Supporting Information for

Hydroxylammonium Derivatives for Selective Active-site Lysine Modification in the Anti-virulence Bacterial Target DHQ1 Enzyme

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EXPERIMENTAL SECTION

General. All starting materials and reagents were commercially available and were used without further purification. ^{1}H NMR spectra (250 and 500 MHz) and ^{13}C NMR spectra (63 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. $[\alpha]_D^{20}$ = values are given in 10^{-1} deg cm^2 g^{-1}. MilliQ deionized water was used in all the buffers. 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. Protein analysis was performed using an Ultraflex III TOF/TOF Bruker mass spectrometer.

Methyl (1*R*,3*S*,4*S*,6*R*,7*S*,9*R*)-7-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (8). A solution of the α,β-unsaturated ester **7**[34] (300 mg, 1.00 mmol) in methanol (3 mL) was added *via* canula to a suspension of Pd/C (30 mg, 10%) in methanol (7 mL) under hydrogen atmosphere. The resultant suspension was shaken under hydrogen atmosphere at room temperature for 24 h. Hydrogen was removed under vacuum, the mixture was filtered over a plug of Celite and the residue was washed with methanol. The filtrate and washings were concentrated under reduced pressure to yield a colourless oil which was purified by flash chromatography, eluting with (40:60) ethyl acetate/hexane, to yield the saturated ester **8** (236 mg, 78%) as a colourless oil. $[\alpha]_D^{20}$ = +180.5° (*c*1.0, $CHCl_3$). Mp: 113.0–116.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.65 (s, 3H, OCH_3), 3.64 (m, 1H, H7), 3.56 (m, 1H, H1), 3.35 (t, *J* = 9.3 Hz, 1H, H6), 3.24 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 2.42 (tt, *J* = 3.6 and 12.6 Hz, 1H, H9), 2.36 (s, 1H, OH), 2.22 (m, 1H, C*H*H), 2.05 (m, 1H, CH*H*), 1.56 (m, 2H, CH_2), 1.29 (s, 3H, CH_3) and 1.26 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8 (C), 99.5 (2×C), 75.9 (CH), 69.1 (CH), 67.4 (CH), 51.9 (OCH_3), 47.9 (OCH_3), 47.8 (OCH_3), 37.9 (CH), 34.0 (CH_2), 31.5 (CH_2) and 17.6 (2×CH_3) ppm. FTIR (KBr) υ: 3500 (OH) and 1733 (CO) cm^{-1}. MS (ESI) *m/z* = 327 (MNa^+). HRMS calcd for $C_{14}H_{24}O_7Na$ (MNa^+): 327.1414; found: 327.1417.

(1*R*,3*S*,4*S*,6*R*,7*S*,9*R*)-7-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicyclo[4.4.0]decane-9-carboxylic acid (9) – A solution of the methyl ester **8** (150 mg, 0.5 mmol) in THF (1.25 mL) was treated with an aqueous solution of lithium hydroxide (1.7 mL, 2.0 M). The resulting mixture was stirred at room temperature for 30 min and then diluted with MilliQ water. THF was removed under reduced pressure and the resulting aqueous solution was washed with diethyl ether (×3). The aqueous layer was treated with Amberlite IR-120 (H^+) until pH 6. The resin was filtered off and washed with MilliQ water. The filtrate and the washings were lyophilized to give the acid **9** (142 mg, 98%) as a colorless oil. $[\alpha]_D^{20}$ = +150.1° (*c*1.0, H_2O). ^{1}H NMR (300 MHz, D_2O) δ: 3.63 (m, 2H, H1+H7), 3.37 (t, *J* = 9.6 Hz, 1H, H6), 3.28 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 2.47 (tt, *J* = 3.0 and 12.6 Hz, 1H, H9), 2.19 (m, 1H, C*H*H), 2.04 (m, 1H, C*H*H), 1.48 (q, *J* = 12.6 Hz, 2H, CH*H*+CH*H*), 1.32 (s, 3H, CH_3) and 1.30 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, D_2O) δ: 182.6 (C), 102.5 (C), 102.4 (C), 78.2 (CH), 71.1 (CH), 70.8 (CH), 50.2 (2×OCH_3), 41.6 (CH), 37.7 (CH_2), 33.9 (CH_2), 19.5 (CH_3) and 19.4 (CH_3) ppm. FTIR (film) υ: 3460 (OH) and 1714 (CO) cm^{-1}. MS (ESI) *m/z* = 289 (M–H). HRMS calcd for $C_{13}H_{21}O_7$ (M–H): 289.1293; found: 289.1294.

Benzyl (1*R*,3*S*,4*S*,6*R*,7*S*,9*R*)-7-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicyclo[4.4.0]decane-9-carboxylate (10) – A solution of the acid **9** (212 mg, 0.73 mmol) in dry DMF (7.3 mL), at room temperature and under argon, was treated with dry triethylamine (0.28 mL, 1.97 mmol), 4-*N*,*N*-dimethylaminopyridine (143 mg, 1.17 mmol) and tetrabutylammonium iodide (11.1 mg, 0.03 mmol). The resulting yellow solution was stirred for 10 min and then treated with benzyl bromide (0.2 mL, 1.68 mmol). After stirring for 12 h, dichloromethane and water were added. The aqueous layer was acidified with HCl (10%), the organic layer was separated and washed with water (×3). The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulted residue was redisolved in (1:4) diethyl ether/water, the organic layer was separated and washed with brine. The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure to afford the benzyl ether **10** (190 mg, 69%) as a colorless oil. $[\alpha]_D^{20}$ = +75.0° (*c*1.64, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (m, 5H, 5×ArH), 5.12 (s, 2H, CH_2), 3.68 (ddd, *J* = 4.8, 9.6 and 11.1 Hz, 1H, H1), 3.56 (ddd, *J* = 4.2, 9.9 and 12.0 Hz, 1H, H7), 3.39 (t, *J* = 9.6 Hz, 1H, H6), 3.28 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 2.51 (tt, *J* = 3.3 and 12.6 Hz, 1H, H9), 2.29 (m, 2H, C*H*H+OH), 2.11 (m, 1H, C*H*H), 1.63 (m, 2H, CH*H*+CH*H*), 1.33 (s, 3H, CH_3) and 1.30 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.0 (C), 135.6 (C), 128.5 (2×CH), 128.2 (CH), 128.0 (2×CH), 99.5 (2×C), 75.9 (CH), 69.0 (CH), 67.4 (CH), 66.5 (CH_2), 47.9 (OCH_3), 47.8 (OCH_3),

38.1 (CH), 34.0 (CH_2), 31.5 (CH_2) and 17.6 (2×CH_3) ppm. FTIR (film) υ: 3501 (OH) and 1735 (CO) cm^{-1}. MS (ESI) *m/z* = 403 (MNa^+). HRMS calcd for $C_{20}H_{28}O_7Na$ (MNa^+): 403.1727; found: 403.1730.

Benzyl (1*R*,3*S*,4*S*,6*S*,9*S*)-7-oxo-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicyclo[4.4.0]decane-9-carboxylate (11) – A solution of the alcohol **10** (86 mg, 0.23 mmol) in dry dichloromethane (3.3 mL), under inert atmosphere and at room temperature, was treated with Dess-Martin periodinane (106 mg, 0.25 mmol). The resulting solution was stirred for 6 h and the solvent was then removed under reduced pressure. The resulted residue was purified by flash chromatography, eluting with (25:75) ethyl acetate/hexanes, to give the ketone **11** (74 mg, 85%) as a colorless oil. $[\alpha]_D^{20}$ = +80.2° (*c*0.8, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34 (m, 5H, 5×ArH), 5.17 (d, *J* = 14.7 Hz, 1H, OC*H*H), 5.13 (d, *J* = 14.7 Hz, 1H, OCH*H*), 4.33 (d, *J* = 10.5 Hz, 1H, H6), 3.83 (dt, *J* = 4.2 and 10.5 Hz, 1H, H1), 3.25 (s, 3H, OCH_3), 3.22 (s, 3H, OCH_3), 2.71 (m, 3H, H9+C*H*H+C*H*H), 2.35 (m, 1H, CH*H*), 2.04 (q, *J* = 12.3 Hz, 1H, CH*H*), 1.38 (s, 3H, CH_3) and 1.29 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 201.0 (C), 171.9 (C), 135.2 (C), 128.5 (2×CH), 128.4 (CH), 128.1 (2×CH), 100.2 (C), 99.4 (C), 76.7 (CH), 68.7 (CH), 66.9 (OCH_2), 48.2 (OCH_3), 47.9 (OCH_3), 41.6 (CH_2), 38.3 (CH), 31.6 (CH_2), 17.5 (CH_3) and 17.4 (CH_3) ppm. FTIR (film) υ: 1731 (CO) cm^{-1}. MS (ESI) *m/z* = 379 (MH^+). HRMS calcd for $C_{20}H_{27}O_7$ (MH^+): 379.1751; found: 379.1758.

1-Deoxy-1-dehydroquinic acid benzyl ester (12) – A stirred solution of the acetal **11** (111 mg, 0.29 mmol) in a (20:1) TFA/H_2O solution (2.9 mL) was stirred at room temperature for 15 min. The solvent was removed under reduced pressure and the resulting residue resulted was purified by flash chromatography, eluting with (75:25) ethyl acetate/hexanes, to afford the diol **12** (59 mg, 77%) as a colorless oil. $[\alpha]_D^{20}$ = −16.5° (*c*0.8, CH_3OH). ^{1}H NMR (300 MHz, DMSO-d*6*) δ: 7.37 (m, 5H, 5×ArH), 5.15 (d, *J* = 12.6 Hz, 1H, OC*H*H), 5.09 (d, *J* = 12.6 Hz, 1H, OCH*H*), 3.93 (d, *J* = 9.3 Hz, 1H, H4), 3.47 (ddd, *J* = 4.5, 9.3 and 11.1 Hz, 1H, H5), 2.76 (tt, *J* = 3.6 and 12.9 Hz, 1H, H1), 2.68 (dt, *J* = 0.9 and 13.2 Hz, 1H, C*H*H), 2.40 (m, 1H, CH*H*), 2.18 (m, 1H, CH*H*) and 1.78 (q, *J* = 12.0 Hz, 1H, CH*H*) ppm. ^{13}C NMR (75 MHz, DMSO-d*6*) δ: 212.0 (C), 177.8 (C), 141.2 (C), 133.7 (2×CH), 133.3 (CH), 133.0 (2×CH), 85.9 (CH), 77.7 (OCH_2), 71.1 (CH), 46.2 (CH_2), 42.2 (CH) and 40.2 (CH_2) ppm. FTIR (film) υ: 3418 (OH) and 1723 (CO) cm^{-1}. MS (ESI) *m/z* = 287 (MNa^+). HRMS calcd for $C_{14}H_{16}O_5Na$ (MNa^+): 287.0890; found: 287.0888.

1-Deoxy-1-dehydroquinic acid (4) – A suspension of Pd/C (10 mg, 10%) in ethyl acetate (5 mL) under hydrogen atmosphere was treated via canula with a solution of the benzyl ester **12** (100 mg, 0.38 mmol) in ethyl acetate (2.6 mL). The resulting suspension was stirred at room temperature for 3 h. After removal of the hydrogen atmosphere, the suspension was filtered over a plug of Celite. The filtrate and the washings (ethyl acetate) were concentrated under reduced pressure to afford the acid **4** (51 mg, 77%) as a colorless oil. $[\alpha]_D^{20}$ = −27.2° (*c*2.3, H_2O). ^{1}H NMR (500 MHz, DMSO-d*6*) δ: 12.50 (br s, 1H, CO_2H), 5.24 (br s, 1H, OH), 5.01 (br s, 1H, OH), 3.91 (d, *J* = 9.5 Hz, 1H, H4), 4.64 (ddd, *J* = 4.5, 9.5 and 11.0 Hz, 1H, H5), 2.62–2.54 (m, 2H, C*H*H+H1), 2.35 (m, 1H, CH*H*), 2.62–2.54 (m, 1H, CH*H*) and 1.39 (q, *J* = 12.5 Hz, 1H, CH*H*) ppm. ^{13}C NMR (63 MHz, aceton-d*6*) δ: 208.5 (C), 175.5 (C), 83.1 (CH), 75.8 (CH), 42.6 (CH_2), 39.3 (CH) and 36.5 (CH_2) ppm. FTIR (ATR) υ: 3384 (OH), 1708 (CO) and 1672 (CO) cm^{-1}. MS (ESI) *m/z* = 173 (M−H). HRMS calcd for $C_7H_9O_5$ (M−H): 173.0455; found: 173.0460.

Methyl (1*R*,3*S*,4*S*,6*S*,9*S*)-7-oxo-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (13). To a stirred suspension of the alcohol **8** (80 mg, 0.24 mmol) and activated powder molecular sieves 4 Å (200 mg) in dry DCM (2.4 mL) and under inert atmosphere pyridinium dichromate (293 mg, 0.72 mmol) was added. The resultant brown suspension was stirred vigorously at room temperature for 48 h. The solvent was concentrated under reduced pressure and diethyl ether was added. The resulting brown suspension was filtered over a plug of Celite and silica gel. The filtrate and the washings were concentrated under reduced pressure to afford the ketone **13** (71 mg, 90%) as white solid. $[\alpha]_D^{20}$ = +28.5° (*c*1.0, $CHCl_3$). Mp: 127–130 °C. ^{1}H NMR (300 MHz, aceton-d*6*) δ: 4.05 (d, *J* = 4.2 Hz, 1H, H6), 3.64 (s, 3H, OCH_3), 3.56 (m, 1H, H1), 3.22 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 2.55 (tt, *J* = 3.6 and 12.9 Hz, 1H, H9), 2.14 (m, 1H, C*H*H), 1.98 (m, 1H, CH*H*), 1.45 (m, 1H, CH_2), 1.22 (s, 3H, CH_3) and 1.20 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 201.2 (C), 172.7 (C), 100.3 (C), 99.5 (C), 76.9 (CH) 68.9 (CH), 52.4 (OCH_3), 48.3 (OCH_3), 48.0 (OCH_3), 41.7 (CH_2), 38.2 (CH), 31.8 (CH_2), 17.6 (CH_3) and 17.5 (CH_3)

ppm. FTIR (KBr) υ: 1726 (CO) cm^{-1}. MS (ESI) *m/z* = 325 (MNa^+). HRMS calcd for $C_{14}H_{22}O_7Na$ (MNa^+): 325.1258; found, 325.1265.

Methyl (1*R*,3*S*,4*S*,6*R*,9*R*)-7-methylene-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (14). A suspension of activated Zn dust (270 mg, 4.14 mmol) in dry THF (2.5 mL), under argon and at room temperature, was treated with diiodomethane (0.2 mL, 2.3 mmol). After stirring for 30 min, the reaction mixture was cooled to 0 °C and treated dropwise with a solution of $TiCl_4$ (0.51 mL, 0.51 mmol, *ca* 1 M in DCM). The resulting mixture was stirred at room temperature for 30 min, cooled to 0 °C and then treated *via canula* with a solution of the ketone **13** (140 mg, 0.46 mmol) in dry THF (1.6 mL). The resulted suspension was stirred for 3 h at room temperature, then diluted with diethyl ether and poured into cool HCl (1 M). The organic layer was separated and the aqueous phase was extracted with diethyl ether (×3). The combined organic extracts were washed with saturated $NaHCO_3$, dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography, eluting with (25:75) diethyl ether/hexanes, to give the alkene **14** (83 mg, 60%) as a light yellow oil. $[\alpha]_D^{20}$ = +142.3° (*c*3.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.14 (d, *J* = 1.5 Hz, 1H, C=C*H*H), 4.87 (d, *J* = 1.5 Hz, 1H, C=CH*H*), 3.96 (d, *J* = 9.6 Hz, 1H, H6), 3.66 (s, 3H, OCH_3), 3.47 (ddd, *J* = 4.5, 9.6 and 12.0 Hz, 1H, H1), 3.20 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 2.59–2.52 (m, 1H, C*H*H), 2.41 (tt, *J* = 3.6 and 12.6 Hz, 1H, H9), 2.21 (d, *J* = 13.2 Hz, 1H, C*H*H), 2.14–2.02 (m, 1H, CH*H*), 1.74 (q, *J* = 12.3 Hz, 1H, CH*H*), 1.32 (s, 3H, CH_3) and 1.27 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.0 (C), 141.4 (C), 107.9 (CH_2), 99.7 (C), 99.4 (C), 72.3 (CH), 70.6 (CH), 51.8 (OCH_3), 47.8 (2×OCH_3), 41.3 (CH), 36.0 (CH_2), 32.0 (CH_2) and 17.7 (2×CH_3) ppm. IR (ATR) υ: 1730 (CO) cm^{-1}. MS (ESI) *m/z* = 323 (MNa^+). HRMS calcd for $C_{15}H_{24}O_6Na$ (MNa^+): 323.1465; found: 323.1465.

Methyl (1*R*,3*S*,4*S*,6*S*,7*S*,9*S*)-7-hydroxy-3,4-dimethoxy-3,4-dimethyl-7-vinyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (16). A stirred solution of the ketone **13** (100 mg, 0.33 mmol) in dry THF (1.3 mL), under argon at −78 °C, was treated with a solution of vinylmagnesium bromide (0.43 mL, 0.43 mmol, *ca* 1.0 M in THF). After stirring for 2 h at −78 °C, the reaction mixture was allowed to warm up to room temperature. Diethyl ether and water were added. The organic layer was separated and the aqueous phase was extracted with diethyl ether (×3). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, eluting with (50:50) diethyl ether/hexanes, to give the vinyl derivative **16** (106 mg, 98%) as a white solid. $[\alpha]_D^{20}$ = +147.5° (*c*1.0, $CHCl_3$). Mp: 82.0–82.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.84 (dd, *J* = 10.5 and 17.1 Hz, 1H, H_2C=C*H*), 5.34 (dd, *J* = 0.9 and 17.1 Hz, 1H, CH=C*H*H), 5.13 (dd, *J* = 0.9 and 10.5 Hz, 1H, CH=CH*H*), 3.94 (ddd, *J* = 4.5, 9.6 and 12.0 Hz, 1H, H1), 3.63 (s, 3H, OCH_3), 3.40 (d, *J* = 9.6 Hz, 1H, H6), 3.19 (s, 3H, OCH_3), 3.16 (s, 3H, OCH_3), 2.86 (tt, *J* = 4.0 and 12.6 Hz, 1H, H9), 2.66 (d, *J* = 2.1 Hz, 1H, OH), 2.11–2.04 (m, 1H, C*H*H), 1.95–1.88 (m, 1H, C*H*H), 1.62–1.49 (m, 2H, CH*H*+CH*H*) and 1.23 (s, 6H, 2×CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.7 (C), 141.9 (CH), 114.0 (CH_2), 100.1 (C), 99.4 (C), 74.9 (CH), 73.5 (C), 66.0 (CH), 51.7 (OCH_3), 47.7 (2×OCH_3), 38.0 (CH_2), 37.0 (CH), 31.8 (CH_2), 17.7 (CH_3) and 17.5 (CH_3) ppm. FTIR (ATR) υ: 3441 (OH) and 1737 (CO) cm^{-1}. MS (ESI) *m/z* = 353 (MNa^+). HRMS calcd for $C_{16}H_{26}O_7Na$ (MNa^+): 353.1571; found: 353.1570.

Methyl (1*R*,3*S*,4*S*,6*S*,7*S*,9*S*)-7-trimethylsilyloxy-3,4-dimethoxy-3,4-dimethyl-7-vinyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (17). A solution of the alcohol **16** (188 mg, 0.57 mmol) in dry pyridine (1.2 mL), under argon and at room temperature, was treated sucessively with hexamethyldisilazane (0.44 mL, 2.11 mmol) and trimethylsilyl chloride (0.72 mL, 5.7 mmol). The resulting white suspension was stirred for 24 h and then more trimethylsilyl chloride (0.72 mL, 5.7 mmol) was added. After stirring for 6 h, diethyl ether and water were added. The aqueous phase was separated and the organic layer was washed succesively with water, saturated solution of $CuSO_4$, water and saturated solution of $NaHCO_3$. The organic layer was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, eluting with (50:50) diethyl ether/hexanes, to give the ether **17** (160 mg, 70%) as a colorless oil. $[\alpha]_D^{20}$ = +102.4° (*c*1.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.19 (dd, *J* = 10.8 and 17.7 Hz, 1H, H_2C=C*H*), 5.19 (d, *J* = 17.7 Hz, 1H, CH=C*H*H), 5.09 (d, *J* = 10.8 Hz, 1H, CH=CH*H*), 3.85 (ddd, *J* = 4.8, 9.9 and 12.0 Hz, 1H, H1), 3.64 (s, 3H, OCH_3), 3.30 (d, *J* = 9.6 Hz, 1H, H6), 3.16 (s, 6H, 2×OCH_3), 2.80 (tt, *J* = 3.6 and 12.6 Hz, 1H, H9), 2.08–1.92 (m, 2H, C*H*H+C*H*H), 1.59–1.45 (m, 2H, CH*H*+CH*H*), 1.24 (s, 3H, CH_3), 1.21 (s, 3H, CH_3) and –0.09 (s, 9H, $Si(CH_3)_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 175.2 (C), 141.6 (CH), 114.0 (CH_2), 99.9 (C), 99.1 (C), 75.8 (CH), 75.7 (C), 66.0 (CH), 51.6 (OCH_3), 47.8 (OCH_3), 47.3 (OCH_3), 39.6 (CH_2), 36.9 (CH),

32.0 (CH_2), 17.8 (CH_3), 17.5 (CH_3) and 2.4 ($Si(CH_3)_3$) ppm. FTIR (ATR) υ: 1737 (CO) cm^{-1}. MS (ESI) *m/z* = 425 (MNa^+). HRMS calcd for $C_{19}H_{34}O_7SiNa$ (MNa^+): 425.1966; found: 425.1963.

Methyl (1*R*,3*S*,4*S*,6*S*,7*R*,9*S*)-7-formyl-7-trimethylsilyloxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (18). A stream of ozone/oxygen was passed through a solution of the alkene **17** (100 mg, 0.25 mmol) in dry dichloromethane (100 mL), under nitrogen atmosphere and at −78°C, until persistence of blue coloration (~5 min). The reaction flask was purged with a stream of nitrogen for 30 min. Dimethyl sulfide (0.20 mL, 2.7 mmol) was added and after 30 min of stirring the reaction mixture was allowed to warm up to room temperature. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography on silica gel, eluting with (20:80) diethyl ether/hexanes, to give the aldehyde **18** (45 mg, 45%) as a colorless oil. $[\alpha]_D^{20}$ = +98.2° (*c*2.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.58 (s, 1H, CHO), 3.92–3.81 (m, 2H, H1+H6), 3.67 (s, 3H, OCH_3), 3.20 (s, 3H, OCH_3), 3.19 (s, 3H, OCH_3), 2.83 (tt, *J* = 3.6 and 12.6 Hz, 1H, H9), 2.17–2.09 (m, 1H, C*H*H), 1.88–1.82 (m, 1H, C*H*H), 1.64–1.51 (m, 2H, CH*H*+CH*H*), 1.25 (s, 3H, CH_3), 1.23 (s, 3H, CH_3) and 1.26 (s, 9H, $Si(CH_3)_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 202.0 (CHO), 174.3 (C), 100.1 (C), 99.5 (C), 82.5 (C), 71.9 (CH), 65.6 (CH), 51.9 (OCH_3), 48.0 (OCH_3), 47.3 (OCH_3), 36.4 (CH), 36.0 (CH_2), 31.8 (CH_2), 17.9 (CH_3), 17.4 (CH_3) and 2.0 ($Si(CH_3)_3$) ppm. FTIR (ATR) υ: 1737 (CO) cm^{-1}. MS (ESI) *m/z* = 427 (MNa^+). HRMS calcd for $C_{18}H_{32}O_8SiNa$ (MNa^+): 427.1759; found: 427.1756.

Methyl (1*R*,3*S*,4*S*,6*S*,7*S*,9*S*)-7-(hydroxyamino)methyl-7-trimethylsilyloxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (19). A suspension of the aldehyde **18** (40 mg, 0.10 mmol), hydroxylamine hydrochloride (7 mg, 0.10 mmol), anhydrous sodium acetate (16 mg, 0.20 mmol) and 4 Å molecular sieves (40 mg) in dry methanol (0.5 mL), at room temperature and under inert atmosphere, was stirred for 1 h. The reaction mixture was filtered through a plug of Celite® and washed with methanol. The filtrate and the washings were concentrated under reduced pressure. The resulting residue was dissolved, at room temperature and under inert atmosphere, in glacial acetic acid (0.25 mL) and treated with sodium cyanoborohydride (63 mg, 1 mmol). After stirring for 1 h, the solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate and saturated $NaHCO_3$. The aqueous layer was separated, dried (Na_2SO_4 anh.), filtered and concentrated under reduced pressure. The reaction mixture was purified by flash chromatography, eluting with (50:50) diethyl ether/hexanes, to yield the hydroxylamine **19** (27 mg, 64%) as a colorless oil. $[\alpha]_D^{20}$ = +91.8° (*c*1.35, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 5.54 (br s, 1H, NH), 3.82 (m, 1H, H1), 3.66 (s, 3H, OCH_3), 3.40 (d, *J* = 9.8 Hz, 1H, H6), 3.30 (d, *J* = 12.8 Hz, 1H, NC*H*H), 3.21 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 3.04 (d, *J* = 12.8 Hz, 1H, NCH*H*), 2.77 (tt, *J* = 3.3 and 12.5 Hz, 1H, H9), 2.07 (m, 2H, CH_2), 1.61–1.46 (m, 2H, CH_2), 1.28 (s, 3H, CH_3), 1.25 (s, 3H, CH_3) and 0.17 (s, 9H, $Si(CH_3)_3$) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 175.2 (C), 100.1 (C), 99.1 (C), 73.7 (CH), 65.9 (CH), 64.0 (C), 58.9 (NCH_2), 51.8 (OCH_3), 47.9 (OCH_3), 47.3 (OCH_3), 38.8 (CH_2), 36.7 (CH), 32.0 (CH_2), 17.9 (CH_3), 17.6 (CH_3) and 2.3 ($Si(CH_3)_3$) ppm. FTIR (ATR) υ: 3470 (OH), 3272 (NH) and 1730 (CO) cm^{-1}. MS (ESI) *m/z* = 444 (MNa^+). HRMS calcd for $C_{18}H_{35}NO_8SiNa$ (MNa^+): 444.2024; found: 444.2022.

(1*S*,3*S*,4*S*,5*R*)-3,4,5-trihydroxy-3-(hydroxyamino)methylcyclohexane-1-carboxylic acid, hydrochloride form (1). A solution of compound **19** (27 mg, 0.064 mmol) in HCl (0.64 mL, 0.3 M) was heated at 100 °C for 24 h. After cooling to room temperature, the mixture was diluted with water and ethyl acetate. The organic layer was separated, the aqueous layer was washed with ethyl acetate (×2) and the aqueous extract was lyophilized to give compound **1** (13 mg, 92%) as a yellow foam. $[\alpha]_D^{20}$ = −25.5° (*c*2.7, H_2O). ^{1}H NMR (300 MHz, D_2O) δ: 3.81 (m, 1H, H5), 3.68 (d, *J* = 12.9 Hz, 1H, NC*H*H), 3.41 (m, 2H, NCH*H*+H4), 2.86 (m, 1H, H1), 2.27 (d, *J* = 11.7 Hz, 1H, C*H*H), 2.10 (d, *J* = 14.1 Hz, 1H, C*H*H), 1.72 (t, *J* = 13.5 Hz, 1H, CH*H*) and 1.49 (q, *J* = 12.3 Hz, 1H, CH*H*) ppm. ^{13}C NMR (75 MHz, D_2O) δ: 180.8 (C), 79.8 (CH), 74.2 (C), 71.8 (CH), 62.6 (CH_2), 38.4 (CH), 38.3 (CH_2) and 36.6 (CH_2) ppm. FTIR (ATR) υ: 3328 (OH), 3102 (OH), 2939 (NH) and 1702 (CO) cm^{-1}. MS (ESI) *m/z* = 220 (M–H–HCl). HRMS calcd for $C_8H_{14}NO_6$ (M–H–HCl): 220.0827; found: 220.0827.

Methyl (1*R*,3*S*,4*S*,6*R*,7*S*,9*S*)-9-hydroxy-7-hydroxymethyl-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (20) – A stirred solution of the alkene **15**[32] (100 mg, 0.32 mmol) in dry THF (1.6 mL), under argon at 0 °C, was treated with a BH_3-THF solution (3.2 mL, 3.2 mmol, *ca* 1.0 M in THF). The mixture was stirred for 1 h. An aqueous solution of $NaBO_3$ (8 mL, 3.2 mmol, 0.4 M) was added and the resulting solution was stirred for 2 h at this temperature. The mixture was

warmed up to room temperature and ethyl acetate and water were added. 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 was purified by flash chromatography on silica gel, eluting with diethyl ether, to give the alcohol **20** (57 mg, 53%) as a white foam. $[\alpha]_D^{20}$ = +113.9° (*c*1.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.24–4.11 (m, 2H, H1+OC*H*H), 3.83 (dd, *J* = 5.7 and 10.2 Hz, 1H, H6), 3.75 (s, 3H, OCH_3), 3.64 (d, *J* = 10.2 Hz, 1H, OCH*H*), 3.24 (s, 3H, OCH_3), 3.22 (s, 3H, OCH_3), 2.32 (m, 1H, H7), 2.06 (dd, *J* = 6.3 and 14.7 Hz, 1H, C*H*H), 1.88 (m, 2H, CH_2), 1.79 (d, *J* = 14.7 Hz, 1H, CH*H*), 1.27 (s, 3H, CH_3) and 1.25 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 176.0 (C), 99.8 (C), 99.5 (C), 74.4 (C), 73.1 (CH), 63.6 (OCH_2), 63.3 (CH), 53.1 (OCH_3), 47.9 (OCH_3), 47.8 (OCH_3), 39.2 (CH), 38.5 (CH_2), 36.2 (CH_2), 17.8 (CH_3) and 17.7 (CH_3) ppm. FTIR (ATR) υ: 3522 (OH), 3374 (OH) and 1736 (CO) cm^{-1}. MS (ESI) *m/z* = 357 (MNa^+). HRMS calcd for $C_{15}H_{26}O_8Na$ (MNa^+): 357.1520; found: 357.1519.

Methyl (1*R*,3*S*,4*S*,6*R*,7*S*,9*R*)-7-hydroxymethyl-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (21). This compound was prepared following a similar procedure as for **20** using the alkene **14** (80 mg, 0.27 mmol), BH_3·THF (2.7 mL, 2.7 mmol, *ca* 1.0 M in THF), aqueous $NaBO_3$ (6.8 mL, 2.7 mmol, 0.4 M). Eluent for chromatography = (75:25) diethyl ether/hexanes. Yield = 67% (57 mg). Colorless oil. $[\alpha]_D^{20}$ = +116.2° (*c*1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.05 (dd, *J* = 9.3 and 10.8 Hz, 1H, OC*H*H), 3.89 (dt, *J* = 4.5 and 10.2 Hz, 1H, H1), 3.77 (dd, *J* = 5.4 and 10.2 Hz, 1H, H6), 3.66 (s, 3H, OCH_3), 3.50 (m, 1H, OCH*H*), 3.24 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 2.85 (br s, 1H, OH), 2.49 (tt, *J* = 3.6 and 12.9 Hz, 1H, H9), 2.32 (m, 1H, H7), 2.10–2.05 (m, 1H, $H10_{ax}$), 1.99–1.93 (m, 1H, $H8_{ax}$), 1.70 (dd, *J* = 5.1 and 13.2 Hz, 1H, $H8_{eq}$), 1.62–1.49 (q, *J* = 12.3 Hz, 1H, $H10_{eq}$) and 1.27 (s, 6H, 2×CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.5 (C), 99.8 (C), 99.4 (C), 73.5 (CH), 65.5 (CH), 63.1 (OCH_2), 51.8 (OCH_3), 47.9 (OCH_3), 47.8 (OCH_3), 38.8 (CH), 37.8 (CH), 32.4 (CH_2), 29.9 (CH_2) and 17.7 (2×CH_3) ppm. FTIR (ATR) υ: 3491 (OH) and 1730 (CO) cm^{-1}. MS (ESI) *m/z* = 341 (MNa^+). HRMS calcd for $C_{15}H_{26}O_7Na$ (MNa^+): 341.1571; found: 341.1571.

Methyl (1*R*,3*S*,4*S*,6*R*,7*S*,9*S*)-7-formyl-9-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (22). A solution of the alcohol **20** (377 mg, 1.13 mmol) in dry dichloromethane (11.3 mL), under inert atmosphere and at room temperature, was treated with Dess-Martin periodinane (527 mg, 1.24 mmol). The resulting solution was stirred for 30 min and saturated solution of Na_2SO_3 was then added. After stirring for 10 min, the organic layer was separated and the aqueous layer was extracted with dichloromethane (×3). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography, eluting with (75:25) diethyl ether/hexanes, to give the corresponding aldehyde as a mixture of diastereoisomers (316 mg) as white foam. A solution of the mixture of aldehydes (316 mg) in methanol (16 mL) and pyridine (7 mL), both dry, was heated at 60 °C for 48 h. After cooling to room temperature, the solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate. The organic solution was successively washed with saturated $CuSO_4$ and water, dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with (75:25) diethyl ether/hexanes, to yield the aldehyde **22** (230 mg, 61%), as a white foam. $[\alpha]_D^{20}$ = +26.1° (*c*0.9, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.92 (d, *J* = 1.5 Hz, 1H, CHO), 4.09 (ddd, *J* = 4.5, 9.5 and 11.5 Hz, 1H, H1), 3.81 (m, 1H, H6), 3.80 (s, 3H, OCH_3), 3.27 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 3.16 (s, 1H, OH), 3.07 (dddd, *J* = 1.5, 5.0, 8.0 and 11.5 Hz, 1H, H7), 1.95–1.81 (m, 4H, 2×CH_2), 1.32 (s, 3H, CH_3) and 1.29 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 202.2 (C), 175.5 (C), 99.7 (C), 99.6 (C), 73.1 (C), 71.5 (CH), 66.4 (CH), 53.1 (OCH_3), 47.9 (2×OCH_3), 47.2 (CH), 37.3 (CH_2), 33.5 (CH_2), 17.6 (CH_3) and 17.5 (CH_3) ppm. FTIR (ATR) υ: 3477 (OH) and 1730 (CO) cm^{-1}. MS (ESI) *m/z* = 333 (MH^+). HRMS calcd for $C_{15}H_{25}O_8$ (MH^+): 333.1544; found: 333.1541.

Methyl (1*R*,3*S*,4*S*,6*R*,7*R*,9*S*)-7-(hydroxyamino)methyl-9-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (23). This compound was prepared following a similar procedure as for **19** using: (i) for the hydroxyimine preparation: the aldehyde **22** (140 mg, 0.42 mmol), hydroxylamine hydrochloride (29 mg, 0.42 mmol), anhydrous sodium acetate (69 mg, 0.84 mmol), 4 Å molecular sieves (140 mg) and methanol (2.1 mL); reaction time = 2 h; (ii) for the reduction: $NaBH_3CN$ (32 mg, 0.50 mmol) and glacial acetic acid (1.1 mL); reaction time = 30 min; Eluent for chromatography = ethyl acetate. Yield = 41% (60 mg). Colorless oil. $[\alpha]_D^{20}$ = +95.8° (*c*1.1, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3OD) δ: 3.94 (ddd, *J* = 4.8, 9.6 and 11.7 Hz, 1H, H1), 3.73 (s, 3H, OCH_3), 3.35 (dd, *J* = 10.8 and 12.6 Hz, 1H, H6), 3.24 (s,

3H, OCH_3), 3.21 (s, 3H, OCH_3), 3.20 (m, 1H, NC*H*H), 2.71 (dd, *J* = 6.3 and 12.6 Hz, 1H, NCH*H*), 2.24–2.11 (m, 1H, H7), 1.98–1.74 (m, 3H, CH_2+C*H*H), 1.59 (t, *J* = 13.5 Hz, 1H, CH*H*), 1.27 (s, 3H, CH_3) and 1.24 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.0 (C), 99.8 (C), 99.5 (C), 75.9 (CH), 73.7 (C), 66.7 (CH), 57.4 (CH_2), 53.0 (OCH_3), 47.9 (2×OCH_3), 47.8 (OCH_3), 39.3 (CH_2), 37.0 (CH_2), 32.5 (CH) and 17.8 (2×CH_3) ppm. FTIR (ATR) υ: 3453 (OH), 3291 (NH) and 1734 (CO) cm^{-1}. MS (ESI) *m/z* = 350 (MH^+). HRMS calcd for $C_{15}H_{28}NO_8$ (MH^+): 350.1809; found: 350.1808.

Methyl (1*R*,3*S*,4*S*,6*R*,7*S*,9*R*)-7-formyl-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (24). To a stirred suspension of the alcohol **21** (40 mg, 0.13 mmol) and activated powder molecular sieves 4 Å (40 mg) in dry DCM (1.3 ml), under inert atmosphere and at room temperature, pyridinium dichromate (59 mg, 0.16 mmol) was added. The resultant brown suspension was stirred vigorously at room temperature for 2 h. The solvent was concentrated under reduced pressure and diethyl ether was added. The resulting brown suspension was filtered over a plug of Celite® and silica gel. The filtrate and the washings were concentrated under reduced pressure and the resulting residue was dissolved in methanol (2.2 mL) and pyridine (1 mL) and heated at 60 ºC for 48 h. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography, eluting with (50:50) diethyl ether/hexanes, to afford the aldehyde **24** (20 mg, 49%) as colorless oil. $[\alpha]_D^{20}$ = +199.1° (*c*1.3, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.81 (d, *J* = 1.8 Hz, 1H, CHO), 3.78 (dd, *J* = 9.6 and 10.5 Hz, 1H, H6), 3.68 (s, 3H, OCH_3), 3.67 (m, 1H, H1), 3.27 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 2.59 (m, 1H, H7), 2.48 (tt, *J* = 3.6 and 12.6 Hz, 1H, H9), 2.16–2.10 (m, 2H, CH_2), 1.67–1.48 (m, 2H, CH_2), 1.30 (s, 3H, CH_3) and 1.27 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 201.3 (CH), 173.7 (C), 99.8 (2×C), 71.1 (CH), 69.3 (CH), 52.0 (OCH_3), 50.7 (CH), 48.1 (OCH_3), 48.0 (OCH_3), 40.0 (CH), 31.6 (CH_2), 27.1 (CH_2), 17.8 (CH_3) and 17.6 (CH_3) ppm. FTIR (ATR) υ: 1730 (CO) cm^{-1}. MS (ESI) *m/z* = 317 (MH^+). HRMS calcd for $C_{15}H_{25}O_7$ (MH^+): 317.1595; found: 317.1595.

Methyl (1*R*,3*S*,4*S*,6*R*,7*R*,9*R*)-7-(hydroxylamino)methyl-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicycle[4.4.0]decane-9-carboxylate (25). This compound was prepared following a similar procedure as for **21** using: (i) for the hydroxyimine preparation: the aldehyde **21** (110 mg, 0.35 mmol), hydroxylamine hydrochloride (24 mg, 0.35 mmol), anhydrous sodium acetate (57 mg, 0.70 mmol), 4 Å molecular sieves (110 mg) and methanol (1.8 mL); reaction time = 3.5 h; (ii) for the reduction: $NaBH_3CN$ (26 mg, 0.42 mmol) and glacial acetic acid (0.9 mL); reaction time = 30 min; Eluent for chromatography = diethyl ether. Yield = 76% (88 mg). Colorless oil. $[\alpha]_D^{20}$ = +110.7° (*c*1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.39 (br s, 2H, NH+OH), 3.64 (s, 3H, OCH_3), 3.56 (m, 1H, H1), 3.30 (t, *J* = 10.2 Hz, 1H, H6), 3.22 (s, 6H, 2×OCH_3), 3.20 (m, 1H, NC*H*H), 2.80 (dd, *J* = 5.4 and 12.6 Hz, 1H, NCH*H*), 2.45 (tt, *J* = 3.3 and 12.6 Hz, 1H, H9), 2.06 (m, 2H, C*H*H+C*H*H), 1.84 (m, 1H, H7), 1.55 (q, *J* = 12.6 Hz, 1H, CH*H*), 1.27 (s, 3H, CH_3), 1.25 (m, 1H, CH*H*) and 1.25 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.2 (C), 99.6 (C), 99.3 (C), 74.9 (CH), 69.5 (CH), 56.8 (NCH_2), 51.8 (OCH_3), 47.8 (2×OCH_3), 40.3 (CH), 36.2 (CH), 31.7 (2×CH_2), 17.8 (CH_3) and 17.7 (CH_3) ppm. FTIR (ATR) υ: 2953 (NH) and 1735 (CO) cm^{-1}. MS (ESI) *m/z* = 334 (MH^+). HRMS calcd for $C_{15}H_{28}NO_7$ (MH^+): 334.1860; found: 334.1860.

(1*S*,3*R*,4*R*,5*R*)-1,3,4-trihydroxy-5-(hydroxyamino)methylcyclohexane-1-carboxylic acid, hydrochloride form (2) – This compound was prepared following a similar procedure as for **1** using compound **23** (20 mg, 0.06 mmol), HCl (0.6 mL, 0.3 M). Reaction time = 1 h. Yield = 96% (14 mg). Beige foam. $[\alpha]_D^{20}$ = +4.40° (*c*2.0, H_2O). ^{1}H NMR (300 MHz, D_2O) δ: 3.78 (m, 1H, H3), 3.58 (dd, *J* = 8.1 and 12.9 Hz, 1H, NC*H*H), 3.42 (t, *J* = 9.9 Hz, 1H, H4), 3.31 (dd, *J* = 4.5 and 12.9 Hz, 1H, NCH*H*), 2.34 (m, 1H, H5), 2.14 (m, 1H, C*H*H) and 1.92–1.79 (m, 3H, CH*H*+CH_2) ppm. ^{13}C NMR (75 MHz, D_2O) δ: 180.4 (C), 79.6 (CH), 76.2 (C), 72.6 (CH), 57.0 (CH_2), 41.9 (CH_2), 38.2 (CH_2) and 35.4 (CH) ppm. FTIR (ATR) υ: 3307 (OH), 2925 (NH_2) and 1716 (CO) cm^{-1}. MS (ESI) *m/z* = 220 (M–H–HCl). HRMS calcd for $C_8H_{14}NO_6$ (M–H–HCl): 220.0827; found: 220.0827.

(1*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxyamino)methylcyclohexane-1-carboxylic acid, hydrochloride salt (3). This compound was prepared following a similar procedure as for **1** using compound **25** (97 mg, 0.29 mmol), HCl (2.9 mL, 0.3 M). Reaction time = 18 h. Yield = 64% (38 mg). Beige foam. $[\alpha]_D^{20}$ = +2.4° (*c*1.3, H_2O). ^{1}H NMR (300 MHz, D_2O) δ: 3.59–3.51 (m, 2H, NC*H*H+H3), 3.35–3.27 (m, 2H, NCH*H*+H4), 2.64 (tt, *J* = 3.0 and 12.6 Hz, 1H, H1), 2.25 (m, 1H, H5), 2.06 (m, 2H, C*H*H+C*H*H), 1.48 (q, *J* = 12.3 Hz, 1H, CH*H*) and 1.38 (q, *J* = 13.2 Hz, 1H, CH*H*) ppm. ^{13}C NMR (75 MHz, D_2O) δ: 180.8 (C),

79.4 (CH), 75.2 (CH), 57.1 (CH_2), 42.1 (CH), 38.7 (CH), 36.8 (CH_2) and 32.6 (CH_2) ppm. FTIR (ATR) υ: 3357 (OH+NH) and 1709 (CO) cm^{-1}. MS (ESI) m/z = 204 (M–H–HCl). HRMS calcd for $C_8H_{14}NO_5$ (M–H–HCl): 204.0877; found: 204.0877.

Crystallization of the *St*-DHQ1/1–3 Adducts Crystals. The *St*-DHQ1 enzyme was purified as described previously.[39] *St*-DHQ1 (0.85 mg mL^{-1}) was concentrated to 9.7 mg mL^{-1} in 50 mM PPB pH 7.0 and 0.5 mM DDT. Compounds **1**, **2** and **3** were dissolved at 250 mM in methanol and added at a ratio of 1:20 (v/v) to the protein solution to give a solution of approximately 10 equivalents of ligand per protein monomer. Plate-shaped crystals of up to: (a) 0.1 mm × 0.1 mm of the *St*-DHQ1/**1**; (b) 0.08 mm × 0.08 mm of the *St*-DHQ1/**2**; (c) 0.1 mm × 0.1 mm of the *St*-DHQ1/**3** adducts were obtained after three weeks of vapor diffusion in sitting drops comprised of 2.0 μL protein/inhibitor solution mixed with 2.0 μL reservoir solution against 0.15 mL reservoirs containing: (i) for **1**: 26% (w/v) PEG 2000 MME and 0.1 M Hepes pH 7.0. (ii) for **2**: 26% (w/v) PEG 2000 MME and 0.1 M Hepes pH 7.0. (iii) for **3**: 24% (w/v) PEG 2000 MME and 0.1 M Hepes pH 7.0.

Crystallization of the *St*-DHQ1/4 Adduct Crystal. Prism-shaped apo-*St*-DHQ1 crystals of up to 0.1 mm × 0.1 mm were obtained from a freshly purified solution of *St*-DHQ1 (0.85 mg mL^{-1}) concentrated to 8 mg mL^{-1} in buffer A (10 mM Tris.HCl pH 7.4 and 40 mM KCl) after 4 weeks of vapor diffusion in sitting drops comprised of 2.0 μL of protein solution mixed with 2.0 μL of reservoir solution and equilibrated against 0.15 mL reservoirs containing the crystallization mixture [24% (w/v) PEG 4000, 0.1 M citrate-phosphate pH 5.6]. *St*-DHQ1/**4** adduct complex crystals were obtained after soaking during 30 seconds of apo-*St*-DHQ1 crystals in 10 mM solutions of ligand **4** in the crystallization mixture.

Structure Determination of the *St*-DHQ1/1–4 Adducts. Crystals were mounted into cryoloops and directly flash frozen by rapid immersion in liquid nitrogen. X-ray diffraction data for the *St*-DHQ1/ligand adducts were collected on beamline BL13-Xaloc[40] (Alba Synchrotron, Barcelona, Spain; Detector: PILATUS 6M - Dectris) from a crystal maintained at 100 K. For the *St*-DHQ1/**1** adduct, the diffraction data were processed and scaled using XDS,[41] AIMLESS[42] and other programs within the CCP4 software suite.[43] For the *St*-DHQ1/**2** and *St*-DHQ1/**3** adducts, the diffraction data were processed, scaled, corrected for absorption effects and the crystal unit-cell parameters were calculated by global refinement with AUTOPROC,[44] which uses XDS, AIMLESS and other programs from the CCP4 software suite. The structures were solved by molecular replacement, using the program PHASER[45] with a search model generated from PDB entry 4UIO[33] (for *St*-DHQ1/**3**), PDB entry 4CNO[46] (for *St*-DHQ1/**2**) and PDB entry 1QFE[28] (for *St*-DHQ1/**4**), from which the ligand and solvent atoms were removed. The structure of the *St*-DHQ1/**3** adduct was solved by direct refinement of a model based on the *St*-DHQ1/**1** adduct, from which the ligand and solvent molecules were removed. The ligand structure and geometrical restraints, including the covalent link to the protein, were generated with the PRODRG2[47] server and JLIGAND[48]. The ligand was manually placed during the model building, which was performed with COOT[49]. Reflections used to calculate Rfree[50] were selected randomly. Refinement of the model was performed with REFMAC5[51] and final structure validation was performed with MOLPROBITY[52] and the PDB validation server[53]. Data collection, refinement and model statistics are summarized in Table S1. Structure figures were prepared using PYMOL.[54]

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Dehydroquinase Assays. The *Sa*-DHQ1 enzyme was purified as described previously.[55] Concentrated solutions of *St*-DHQ1 (0.85 mg mL^{-1}, 30.74 μM) and *Sa*-DHQ1 (4 mg mL^{-1}, 148.13 μM) were stored in potassium phosphate buffer (50 mM) and DTT (1 mM) at pH 6.6 and −80 °C. When required for assays, aliquots of the enzyme stocks were diluted in water and buffer and stored on ice. DHQ1 was assayed in the forward direction by monitoring the increase in absorbance at 234 nm in the UV spectrum due to the absorbance of the enone-carboxylate chromophore of 3-dehydroshikimic acid (ε/M^{-1} cm^{-1} 12 000). Standard assay conditions were PPB (50 mM, pH 7.2) at 25 °C. Each assay was initiated by addition of the substrate. Solutions of 3-dehydroquinic acid were calibrated by equilibration with DHQ1 and measurement of the change in the UV absorbance at 234 nm due to the formation of the enone-carboxylate chromophore of 3-dehydroshikimic acid.

Incubation studies. *St*-DHQ1 (3.1 μM from a stock protein concentration of 0.85 mg mL^{-1}) and *Sa*-DHQ1 (3.0 μM from a stock protein concentration of 4 mg mL^{-1}) was incubated with various aqueous solutions of ligands **1**–**3** in PPB (0.5 mL, 50 mM) at pH 7.0, 25 °C. The activity was progressively determined over a 24 h period under the standard assay conditions using aliquots from the incubation samples and the control. The activity was measured at a substrate concentration of 14 μM. The initial rate of each assay was measured.

Docking Studies. They were carried out using program GOLD 5.2.2[36] and the protein coordinates found in the crystal structure of *St*-DHQ1 PDB code 4UIO[33]. Ligand geometries were minimized using the AM1 Hamiltonian as implemented in the program Gaussian 09[56] and used as MOL2 files. Each ligand was docked in 25 independent genetic algorithm (GA) runs, and for each of these a maximum number of 100000 GA operations were performed on a single population of 50 individuals. Operator weights for crossover, mutation and migration in the entry box were used as default parameters (95, 95, and 10, respectively), as well as the hydrogen bonding (4.0 Å) and van der Waals (2.5 Å) parameters. The position of the side chain of the experimentally observed modified residue was used to define the active-site and the radius was set to 6 Å. All crystallographic water molecules and the ligands were removed for docking. The "flip ring corners" flag was switched on, while all the other flags were off. The GOLD scoring function was used to rank the ligands in order to fitness.

Molecular Dynamics Simulations. *Ligand preparation*. Lysine bound ligands were capped with the usual acetyl and methylamino groups. No bounded ligands were manually docked into the active site. Partial charges were derived by quantum mechanical calculations (HF/6-31G*) using Gaussian 09, as implemented in the R.E.D. Server (version 3.0),[57] according to the RESP[58] model. The missing bonded and non-bonded parameters were assigned, by analogy or through interpolation from those already present in the AMBER database (GAFF).[59]

Generation and minimization of the St-DHQ1/ligand complexes. These studies were carried out using the enzyme coordinates found in the crystal structure of the corresponding *St*-DHQ1/ligand adduct. Crystallographic water molecules were maintained. Hydrogens were added and protonation states to all titratable residues at the chosen pH of 7.0 were assigned using web-based H++ (version 3.1),[60] and WHAT-IF Optimal Hydrogen Bonding Network tool.[61] As a result of this analysis, His51, His96, His146, His179, His194 were protonated in ε position and His132, His134 and His250 in both positions. Two protonation states for His143 (δ or δ and ε) were considered as well as for the amino group of the ligand. The results showed that for covalent

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modification His143 is protonated in δ and ε and Lys170 is neutral. In addition, for covalent enzyme/inhibitor adducts, His143 is only protonated in δ and the covalently modified lys170 is protonated. Molecular mechanics parameters from the ff12SB[62] were assigned to the protein and the ligands using the LEaP module of AMBER 12.[63] Each molecular system was immersed in a truncated octahedron containing TIP3P[64] water molecules (10 Å radius) and Na^+ ions[65] to achieve electroneutrality. All systems were minimized in four stages: a) initial minimization of the ligand applying a restraint mask to the rest of the atoms; b) minimization of the solvent and ions with a restraint mask to the protein and ligand; c) minimization of the side chains, waters and ions by applying a restraint mask to all α carbons; d) final minimization of the whole system. For all the cases a positional restraint force of 50 kcal mol^{-1} $Å^{-2}$ was used. 1000 steps were used for the first minimization and 5000 in the rest, with the first half of them using steepest descent method, the second half using conjugate gradient method.

Simulations. MD simulations were performed using the pmemd.cuda_SPFP module[66] in AMBER 14[67] suite of programs and Amber ff12SB force field. Periodic boundary conditions were applied and electrostatic interactions were treated using the smooth particle mesh Ewald method (PME)[68] with a grid spacing of 1 Å. The cutoff distance for the non-bonded interactions was 9 Å. The SHAKE algorithm[69] was applied to all bonds containing hydrogen, using a tolerance of 10^{-5} Å and an integration step of 2.0 fs. The minimized system was heated at 300 K (1 atm, 200 ps, a positional restraint force constant of 50 kcal mol^{-1} $Å^{-2}$ applied to all α carbons). Following equilibration under a constant volume (NVT) ensemble (200 ps, a positional restraint force constant of 5 kcal mol^{-1} $Å^{-2}$ applied to all α carbons); and six stages of equilibrations under a constant pressure (NPT) ensemble were carried out for 100 ps each, reducing gradually the initial force constraint from 5 kcal mol^{-1} $Å^{-2}$ to 0 kcal mol^{-1} $Å^{-2}$ in the final stage. MD simulations were carried out for 50 ns collecting system coordinates every 10 ps for further analysis.

QM/MM MD simulations. For the mechanistic studies, the structure obtained at 50 ns of the MD simulation of *St*-DHQ1/**2** complex was used. The QM region included the ligand **2**, the catalytic water W303, and the side chains of residues: Glu46, Arg82, Asp114, His143, Lys170 and Phe225. Hydrogen 'link atoms'[70] [71] were used to model bonds across the QM/MM boundary, specifically between Cβ and Cγ of Glu46, Cγ and Cδ of Arg82 and Lys170, Cα and Cβ of Asp114, His143 and Phe225. QM/MM calculations were performed using sander from AMBER 17 (version 17.10)[72]. SCC-DFTB[73] was used for the QM region.

QM/MM umbrella sampling MD simulations were run for each reaction step, harmonically restraining the reaction coordinate with a force constant of 100 kcal mol^{-1} $Å^{-2}$. Each simulation (window) consisted of 5 ps (10000 steps with an integration step of 0.5 fs) of sampling and the reaction coordinate was increased (or decreased) by 0.1 Å between neighbouring windows, using the last geometry of the previous window as starting point. Values of the reaction coordinate were collected for all simulation steps. The free-energy profiles for each step were obtained by combining the statistics from all simulations for that reaction using the weighted histogram analysis method (WHAM).[74] [75] [76] The following reaction coordinates were used: *(a)* formation of the C–N bond, $rc1 = d(NZ_{Lys170}–C8_2)$ between 3.0 and 1.5 Å; *(b)* a linear combination of distances for the proton transfer from Lys170 and

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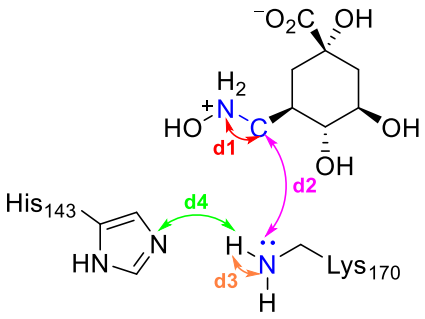
His143, *rc2* = [d(NZ_{Lys170}–$HZ3_{Lys170}$) – d($HZ3_{Lys170}$–$NE2_{His143}$)] between −1.0 and 1.0 Å. To avoid that the attack of the Lys170 happened from the same face as the leaving group, an additional restraint was employed: an angle restriction between NZ_{Lys170} $C8_2$ and NZ_2 with a force constant of 100 kcal mol^{-1} degree^{-2} and a minimum value of 150 degrees.

Results of the reaction coordinates explored: The geometries at 19, 32, 50 and 90 ns on the non-covalent binary *St*-DHQ1/**2** complex MD simultation (100 ns) were choosen. Four possible reaction coordinates were explored, which are indicated below. Two possible protonation states of His143 (δ and dual) were initially employed. However, the latter possibility (dual) was ruled out because it caused an opening of the active site. The results discussed below involve the use of neutral His143 (free N pair in position ε):



(i) *Breakdown of the C–N bond in* **2** *(d1).* This possibility afforded very high energies due to the formation of a carbocation and HNO as leaving group.

(ii) *Reduction of the distance between C8(***2***)–NZ(K170) (d2) until 1.5 Å.* The results showed that the energy increases without reaching a maxima. The formation of the new C-N bond and the release of NH_2OH was not observed. A restriction of the angle of attack of the NZ atom of Lys170 to carbon C8 in **2** of 120° and 150° was applied. The best results were obtained with the latter restriction (150°), not only in terms of energy, but also in the chemical processes observed. Thus, for the structure at 50 ns, an activation energy of 39.0 kcal mol^{-1} and a ΔG of 27.7 kcal mol^{-1} were obtained. Once Lys170 is covalently modified, the release of NH_2OH and the deprotonation of the modified Lys170 by His143 were observed. However, a lack of overlap in the population histogram for windows at 1.9 and 1.8 Å was identified, which usually leads to higher energy values. To avoid this issue, a two-dimensional Umbrella Sampling study (approach iii) was then performed.

(iii) *Two-dimensional US:* The first reaction coordinate (rc1) was the decrease of d2 from 3.0 until 1.5 Å and the second reaction coordinate (rc2) was the difference of the distances d3–d4, which corresponds to the proton transfer from Lys170 to His143, with values for the difference of distances from -1.0 until 1.0 Å. The structure at 50 ns of the non-covalent binary *St*-DHQ1/**2** complex MD simultation was used. The results revealed that the process is sequential with the nucleophilic attack of the NZ atom of Lys170 with the release of NH_2OH occurring first. An energy barrier for the **TS1** of +27.2 kcal mol^{-1} was obtained, wth an energy of +11.8 kcal mol^{-1} for the intermediate **INT** formation (see Fig. 4 main text). The deprotonation of the modified Lys170 involved a very low energy barrier for the **TS2** (+0.9 kcal mol^{-1} relative to **INT**). The resulting product has a similar energy than the reactants (−0.1 kcal mol^{-1}).

(iv) *Lineal combination of d1–d2.* The structures at 19 and 50 ns of the non-covalent binary *St*-DHQ1/**2** complex MD simultation were used. The reaction coordinate explored varies from −1.9 to 1.9 Å (19 ns structure) and −1.7 to 1.7 Å (50 ns structure). A restriction of the angle of attack of the NZ atom of Lys170 of 150° was employed. The structure at 50 ns provides the best results with a energy barrier for the TS of +34.1 kcal mol^{-1} and +16.2 kcal mol^{-1} for the reaction. The results were similar to those obtained in (ii). A two-dimensional Umbrella Sampling study was then explored with the structure at 50 ns. The rc1 was the aforementioned linear combination *d1–d2* and rc2 was the difference of the distances d3–d4, which corresponds to the proton transfer from Lys170 to His143 from −1.0 to 1.0 Å. The results were similar to approach (iii) in terms of energy and the chemical processes observed.

Table S1. Crystallographic data collection and refinement statistics for the *St*-DHQ1/**1**–**4** adducts[a]

Data processing[a]	*St*-DHQ1/**1**	*St*-DHQ1/**2**	*St*-DHQ1/**3**	*St*-DHQ1/**4**
space group	*P*1	*P*1 2_1 1	*P*2_1 2_1 2	P2 2_1 2_1
cell parameters				
a, b, c (Å)	a = 42.88, b = 43.84, c = 73.26	a = 60.22, b = 44.06, c = 84.60	a = 46.73, b = 114.63, c = 42.55	a = 42.50, b = 46.60, c = 114.49
α, β, γ (°)	α = 92.26, β = 93.05, γ = 119.25	α = γ = 90.00, β = 95.25	α = β = γ = 90.00	α = β = γ = 90.00
wavelength (Å)	0.97918	0.97918	0.97918	0.97925
observed reflections[b]	150270 (21721)[c]	115498 (5854)[c]	109621 (15714)[c]	247132 (15865)[c]
resolution range (Å)	38.14 – 1.14 (1.20 – 1.14)	59.97 – 1.25 (1.27 – 1.25)	46.73 – 1.04 (1.10 – 1.04)	46.60 – 1.40 (1.48 – 1.40)
Wilson B (Å^2)	12.4	7.9	10.3	13.4
multiplicity	1.8 (1.7)	3.1 (2.8)	10.2 (7.9)	6.1 (4.7)
completeness	0.886 (0.876)	0.944 (0.972)	0.999 (0.995)	0.894 (0.528)
Mean((I)/sd(I))	6.3 (1.4)	8.5 (2.1)	16.9 (2.2)	24.0 (4.1)
Mn(I) half-set correlation CC(1/2)	0.996 (0.610)	0.984 (0.538)	0.999 (0.767)	0.999 (0.918)
R_{merge}	0.047 (0.496)	0.090 (0.719)	0.063 (0.832)	0.040 (0.387)
Refinement[d]				
resolution range (Å)	38.18 –1.14 (1.17 – 1.14)	59.97 – 1.25 (1.28 – 1.25)	46.73 – 1.04 (1.07 – 1.04)	42.53 – 1.40 (1.48 – 1.40)
reflections used in refinement[c]	142383 (10395)	109028 (8247)	104069 (7543)	38638 (3233)
reflections used for R_{free}	7885 (577)	5737 (435)	5426 (391)	2040 (172)
R factor[e]	0.151 (0.285)	0.158 (0.293)	0.124 (0.251)	0.126 (0.180)
R_{free}[f]	0.182 (0.281)	0.200 (0.321)	0.144 (0.248)	0.177 (0.265)
rmsd (bonds (Å)/angles (°))	0.010/1.498	0.011/1.5	0.009/1.466	0.013/1.6
Final Model				
Protein/inhibitor/water atoms	3967/26/703	4056/26/759	2043/12/448	1996/12/249
average B protein/inhibitor/water (Å^2)	13.0/9.4/27.5	12.1/10.1/26.8	14.4/11.4/30.0	16.7/13.7/29.7
Ramachandran statistics[g] (%)	97.7/100	98.9/100	98.9/100	98.1/100.0
PDB accession code	6H5C	6H5D	6H5G	6H5J

[a]Results from AIMLESS.[41] [b]No sigma cut-off or other restrictions were used for inclusion of reflections. [c]Values in parentheses are for the highest resolution bin, where applicable. [d]Results from REFMAC5.[50] [e]*R*-factor = Σ ||*Fobs(hkl)*| - |*Fcalc(hkl)*|| / Σ |*Fobs(hkl)*|. [f]According to Brünger.[49] [g]According to the program MOLPROBITY.[51] The percentages indicated are for residues in favored and total allowed regions, respectively.

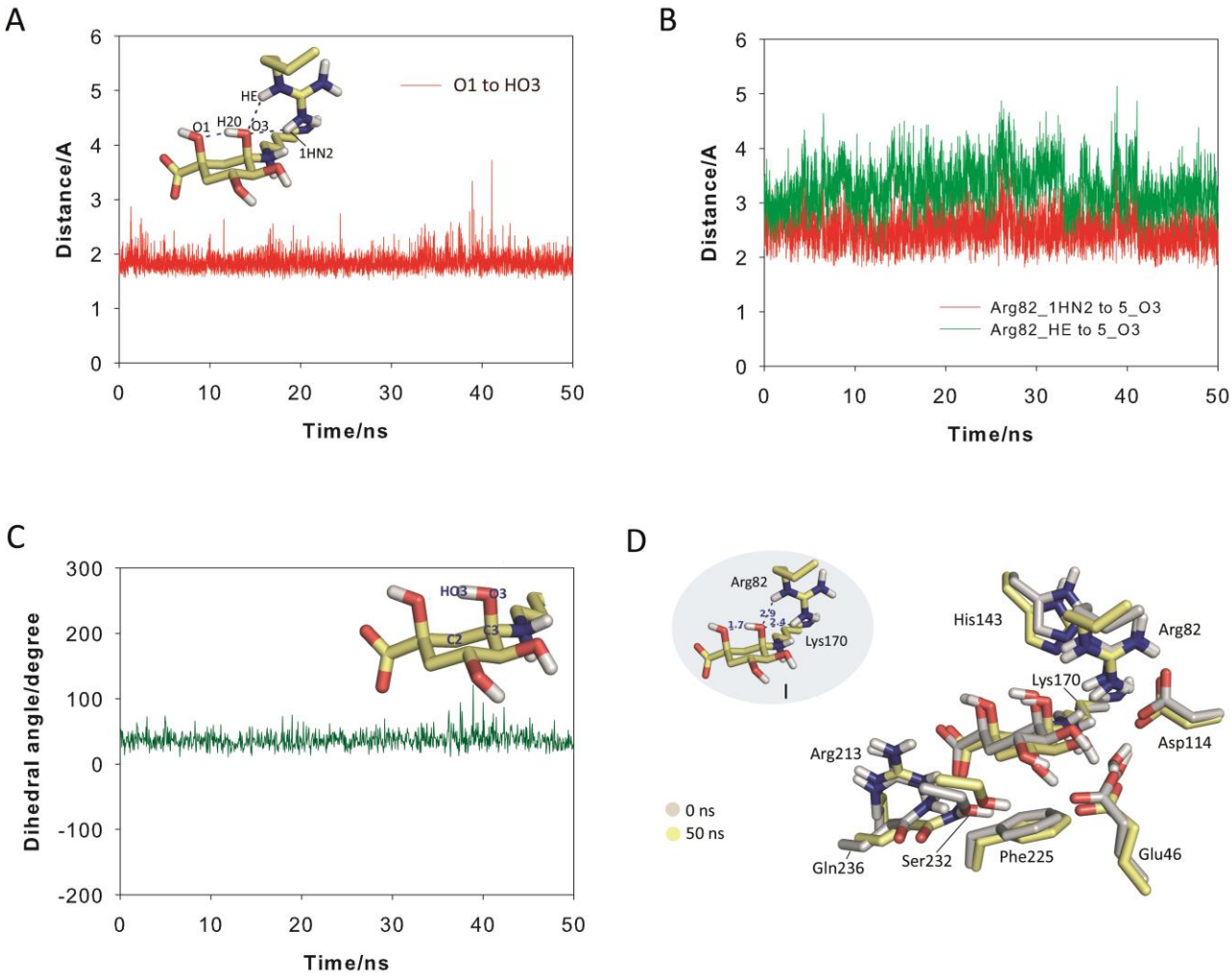


Figure S1. Variation of the relative distances between: (A) the O1 and HO3 atoms of modified natural substrate in addition intermediate adduct I (average distance of 1.8 Å)**;** and (B) the O3 atom of modified natural substrate and the 1HN2 and HE atoms of Arg82 (average distances of 2.5 and 3.3 Å, respectively). Note how these interactions remain very stable during the whole simulation (50 ns). (C) Variation of the dihedral angle between the C2, C3, O3 and HO3 atoms of modified natural substrate in addition intermediate adduct I during the whole simulation. Note how the oxygen´s lone pairs of the C3 hydroxyl group are geometrically well engaged by the guanidinium group of Arg82. The dihedral angle remains stable during the simulation with an average dihedral angle of ~37.2°. (D) Comparison of the binding mode of the addition intermediate I in the *St*-DHQ1/I adduct after minimization and prior to simulation (gray) and after 50 ns of dynamic simulation (yellow). Relevant hydrogen bonding interactions and side chain residues are shown and labeled.

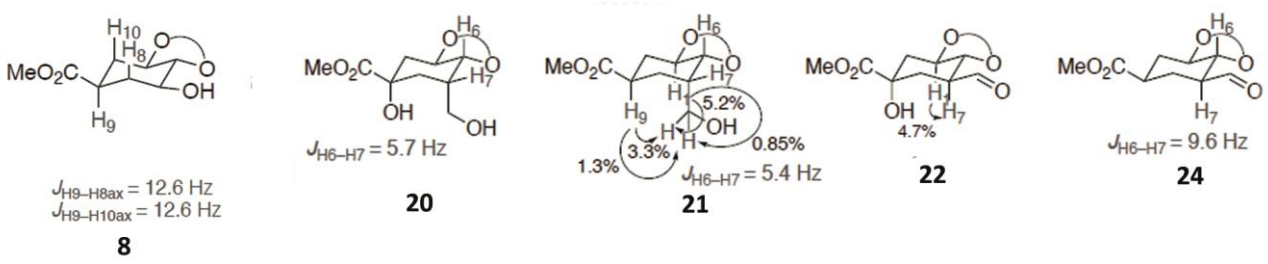


Figure S2. Determination of the stereochemistry of positions C7 and C9 in compounds **8**, **20**−**22** and **24** based on the coupling constants between H6 and H7 and H9 and $H8_{axial}$ and $H10_{axial}$ in their ^{1}H NMR spectra and NOE experiments. The $J_{H6,H7}$ of 5.7 Hz and 5.4 Hz in compounds **20** and **21**, respectively, and of 9.6 Hz in compound **26** are in agreement with an axial and equatorial disposition of the CH_2OH and CHO groups in **20**−**22** and **24**, respectively. The $J_{H9,H8ax}$ and $J_{H9,H10ax}$ of 12.6 Hz in compound **8** is in agreement with an axial disposition of H9 hydrogen atom. In addition, the equatorial disposition of the aldehyde group in **22** and the axial position of the CH_2OH group in **21** were confirmed by NOE experiments. Irradiation of H1 in **22** led to enhancement of the signal for H7 (4.7%). Irradiation of H1 in **21** led to enhancement of the signal for the methylene group (0.85%, 5.2%). Irradiation of H9 in **21** led to enhancement of the signal for the methylene group (1.3%, 3.3%).

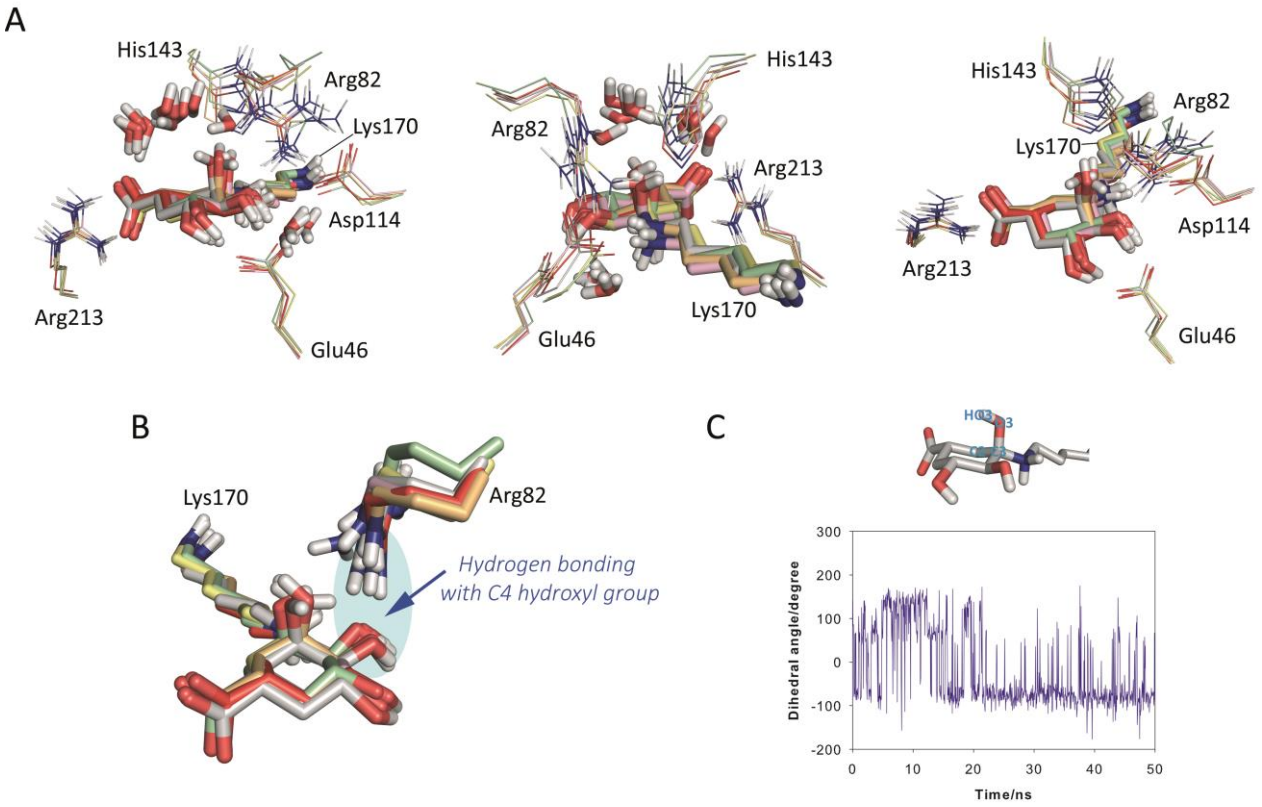


Figure S3. (A−B) Comparison of the several snapshots of the *St*-DHQ1/**4** adduct during 50 ns of MD simulations [after 1 ns (grey), 10 ns (pale green), 20 ns (pale yellow), 30 ns (light pink), 40 ns (pale orange) and 50 ns (red)]. Relevant side chain residues and water molecules are shown and labeled. (B) Comparison of the position of the side chain of Arg82 and the modified Lys170 after 1, 10, 20, 30, 40 and 50 ns of simulation. Note how there is free rotation of the C3 hydroxyl group of the modified ligand **4** and how the guanidinium group of Arg82 is mainly interacting with the C4 hydroxyl group of the modified ligand **4** instead of with the C3 hydroxyl group as for the natural substrate (Figure 3B, main text). Note how the oxygen´s lone pairs of the C3 hydroxyl group are not engaged by the guanidinium group of Arg82 as for the natural substrate. (C) Variation of the dihedral angle between the C2, C3, O3 and HO3 atoms of modified substrate analogue **4** during the whole simulation. Note the large variability of the dihedral angle for this adduct compared with the corresponding one for the addition adduct intermediate I (average dihedral angle is ~37.2°, see Figure S1).

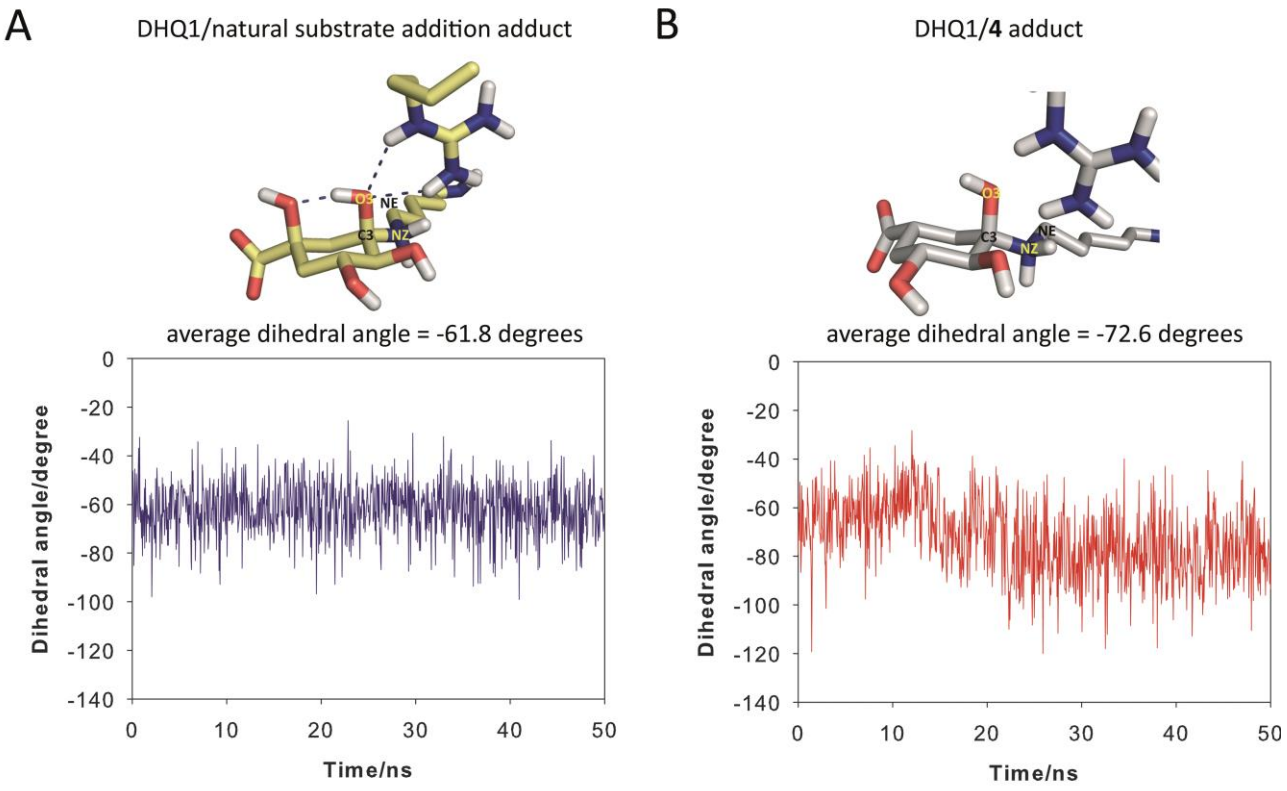


Figure S4. Variation of the dihedral angle between the O3, C3, NZ and NE atoms of the natural substrate addition adduct (A) and the modified substrate analogue **4** (B) during the whole simulation.

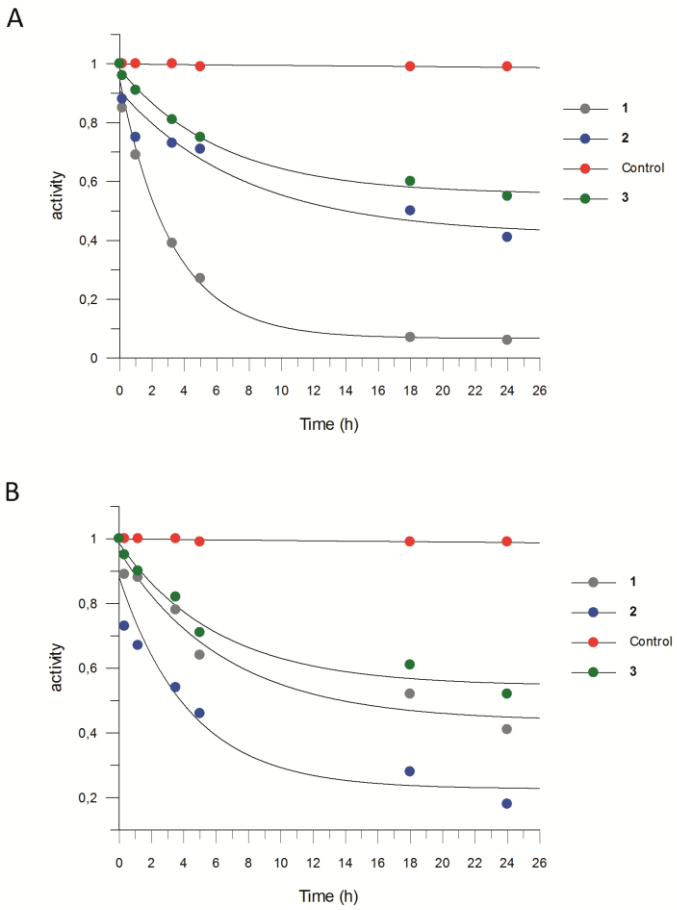


Figure S5. Variation of the enzymatic activity of *Sa*-DHQ1 (A) and *St*-DHQ1 (B) with time without incubation and after incubation with ligands **1**−**3**. Results are the average of three observations. Assay conditions were PPB (50 mM, pH 7.0) at 25 °C, ligand concentration: [**1**] = 406 µM, [**2**] = 373 µM, [**3**] = 630 µM; enzyme concentration [*Sa*-DHQ1] = 3.0 µM and [*St*-DHQ1] = 3.1 µM.

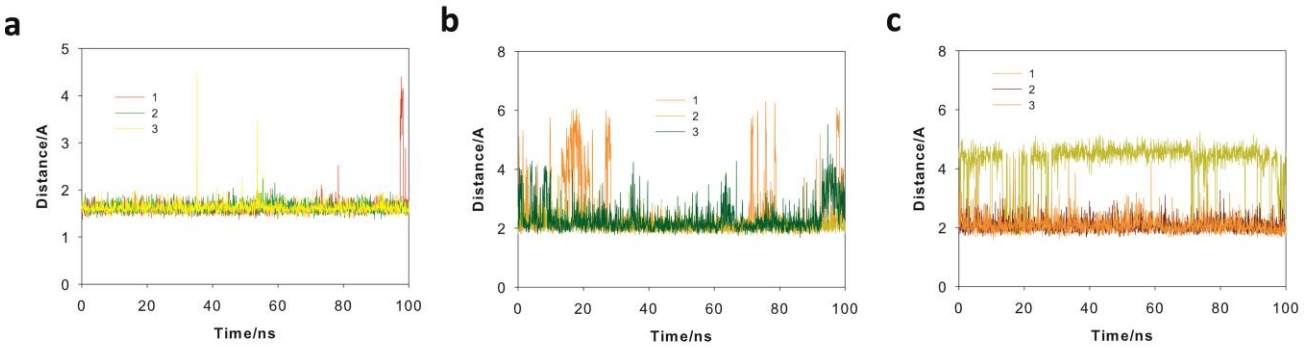


Figure S6. **a** Variation of the relative distance between residue Asp114 (OD1 atom) and the $NH_2O\underline{H}$ group (H04 atom) of the ligands **1**–**3** during the whole simulation. **b** Variation of the relative distance between residue His143 (NE2 atom) and the $N\underline{H}_2OH$ group (H02 or NZ atom) of the ligands **1**–**3** during the whole simulation. **c** Variation of the relative distance between the structural water molecule (O atom) and the $N\underline{H}_2OH$ group (NZ atom) of the ligands **1**–**3** during the whole simulation.

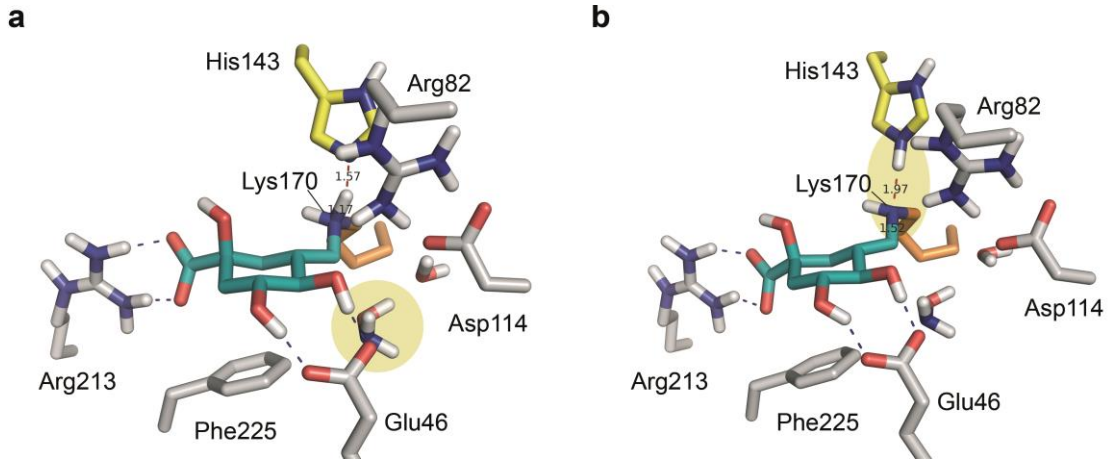
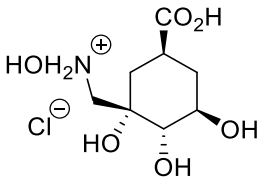


Figure S7. Representative geometries of the **TS2** (**a**) and the products (**b**). Relevant residues and water molecule are shown and labeled. Key hydrogen bonding interactions and bonds broken/formed (distances included) are indicated as blue and red dashed lines, respectively. The residues Lys170 and His143 are highlighted in orange and yellow, respectively. Geometries were taken from the potential energy surface (see Fig. 5 main text).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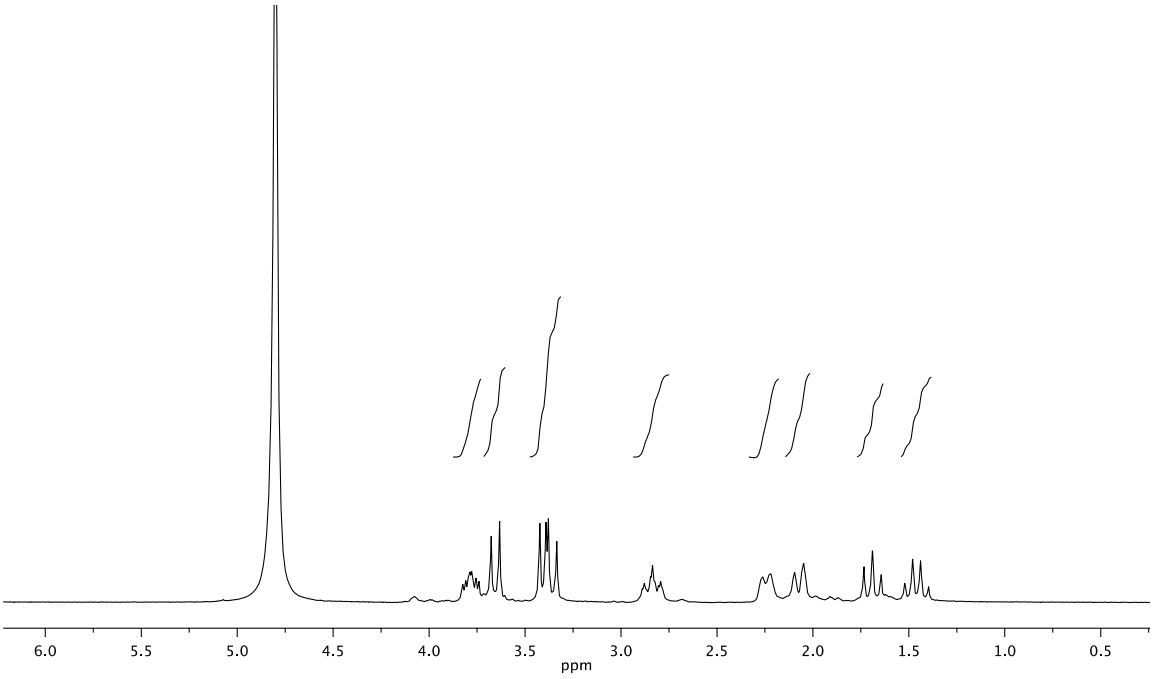
Cl
HO
OH
OH

1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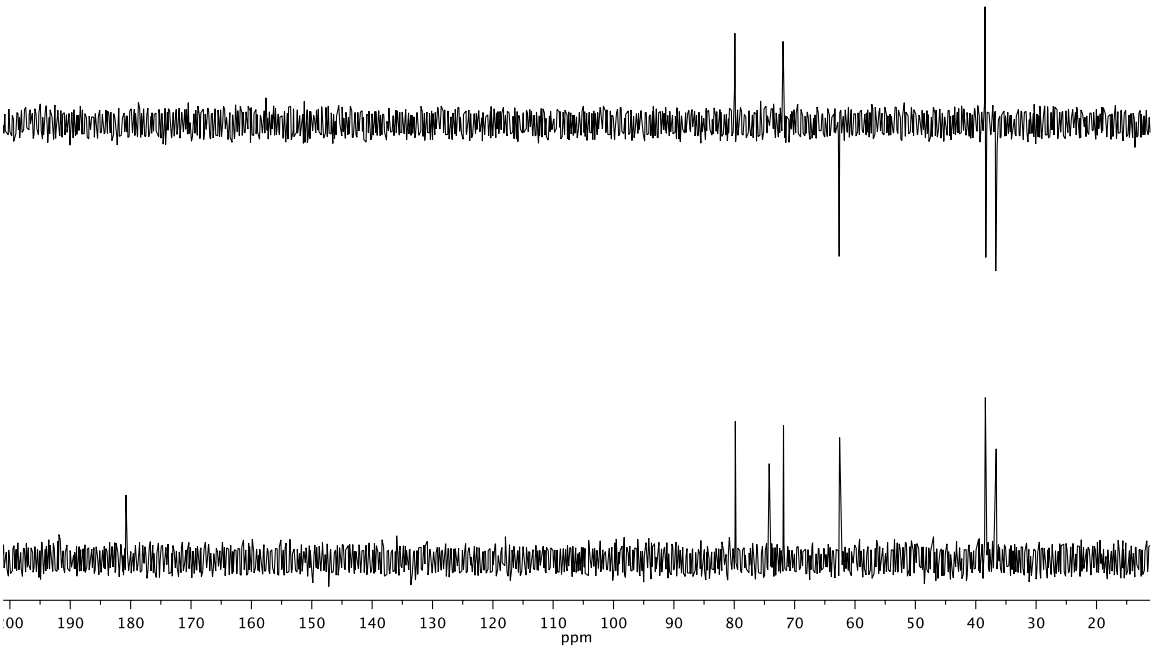
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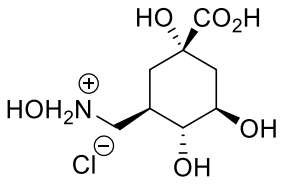
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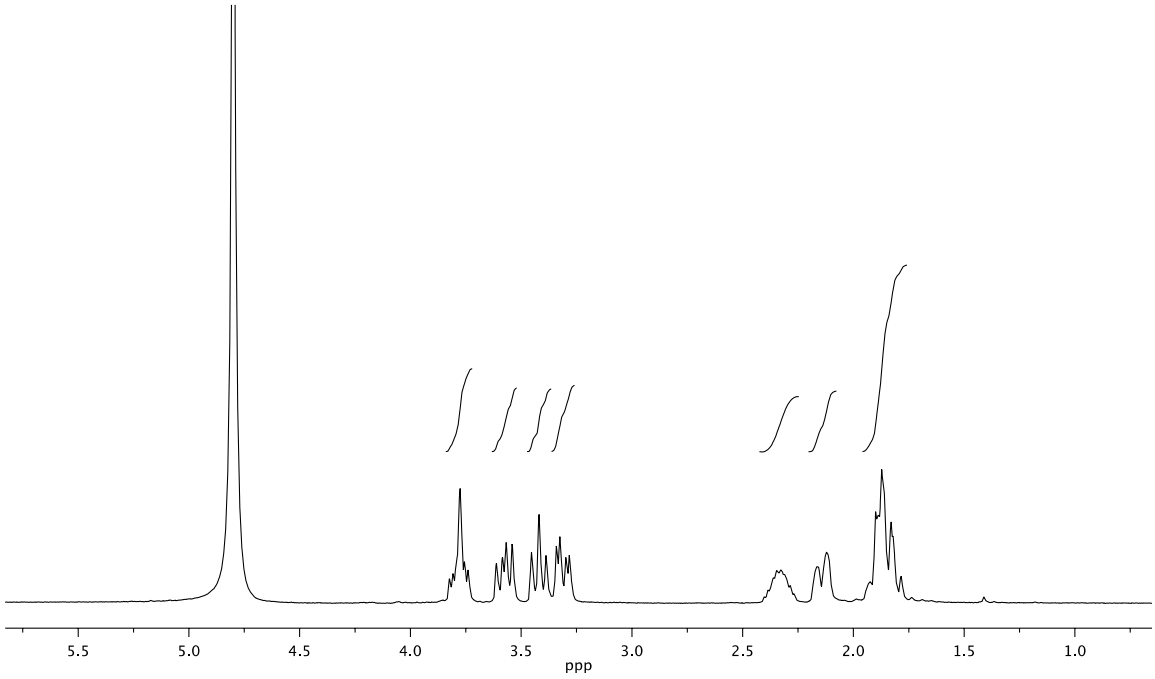
2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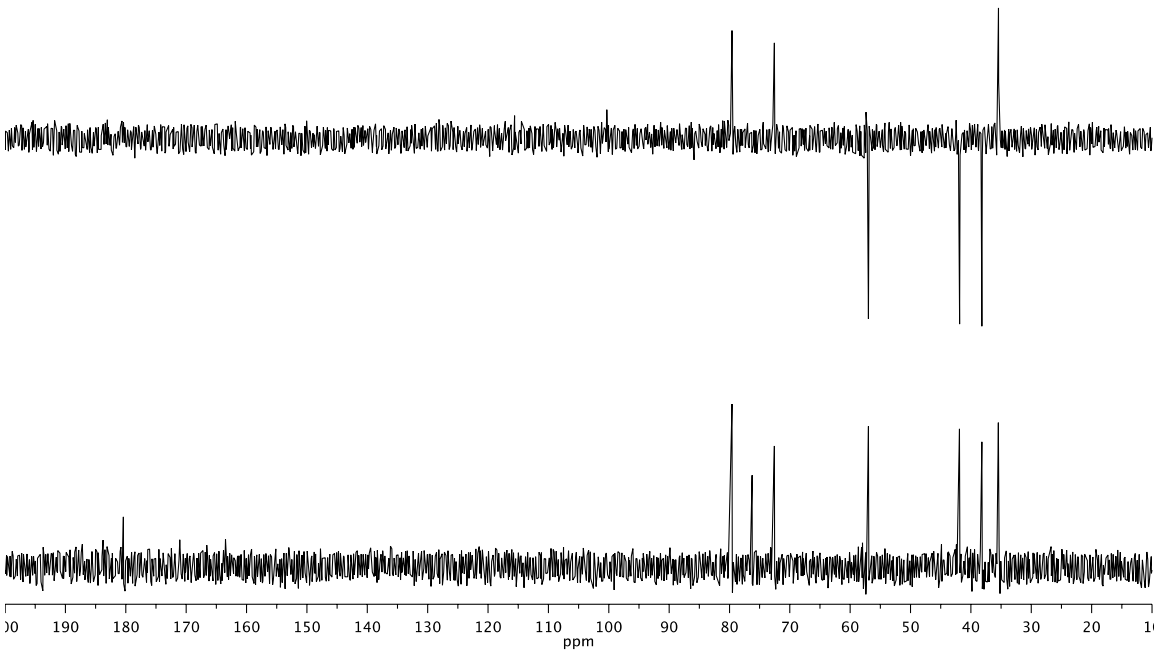
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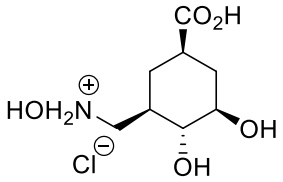
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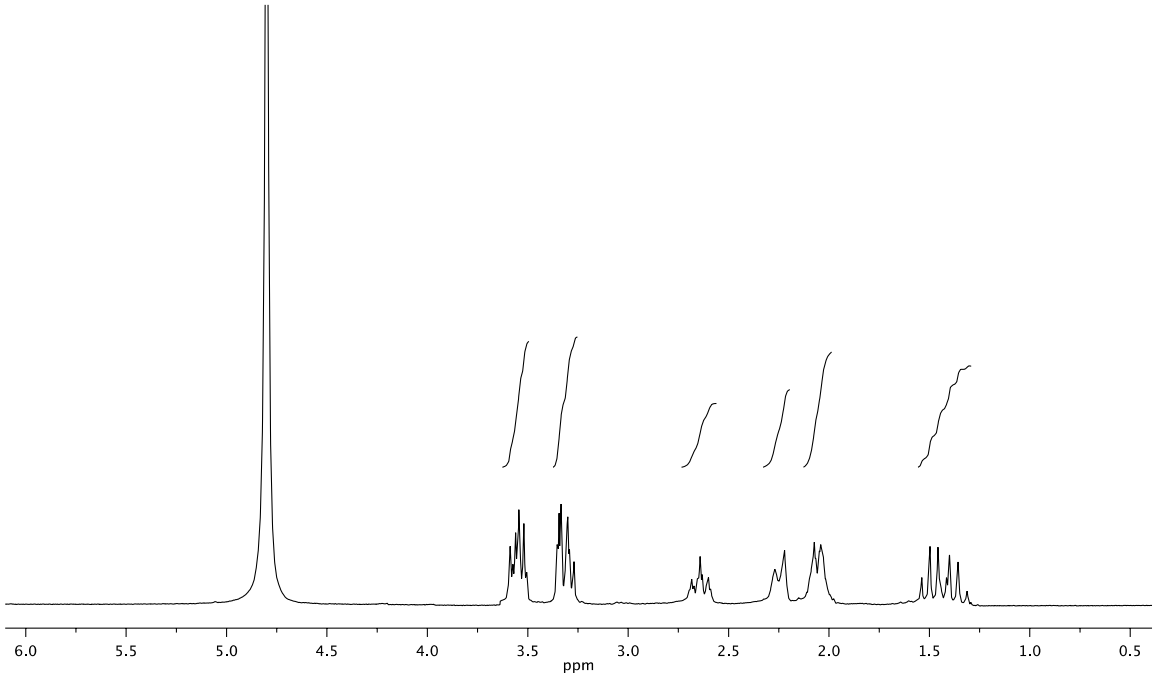
Cl
OH

3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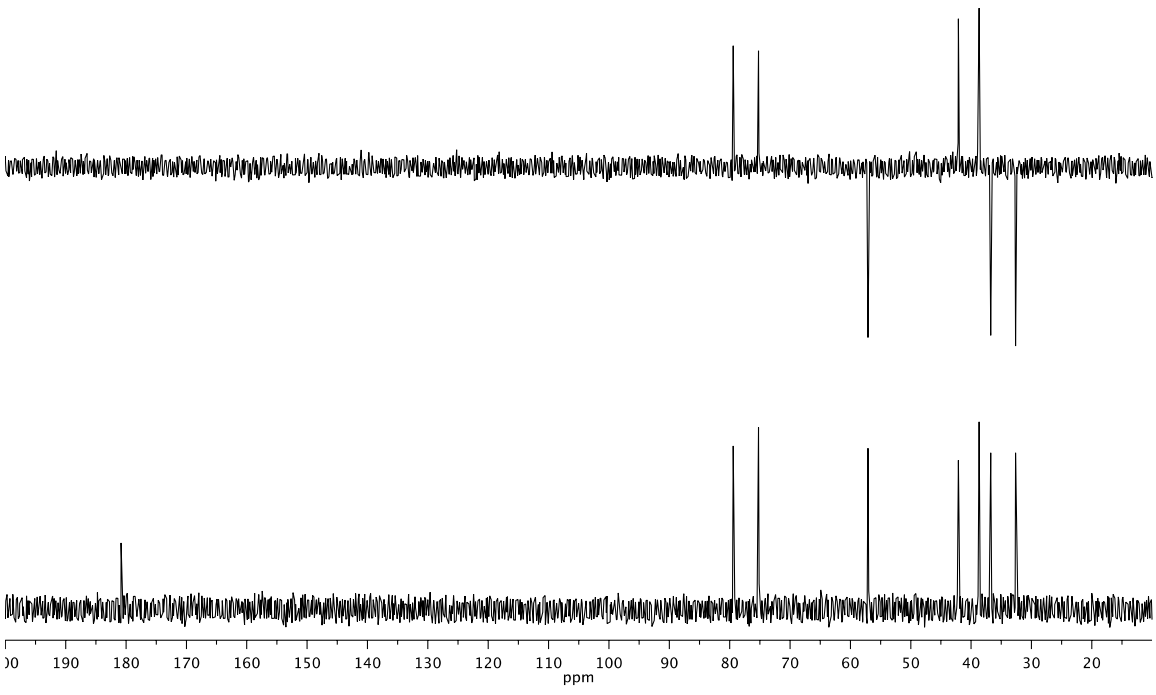
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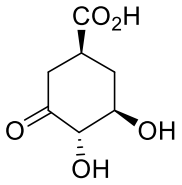
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OH
OH

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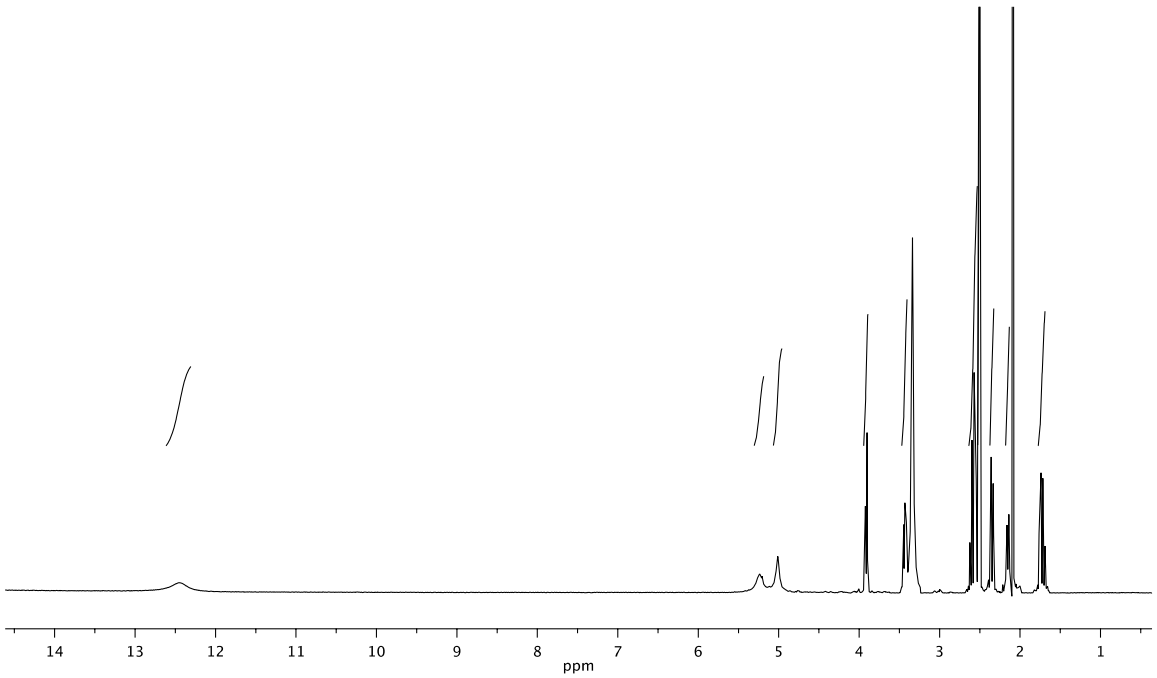
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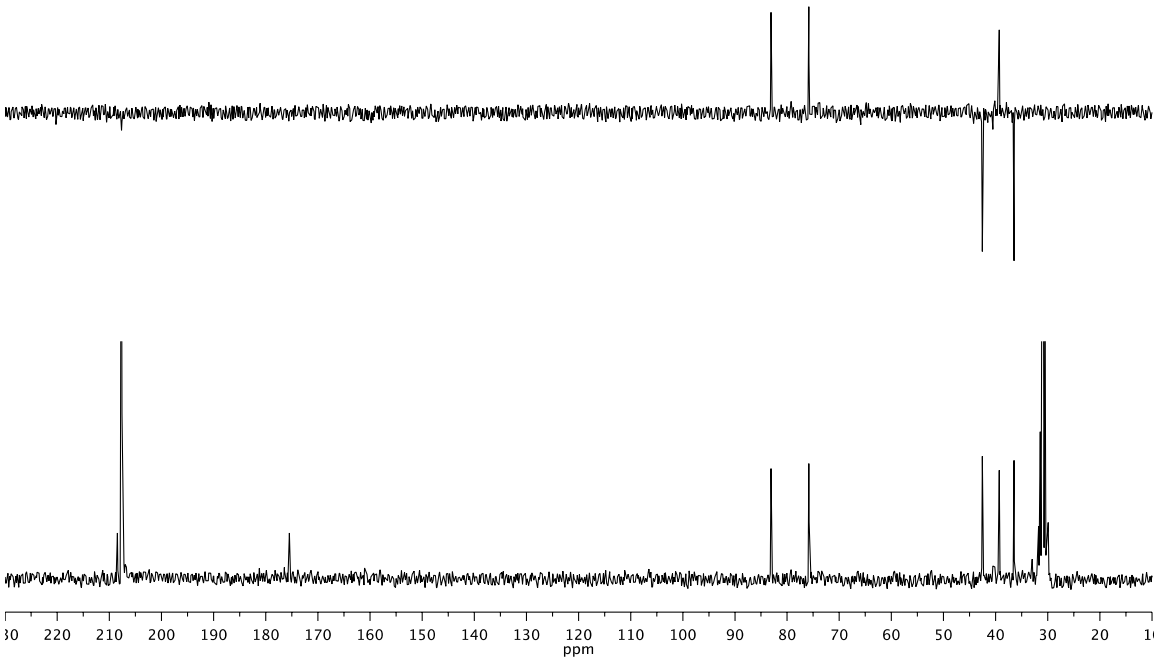
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ppm