Electronic Supplementary Information (ESI) for:

Irreversible Covalent Modification of Type I Dehydroquinase by a Stable Schiff Base

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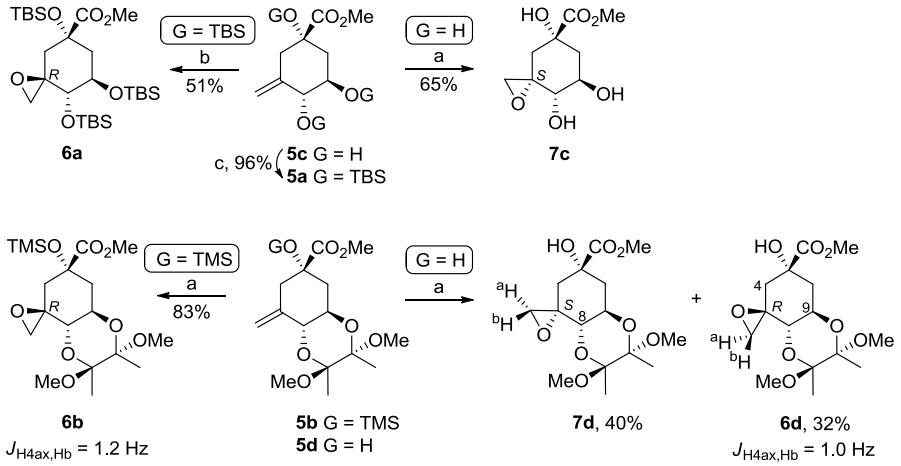
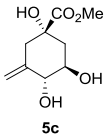
Table of Contents:

Supplemental Experimental Procedures



Scheme S1. Epoxidation of other alkenes related to Alkene **9**: effects on diastereoselectivity. *Reagents and conditions*: a) MCPBA, $NaHCO_3$, DCM, RT. b) UHP, TFAA, Na_2HPO4, DCM, -78 ºC. c) TBSOTf, Py, DCM, 0 ºC to RT.

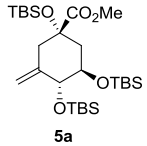
Methyl (1*S*,3*R*,4*R*)-1,3,4-trihydroxy-5-methylenecyclohexane-1-carboxylate (5c)



5c

A stirred solution of acetal **5d** (1.0 g, 3.29 mmol) in a (20:1) TFA/H_2O solution (33 mL) was stirred at room temperatura for 15 min. The solvent was removed under reduced pressure and the residue resulted was purified by flash chromatography eluting with ethyl acetate to afford compound **5c** (630 mg, 95%) as a colorless oil. $[\alpha]_D^{20}$ = –3° (*c* 1.0, MeOH). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 5.25 (s, 1H, C*H*HC), 4.99 (s, 1H, CH*H*C), 3.97 (d, *J* = 8.7 Hz, 1H, H4), 3.81 (s, 3H, OCH_3), 3.74 (m, 1H, H3), 2.95 (s, 1H, OH), 2.64 (d, *J* = 13.7 Hz, 1H, $H6_{ax}$), 2.39 (dd, *J* = 13.7 and 2.7 Hz, 1H, $H6_{eq}$), 2.13 (ddd, *J* = 13.0, 5.0 and 3.0 Hz, 1H, $H2_{eq}$) and 1.99 (dd, *J* = 13.0 and 11.2 Hz, 1H, $H2_{ax}$) ppm. ^{13}C NMR (75 MHz, CD_3OD) δ: 176.7 (C), 145.1 (C), 110.8 (CH_2), 78.2 (CH), 75.5 (C), 72.7 (CH), 53.0 (CH_3), 43.4 (CH_2) and 42.0 (CH_2) ppm. IR (film) υ: 3400 (OH) and 1730 (CO) cm^{-1}. MS (ESI) *m/z* = 225 (M + Na$^+$). HRMS calcd for $C_9H_{14}O_5Na$ (M + Na$^+$): 225.0733; found, 225.0737.

Methyl (1*S*,3*R*,4*R*)-1,4,5-tri(*tert*-butyldimethylsilyloxy)-3-methylenecyclohexane-1-carboxylate (5a)

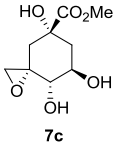


5a

A stirred solution of the triol **5c** (423 mg, 2.09 mmol) and dry pyridine (0.9 mL, 10.99 mmol) in dry dichloromethane (7.0 mL) under argon was cooled at 0 ºC and then it was treated with TBSOTf (2.5 mL, 10.99 mmol). The resulting mixture was stirred at room temperature for 20 h and then diluted succesively with dichloromethane and water. The aqueous layer was acidified with HCl (10%) and the organic phase was separated. The aqueous layer was extracted with dichloromethane (×3). All combined organic extracts were dried (anh. Na_2SO_4), filtered and evaporated under reduced pressure. The obtained residue was purified by flash chromatography eluting with (50:50)

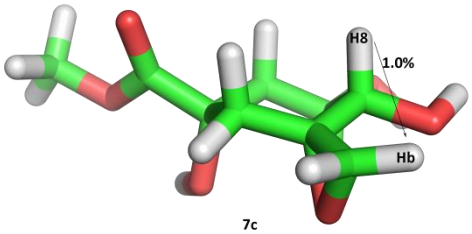
ethyl acetate-hexane to afford silyl ether **5a** (1.1 g, 96%) as a white solid. Mp: 66–69 °C. $[\alpha]_D^{20}$ = –22° (*c* 0.7, CH_3OH). ^1H NMR (250 MHz, $CDCl_3$) δ: 5.15 (br s, 1H, C*H*HC), 4.90 (br s, 1H, CH*H*C), 3.77 (m, 2H, H5+H4), 3.69 (s, 3H, OCH_3), 2.62 (d, *J* = 13.5 Hz, 1H, $H2_{ax}$), 2.45 (dd, *J* = 13.5 and 1.2 Hz, 1H, $H2_{eq}$), 2.11 (m, 1H, $H6_{eq}$), 1.96 (dd, *J* = 13.5 and 8.0 Hz, 1H, $H6_{ax}$), 0.92 (s, 9H, $C(CH_3)_3$), 0.87 (s, 9H, $C(CH_3)_3$), 0.84 (s, 9H, $C(CH_3)_3$), 0.08 (s, 3H, CH_3), 0.08 (s, 3H, CH_3), 0.07 (s, 3H, CH_3), 0.06 (s, 6H, 2×CH_3) and 0.02 (s, 3H, CH_3) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 173.8 (C), 143.7 (C), 113.2 (CH_2), 77.7 (C), 77.5 (CH), 72.8 (CH), 52.1 (OCH_3), 43.0 (CH_2), 42.7 (CH_2), 26.3 ($C(CH_3)_3$), 26.1 ($C(CH_3)_3$), 25.9 ($C(CH_3)_3$), 18.8 ($C(CH_3)_3$), 18.5 ($C(CH_3)_3$), 18.3 ($C(CH_3)_3$), –2.6 (CH_3), –2.7 (CH_3), –3.1 (CH_3), –3.9 (CH_3), –4.2 (CH_3) and –4.6 (CH_3) ppm. IR (KBr) υ: 1755 (CO) cm^{-1}. MS (ESI) *m/z* = 567 ($M + Na^+$). HRMS calcd for $C_{27}H_{56}O_5Si_3Na$ ($M + Na^+$): 567.3328; found, 567.3320.

Methyl (3*S*,5*R*,7*R*,8*S*)-5,7,8-trihydroxy-1-oxaspiro[2.5]octane-5-carboxylate (7c)



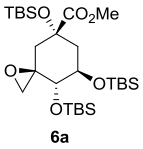
7c

A stirred solution of alkene **5c** (50 mg, 0.25 mmol) in dry dichloromethane (2.5 mL), at room temperature and under argon, was treated with MCPBA (52 mg, 0.62 mmol) and sodium bicarbonate (64 mg, 0.37 mmol). After stirring for 20 h, potassium carbonate was added and the suspension was filtered. The filtrate and the washings were concentrated under reduced pressure. The resulted residue was purified by flash chromatography eluting with a gradient of ethyl acetate/hexanes [(75:25) to (100:0)] to afford epoxide **7c** (35 mg, 65%), as a beige solid. The stereochemistry of C1 in **7c** is supported by the NOE enhancement of the H_b signal (1%) upon irradiation of the H8 signal. Mp: 125–128 °C. $[\alpha]_D^{20}$ = –11° (*c* 1.3, CH_3OH). ^1H NMR (250 MHz, CD_3OD) δ: 3.94 (m, 1H, H7), 3.73 (s, 3H, OCH_3), 3.59 (d, *J* = 9.0 Hz, 1H, H8), 2.88 (d, *J* = 5.0 Hz, 1H, C*H*HO(H_b)), 2.47 (d, *J* = 14.7 Hz, 1H, $H4_{ax}$), 2.44 (d, *J* = 5.0 Hz, 1H, CH*H*O(H_a)), 2.17 (ddd, *J* = 13.5, 4.5 and 3.0 Hz, 1H, $H6_{eq}$), 1.90 (dd, *J* = 13.5 and 11.0 Hz, 1H, $H6_{ax}$) and 1.59 (dd, *J* = 14.5 and 3.0 Hz, 1H, $H4_{eq}$) ppm. ^{13}C NMR (63 MHz, CD_3OD) δ: 176.4 (C), 75.8 (C), 73.8 (CH), 70.2 (CH), 59.0 (C), 53.0 (OCH_3), 47.1 (OCH_2), 41.8 (CH_2) and 40.6 (CH_2) ppm. IR (KBr) υ: 3419 (OH) and 1732 (CO) cm^{-1}. MS (ESI) *m/z* = 241 ($M + Na^+$). HRMS calcd for $C_9H_{14}O_6Na$ ($M + Na^+$): 241.0683; found, 241.0671.



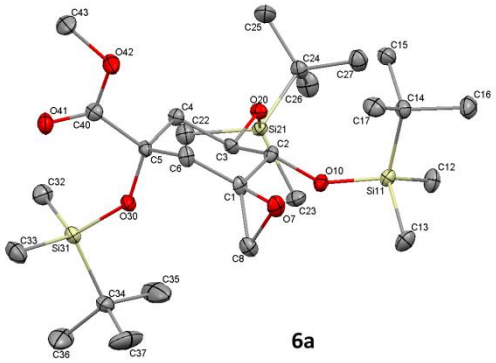
7c

Methyl (3*S*,5*R*,7*R*,8*S*)-5,7,8-tri(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-5-carboxylate (6a)



6a

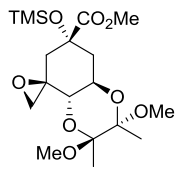
A stirred suspension of alkene **5a** (80 mg, 0.15 mmol), urea hydrogen peroxide adduct (UHP) (128 mg, 1.32 mmol) and Na_2HPO_4 (209 mg, 1.47 mmol) in dry dichloromethane, at –78 ºC and under inert atmosphere, was treated with freshly distilled trifluoroacetic anhydride (82 μL, 0.59 mmol). The resultant solution was stirred for 18 h during which time the reaction mixture was slowly warming up to room temperature. Powdered K_2CO_3 was added and after 15 min, the suspension was filtered and washed with dichloromethane. The filtrate and the washings were concentrated under reduced pressure to give a solid which was purified by flash chromatography eluting with a gradient of diethyl ether/hexanes [1) (0:100), 2) (5:95)], to yield epoxide **6a** (43 mg, 51%) as a white solid. The compound was crystallized from diethyl ether/hexanes and the stereochemistry of the new chiral center was determined by X-ray crystallography (see Figure S1). Mp: 121–123 °C. $[\alpha]_D^{20}$ = –12 ° (*c* 1.1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 3.88 (m, 1H, H7), 3.72 (s, 3H, OCH_3), 3.61 (d, *J* = 8.2 Hz, 1H, H8), 2.98 (br d, *J* = 5.7 Hz, 1H, C*H*HO(H_b)), 2.51 (d, *J* = 5.7 Hz, 1H, CH*H*O(H_a)), 2.30 (br d, *J* = 13.7 Hz, 1H, $H4_{ax}$), 2.16 (ddd, *J* = 13.5, 4.2 and 3.0 Hz, 1H, $H6_{eq}$), 1.86 (dd, *J* = 13.7 and 10.7 Hz, 1H, $H6_{ax}$), 1.58 (dd, *J* = 13.7 and 3.0 Hz, 1H, $H4_{eq}$), 0.88 (s, 18H, 2×$C(CH_3)_3$), 0.87 (s, 9H, $C(CH_3)_3$), 0.09 (s, 3H, CH_3), 0.08 (s, 6H, 2×CH_3), 0.08 (s, 3H, CH_3), 0.07 (s, 3H, CH_3) and 0.06 (s, 3H, CH_3) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 173.4 (C), 76.4 (C), 75.4 (CH), 71.7 (CH), 57.7 (C), 52.3 (OCH_3), 49.1 (OCH_2), 43.5 (CH_2), 42.4 (CH_2), 26.2 ($C(CH_3)_3$), 26.1 ($C(CH_3)_3$), 26.1 ($C(CH_3)_3$), 18.7 ($C(CH_3)_3$), 18.3 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), –3.1 (CH_3), –3.2 (CH_3), –3.6 (CH_3), –3.7 (CH_3), –4.4 (CH_3) and –4.4 (CH_3) ppm. IR (KBr) υ: 1755 (CO) cm^{-1}. MS (ESI) *m/z* = 583 (M + Na^+). HRMS calcd for $C_{27}H_{56}O_5Si_3Na$ (M + Na^+): 583.3277; found, 583.3275.



6a

Figure S1. X-ray structure of epoxide **6a**. Ellipsoids are shown at the 50% probability level. Hydrogen atoms are not shown for clarity. Structure figure was prepared using Mercury 2.3 (http://www.ccdc.cam.ac.uk/mercury/). CCDC 993317 contains the supplementary crystallographic data for this structure. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (+44)-1223-336033; E-mail: deposit@ccdc.cam.ac.uk].

Epoxide 6b

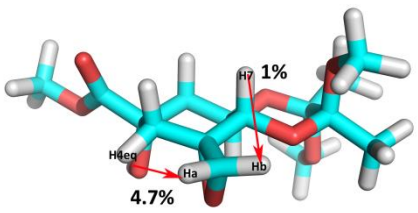


6b

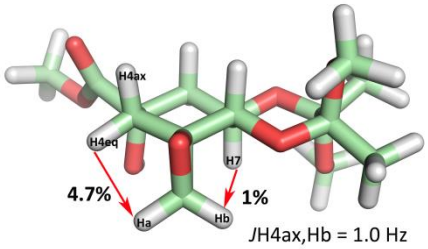
A stirred solution of alkene **5b**[20] (234 mg, 0.60 mmol) in dry dichloromethane (6.0 mL), at room temperature and under argon, was treated with MCPBA (258 mg, 1.50 mmol) and sodium bicarbonate (75 mg, 0.90 mmol). After stirring for 20 h, potassium carbonate was added and the suspension was filtered. The filtrate and the washings were concentrated under reduced pressure. The resulted residue was purified by flash chromatography eluting with a gradient of ethyl acetate/hexanes [(15:85) to (20:80)] to afford epoxide **6b** (202 mg, 83%), as a white solid. The w-coupling constant between $H4_{ax}$ and H_b (1.2 Hz) supports the C1 stereochemistry of **6b** as for epoxide **11**. $[\alpha]_D^{20}$ = +77° (*c* 1.0, $CDCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 4.01 (ddd, *J* = 11.5, 9.5 and 5.0 Hz, 1H, H7), 3.81 (d, *J* = 11.5 Hz, 1H, H8), 3.76 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 3.11 (dd, *J* = 5.5 and 1.2 Hz, 1H, C*H*HO(H_b)), 2.62 (d, *J* = 5.5 Hz, 1H, CH*H*O(H_a)), 2.36 (dd, *J* = 13.5 and 1.2 Hz, 1H, $H4_{ax}$), 2.03 (ddd, *J* = 13.0, 4.7 and 2.5 Hz, 1H, $H6_{eq}$), 1.93 (dd, *J* = 13.0 and 11.5 Hz, 1H, $H6_{ax}$), 2.72 (dd, *J* = 13.5 and 2.5 Hz, 1H, $H4_{eq}$), 1.26 (s, 3H, CH_3), 1.24 (s, 3H, CH_3) and 0.15 (s, 9H, 3×CH_3) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 173.7 (C), 99.7 (C), 99.5 (C), 77.5 (C), 69.5 (CH), 66.5 (CH), 52.4 (OCH_3), 50.2 (CH_2), 48.0 (2×CH_3), 42.9 (OCH_2), 38.4 (CH_2), 17.8 (CH_3), 17.6 (CH_3) and 1.7 (3×CH_3) ppm. IR (KBr) υ: 1743 (CO) cm^{-1}. MS (ESI) *m/z* = 427 (M + Na^+). HRMS calcd for $C_{18}H_{32}O_8SiNa$ (M + Na^+): 427.1759; found, 427.1758.

Epoxidation of alkene 5d

A stirred solution of alkene **5d** (150 mg, 0.49 mmol) in dry dichloromethane (4.9 mL), at room temperature and under argon, was treated with MCPBA (211 mg, 1.23 mmol) and sodium bicarbonate (62 mg, 0.74 mmol). After stirring for 20 h, potassium carbonate was added and the suspension was filtered. The filtrate and the washings were concentrated under reduced pressure. The resulted residue was purified by flash chromatography eluting with (30:70) ethyl acetate/hexanes to afford epoxides **7d** (65 mg, 40%) and **6d** (53 mg, 32%). The stereochemistry of new chiral center of both epoxides was determined by NOE experiments. The stereochemistry of C1 in **7d** and **6d** is supported by the NOE enhancement of the respective H_b signals (1%) upon irradiation of the H8 and H7 signals, respectively.



7d



6d

7d: Brown oil. $[\alpha]_D^{20}$= +84° (*c* 1.5, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$) δ: 4.25 (ddd, *J* = 12.5, 10.5 and 4.5 Hz, 1H, H7), 3.89 (d, *J* = 10.5 Hz, 1H, H8), 3.78 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 3.07 (d, *J* = 5.5 Hz, 1H, C*H*HO), 2.54 (d, *J* = 14.5 Hz, 1H, $H4_{ax}$), 2.48 (d, *J* = 5.5 Hz, 1H, CH*H*O), 2.13 (ddd, *J* = 12.5, 4.5 and 3.0 Hz, 1H, $H6_{eq}$), 2.02 (t, *J* = 12.5 Hz, 1H, $H6_{ax}$), 1.52 (dd, *J* = 14.5 and 3.0 Hz, 1H, $H4_{eq}$), 1.30 (s, 3H, CH_3) and 1.28 (s, 3H, CH_3) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 174.6 (C), 100.1 (C), 99.4 (C), 74.8 (C), 68.3 (CH), 65.1 (CH), 56.8 (C), 53.0 (OCH_3), 47.9 (2×OCH_3), 45.1 (OCH_2), 40.4 (CH_2), 38.2 (CH_2), 17.7 (CH_3) and 17.6 (CH_3) ppm. IR (film) υ: 3435 (OH) and 1728 (CO) cm^{-1}. MS (ESI) *m/z* = 355 ($M + Na^+$). HRMS calcd for $C_{15}H_{24}O_8Na$ ($M + Na^+$): 355.1363; found, 355.1358.

6d: White solid. Mp: 111–113 °C. $[\alpha]_D^{20}$= +79° (*c* 1.4, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$) δ: 4.10 (ddd, *J* = 12.5, 9.5 and 4.5 Hz, 1H, H7), 3.87 (d, *J* = 9.5 Hz, 1H, H7), 3.81 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.24 (m, 1H, C*H*HO(H_b)), 3.23 (s, 3H, OCH_3), 3.18 (br s, 1H, OH), 2.67 (d, *J* = 5.5 Hz, 1H, CH*H*O(H_a)), 2.46 (dd, *J* = 13.5 and 1.0 Hz, 1H, $H4_{ax}$), 2.04 (dd, *J* = 13.0 and 12.5 Hz, 1H, $H6_{ax}$), 1.91 (ddd, *J* = 13.0, 4.5 and 2.5 Hz, 1H, $H6_{eq}$), 1.42 (dd, *J* = 13.5 and 2.5 Hz, 1H, $H4_{eq}$), 1.28 (s, 3H, CH_3) and 1.26 (s, 3H, CH_3) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 175.5 (C), 99.7 (C), 99.5 (C), 73.6 (C), 69.4 (CH), 66.3 (CH), 56.5 (C), 53.3 (OCH_3), 50.4 (OCH_2), 47.9 (OCH_3), 47.9 (OCH_3), 41.3 (CH_2), 37.8 (CH_2), 17.7 (CH_3) and 17.5 (CH_3) ppm. IR (KBr) υ: 3438 (OH) and 1745 (CO) cm^{-1}. MS (ESI) *m/z* = 355 ($M + Na^+$). HRMS calcd for $C_{15}H_{24}O_8Na$ ($M + Na^+$): 355.1363; found, 355.1361.

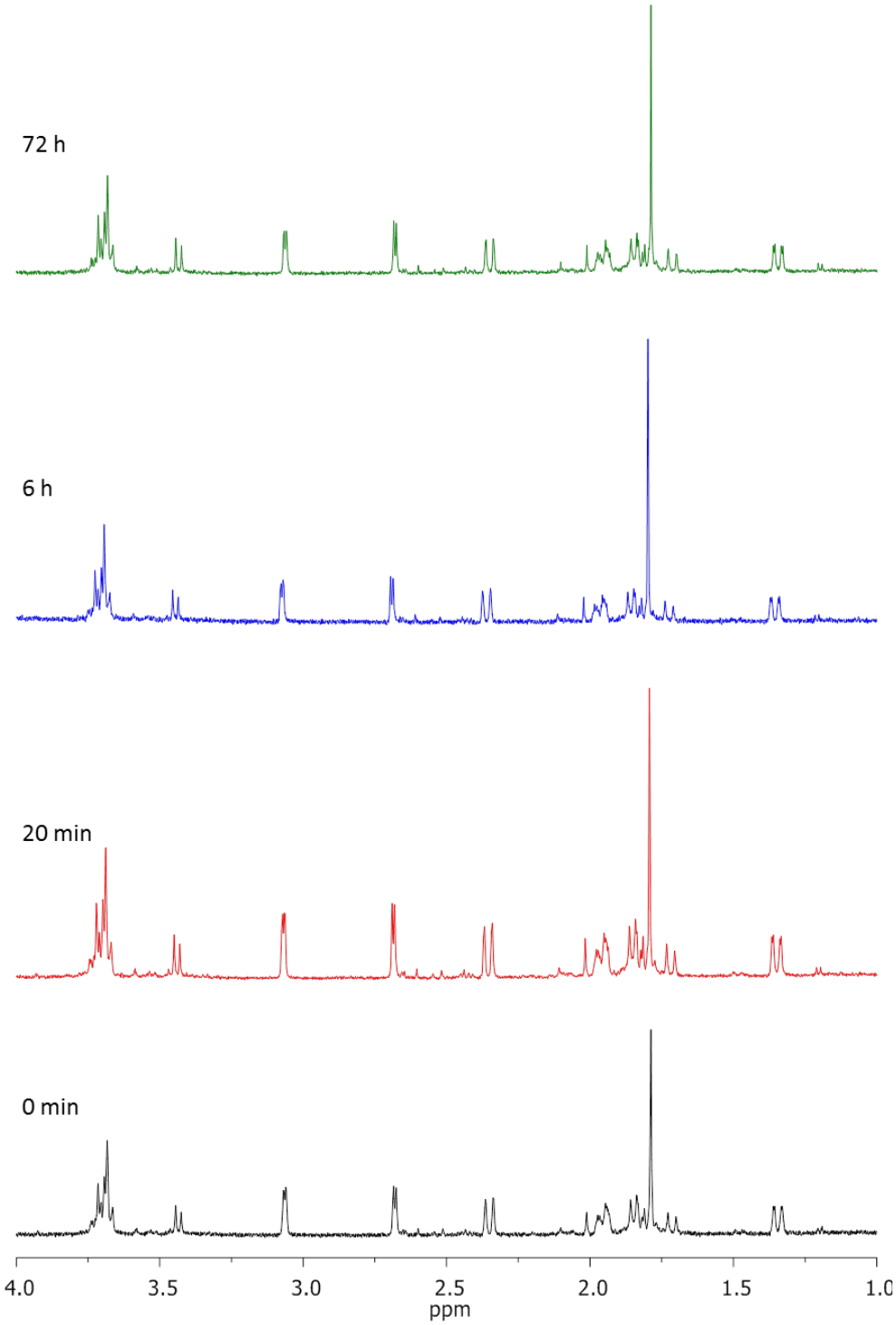


Figure S2. Monitoring incubation of epoxide **4** with *St*-DHQ1. ^{1}H NMR (500 MHz, 25 ºC) spectra of a sample of epoxide **4** in potassium phosphate buffer (50 mM, pH 7.2) after incubation over 72 h with *St*-DHQ1 (3 units). The sample contains 80% D_2O.

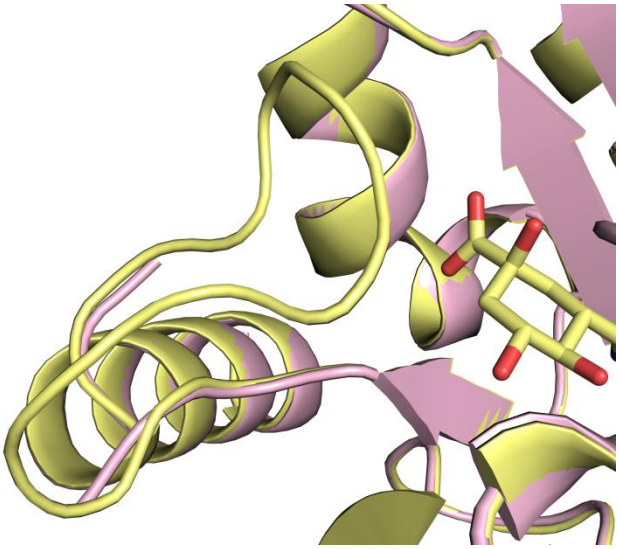


Figure S3. *St*-DHQ1 crystal structure covalently modified by compound **3**. Superposition of chains A (pink) and B (yellow) of *St*-DHQ1 crystal structure covalently modified by compound **3** (PDB entry 4CLM). Note how residues 228–236 are not solved in chain A. Modified compound **3** is not visible in chain A.

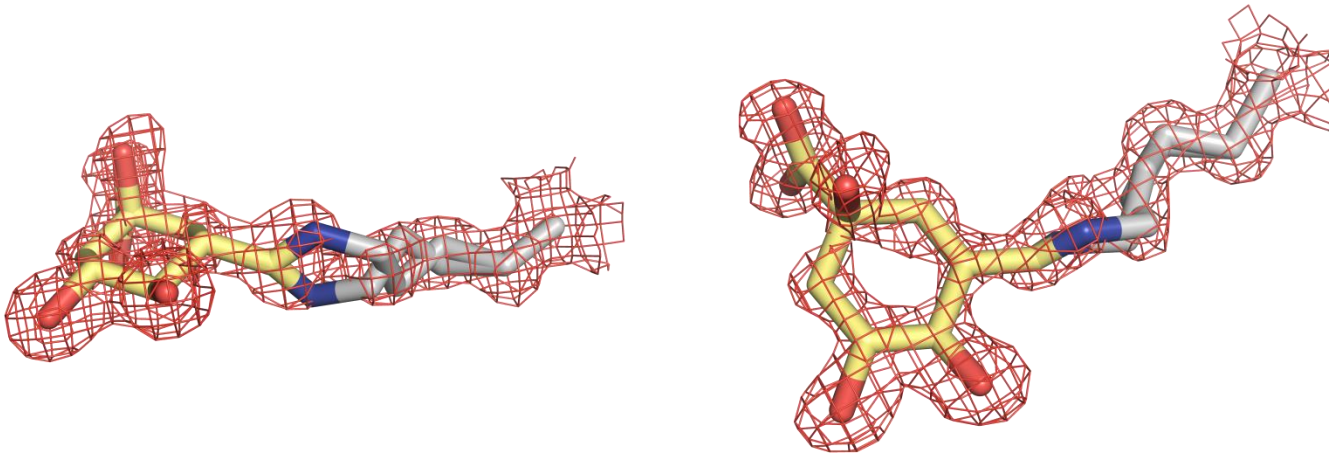


Figure S4. Close view of the modified ligand covalently attached to the essential Lys170 in the reported crystal structure of *St*-DHQ1 (PDB entry 4CLM).

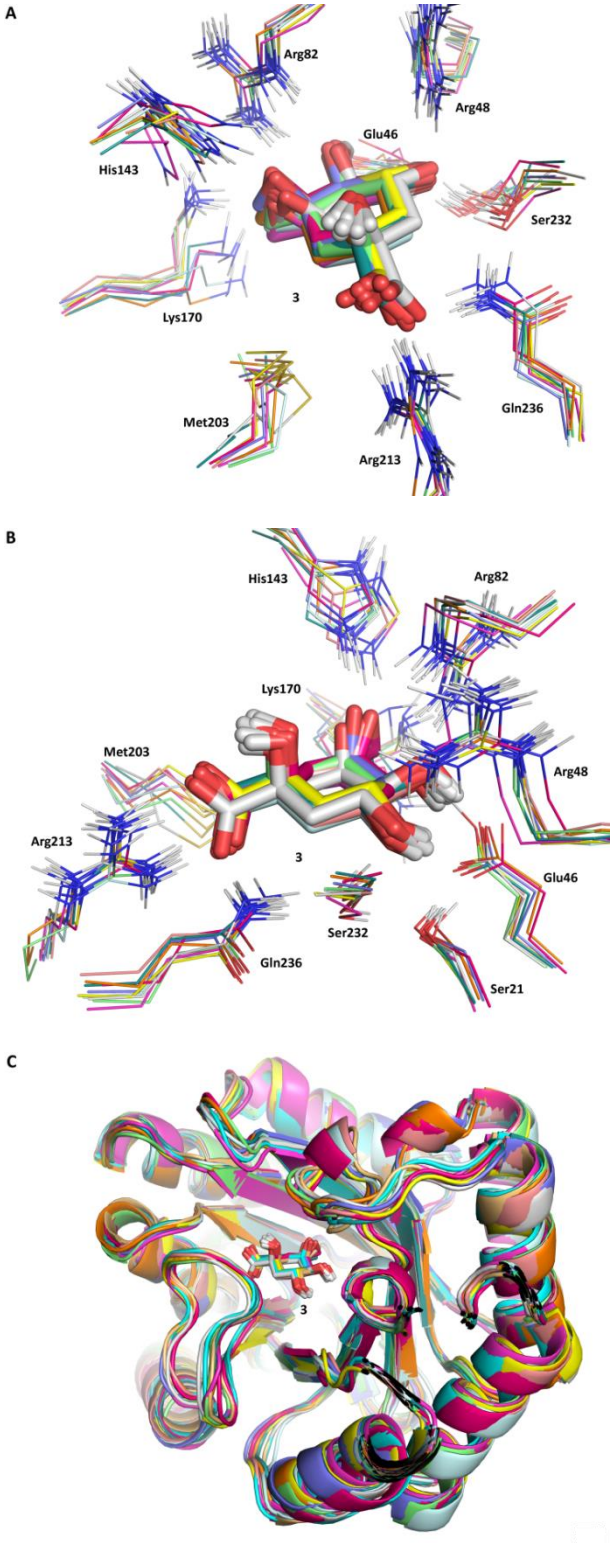


Figure S5. Simulation of *St*-DHQ1/**3** binary complex. Comparison of the several snapshots of *St*-DHQ1/**3** binary complex during 10 ns of MD simulations. Note how no significant changes were observed in the binding mode of the epoxide **3**. Relevant side chain residues are shown and labeled.

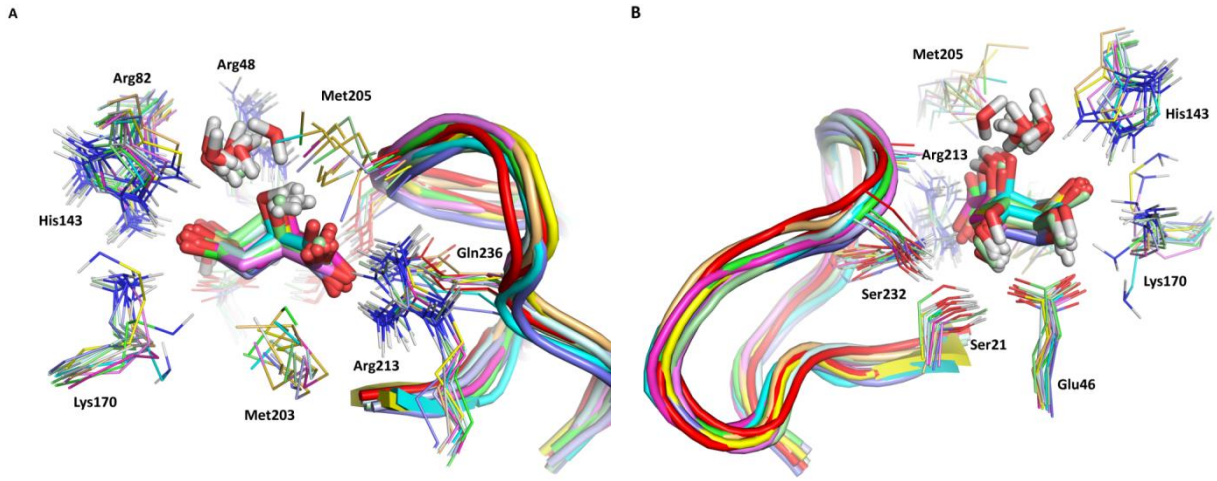


Figure S6. Simulation of Michaelis complex. Comparison of the several snapshots of the *St*-DHQ1 Michaelis complex (*St*-DHQ1/**1**) during 10 ns of MD simulations. Note how no significant changes were observed in the binding mode of the natural substrate, 3-dehydroquinic acid (**1**). Relevant side chain residues are shown and labeled.

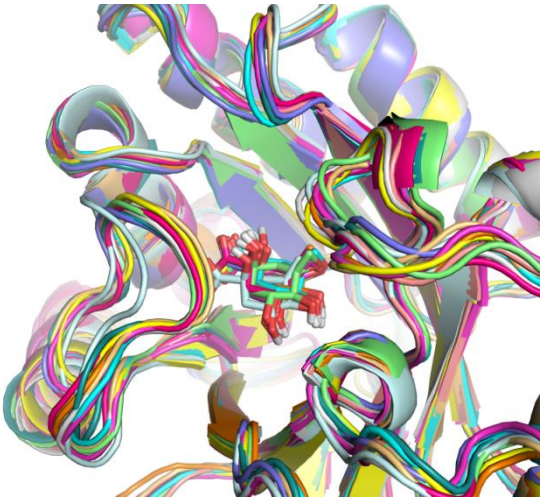


Figure S7. Simulation of *St*-DHQ1/**4** binary complex. Comparison of the several snapshots of *St*-DHQ1/**4** binary complex during 10 ns of MD simulations. Note how the epoxide **4** caused the opening of the flexible loop.

Table S1. Relative binding free energy of compounds **1**, **3**, and **4** in the active site of *St*-DHQ1

Compound	Energy (kcal mol^{-1})
1	–50.15 ± 5.30
3	–55.26 ± 4.15
4	–48.30 ± 4.58