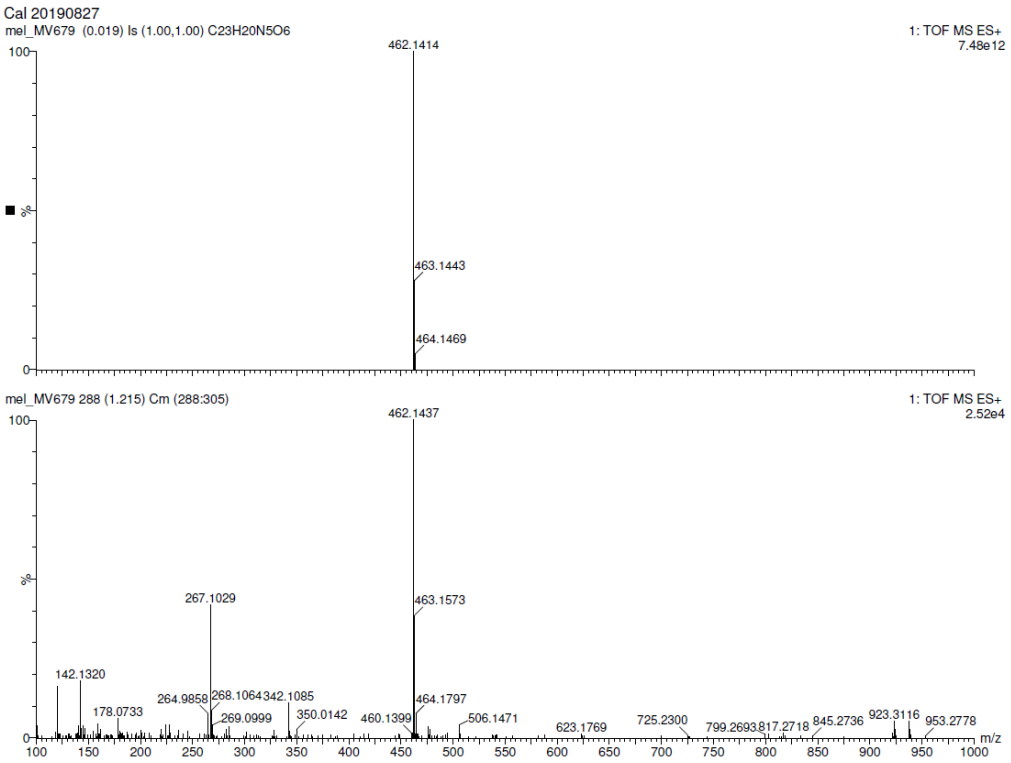
909.2526
%
m/z



25


L (s) 12.85
K (d) 10.75
J (s) 10.15
I (s) 10.03
H (s) 8.05
G (m) 7.83
F (dd) 7.75
E (m) 7.43
D (d) 6.37
C (s) 6.03
B (s) 4.01
A (s) 3.74
f1 (ppm)


f1 (ppm)


Cal 20190827
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463.1443
464.1469
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267.1029
463.1573
142.1320
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178.0733
269.0999
350.0142
460.1399
464.1797
506.1471
623.1769
725.2300
799.2693
817.2718
845.2736
923.3116
953.2778
%
m/z

3. Table S1. IC_{50} (μM) for ChTS and human TS[a]

Compound	*Ch*TS IC_{50}	*h*TS IC_{50}	Fold-Change
1[b]	0.38 ± 0.04	1.8 ± 0.5	5
2	20 ± 12	1.3 ± 0.2	15
3	96 ± 10	0.13 ± 0.03	738
4	>100	0.47 ± 0.06	213
5	16 ± 1	1.7 ± 0.2	9
6	114 ± 21	3.5 ± 0.5	33
7	159 ± 19	1.2 ± 0.2	133
8	123 ± 7	1.3 ± 0.1	95
9	210 ± 81	ND	~~
10	>100	7.3 ± 0.9	14
11	119 ± 13	1.8 ± 0.2	66
12	>500	98 ± 29	5
13	63 ± 8	3.8 ± 0.5	17
14	28 ± 6	3.2 ± 0.4	9
15	18 ± 2	0.26 ± 0.02	69
16	50 ± 8	0.6 ± 0.1	83
17	11 ± 1	0.46 ± 0.09	24
18	181 ± 32	2.8 ± 0.5	65
19	68 ± 12	0.39 ± 0.07	174
20	20± 4	1.0 ± 0.3	20
21	222 ± 77	6 ± 2	37
22	>100	0.7 ± 0.2	143
23	11 ± 1	0.8 ± 0.1	14
24	9.1 ± 0.7	2.2 ± 0.2	4
25	10 ± 1	0.26 ± 0.02	38
26	97 ± 44	ND	~~
27	>500	52 ± 14	10

[a] SD from triplicate measurement. [b] Results from reference [6]

4. Table S2. X-Ray crystallography data collection and refinement statistics

	mhTS:**5**	mhTS:**14**	mhTS:**15**	mhTS:**23**	*Ch*TS-DHFR:**2**	*Ch*TS-DHFR:**5**	*Ch*TS-DHFR:**6**	*Ch*TS-DHFR:**11**
PDB Code	6PF3	6PF4	6PF5	6PF6	6PF7	6PF8	6PF9	6PFA
X-Ray Source	APS 24ID-E	APS 24ID-E	APS 24ID-E	APS 24ID-E	APS 24ID-E	APS 24ID-E	APS 24ID-C	APS 24ID-E
Wavelength (Å)	0.979180	0.979180	0.979180	0.979180	0.979180	0.979180	0.979300	0.979180
Space group	P21 21 21	C 1 2 1	P41 21 2	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1
Unite-cell parameters (Å)	a=92.41, b=96.78, c=136.20 α=90, β=90, γ=90	a=89.09, b=89.30, c=163.47 α=90, β=89.98, γ=90	a=151.59, b=151.59, c=106.73 α=90, β=90, γ=90	a=85.03, b=85.10, c=175.73 α=90, β=90.12, γ=90	a=214.74, b=115.72, c=220.04 α=90, β=94.65, γ=90	a=214.22, b=116.47, c=221.39 α=90, β=94.87, γ=90	a=213.60, b=116.10, c=221.32 α=90, β=94.74, γ=90	a=213.19, b=117.36, c=222.89 α=90, β=95.70, γ=90
Resolution range (Å)	50.0-2.84 (3.02-2.84)	50.0-2.39 (2.54-2.39)	50.0-2.50 (2.65-2.50)	50.0-2.39 (2.53-2.39)	50.0-2.80 (2.96-2.80)	50.0-2.89 (3.06-2.89)	50.0-3.09 (3.28-3.09)	50.0-2.79 (2.95-2.79)
Completeness (%)	97.6 (86.0)	98.9 (95.1)	99.8 (98.8)	98.7 (93.2)	99.6 (98.4)	99.2 (95.5)	97.8 (91.9)	99.2 (96.3)
R_{sym} (%)	0.176 (0.737)	0.072 (0.941)	0.181 (4.015)	0.074 (0.777)	0.112 (1.116)	0.183 (1.132)	0.280 (1.954)	0.106 (0.845)
CC(1/2) (%)	99.5 (92.8)	99.8 (82.8)	99.9 (58.0)	99.8 (78.0)	99.6 (66.6)	98.0 (78.2)	97.8 (44.2)	99.6 (78.2)
Avg. I/σ	10.29 (3.20)	12.95 (1.46)	17.60 (1.07)	13.25 (1.98)	11.64 (1.26)	5.07 (1.03)	4.81 (0.74)	10.30 (1.26)
Redundancy	7.0 (6.0)	3.7 (3.6)	26.5 (26.2)	3.6 (3.5)	3.8 (3.8)	3.8 (3.7)	3.9 (3.8)	3.7 (3.6)
No. Reflections (Unique Reflections)	199233 (28415)	186029 (50336)	1162478 (43817)	179082 (49247)	499613 (132650)	455977 (120860)	375681 (97343)	504700 (135631)
R_{free}/R_{work}	0.2537/0.2395	0.2888/0.2583	0.2644/0.2266	0.2582/0.2271	0.2534/0.2227	0.2418/0.2142	0.2707/0.2373	0.2490/0.2222
No. of Atoms	9198	9080	9001	9170	21040	20984	20535	21045
Protein	8968	8834	8833	8836	20221	20282	19865	20254
Ligands	204	204	146	212	660	665	670	665
Solvent	26	42	22	122	139	12		101
R.M.S. deviation								
Bond lengths (Å)	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Bond angles (°)	0.615	0.487	0.502	0.575	0.517	0.468	0.483	0.494
Avg. B-factor	35.70	62.91	71.09	48.77	80.04	82.31	98.82	78.42
Protein	36.72	62.29	70.94	50.07	74.87	78.96	103.96	73.71
Ligand	35.24	63.80	72.41	48.36	81.75	89.97	89.97	81.25
Solvent	35.26	58.23	63.73	46.85	98.30	53.31		57.35
Ramachandran								
Plot	95.12	95.23	96.54	95.94	95.27	93.82	92.90	94.26
Favored region (%)	4.88	4.77	3.46	4.06	4.73	6.18	7.10	5.74
Allowed region (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Outliers (%)								

Table S2. Continued

	*Ch*TS-DHFR:**14**	*Ch*TS-DHFR:**15**	*Ch*TS-DHFR:**16**	*Ch*TS-DHFR:**17**	*Ch*TS-DHFR:**20**	*Ch*TS-DHFR:**23**	*Ch*TS-DHFR:**24**	*Ch*TS-DHFR:**25**
PDB Code	6PFB	6PFC	6PFD	6PFE	6PFF	6PFG	6PFH	6PFI
X-Ray Source	APS 24ID-E	APS 24ID-E	APS 24ID-C	APS 24ID-E	APS 24ID-E	APS 24ID-E	NSLS-II 17ID-FMX	APS 24ID-C
Wavelength (Å)	0.979180	0.979180	0.979200	0.979180	0.979180	0.979180	0.979303	0.979200
Space group	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1
Unite-cell parameters (Å)	a=214.82, b=117.55, c=222.99 α=90, β=95.71, γ=90	a=212.54 b=113.46, c=220.67 α=90, β=94.33, γ=90	a=213.62, b=116.56, c=220.85 α=90, β=95.00, γ=90	a=212.75, b=116.81, c=220.45 α=90, β=95.37, γ=90	a=212.21, b=115.95, c=220.50 α=90, β=94.92, γ=90	a=215.10, b=117.10, c=221.80 α=90, β=95.30, γ=90	a=214.23, b=117.07, c=221.38 α=90, β=95.53, γ=90	a=214.74, b=116.65, c=222.21 α=90, β=95.30, γ=90
Resolution range (Å)	50.0-2.53 (2.69-2.53)	50.0-3.32 (3.52-3.32)	50.0-2.80 (2.97-2.80)	50.0-2.98 (3.16-2.98)	50.0-2.71 (2.87-2.71)	50.0-2.69 (2.85-2.69)	30.0-2.94 (3.12-2.94)	50.0-2.89 (3.06-2.89)
Completeness (%)	98.5 (92.9)	95.9 (81.6)	95.5 (84.1)	98.2 (89.3)	99.0 (97.2)	98.7 (94.2)	99.3 (96.2)	98.2 (95.1)
R_{sym} (%)	0.125 (0.605)	0.163 (0.714)	0.254 (1.629)	0.128 (0.659)	0.077 (0.456)	0.132 (0.966)	0.152 (0.930)	0.255 (1.324)
CC(1/2) (%)	98.9 (79.3)	98.7 (81.6)	98.4 (50.0)	99.3 (82.9)	99.7 (88.8)	99.2 (73.3)	99.6 (79.1)	98.5 (67.1)
Avg. I/σ	7.49 (1.75)	9.04 (1.86)	5.78 (0.83)	9.62 (1.90)	14.06 (2.45)	8.35 (1.52)	10.51 (1.76)	5.18 (1.16)
Redundancy	3.6 (3.5)	3.7 (3.1)	5.9 (5.3)	3.7 (3.6)	3.7 (3.6)	3.7 (3.7)	7.0 (7.0)	6.9 (7.0)
No. Reflections (Unique Reflections)	659219 (180633)	277121 (75886)	745530 (124669)	402915 (108730)	538269 (146236)	553601 (150400)	801037 (115109)	827894 (119315)
R_{free}/R_{work}	0.2260/0.2027	0.2615/0.2217	0.2572/0.2274	0.2393/0.2100	0.2310/0.2023	0.2396/0.2138	0.2191/0.1899	0.2519/0.2129
No. of Atoms	21656	20790	20966	20924	21541	21366	20968	20959
Protein	20379	20115	20256	20215	20374	20427	20293	20253
Ligands	665	675	670	670	675	675	675	680
Solvent	612		40	14	462	264		26
R.M.S. deviation								
Bond lengths (Å)	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Bond angles (°)	0.531	0.505	0.552	0.534	0.533	0.481	0.504	0.505
Avg. B-factor	52.55	93.14	71.58	71.45	63.46	59.69	73.64	74.78
Protein	49.95	78.43	70.50	63.51	57.31	58.46	69.62	72.01
Ligand	53.95	96.82	72.71	74.53	65.57	60.56	74.65	76.45
Solvent	45.49		54.50	35.73	47.14	48.50		55.13
Ramachandran Plot								
Favored region (%)	95.85	94.18	94.31	94.10	94.7	95.70	94.95	94.39
Allowed region (%)	4.15	5.82	5.69	5.90	5.30	4.30	5.01	5.61
Outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Values in parentheses are from highest resolution shell. One crystal was used for the data set.

5. Predicted binding pose of a biphenyl compound in *C. hominis* TS active site

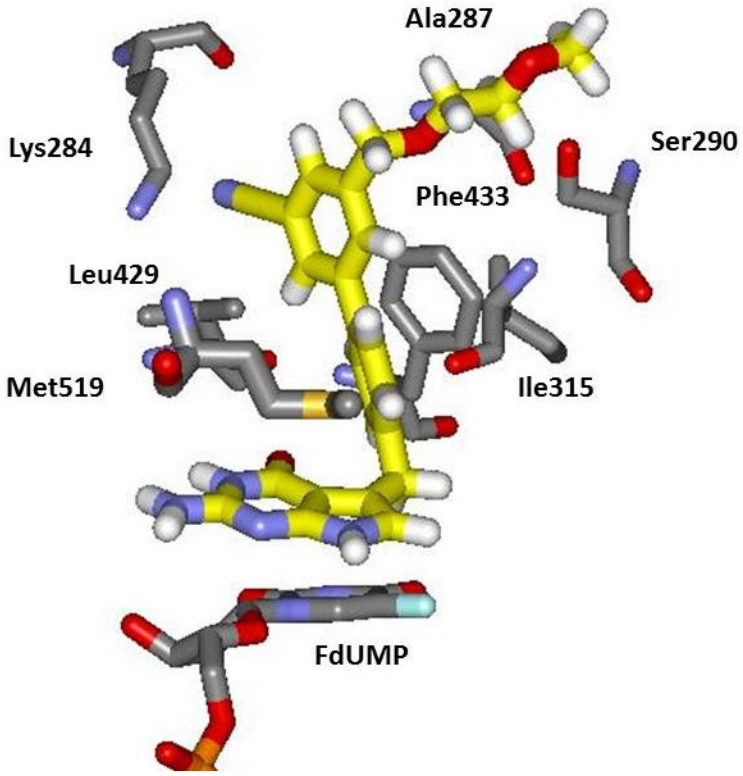

Figure S1. Computed biphenyl structure from BOMB after energy minimization with MCPRO of inhibitor **4** (R_1 = CN, R_2 = $CH_2OCH_2CH_2OCH_3$) and FdUMP bound to *Ch*TS.

6. X-ray crystallography

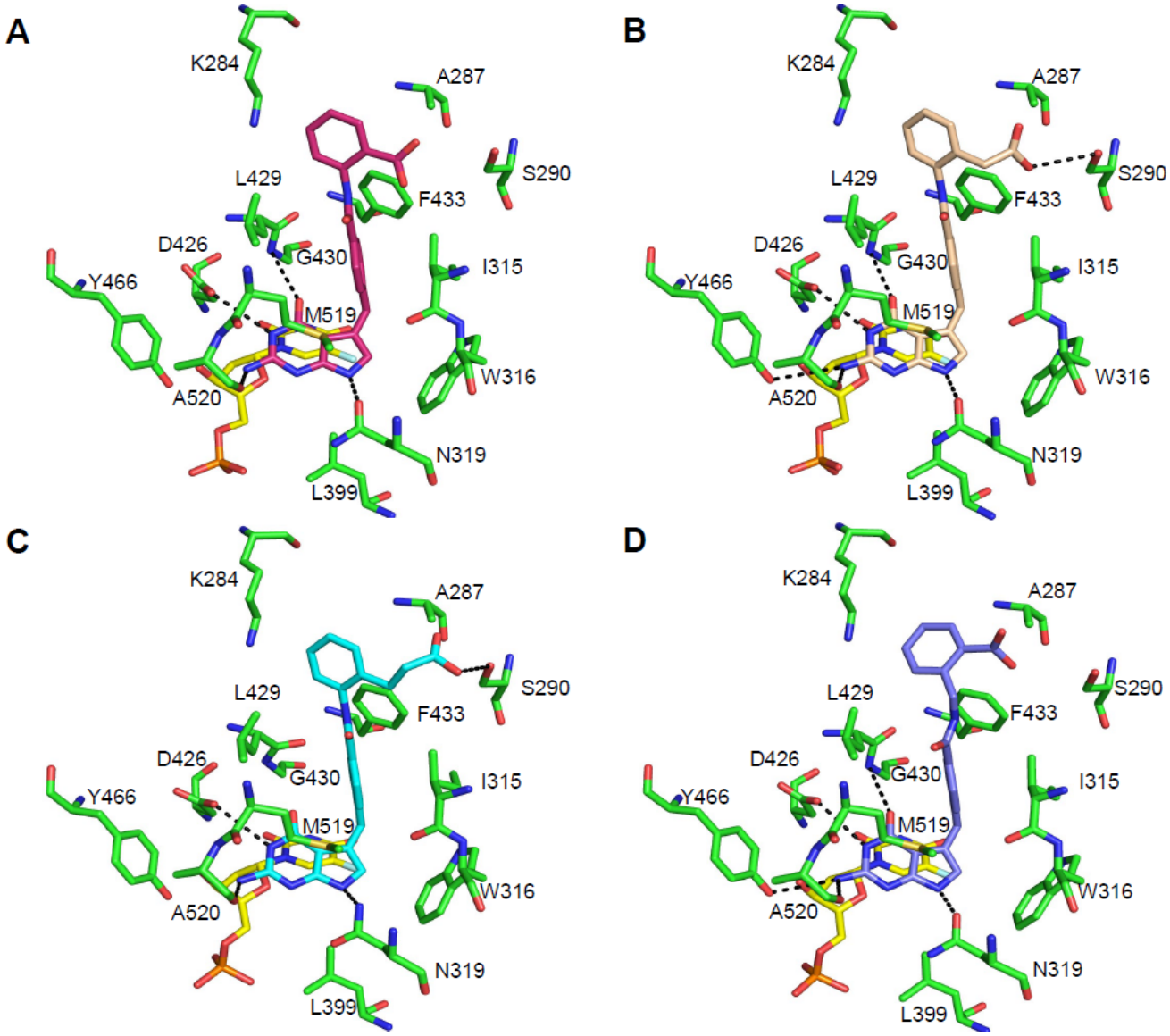

Figure S2. Active site residues of *Ch*TS (green) (PDB: 6PF7, 6PF9, 6PFB, and 6PFA) A. *Ch*TS complexed with **2** (warm pink) and FdUMP (yellow). B. *Ch*TS complexed with **5** (wheat) and FdUMP (yellow). C. *Ch*TS complexed with **6** (cyan) and FdUMP (yellow). D. *Ch*TS complexed with **11** (slate) and FdUMP (yellow). Hydrogen bonding interactions with active site residues designated by black dotted lines.

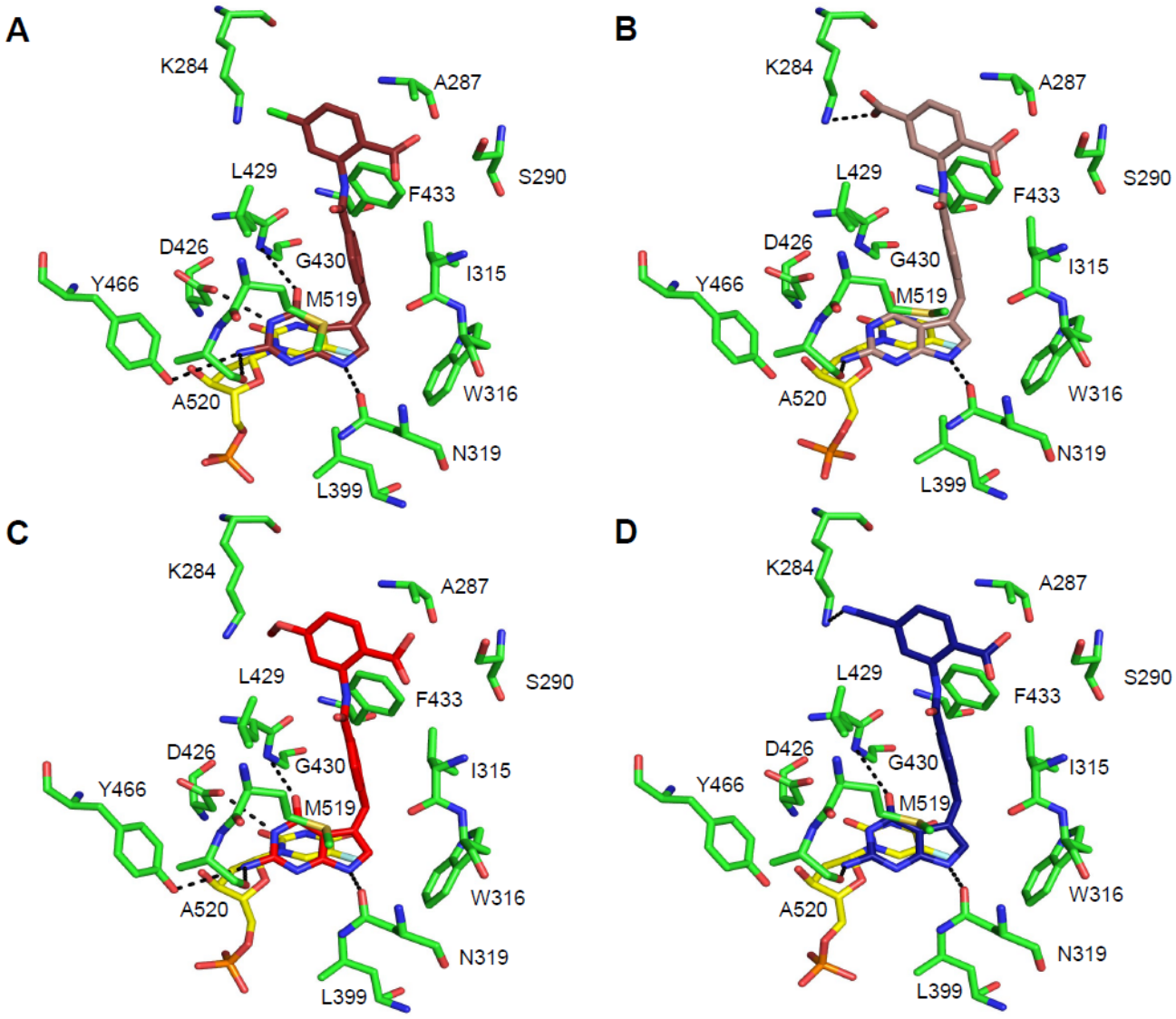

Figure S3. Active site residues of *Ch*TS (green) (PDB: 6PF8, 6PFD, 6PFE, and 6PFF). A. *Ch*TS complexed with **14** (red brick) and FdUMP (yellow). B. *Ch*TS complexed with **15** (light brown) and FdUMP (yellow). C. *Ch*TS complexed with **16** (red) and FdUMP (yellow). D. *Ch*TS complexed with **17** (navy) and FdUMP (yellow). Hydrogen bonding interactions with active site residues designated by black dotted lines.

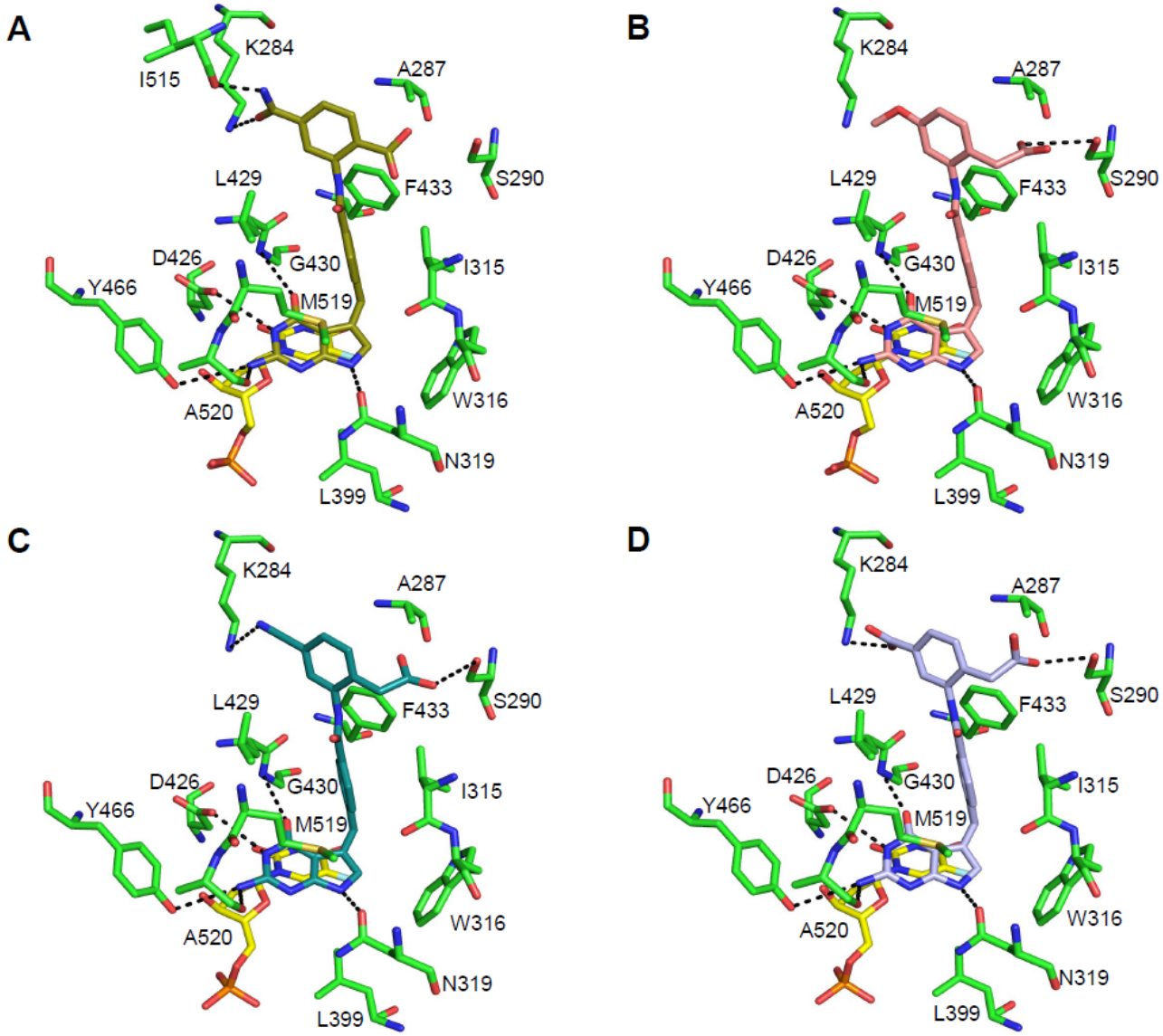

Figure S4. Active site residues of *Ch*TS (green) (PDB: 6PFG, 6PFC, 6PFH, and 6PFI). A. *Ch*TS complexed with **20** (olive) and FdUMP (yellow) (. B. *Ch*TS complexed with **23** (salmon) and FdUMP (yellow). C. *Ch*TS complexed with **24** (dark teal) and FdUMP (yellow). D. *Ch*TS complexed with **25** and FdUMP (yellow). Hydrogen bonding interactions with active site residues designated by black dotted lines.

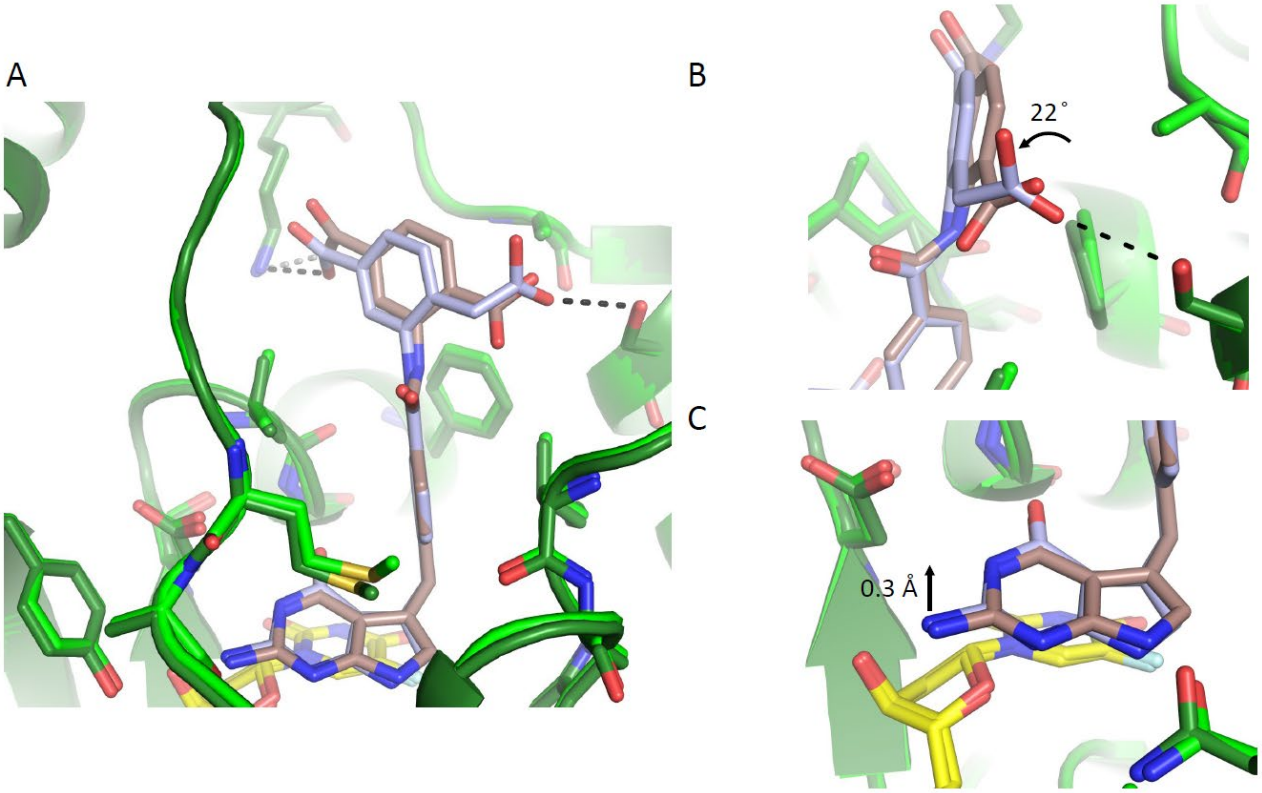

Figure S5. Comparison of *Ch*TS bound to **15** (green/light brown) and *Ch*TS bound to **25** (dark green/lavender) with FdUMP (yellow). (PDB: 6PFD and 6PFI) A. Comparison of **15** and **25** bound in *Ch*TS active site, hydrogen bonds to **15** gray dotted line and to **25** black dotted line. B. Zoom in view rotation of **25** 2-phenylacetic acid moiety with respect to **15** benzoic acid moiety. C. Zoom in view of shift of pyrrolo[2,3-d]pyrimidine moiety of **15** and **25**.

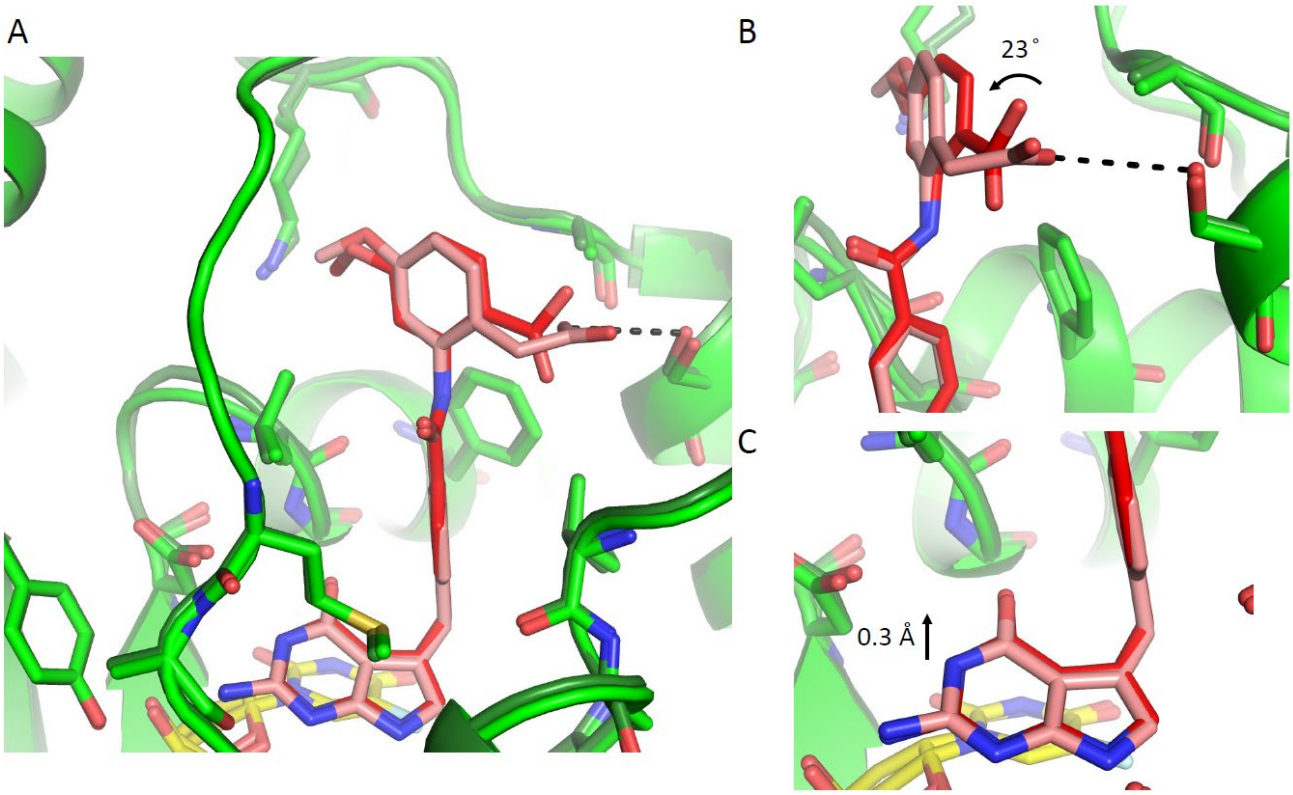

Figure S6. Comparison of *Ch*TS bound to **16** (green/red) and *Ch*TS bound to **23** (dark green/salmon) with FdUMP (yellow) (PDB: 6PFE and 6PFC). A. Comparison of **16** and **23** bound in *Ch*TS active site, hydrogen bonds to **23** black dotted line. B. Zoom in view rotation of **23** 2-phenylacetic acid moiety ith respect to **16** benzoic acid moiety. C. Zoom in view of shift of pyrrolo[2,3-d]pyrimidine moiety of **16** and **23**.

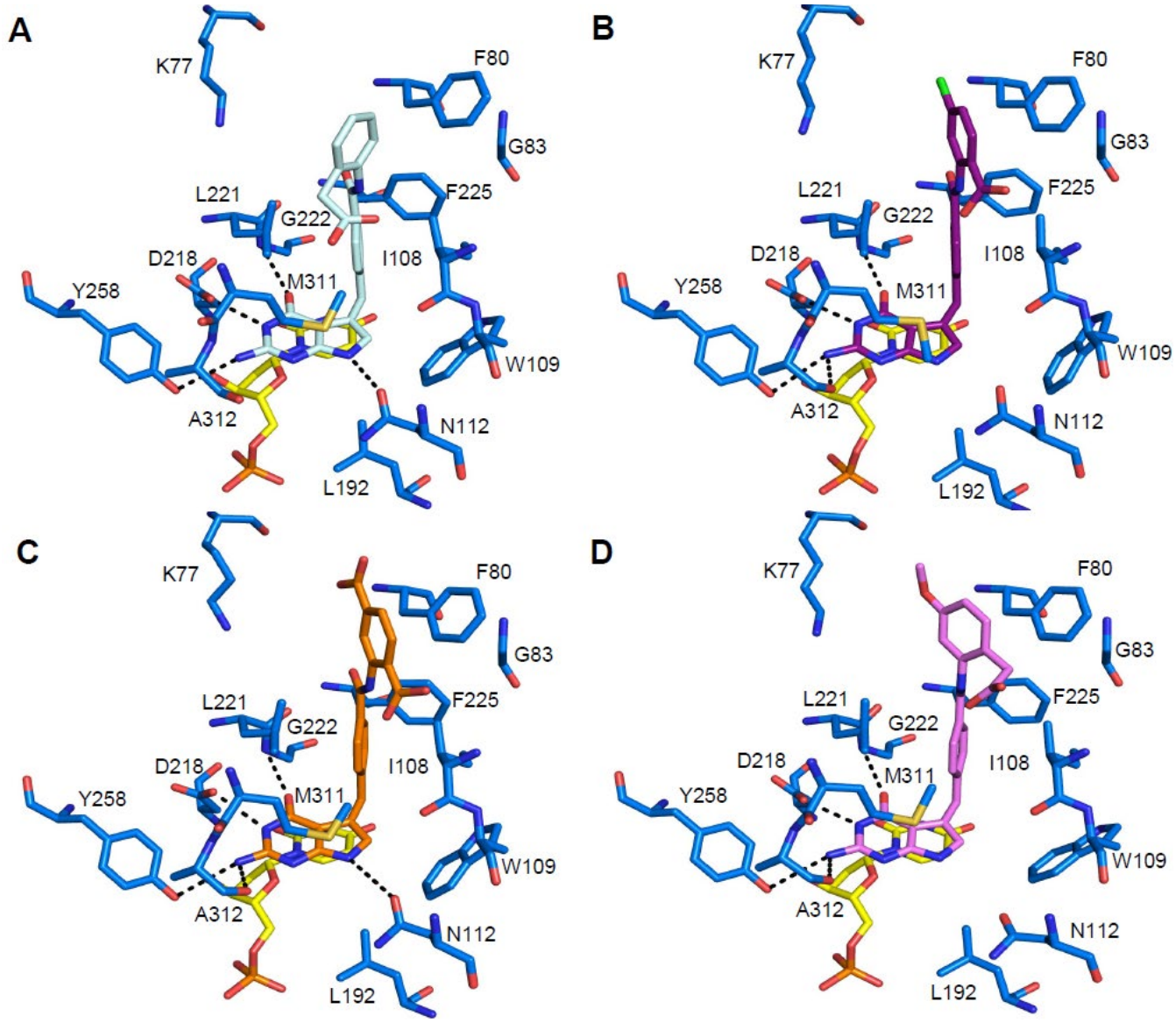

Figure S7. Active site residues of hTS (blue) (PDB: 6PF4, 6PF3, 6PF6 and 6PF5). A. hTS complexed with **5** (light cyan) and dUMP (yellow). B. hTS complexed with **14** (dark purple) and dUMP (yellow). C. hTS complexed with **15** (orange) and dUMP (yellow). D. hTS complexed with **23** (violet) and dUMP (yellow). Hydrogen bonding interactions with active site residues designated by black dotted lines.

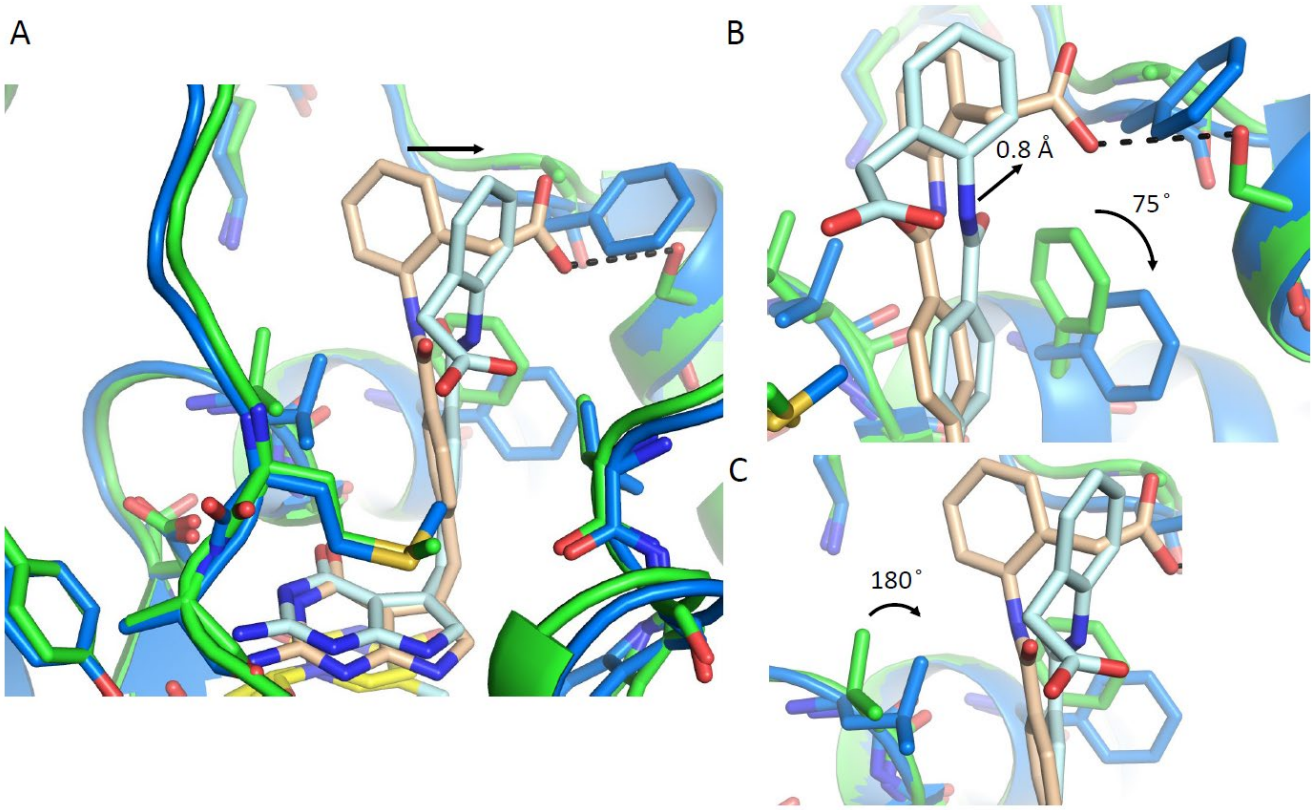

Figure S8. Comparison of hTS bound to **5** (blue/light cyan) and *Ch*TS bound to **5** (green/wheat) with dUMP or FdUMP, respectively (yellow) (PDB: 6PF9 and 6PF4). A. Comparison of **5** bound in hTS and *Ch*TS active site, hydrogen bond between *Ch*TS and **5** black dotted line. B. Zoom in view hTS residue F225 and ChTS residue F433 position upon binding **5**. C. Zoom in view hTS residue L221 and ChTS residue L429 position upon binding **5**.

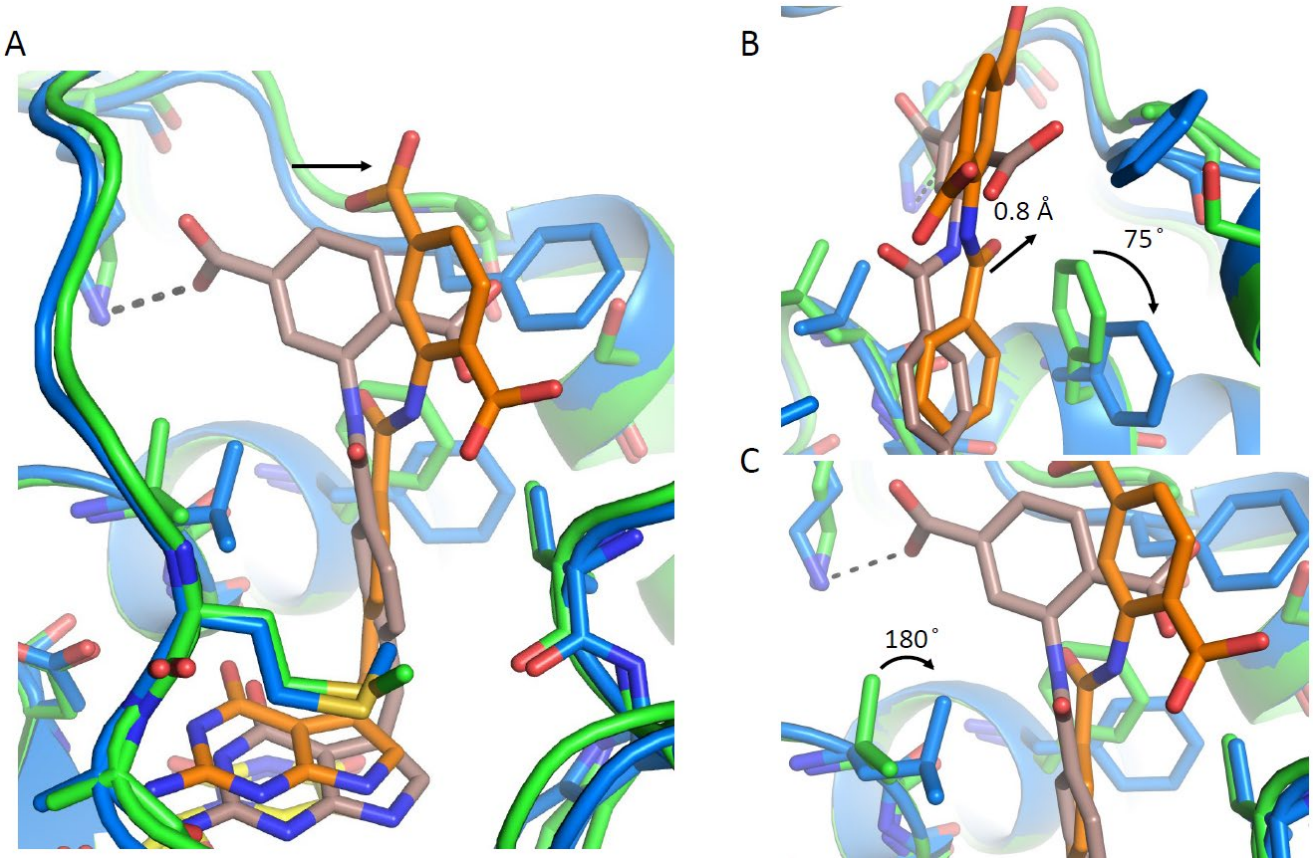

Figure S9. Comparison of hTS bound to **15** (blue/orange) and *Ch*TS bound to **15** (green/light brown) with dUMP or FdUMP, respectively (yellow) (PDB: 6PFD and 6PF6). A. Comparison of **15** bound in hTS and *Ch*TS active site, hydrogen bond between *Ch*TS and **15** black dotted line. B. Zoom in view hTS residue F225 and ChTS residue F433 position upon binding **15**. C. Zoom in view hTS residue L221 and ChTS residue L429 position upon binding **15**.

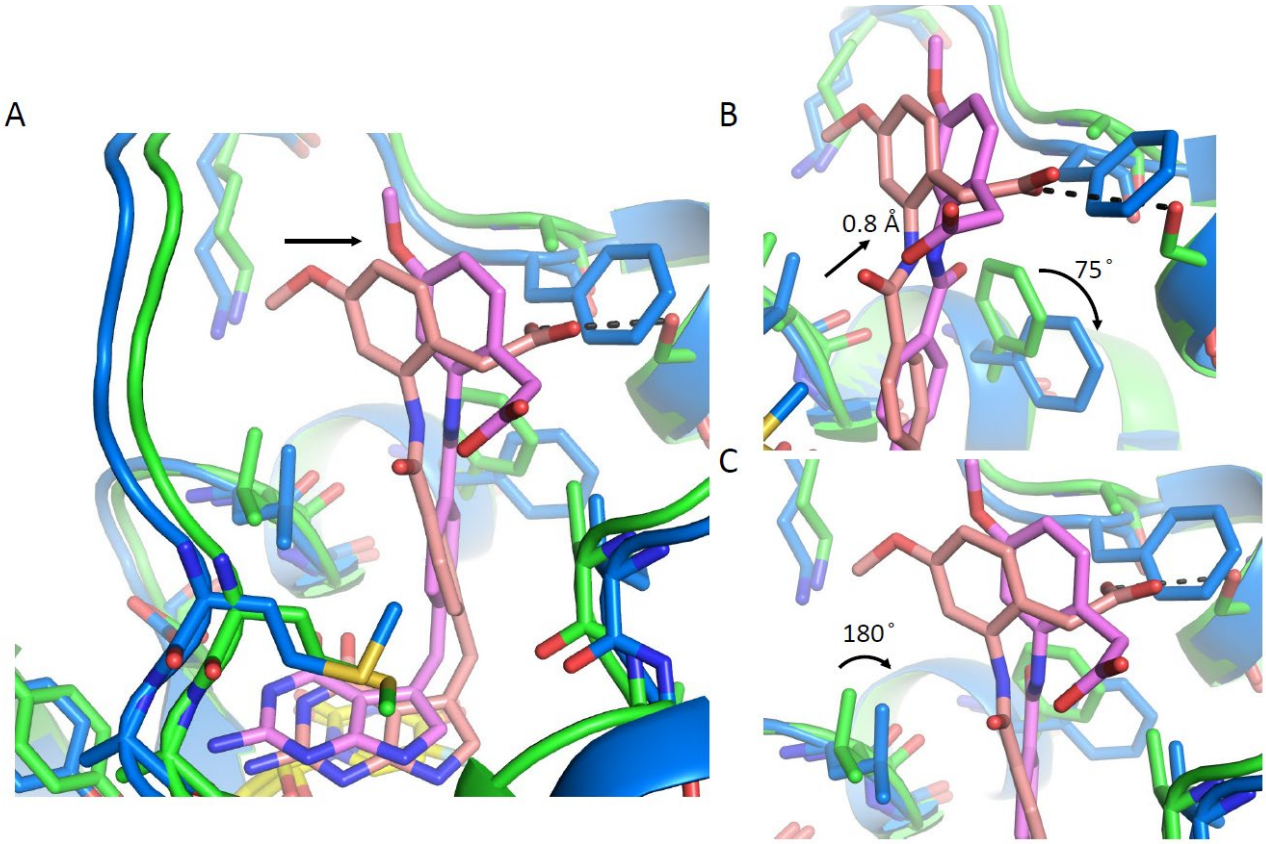

Figure S10. Comparison of hTS bound to **23** (blue/violet) and *Ch*TS bound to **23** (green/salmon) with dUMP or FdUMP, respectively (yellow) (PDB: 6PFC and 6PF5). A. Comparison of **23** bound in hTS and *Ch*TS active site, hydrogen bond between *Ch*TS and **23** black dotted line. B. Zoom in view hTS residue F225 and ChTS residue F433 position upon binding **23**. C. Zoom in view hTS residue L221 and ChTS residue L429 position upon binding **23**.

7. References

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