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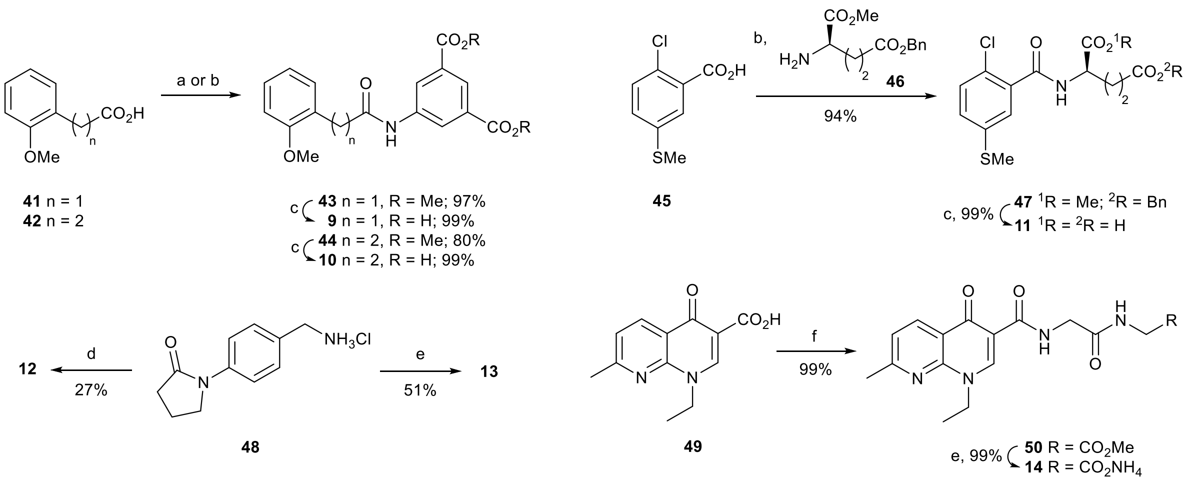
1. **Additional Experimental Section**

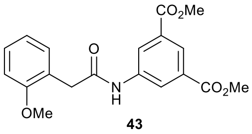
General – All starting materials and reagents were commercially available and were used without further purification. ^{1}H NMR spectra (250, 300, 400 and 500 MHz) and ^{13}C NMR spectra (63, 75, 100 and 125 MHz) were measured in deuterated solvents. *J* values are given in Hertz. NMR assignments were carried out by a combination of 1 D, COSY, and DEPT-135 experiments. FT-IR spectra were recorded as NaCl plates or KBr discs in a PerkinElmer Two FTIR spectrometer with attenuated total reference. $[\alpha]_{D}^{20}$ = values are given in 10^{-1} deg cm^{2} g^{-1}. MilliQ deionized water was used in all the buffers. All procedures involving the use of ion-exchange resins were carried out at room temperature using Milli-Q deionized water. Amberlite IR-120 (H^{+}) (cation exchanger) was washed alternately with water, NaOH (10%), water, HCl (10%), and finally water before use. The spectroscopic measurements were made on a Varian Cary 100 UV-Vis spectrophotometer with a 1 cm pathlength cell fitted with a Peltier temperature controller. The purity of the reported compounds was analyzed by HPLC and by NMR. HPLC was performed on either a Bio-Rad Aminex ion exclusion HPX-87H organic acids column (300 mm × 16 mm), eluting with 100 mM aqueous formic acid at a flow rate of 0.6 mL min^{-1} or a Phenomenex Luna 5 µM C18 column (250 mm × 4.6 mm), eluting with a gradient of acetonitrile/water [from (5:95) to (30:70)] at a flow rate of 1 mL min^{-1}. All tested compounds have a purity ≥95%.

1.1 Synthesis of Compounds 9–14

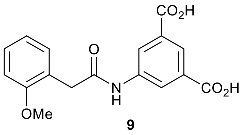
The synthesis of compounds **9–14** was performed as outlined in Scheme S1.



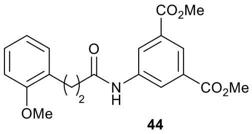
Scheme S1 Synthesis of compounds **9**–**14**. *Reagents and conditions.* (a) 1. SOCl$_2$, CHCl$_3$, Δ. 2. dimethyl 5-aminoisophathalate, CH$_2$Cl$_2$, Et$_3$N, RT. (b) dimethyl 5-aminoisophathalate, HATU, DIPEA, CH$_2$Cl$_2$, RT. (c) 1. LiOH, THF, RT. 2. HCl. (d) *cis*-1,2-cyclohexanedicarboxylic anhydride, DMAP, Et$_3$N, THF, RT. (e) *trans*-1,2-cyclohexanedicarboxylic anhydride, DMAP, Et$_3$N, DMF, RT. (f) methyl glycylglycinate, HATU, DIPEA, CH$_2$Cl$_2$, RT. (e) 1. LiOH, THF, RT. 2. Dowex 50W X8–400 (NH$_4^{+}$ form).


43

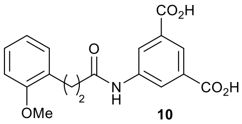
Dimethyl 5-(2-(2-methoxyphenyl)acetamido)isophthalate (43) – A solution of 2-methoxyphenyl acetic acid (**41**) (114 mg, 0.69 mmol) and thionyl chloride (0.10 mL, 1.38 mmol) in chloroform (2.8 mL) was heated under reflux for 2 h. After cooling to room temperature, solvents were removed under reduced pressure. The resulting residue was dissolved in dry dichloromethane (2 mL) and it was added via canula to a suspension of dimethyl 5-aminoisophathalate (148 mg, 0.69 mmol) and triethylamine (0.14 mL, 1.00 mmol) in dry dichloromethane (3.45 mL). After 3 h, the reaction mixture was diluted with dichloromethane and aqueous HCl (10%). The aqueous layer was separated and the organic extract was washed successively with saturated sodium bicarbonate and brine. The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (60:40) diethyl acetate/hexane, to afford the amide **43** (240 mg, 97%) as white solid. Mp: 169–170 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.37 (s, 1H, H2), 8.28 (s, 2H, H4+H6), 7.90 (s, 1H, NH), 7.39–7.27 (m, 2H, H3´+H6´), 6.99 (t, *J* = 8.1 Hz, 2H, H4´+H5´), 3.94 (s, 3H, OCH_3), 3.91 (s, 6H, 2×OCH_3) and 3.75 (s, 2H, CH_2) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 169.8 (C), 165.9 (2×C), 156.9 (C), 138.6 (C), 131.3 (2×C), 131.1 (CH), 129.2 (CH), 125.9 (CH), 124.6 (2×CH), 122.7 (C), 121.4 (CH), 111.0 (CH), 55.6 (OCH_3), 52.4 (2×OCH_3) and 39.8 (CH_2) ppm. IR (KBr) υ: 3300 (NH), 1732 (OCO) and 1678 (NCO) cm^{-1}. MS (ESI) *m/z*: 380 (MNa^+). HRMS calcd for $C_{19}H_{19}NO_6Na$ (MNa^+): 380.1105 found, 380.1103.


9

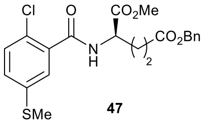
5-(2-(2-Methoxyphenyl)acetamido)isophthalic acid (9) – A stirred solution of diester **43** (31 mg, 0.09 mmol) in THF (1 mL) was treated with an aqueous solution of LiOH (0.9 mL, 0.5 M). The reaction mixture was stirred for 2 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with HCl(c). The resulting precipitate was isolated by filtration and washed with MilliQ water. Diacid **9** (29 mg, 99%) was obtained as a white solid. Mp: 323–324 °C. ^{1}H NMR (250 MHz, CD_3OD) δ: 8.44 (d, *J* = 1.4 Hz, 2H, H4+H6), 8.34 (t, *J* = 1.4 Hz, 1H, H2), 7.29–7.31 (m, 2H, H4´+H5´), 6.95 (br d, *J* = 8.1 Hz, 1H, H6´), 6.89 (dd, *J* = 7.4 and 1.0 Hz, 1H, H3´), 3.82 (s, 3H, OCH_3) and 3.70 (s, 2H, CH_2) ppm. ^{13}C NMR (63 MHz, DMSO-*d6*) δ: 169.8 (C), 166.6 (2×C), 157.3 (C), 140.0 (C), 131.7 (2×CH), 131.0 (2×C), 128.2 (CH), 124.4 (CH), 123.9 (C), 123.5 (CH), 120.2 (CH), 110.7 (CH), 55.5 (OCH_3) and 37.8 (CH_2) ppm. IR (KBr) υ: 3479 (OH), 3263 (NH), 1693 (OCO) and 1658 (NCO) cm^{-1}. MS (ESI) *m/z*: 328 (M–H). HRMS calcd for $C_{17}H_{14}NO_6$ (M–H): 328.0816; found, 328.0812.


44

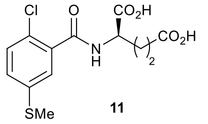
Dimethyl 5-(3-(2-methoxyphenyl)propanamido)isophthalate (44) – A solution of 3-(2-methoxyphenyl)propionic acid (**42**) (212 mg, 1.16 mmol), dimethyl 5-aminoisophthalate (247 mg, 1.16 mmol), *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (483 mg, 1.28 mmol) and *N*,*N*-diisopropylethylamine (0.2 mL, 4.64 mmol) in dry dichloromethane (10 mL) was stirred at room temperature for 5 h. The reaction mixture was diluted with dichloromethane and washed with saturated solution of ammonium chloride, sodium bicarbonate and brine. The organic layer was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (40:60) ethyl acetate/hexane, to give the amide **44** (344 mg, 80%) as white solid. Mp: 138–139 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.36 (s, 3H, H2+H4+H6), 8.09 (s, 1H, NH), 7.21–7.13 (m, 2H, H3´+H6´), 6.84 (m, 2H, H4´+H5´), 3.88 (s, 6H, 2×OCH_3), 3.78 (s, 3H, OCH_3), 3.04 (t, *J* = 7.5 Hz, 2H, CH_2) and 2.71 (t, *J* = 7.5 Hz, 2H, CH_2) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 171.5 (C), 166.0 (2×C), 157.1 (C), 138.7 (C), 131.0 (2×C), 130.0 (CH), 128.5 (C), 127.7 (CH), 125.9 (CH), 124.7 (2×CH), 120.6 (CH), 110.2 (CH), 55.2 (OCH_3), 52.4 (2×OCH_3), 37.5 (CH_2) and 26.2 (CH_2) ppm. IR (KBr) υ: 3361 (NH), and 1699 (CO) cm^{-1}. MS (ESI) *m/z*: 394 (MNa^+). HRMS calcd for $C_{20}H_{21}NO_6Na$ (MNa^+): 394.1261; found, 394.1267.



5-(3-(2-methoxyphenyl)propanamido)isophthalic acid (10) – A stirred solution of diester **44** (389 mg, 1.05 mmol) in THF (10 mL) was treated with an aqueous solution of LiOH (11 mL, 0.5M). The reaction mixture was stirred for 2 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with HCl(c). The resulting precipitate was isolated by filtration and washed with MilliQ water. Diacid **10** (356 mg, 99%) was obtained as a white solid. Mp: 305–306 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ: 13.20 (br s, 2H, 2×OH), 10.26 (s, 1H, H2), 8.44 (s, 2H, H4+H6), 8.15 (s, 1H, NH), 7.17 (m, 2H, H3'+H6'), 6.94 (d, *J* = 8.3 Hz, 1H, H4'), 6.85 (t, *J* = 7.3 Hz, 1H, H5'), 3.79 (s, 3H, OCH_3), 2.89 (t, *J* = 7.6 Hz, 2H, CH_2) and 2.61 (t, *J* = 7.6 Hz, 2H, CH_2) ppm. ^{13}C NMR (100 MHz, DMSO-*d6*) δ: 171.1 (C), 166.5 (2×C), 157.0 (C), 139.8 (C), 131.6 (2×C), 129.4 (2×CH), 128.6 (C), 127.4 (CH), 124.3 (CH), 123.4 (CH), 120.2 (CH), 110.5 (CH), 55.2 (OCH_3), 36.3 (CH_2) and 25.4 (CH_2) ppm. IR (KBr) υ: 3479 (OH), 3280 (NH), 1718 (CONH), 1697 (CO) and 1660 (CO) cm^{-1}. MS (ESI) *m/z*: 342 (M–H). HRMS calcd for $C_{18}H_{16}NO_6$ (M–H): 342.0983; found, 342.0980.

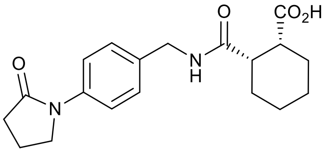


1-Methyl-5-benzyl (2*R*)-2-[2-chloro-5-(methylthio)benzamido]pentanedioate (47) – A solution of 2-chloro-5-(methylthio)benzoic acid (**45**) (146 mg, 0.72 mmol), 5-benzyl 1-methyl D-glutamate (**46**)[46] (182 mg, 0.72 mmol), HATU (330 mg, 0.87 mmol) and *N*,*N*-diisopropylethylamine (0.5 mL, 2.87 mmol) in dry dichloromethane (7.2 mL) was stirred at room temperature for 3 h. The reaction mixture was diluted with ethyl acetate and washed with saturated solution of ammonium chloride, sodium bicarbonate and brine. The organic layer was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (40:60) ethyl acetate/hexane, to give the amide **47** (292 mg, 94%) as a white solid. $[\alpha]_D^{20}$ = –12.5 (*c* 1.0, $CHCl_3$). Mp: 78–79 ºC. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 7.45 (d, *J* = 2.3 Hz, 1H, ArH), 7.30 (m, 5H, ArH), 7.25 (d, *J* = 8.5 Hz, 1H, ArH) 7.16 (dd, *J* = 8.5 and 2.3 Hz, 1H, ArH), 7.00 (d, *J* = 7.6 Hz, 1H, NH), 5.07 (s, 2H, CH_2), 4.81 (td, *J* = 7.6 and 5.2 Hz, 1H, H2), 3.72 (s, 3H, OCH_3), 2.42 (s, 3H, SCH_3), 2.55–2.47 (m, 2H, CH_2), 2.40–2.26 (m, 1H, C*H*H) and 2.17–2.03 (m, 1H, CH*H*) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$) δ: 172.5 (C), 171.9 (C), 165.8 (C), 138.5 (C), 135.7 (C), 134.5 (C), 130.5 (CH), 129.3 (CH), 128.6 (C), 128.3 (CH), 127.4 (CH), 127.0 (C), 66.6 (CH_2), 52.7 (OCH_3), 52.3 (CH), 30.3 (CH_2), 27.3 (CH_2) and 15.7 (SCH_3) ppm. IR (KBr) υ: 3315 (NH), 1749 (OCO) 1728 (OCO) and 1647 (NCO) cm^{-1}. MS (ESI) *m/z*: 458 (MNa$^+$). HRMS calcd for $C_{21}H_{22}NO_5SClNa$ (MNa$^+$): 458.0799; found, 458.0805.



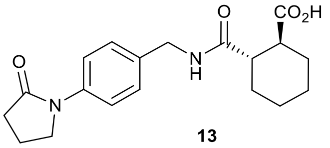
11

(2*R*)-2-[2-chloro-5-(methylthio)benzamido]pentanedioic acid (11) – A stirred solution of diester **47** (64 mg, 0.15 mmol) in THF (1.5 mL) was treated with an aqueous solution of LiOH (1.5 mL, 0.5M). The reaction mixture was stirred at room temperature for 1 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with HCl(c). The resulting precipitate was isolated by filtration and washed with MilliQ water. Diacid **11** (48 mg, 99%) was obtained as a white solid. $[\alpha]_D^{20}$ = +9.4 (*c* 1.1, MeOH). Mp: 207–208 ºC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ: 8.81 (d, *J* = 7.6 Hz, 1H, NH), 7.74 (d, *J* = 8.4 Hz, 1H, H3´), 7.64 (dd, *J* = 8.4 and 2.1 Hz, 1H, H4'), 7.59 (d, *J* = 2.1 Hz, 1H, H6'), 4.65 (q, *J* = 7.8 Hz, 1H, H-2), 2.82 (s, 3H, SCH_3), 2.67 (t, *J* = 7.5 Hz, 2H, CH_2), 2.36 (m, 1H, C*H*H) and 2.20 (m, 1H, CH*H*) ppm. IR (KBr) υ: 3500 (OH), 3309 (NH), 1709 (OCO) and 1643 (NCO) cm^{-1}. MS (ESI) *m/z*: 330 (M–H). HRMS calcd for $C_{13}H_{13}NO_5SCl$ (M–H): 330.0208; found, 330.0204.



12

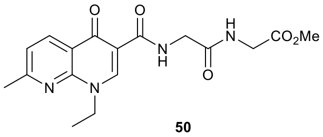
(1*R*,2*S*)-2-[(4-(2-oxopyrrolidin-1-yl)benzyl)carbamoyl]cyclohexanecarboxylic acid (12) – To a solution of *cis*-1,2-cyclohexanedicarboxylic anhydride (39 mg, 0.25 mmol) and dry triethylamine (0.1 mL, 0.76 mmol) in dry DMF (0.8 mL) and under argon was treated with a solution of 1-(4-(aminomethyl)phenyl)pyrrolidin-2-one hydrochloride (**48**) (60 mg, 0.26 mmol) in dry DMF (0.4 mL). The resultant mixture was stirred for 8 h. The solvent was removed under reduced pressure and the resulting residue was partitioned in diluted HCl (0.1M) and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (×2). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (10:90) methanol/ethyl acetate, to give the acid **12** (24 mg, 27%) as a white solid. Mp: 171–172 ºC. ^{1}H NMR (250 MHz, DMSO-*d*6) δ: 11.7 (s, 1H, OH), 8.15 (t, *J* = 5.7 Hz, 1H, NH), 7.50 (d, *J* = 8.5 Hz, 2H, H2´), 7.16 (d, *J* = 8.5 Hz, 2H, H3´), 4.16 (d, *J* = 5.7 Hz, 2H, $ArCH_2$), 3.75 (t, *J* = 7.0 Hz, 2H, NCH_2), 3.56–3.08 (m, 2H, H1+H2), 2.54–2.37 (m, 6H, 3×CH_2), 2.07–1.89 (m, 2H, CH_2) and 1.69–1.40 (m, 4H, 2×CH_2) ppm. ^{13}C NMR (63 MHz, DMSO-*d*6) δ: 175.3 (C), 173.7 (C), 173.4 (C), 138.1 (C), 135.5 (C), 127.1 (2×CH), 119.2 (2×CH), 48.1 (CH_2), 42.0 (CH), 41.9 (CH), 41.3 (CH_2), 32.3 (CH_2), 27.5 (CH_2), 25.6 (CH_2), 23.9 (CH_2), 22.7 (CH_2) and 17.4 (CH_2) ppm. IR (KBr) υ: 3350 (NH), 1693 (OCO) and 1633 (NCO) cm^{-1}. MS (ESI) *m/z*: 343 (M–H). HRMS calcd for $C_{19}H_{23}N_2O_4$ (M–H): 343.1663; found, 343.1659.



13

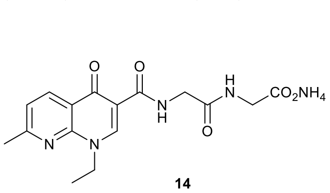
(1*S*,2*S*)-2-[(4-(2-Oxopyrrolidin-1-yl)benzyl)carbamoyl]cyclohexanecarboxylic acid (13) – A solution of *trans*-1,2-cyclohexanedicarboxylic anhydride (109 mg, 0.68 mmol), 1-(4-

(aminomethyl)phenyl)pyrrolidin-2-one hydrochloride (**48**) (161 mg, 0.71 mmol), 4-*N*,*N*-dimethylaminopyridine (8 mg, 0.07 mmol) and dry triethylamine (0.2 mL, 1.43 mmol) in dry THF (1.9 mL) was stirred for 24 h. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography eluting with (10:90) methanol/ethyl acetate to give the acid **13** (118 mg, 51%) as a white solid. $[\alpha]_D^{20}$ = – 4.2 (*c* 1.0, DMSO). Mp: 208–209 ºC. ^{1}H NMR (250 MHz, DMSO-*d6*) δ: 11.62 (s, 1H, CO_2H), 8.25 (t, *J* = 5.9 Hz, 1H, NH), 7.50 (d, *J* = 8.6 Hz, 2H, 2×ArH), 7.15 (d, *J* = 8.6 Hz, 2H, 2×ArH), 4.20 (dd, *J* = 15.5 and 5.9 Hz, 1H, C*H*HN), 4.10 (dd, *J* = 15.5 and 5.9 Hz, 1H, CH*H*N), 3.74 (t, *J* = 7.0 Hz, 2H, CH_2), 2.45 (m, 2H, 2×CH), 2.04 (m, 2H, CH_2), 1.94–1.82 (m, 2H, CH_2) and 1.71 (m, 2H, CH_2) and 1.23 (m, 6H, 3×CH_2) ppm. ^{13}C NMR (63 MHz, DMSO-*d6*) δ: 176.4 (C), 174.5 (C), 173.7 (C), 138.1 (C), 135.3 (C), 127.0 (2×CH), 119.2 (2×CH), 60.5 (NCH_2), 48.1 (NCH_2), 45.4 (CH), 44.4 (CH), 41.2 (CH_2), 32.3 (CH_2), 29.7 (CH_2), 29.0 (CH_2), 25.2 (CH_2) and 17.4 (CH_2) ppm. IR (KBr) υ: 3305 (NH+OH), 1730 (CO) and 1647 (CO) cm^{-1}. MS (ESI) *m/z*: 343 (M–H). HRMS calcd for $C_{19}H_{23}N_2O_4$ (M–H): 343.1652; found, 343.1649.



50

N-[(N-(methoxycarbonyl)methyl)carbamoylmethyl] 1-ethyl-7-methyl-4-oxo-[1,8]naphthyridine-3-carboxamide (50) – A solution of 1-ethyl-7-methyl-4-oxo-[1,8]naphthyridine-3-carboxylic acid (**49**) (370 mg, 1.56 mmol), diglycine methyl ester (251 mg, 1.56 mmol), HATU (652 mg, 1.71 mmol) and *N*,*N*-diisopropylethylamine (1.1 mL, 6.31 mmol) in dry dichloromethane (15 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate and washed with saturated solution of ammonium chloride, sodium bicarbonate and brine. The organic layer was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (10:90) methanol/dichloromethane, to give the amide **50** (574 mg, 99%) as a white solid. Mp: 276–278 °C. ^{1}H NMR (400 MHz, DMSO-*d6*, 70 °C) δ: 10.03 (t, *J* = 5.5 Hz, 1H, NH), 8.93 (s, 1H, H2), 8.57 (d, *J* = 8.1 Hz, 1H, H5), 8.21 (br s, 1H, NH), 7.46 (d, *J* = 8.1 Hz, 1H, H6), 4.58 (q, *J* = 7.1 Hz, 2H, C*H*$_2$CH$_3$), 4.07 (d, *J* = 5.5 Hz, 2H, CH_2), 3.90 (d, *J* = 5.8 Hz, 2H, CH_2), 3.65 (s, 3H, OCH_3) 2.67 (s, 3H, CH_3) and 1.42 (t, *J* = 7.1 Hz, 3H, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (100 MHz, DMSO-*d6*, 70 °C) δ: 175.3 (C), 169.6 (C), 168.8 (C), 163.5 (C), 162.6 (C), 147.9 (C), 147.3 (CH), 135.4 (CH), 120.8 (CH), 119.3 (C), 111.8 (C), 51.1 (CH_2), 45.4 (CH_2), 41.7 (CH_2), 40.3 (CH_2), 24.3 (CH_3) and 14.4 (CH_3) ppm. IR (KBr) υ: 3292 (NH), 1747 (OCO), 1684 (NCO) and 1647 (NCO) cm^{-1}. MS (ESI) *m/z*: 383 (MNa$^+$). HRMS calcd for $C_{17}H_{20}N_4O_5Na$ (MNa$^+$): 383.1326 found, 383.1320.



14

N-[(N-(carboxyl)methyl)carbamoylmethyl] 1-ethyl-7-methyl-4-oxo-[1,8]naphthyridine-3-carboxamide ammonium salt (14) – A stirred solution of the ester **50** (68.6 mg, 0.19 mmol) in THF (0.8 mL) was treated with an aqueous solution of LiOH (1.0 mL, 0.5M). The reaction mixture was stirred for 1 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with Dowex 50W X8-400 (NH_4^+ form). The resulting aqueous phase was evaporated under reduced pressure and freeze-dried to afford the ammonium salt **14** (68 mg, 99%) as a white solid. Mp: 240 ºC (dec.). ^{1}H NMR (250 MHz, DMSO-*d6*) δ: 10.9 (t, *J* = 5.3 Hz, 1H, NH), 8.97 (s, 1H, H2), 8.55 (d, *J* = 8.1 Hz, 1H, H5), 8.00 (t, *J* = 5.1 Hz, 1H, NH), 7.47 (d, *J* = 8.1 Hz, 1H, H6), 4.59 (q, *J* = 7.0 Hz, 2H, C*H*$_2$CH$_3$), 4.03 (d, *J* = 5.3 Hz, 2H, CH_2), 3.62 (d, *J* = 5.1 Hz, 2H, CH_2), 2.65 (s, 3H, CH_3) and 1.38 (t, *J* = 7.0 Hz, 3H, CH_2CH_3) ppm. ^{13}C NMR (63 MHz, DMSO-*d*6) δ: 175.7 (C), 171.3 (C), 168.3 (C), 163.8 (C), 163.1 (C), 148.0 (CH), 136.0 (CH), 121.4 (CH), 119.7 (C), 111.9 (C), 46.0 (CH_2), 42.2 (2×CH_2), 24.9 (CH_3) and 15.6 (CH_3) ppm. IR (KBr) υ : 3566 (N–H), 3269 (NH), 1666 (CO) and 1606 (CO) cm^{-1}. MS (ESI) *m/z*: 345 (M–NH_4). HRMS calcd for $C_{16}H_{17}N_4O_5$ (M–NH_4): 345.1204 found, 345.1194.

1.2 Fragment-based screeing – It was performed following previously described protocol.[27] The enzyme geometries found in PDB code 2WKS[9m] was used. Taking into account that unfolding and refolding studies of DHQ2 have shown that the trimer[47] is the biological unit of the enzyme, the trimer was used for these studies. The trimer composed by chains A, B and E in 2WKS was considered.

1.3 Dehydroquinase Assays – The enzyme was purified and assayed as described previously.[48]

1.4 Molecular dynamics simulations – *Ligand minimization*. Ligand geometries were optimized using a restricted Hartree–Fock (RHF) method and a 6–31G(d,p) basis set, as implemented in the *ab initio* program Gaussian 09.[49] The resulting wavefunctions were used to calculate electrostatic potential-derived (ESP) charges employing the restrained electrostatic potential (RESP)[50] methodology, as implemented in the assisted model building with energy refinement (AMBER) suite of programs. The missing bonded and non-bonded parameters were assigned, by analogy or through interpolation from those already present in the AMBER database (GAFF).[49,51]

Generation and minimization of the DHQ2-ligand complexes. Simulations were carried out using the enzyme geometries found in PDB code 2WKS[9m] (chains A, B and E).[9m] The trimer composed by chains A, B and E in 2WKS was considered. Computation of the protonation state of titratable groups at pH 7.0 was carried out using the H++ Web server.[52] δ and/or ε protonation was manually corrected for His102 (dual) of the active site due to mechanistic considerations. Addition of hydrogen and molecular mechanics parameters from the ff14SB[53] and GAFF force fields, respectively, were assigned to the protein and the ligands using the LEaP module of AMBER Tools 14.[54] [55] The protein was immersed in a truncated octahedron of ~19000 TIP3P water molecules and neutralized by addition of sodium ions.[50] [56] [57] The system was minimized in three stages: (a) minimization of the solvent and ions (5000 steps, first half using steepest descent and the rest using conjugate gradient); (b) minimization of the side chains, waters and ions (5000 steps, first half using steepest descent and the rest using conjugate gradient); (c) final minimization of the whole system (5000 steps, first half using steepest descent and the rest using conjugate gradient). A positional restraint force of 50 kcal mol^{-1} $Å^{-2}$ was applied to the whole protein and α carbons during the first two stages (a–b), respectively.

Simulations. MD simulations were performed using the pmemd.cuda_SPFP[58] [59] [60] module from the AMBER 14 suite of programs. Periodic boundary conditions were applied and electrostatic interactions were treated using the smooth particle mesh Ewald method (PME)[61] with a grid spacing of 1 Å. The cutoff distance for the non-bonded interactions was 9 Å. The SHAKE algorithm[62] was applied to all bonds containing hydrogen, using a tolerance of 10^{-5} Å and an integration step of 2.0 fs. Minimization was carried out in three steps, starting with the octahedron water hydrogens, followed by solvent molecules and sodium counterions and finally the entire system. The minimized system was then heated at 300 K at 1 atm by increasing the temperature from 0 K to 300 K over 100 ps and by keeping the system at 300 K another 100 ps. A positional restraint force of 50 kcal mol^{-1} $Å^{-2}$ was applied to all α carbons during the heating stage. Finally, an equilibration of the system at constant volume (200 ps with positional restraints of 5 kcal mol^{-1} Å^{-2} to α alpha carbons) and constant pressure (another 100 ps with positional restraints of 5 kcal mol^{-1} Å^{-2} to α alpha carbons) were performed. The positional restraints were gradually reduced from 5 to 1 mol^{-1} Å^{-2} (5 steps, 100 ps each), and the resulting systems were allowed to equilibrate further (100 ps). Unrestrained MD simulations were carried out for 100 ns. System coordinates were collected every 10 ps for further analysis. Figures depicting structures were prepared using PYMOL.[63] The cpptraj module in AMBER 14 was used to analyze the trajectories and to calculate the root-mean-square deviations (RMSD) of the protein during the simulation.[64]

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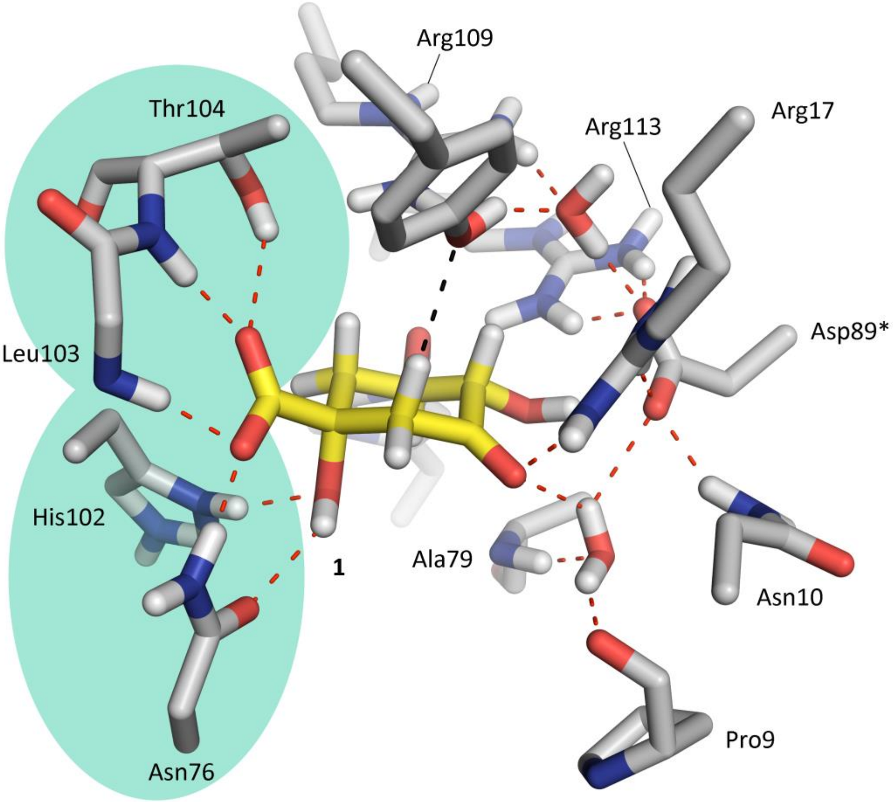


Figure S1. Detailed view of the *Hp*-DHQ2 Michaelis complex obtained by MD simulation studies. The C1 binding pocket is highlighted (cyan). Note how the natural substrate (**1**, yellow) is anchored in the C1 binding pocket by six hydrogen bonds with residues Thr104, Leu103, His102, and Asn76. The essential His102 acts as a proton donor in the final step of the reaction, i.e. the acid-catalyzed elimination of the C1 hydroxyl group. Relevant residues are shown and labeled. Hydrogen-bonding and lipophilic interactions are indicated as red and grey dashed lines, respectively.

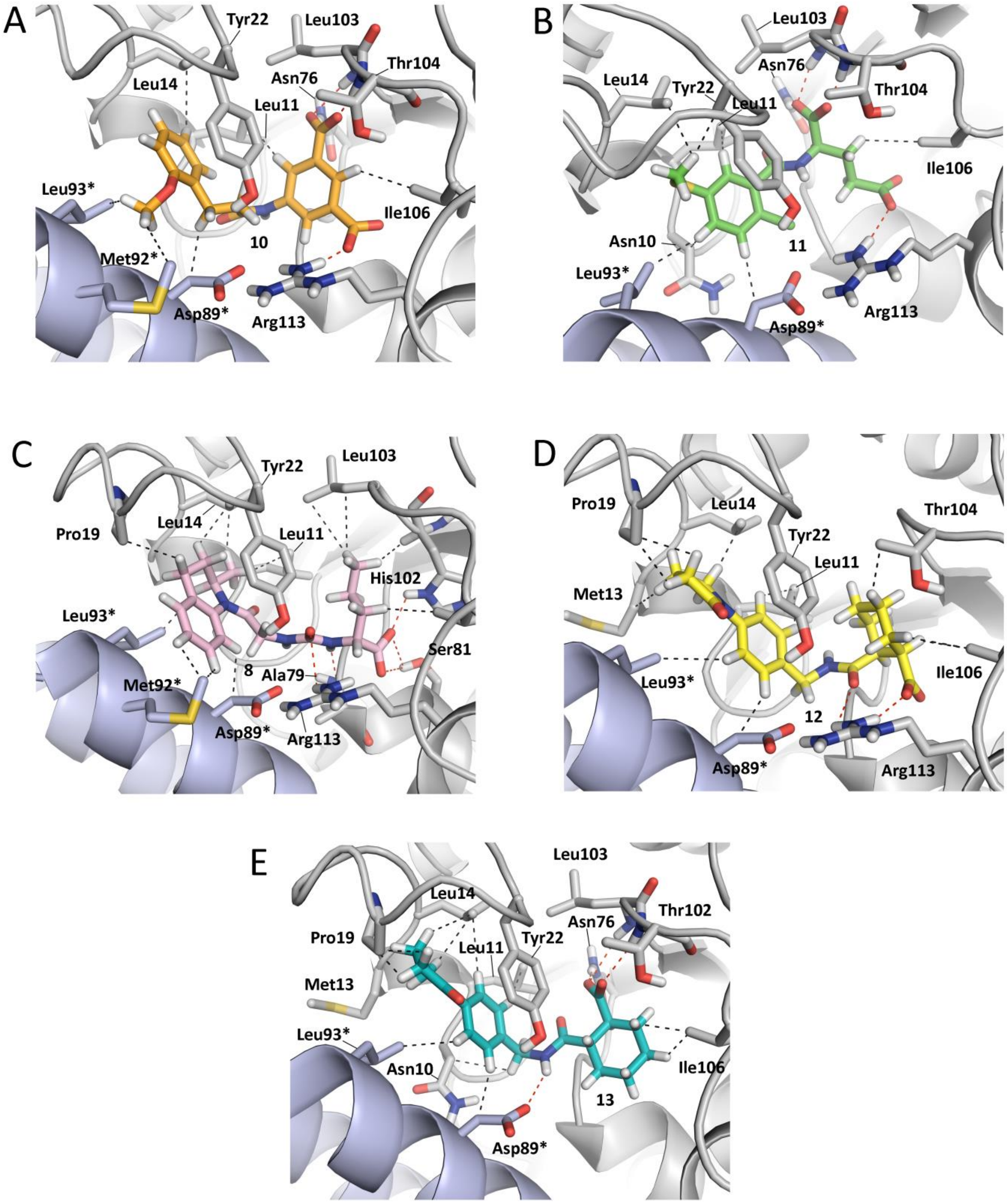
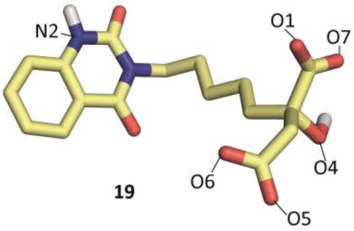


Figure S2. Selected view of the predicted binding mode of ligands: (A) **10** (yellow), (B) **11** (green), (C) **8** (pink), (D) **12** (yellow), and (E) **13** (cyan) in the *Hp*-DHQ2 active site. Note how the less potent inhibitors, i.e. compounds **8** and **12**, do not interact with the C1 binding pocket, which is key for substrate recognition. Relevant side chain residues are shown and labeled. The neighboring chain (blue) close to the active site chain residues (*) are shown and labeled. Hydrogen-bonding and lipophilic interactions are indicated as red and grey dashed lines, respectively.



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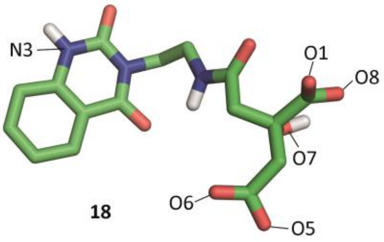

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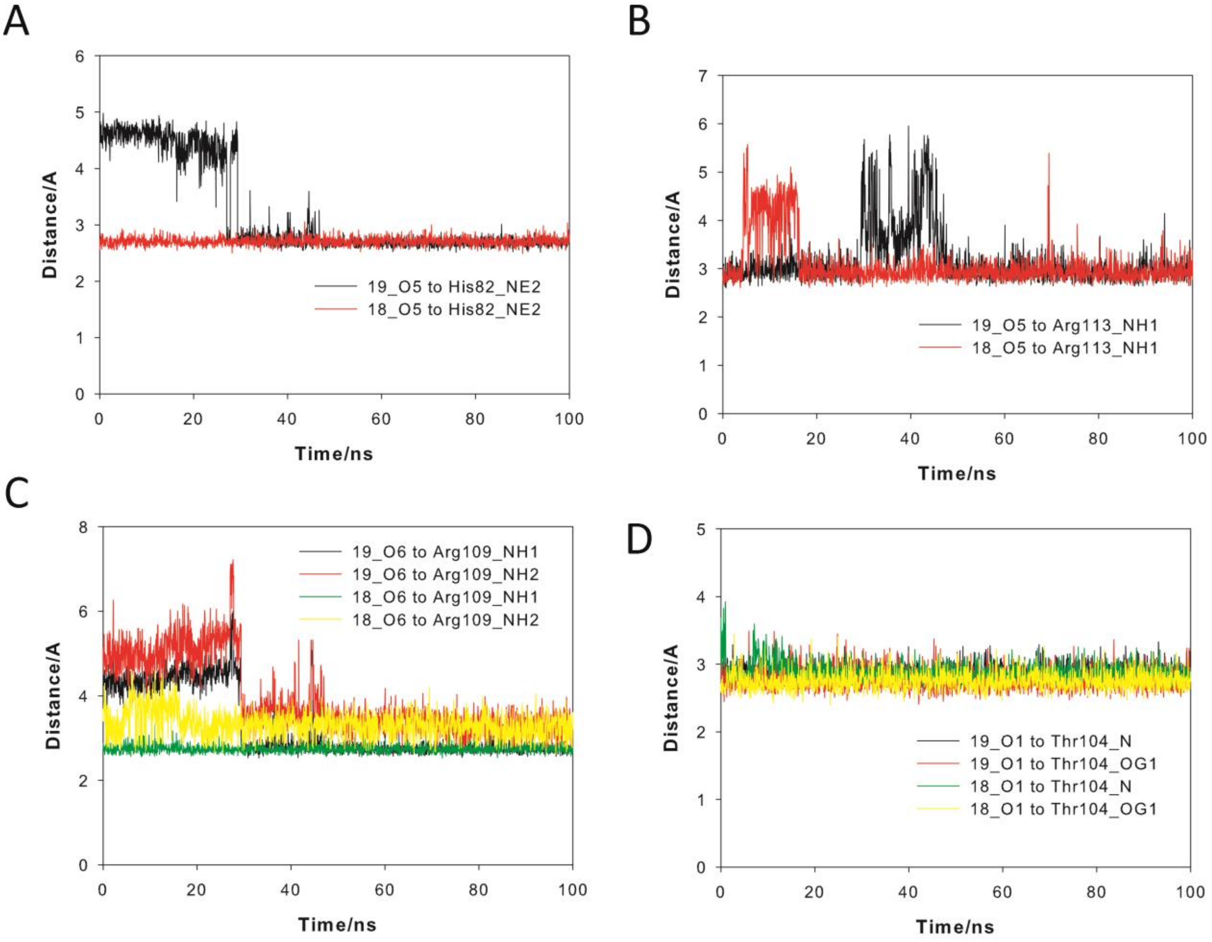


Figure S3 Variation of the relative distance between resides His82 (NE2 atom, A), Arg113 (NH1 atom, B), Arg109 (NH1 and NH2 atoms, C) and Thr104 (N and OG1 atoms, D) and the terminal carboxylate (O5 and O6 atoms) and medium chain carboxylate (O1 atom) in **19** and **18**, respectively, during the whole simulation.

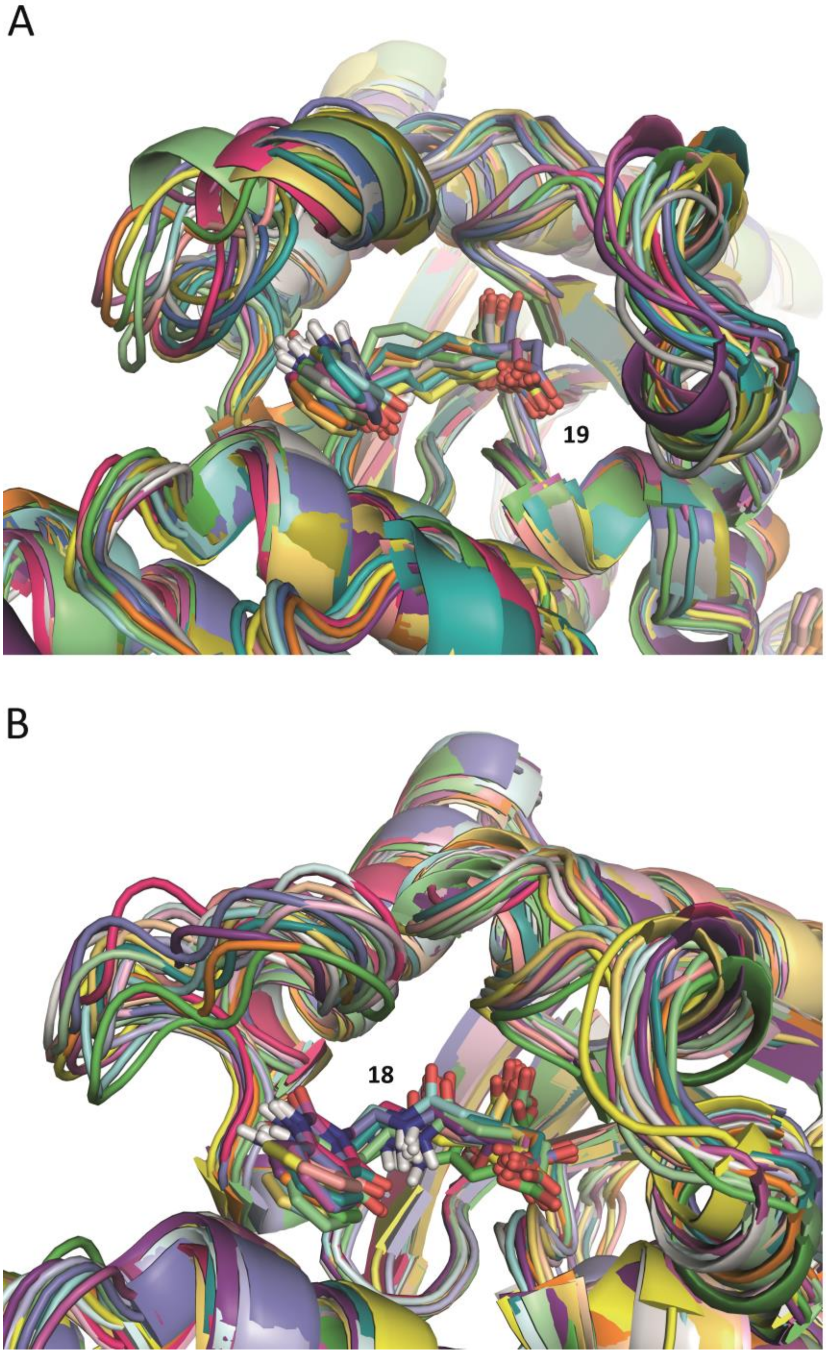
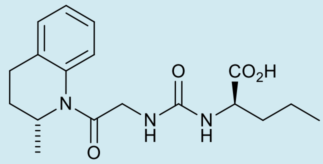
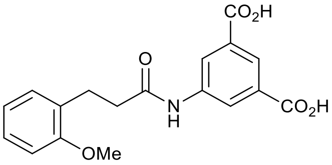
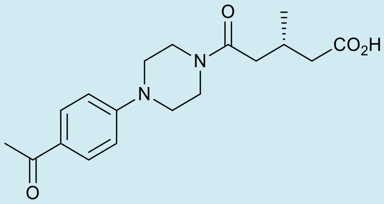
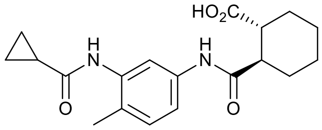
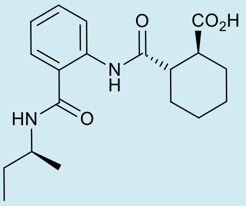
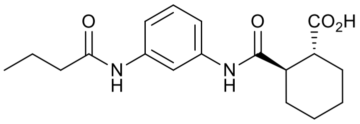
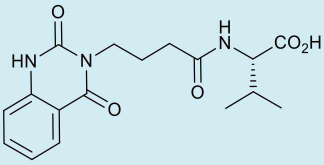
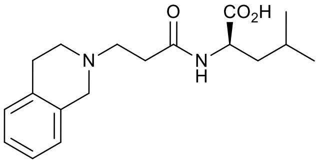
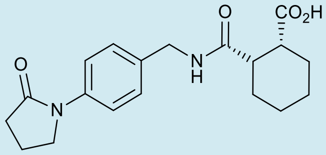
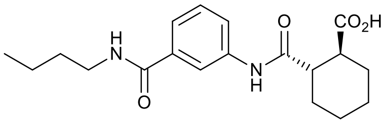
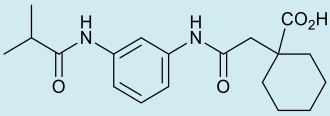
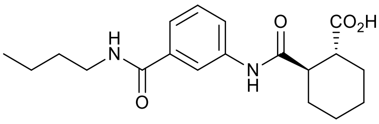
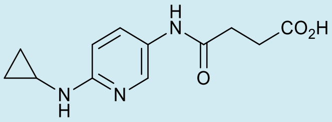
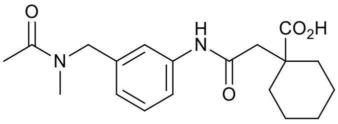
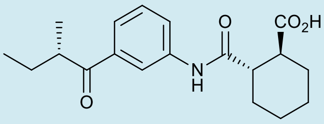
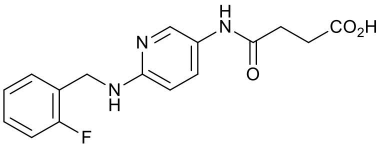
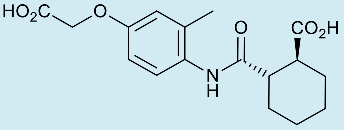
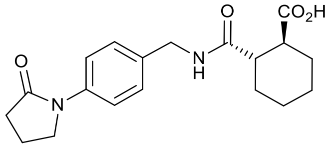
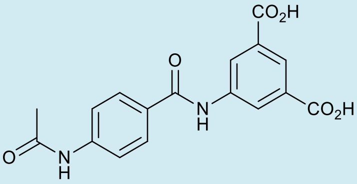
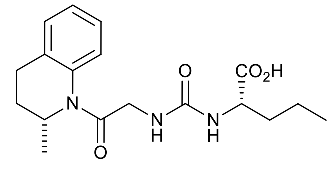
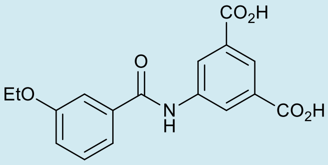
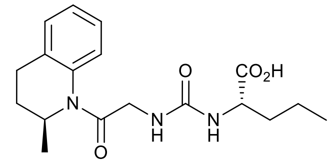
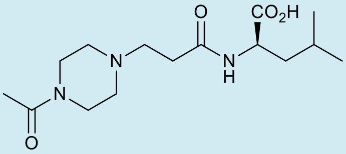
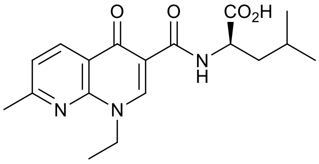
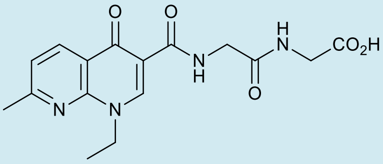
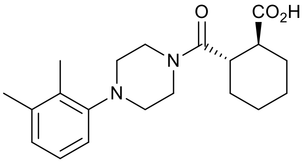
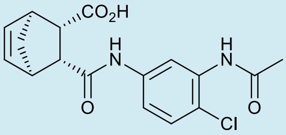
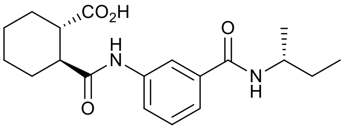
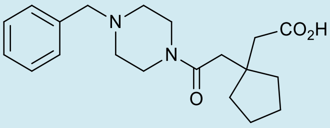
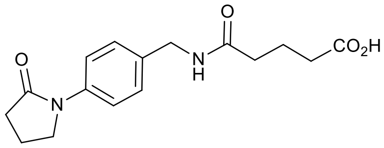
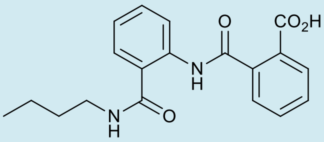
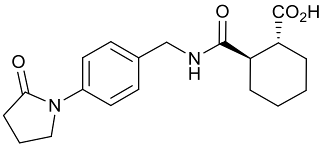
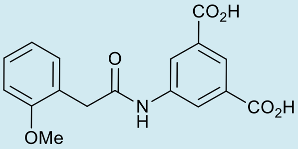
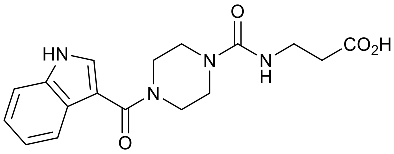


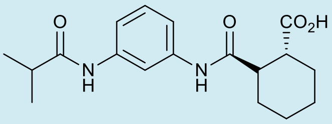
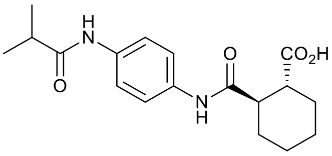
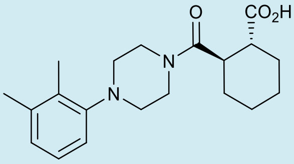
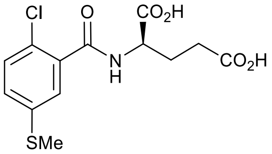
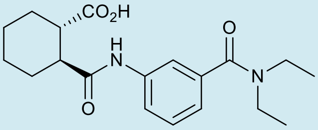
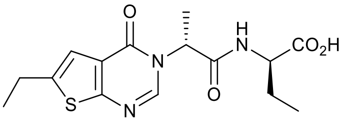
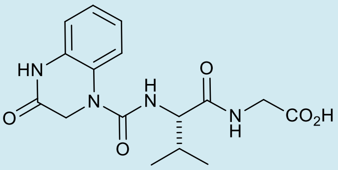
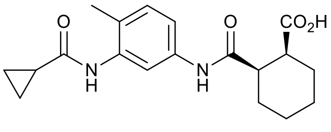
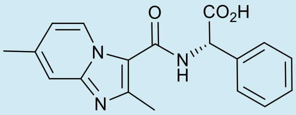
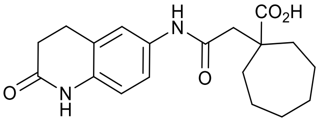
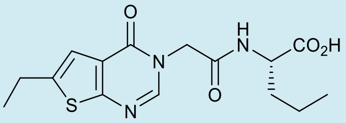
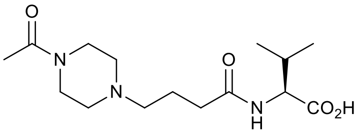
Figure S4 (B) Comparison of several snapshots of the *Hp*-DHQ2/**19** (A) and *Hp*-DHQ2/**18** (B) enzyme complexes during 100 ns of MD. Note the high stability of the enzyme inhibitor complexes and how ligand **19** induces a closer conformation of the substrate-covering loop.

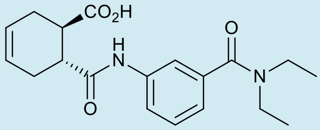
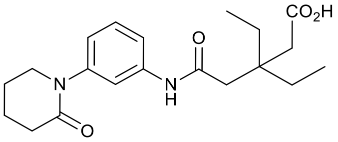
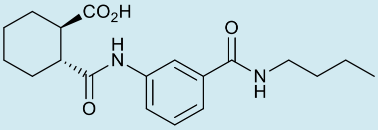
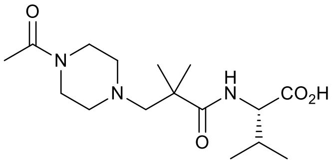
Table S1. The 50 top-ranking compounds obtained with the ALTA approach. The LIECE energy (kcal mol^{-1}) is indicated including their van der Waals and electrostatic components.

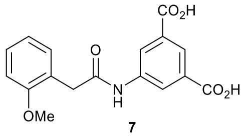
Entry	Compound	Structure	Score	Electrostatic	VdW
1	ZINC12865751		–76,9	–55,5	–21,4
2	ZINC08906157		–76,5	–50,8	–25,7
3	ZINC17068317		–74,7	–56,0	–18,7
4	ZINC30831039		–74,4	–55,8	–18,6
5	ZINC10684193		–73,8	–55,5	–18,3
6	ZINC09229944		–73,7	–53,7	–20,0
7	ZINC20411989		–73,4	–54,0	–19,4
8	ZINC32072562		–73,3	–55,0	–18,3
9	ZINC06701685		–73,3	–53,6	–19,7
10	ZINC12403804		–73,2	–54,2	–19,0

11	ZINC27056567		–73,0	–57,1	–15,8
12	ZINC12403819		–72,9	–49,3	–23,6
13	ZINC31960260		–72,9	–49,3	–23,6
14	ZINC30521263		–72,7	–55,2	–17,5
15	ZINC12403830		–72,7	–50,5	–22,2
16	ZINC31960258		–72,7	–55,8	–16,9
17	ZINC08906241		–72,7	–53,1	–19,6
18	ZINC06701683		–72,5	–48,3	–24,2
19	ZINC05186604		–72,5	–50,7	–21,8
20	ZINC12865758		–72,5	–48,7	–23,8
21	ZINC04701017		–72,5	–52,3	–20,2
22	ZINC12865743		–72,4	–54,9	–17,5

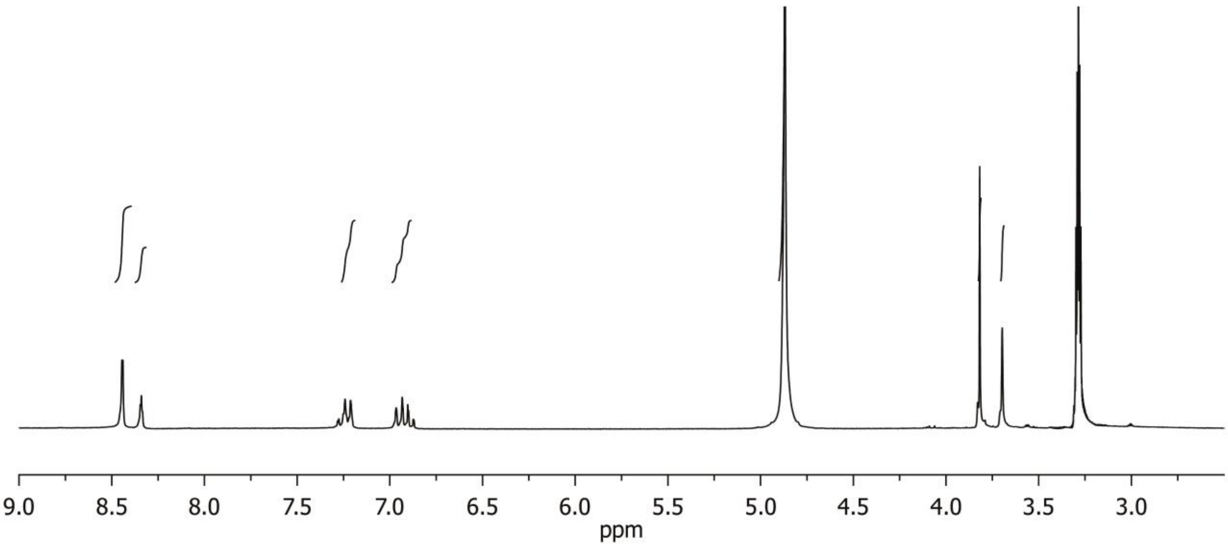
23	ZINC32070669		–72,4	–54,7	–17,7
24	ZINC32603290		–72,4	–51,5	–20,9
25	ZINC32603289		–72,4	–53,8	–18,6
26	ZINC00204936		–72,3	–50,4	–21,9
27	ZINC13154128		–72,3	–55,2	–17,1
28	ZINC12403829		–72.3	–51.2	–21.1
29	ZICNC25337235		–72.2	–51.5	–20.7
30	ZINC1221148856		–72.2	–52.5	–19.7
31	ZINC10473038		–72,1	–54,9	–17,2
32	ZINC06701684		–72.1	–46.9	–25.2
33	ZINC04670525		–72.1	–52.3	–19.8
34	ZINC08991228		–72.1	–51.0	–21.1

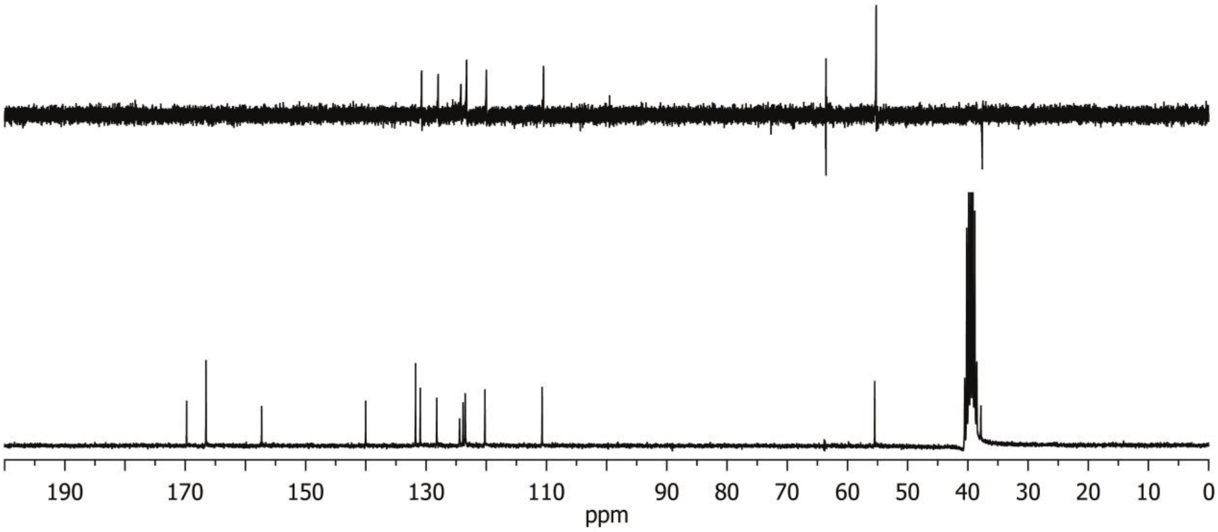
35	ZINC09231696		–72.0	–55.2	–16.8
36	ZINC09230433		–72.0	–55.0	–17.0
37	ZINC00204935		–71.9	–45.8	–26.1
38	ZINC08762439		–71,9	–55,2	–16,7
39	ZINC12403809		–71,8	–53,8	–18,1
40	ZINC31948421		–71.8	–49.3	–22.5
41	ZINC20757920		–71,8	–50,9	–20,9
42	ZINC30831035		–71,8	–50,9	–20,9
43	ZINC31965364		–71.8	–51.5	–20.3
44	ZINC27057409		–71,8	–53,3	–18,5
45	ZINC31948411		–71,8	–55,9	–15,9
46	ZINC32070679		–71,8	–54,1	–17,7

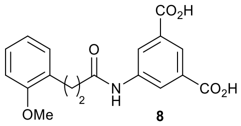
47	ZINC13253841		–71.7	–56.0	–15.7
48	ZINC27058229		–71,7	–55,5	–16,2
49	ZINC12403805		–71,7	–55,5	–16,2
50	ZINC32088058		–71,7	–53,8	–17,9


CO2H
O
N
H
CO2H
OMe

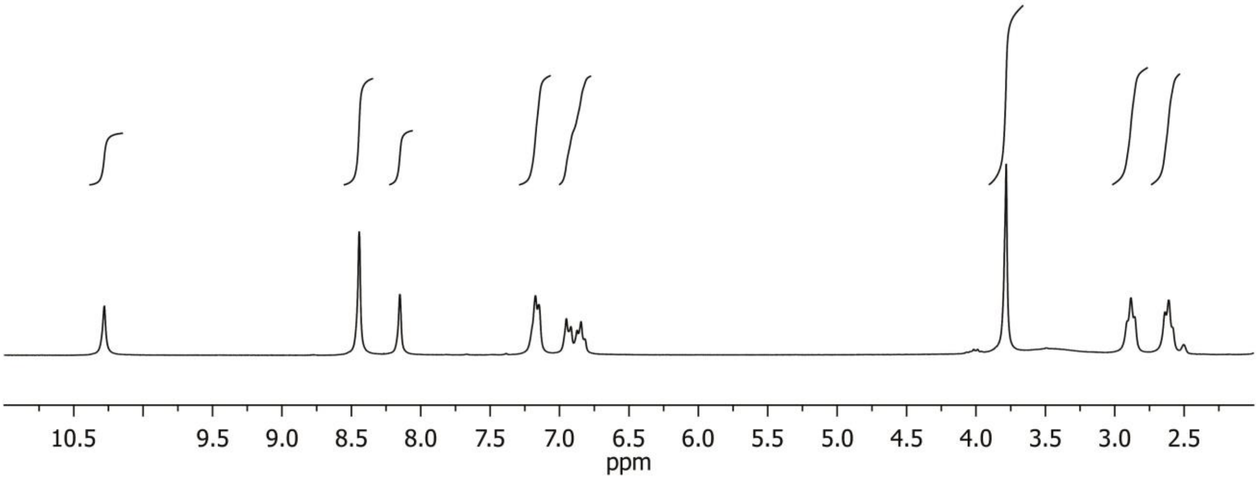
7


9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm


190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
ppm


CO2H
O
N
H
2
CO2H
OMe

8


10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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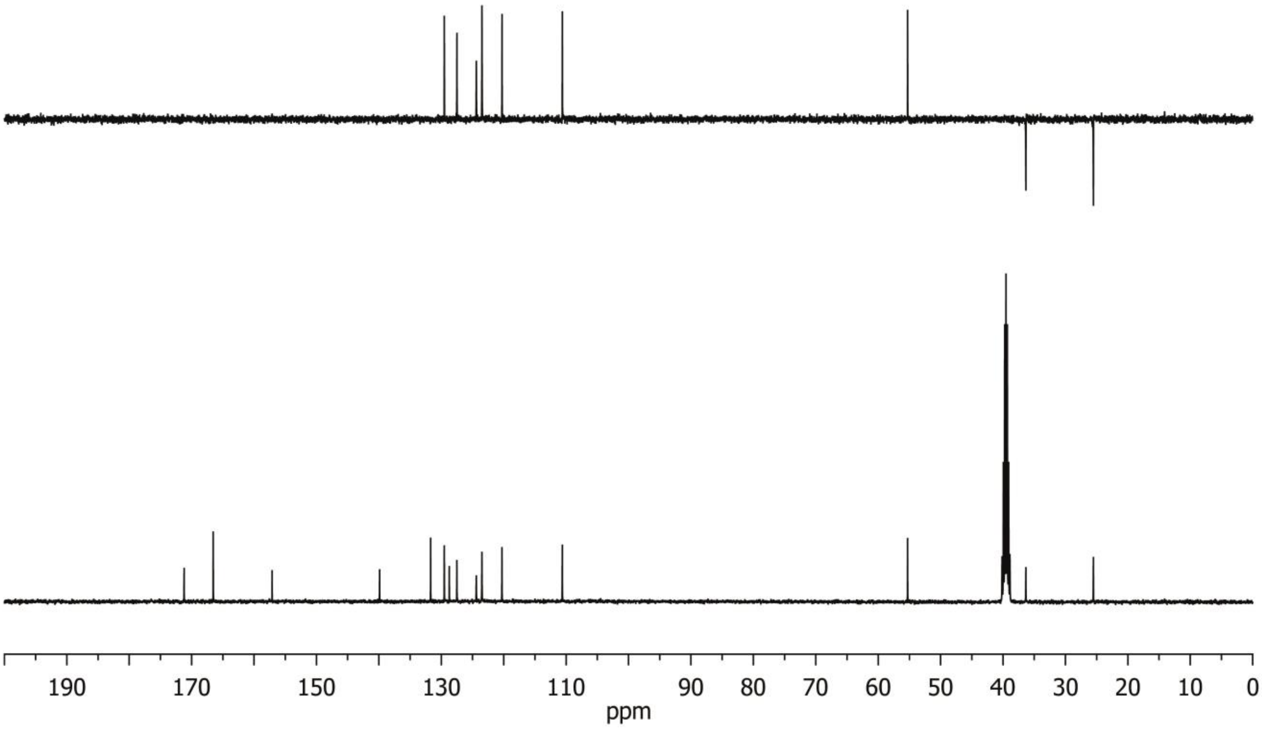
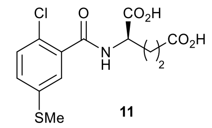
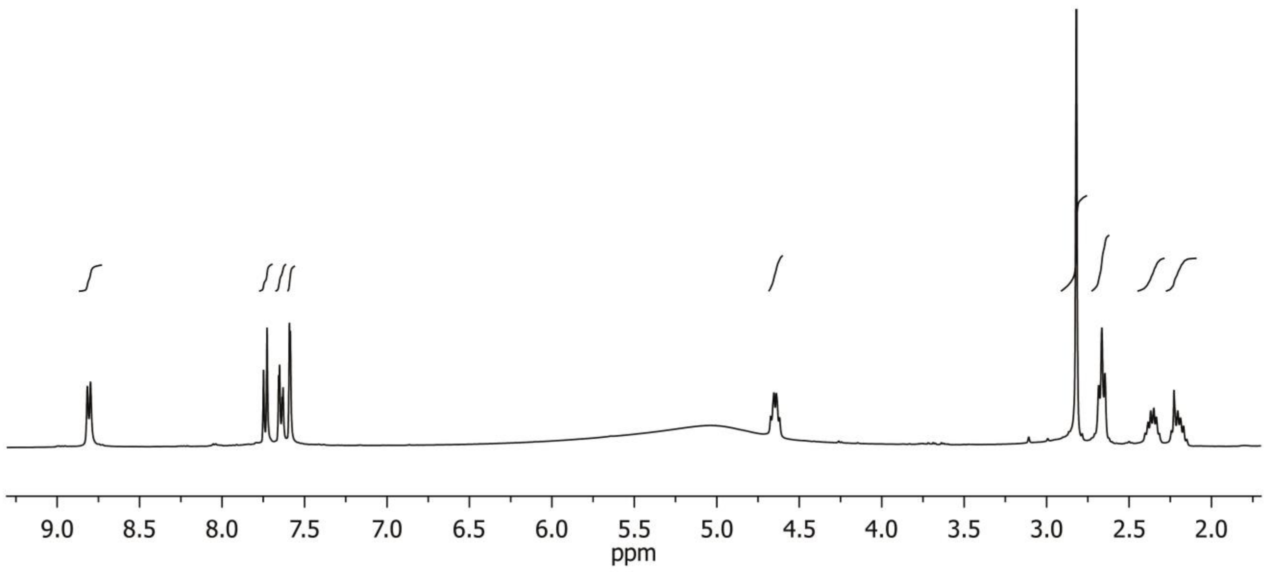
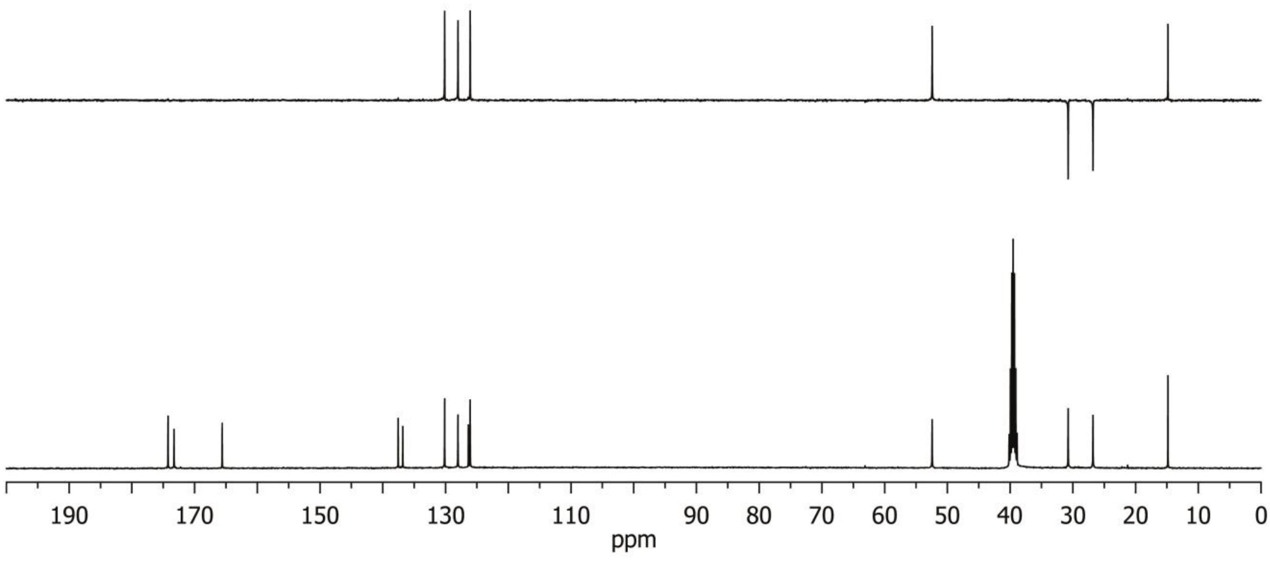
3.5
3.0
2.5
ppm

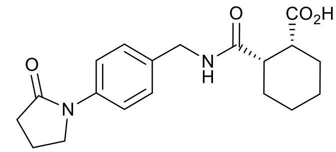

190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
ppm



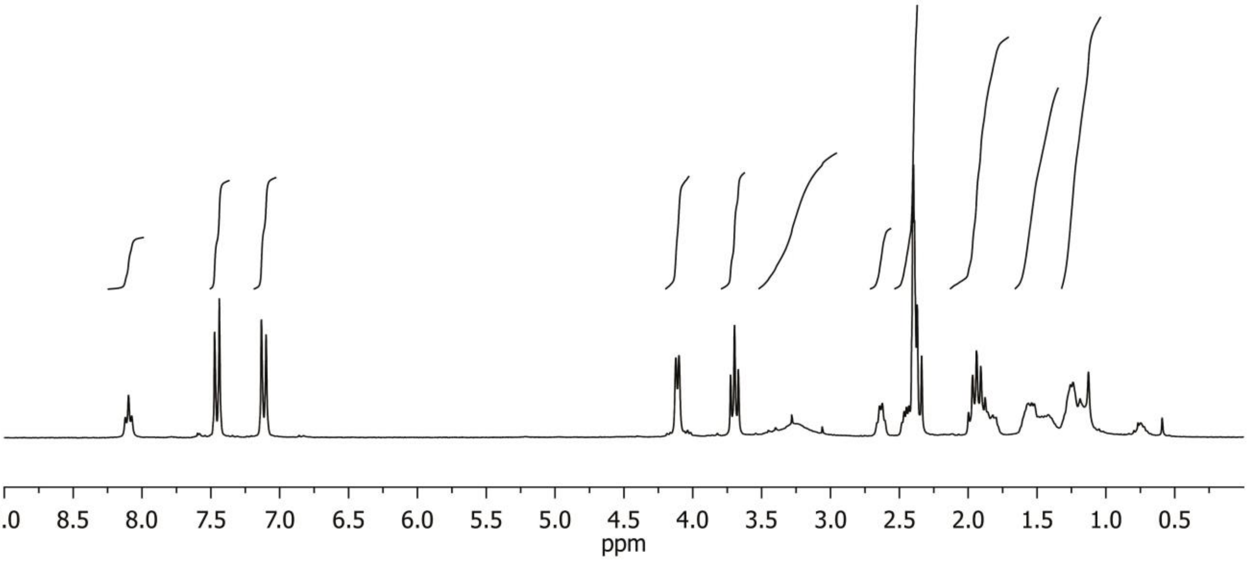
11

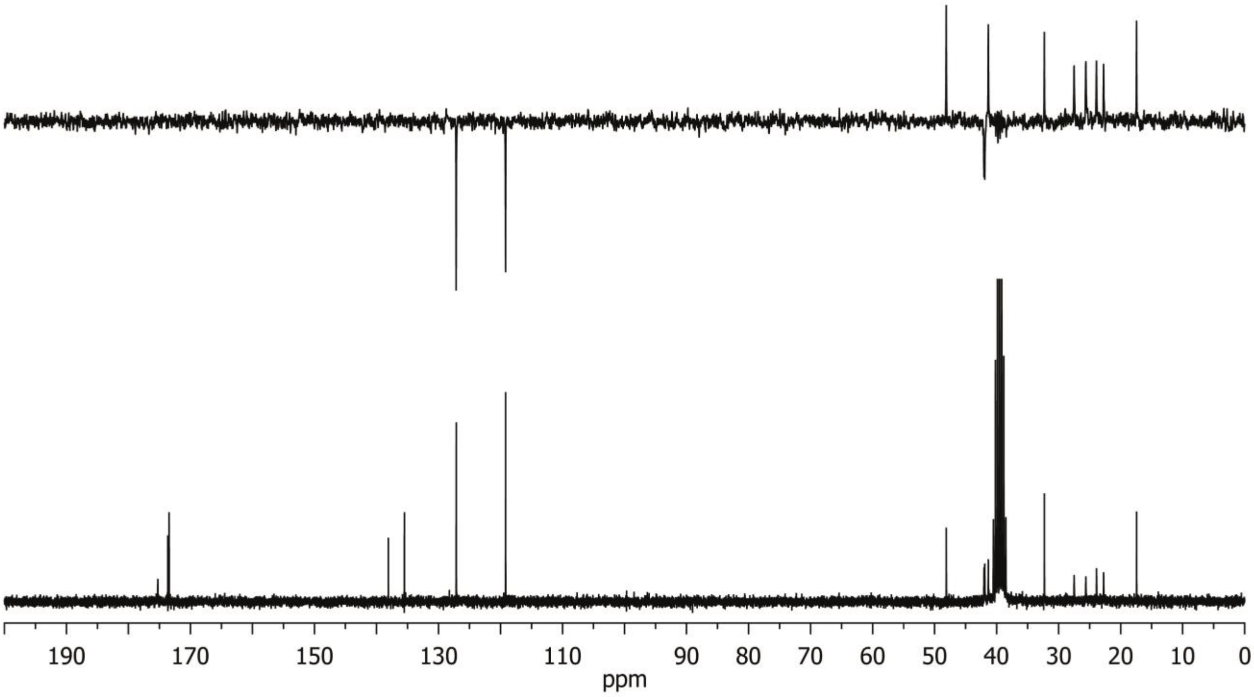

9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm

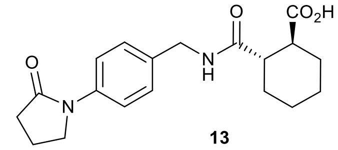

190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
ppm


O
CO2H
O
N
N
H

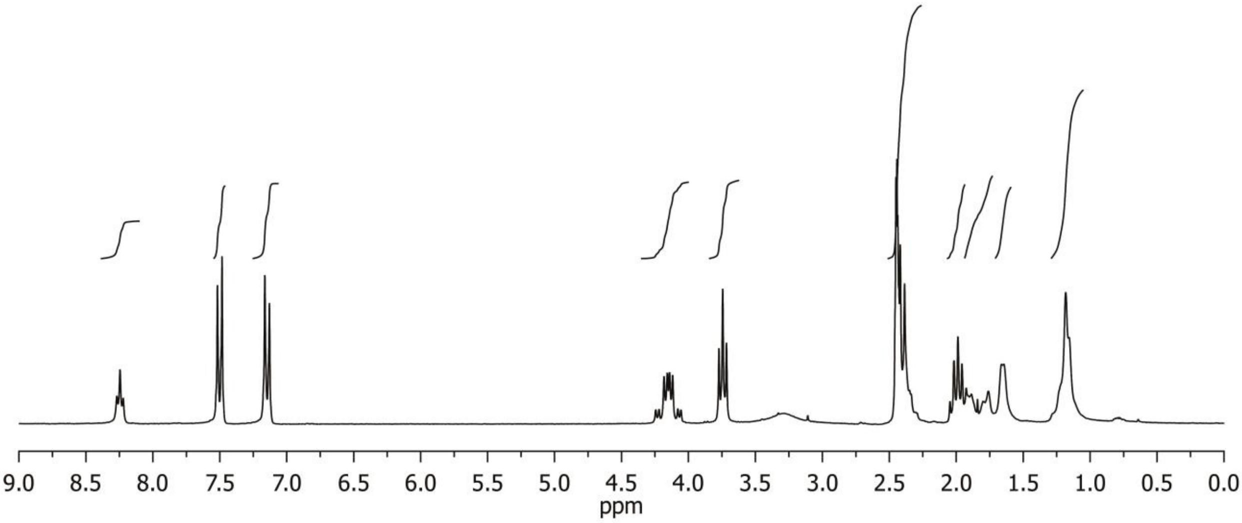
12

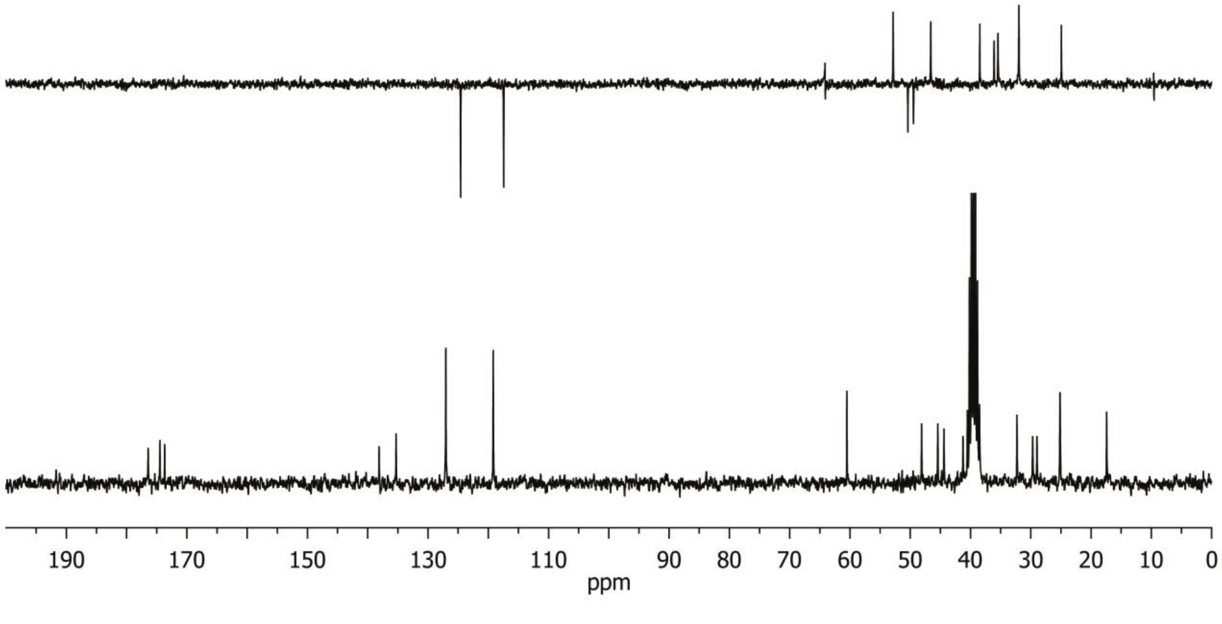

9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

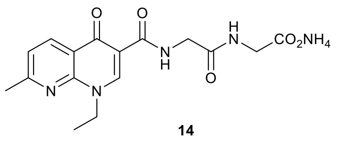

190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
ppm


O
CO2H
N
H
O
N

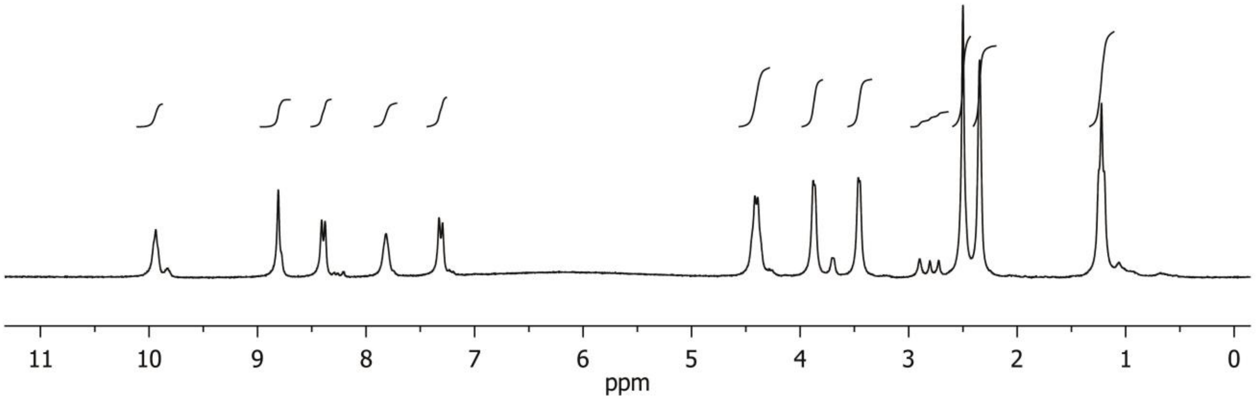
13

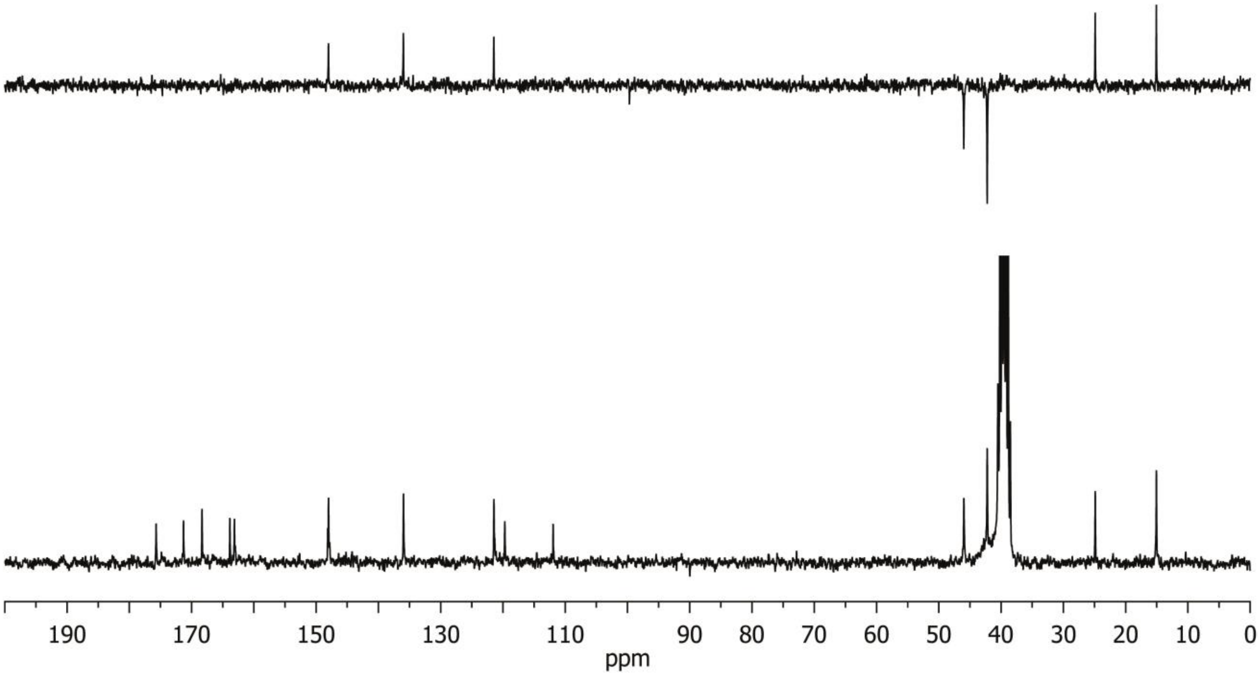

9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

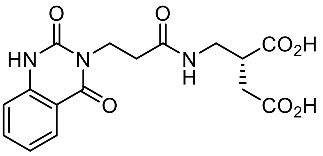

190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
ppm


O
O
H
N
N
H
CO2NH4
O
N
N

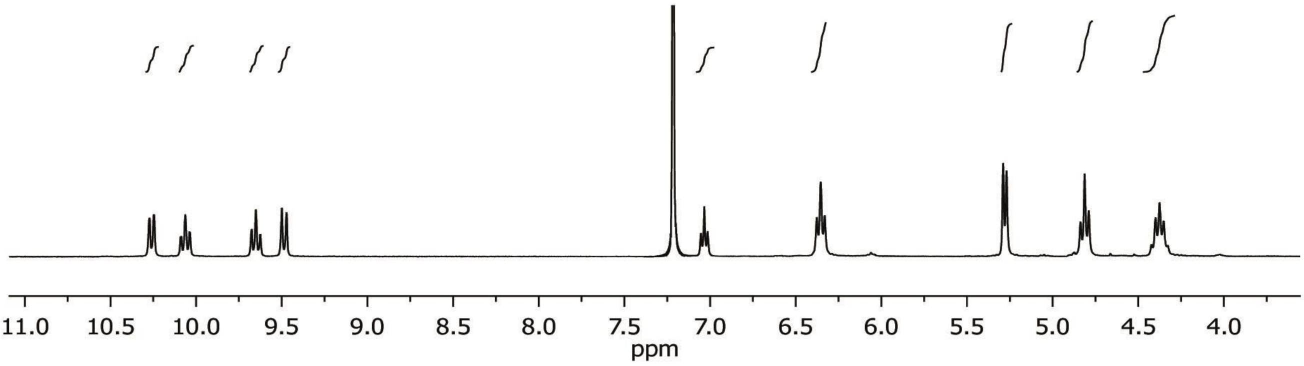
14

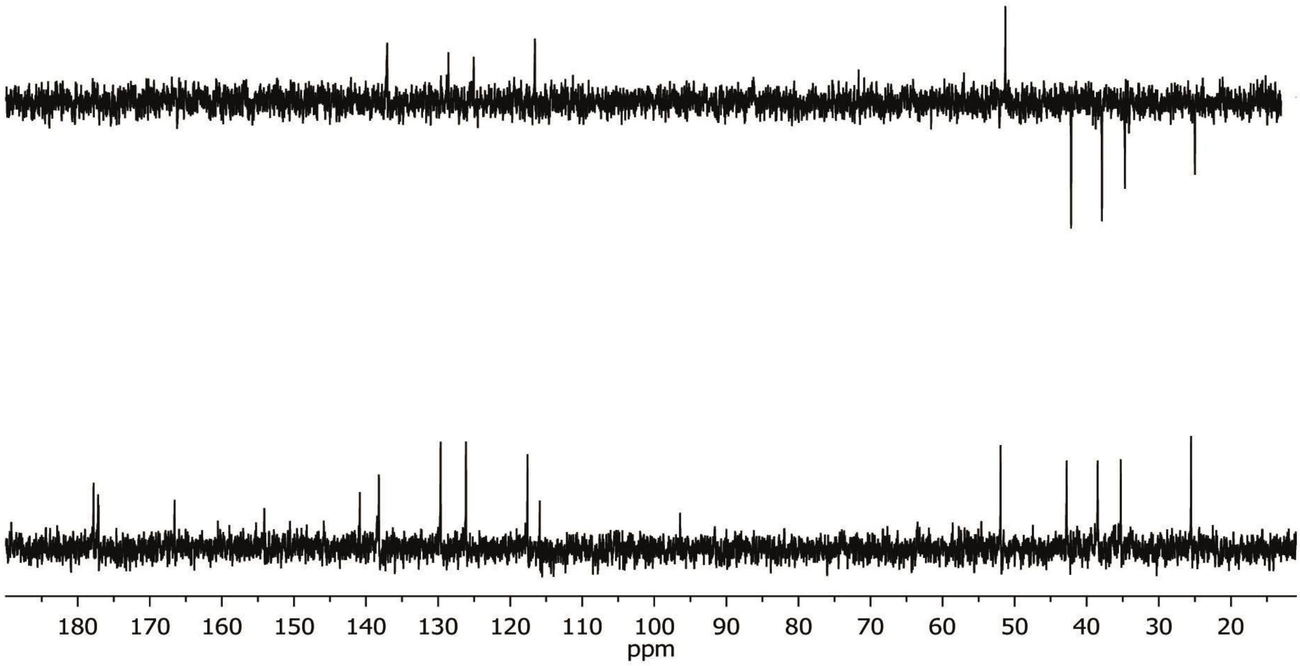

11
10
9
8
7
6
5
4
3
2
1
0
ppm


190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
ppm


O
HN
N
O
O
N
H
CO2H
CO2H

17*S*


11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm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160
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140
130
120
110
100
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80
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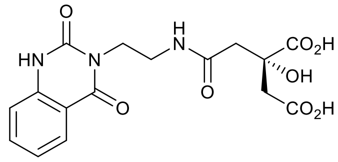
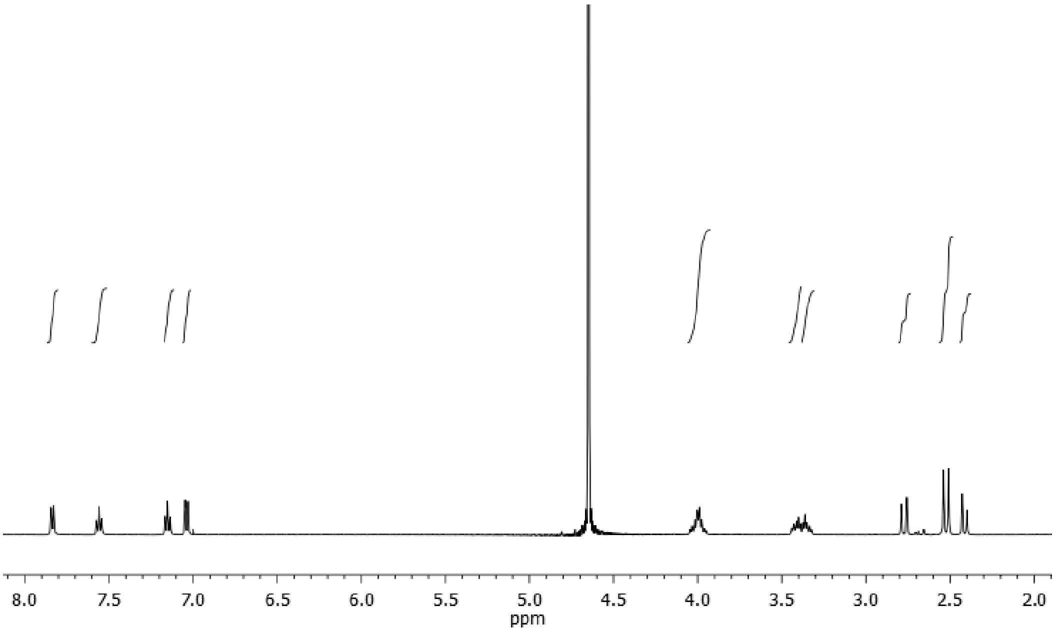
50
40
30
20
ppm



18


8.0
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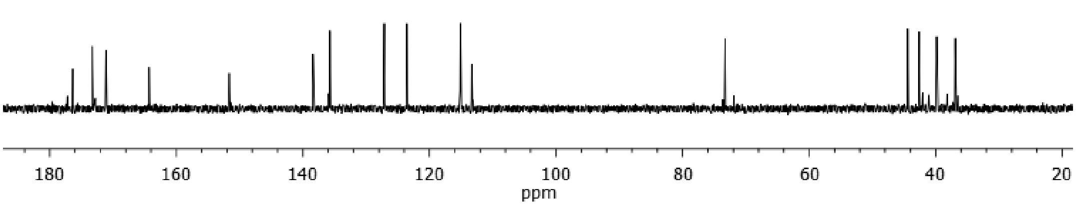
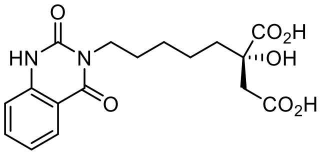
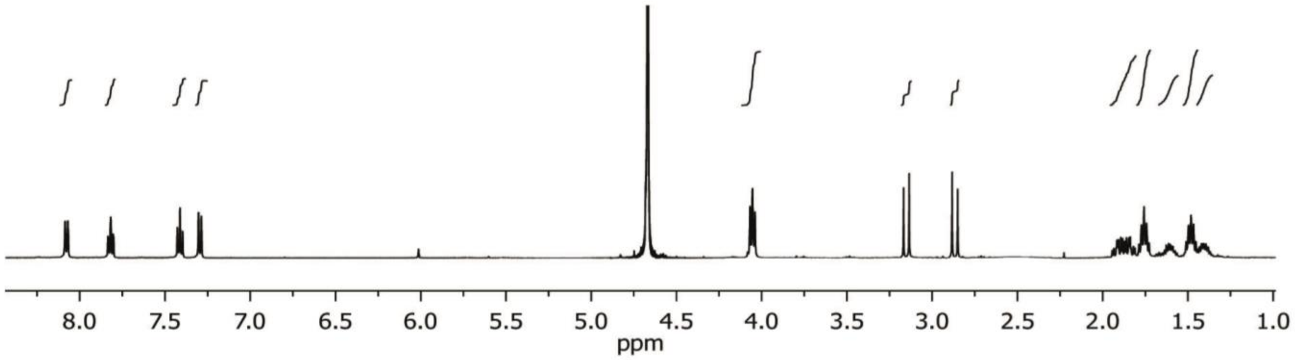
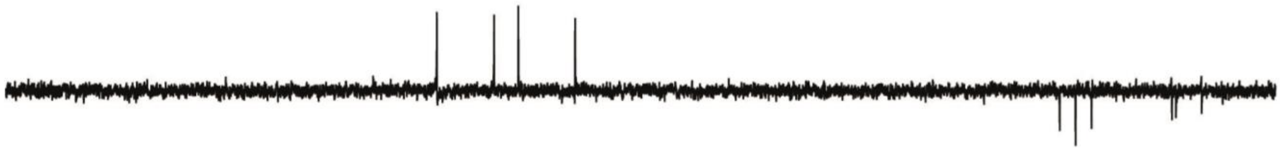
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm

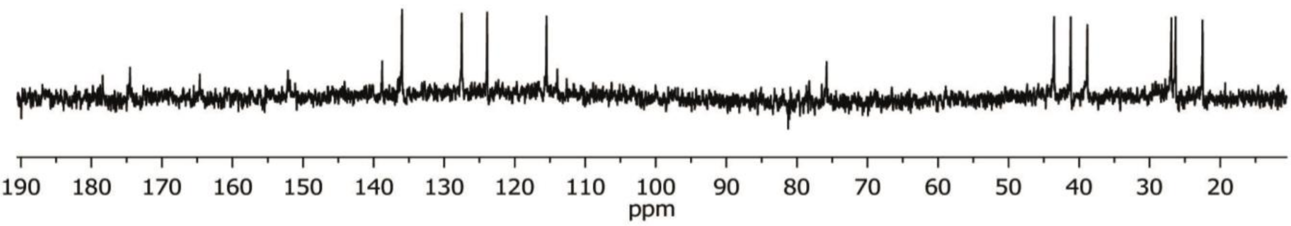

180
160
140
120
100
80
60
40
20
ppm


O
HN
N
O
CO2H
OH
CO2H

19


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm




190
180
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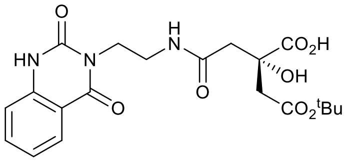
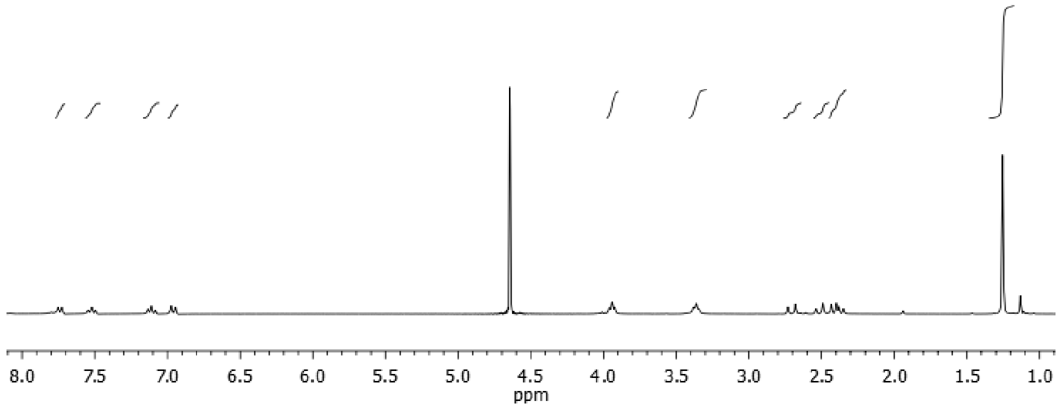
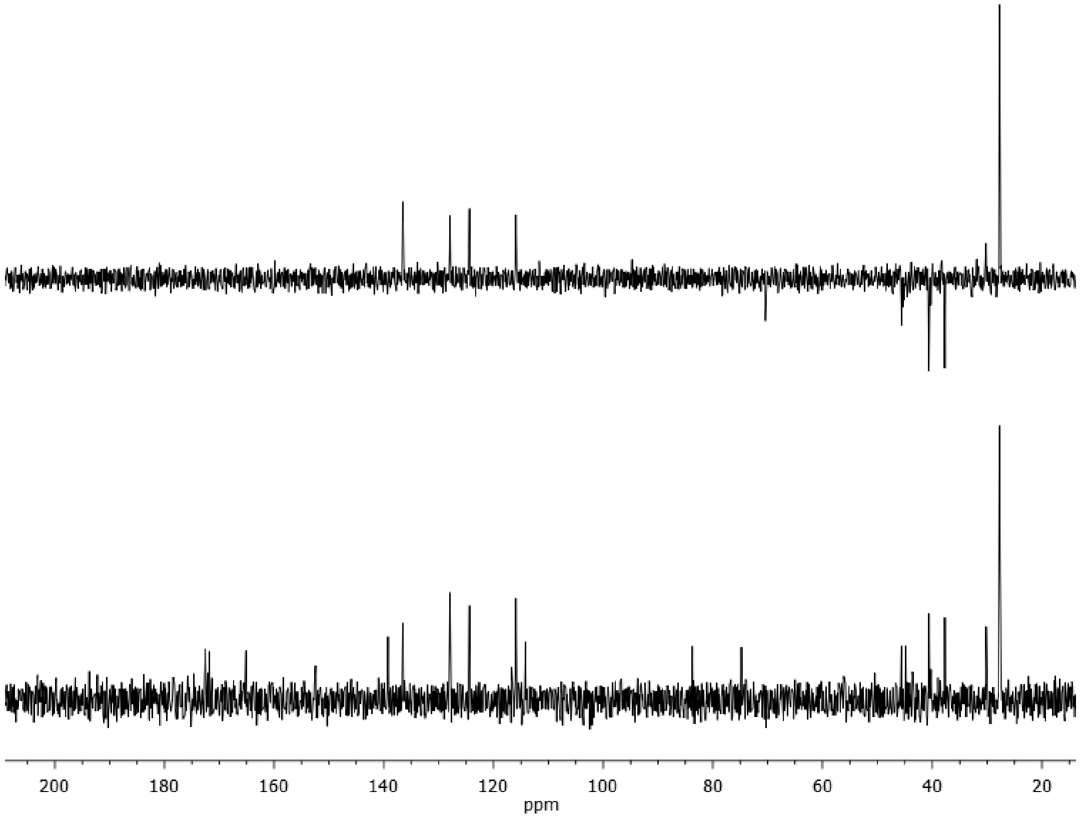
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

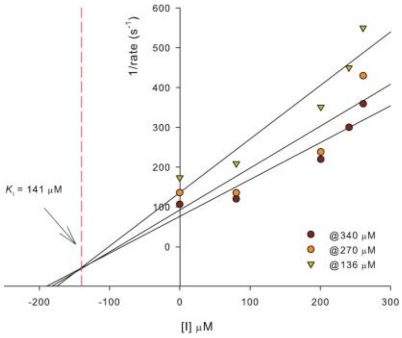

HN
N
H
N
O
O
O
CO2H
OH
CO2tBu

32

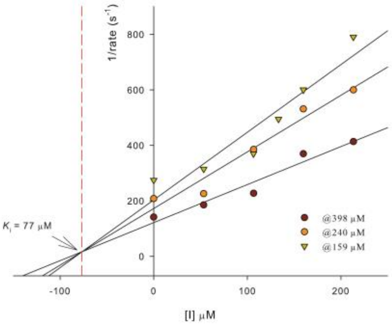

8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


200
180
160
140
120
100
80
60
40
20
ppm

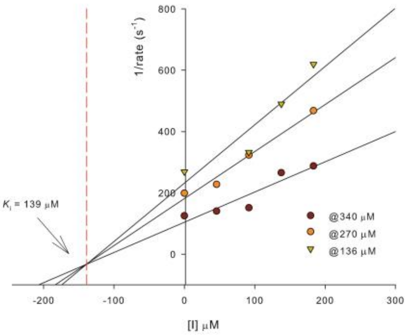
compound 8



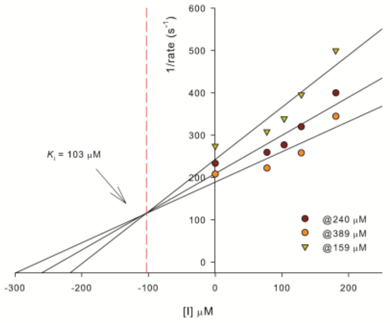
compound 9



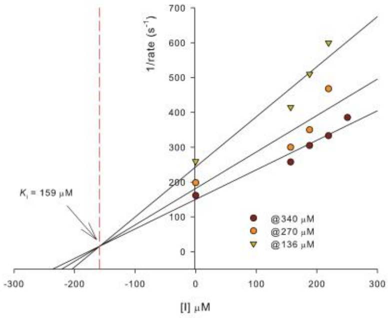
compound 10



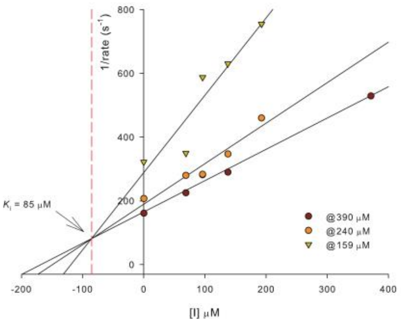
compound **11**



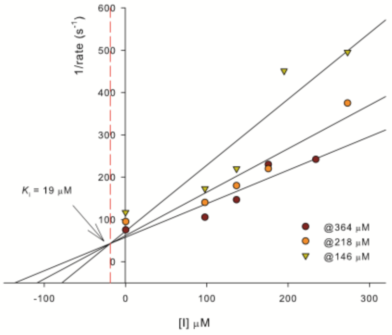
compound 12



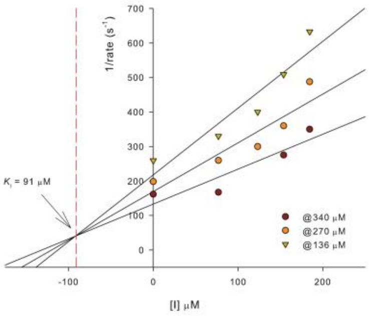
compound 14



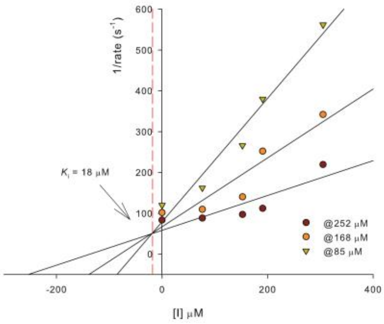
compound 15



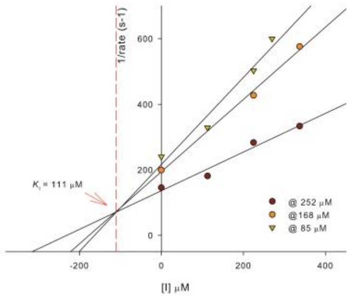
compound 16



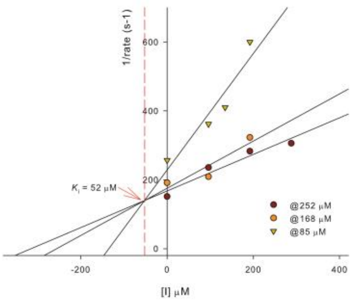
Compound 18



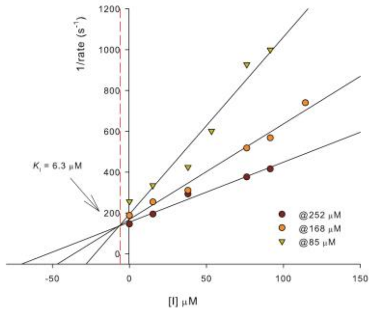
Compound 17S

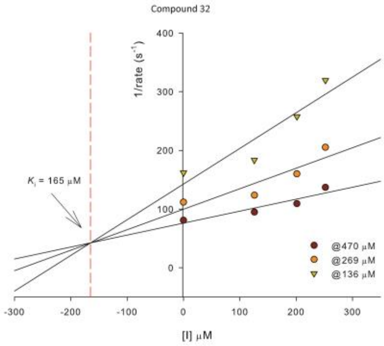


Compound 17R



Compound 19




Compound 32
1/rate (s^{-1})
400
300
200
100
0
K_i = 165 μM
@470 μM
@269 μM
@136 μM
-300
-200
-100
0
100
200
300
[I] μM