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Competitive Inhibitors of *Helicobacter pylori* Type II Dehydroquinase: Synthesis, Biological Evaluation, and NMR Studies

Cristina Sánchez-Sixto,[a] Verónica F. V. Prazeres,[a] Luis Castedo,[a] Se Won Suh,[b] Heather Lamb,[c] Alastair R. Hawkins,[c] Francisco Javier Cañada,[d] Jesús Jiménez-Barbero,[d] and Concepción González-Bello*[a]

*Several 3-heteroaryl analogs of the known dehydroquinase inhibitor (1R,4R,5R)-1,4,5-trihydroxy-2-cyclohexene-1-carboxylic acid (**4**) were synthesized and tested as inhibitors of Helicobacter pylori type II dehydroquinase, the third enzyme of the shikimic acid pathway. All of these compounds proved to be reversible competitive inhibitiors of this enzyme and proved to be, with the exception of nitrile **8e**, more potent than the parent inhibitor **4** (K_i = 370 μM). The 2-thienyl derivative **8b** was found to be the most potent inhibitor of the series and has a K_i value of 540 nM, which is almost seven hundred times lower than that of the parent inhibitor. The 3-nitrothienyl derivative **8d** and 2-furanyl derivative **8a** also had a good affinity of 1 μM. The conformation of the potent competitive inhibitor **8b**, when bound in the active site of the H. pylori enzyme, was elucidated by 1D-selective inversion NOE, Saturation Transfer Difference (STD) and transferred NOESY NMR experiments. One of the conformations that exists in solution for the potent competitive inhibitor 2-thienyl derivative **8b** is selected when it is bound to the active site of the enzyme. In the bound conformation derivative **8b** has the sulfur atom of its thienyl group oriented towards the double bond of the cyclohexene moiety. The large STD effects observed for the aromatic protons of **8b** show that it is the thiophene side of the ligand that makes closest contact with enzyme protons. Docking studies using GOLD3.0.1 suggest that the conformation determined by NMR allows strong lipophilic interactions with the enzyme residues Pro9, Asn10, Ile11, Gly78 and Ala79. Competitive STD experiments carried out with high-, medium- and low-affinity ligands **8b**, **5d** and **5f** show that they all bind in the same site of Helicobacter pylori dehydroquinase.*

Introduction

Bacterial infections have been a scourge of humankind for millennia. The discovery of penicillin and other natural or synthetic antibiotics, which revolutionized the treatment of infectious diseases, was one of the most important achievements of the last century. However, the remarkable success of antibiotic therapies has led to abuse and the appearance of resistant bacterial strains.[1–2] This fact is especially remarkable for important diseases such as tuberculosis or pneumonia, where the current therapies have become less efficient.[3–8] The most vulnerable individuals are those with a compromised immune system such as HIV patients. The synergy between the AIDS epidemic and the increasing surge of multidrug-resistant isolates to antibiotics, leads to the alarming conclusion that antibiotics are losing their effectiveness.[9–11] Therefore, the development of new antibiotics to control these adaptable pathogens is much needed.

Bacteria, as well as fungi, higher plants, and some apicomplexan parasites (which are the cause of malaria),[12–14] biosynthesize the aromatic amino acids L-Phe, L-Tyr, and L-Trp—as well as other important aromatic compounds such as folates, ubiquinone, and vitamins E and K—through a biosynthetic route known as the shikimic acid pathway.[15,16] This route involves seven enzymes that catalyze the sequential conversion of erythrose-4-phosphate and phosphoenol pyruvate to chorismic acid, which is the precursor of these aromatic compounds.[15–17] The absence of the pathway in mammals, com-

[a] C. Sánchez-Sixto, V. F. V. Prazeres, Prof. Dr. L. Castedo, Prof. Dr. C. González-Bello
Laboratorio de Química Orgánica (CSIC) y
Departamento de Química Orgánica
Facultad de Química
Universidad de Santiago de Compostela
Avenida de las Ciencias s/n, 15782 Santiago de Compostela (Spain)
Fax: (+34) 981-59-5012
E-mail: cgb1@usc.es

[b] Prof. Dr. S. W. Suh
Laboratory of Structural Proteomics
School of Chemistry and Molecular Engineering
Seoul National University
Seoul (Korea)

[c] Dr. H. Lamb, Prof. Dr. A. R. Hawkins
Institute of Cell and Molecular Biosciences
Medical School
University of Newcastle upon Tyne
Catherine Cookson Building
Framlington Place, Newcastle upon Tyne NE2 4HH (UK)

[d] Dr. F. J. Cañada, Prof. Dr. J. Jiménez-Barbero
Centro de Investigaciones Biológicas
CSIC, Ramiro de Maeztu 9, 28040 Madrid (Spain)

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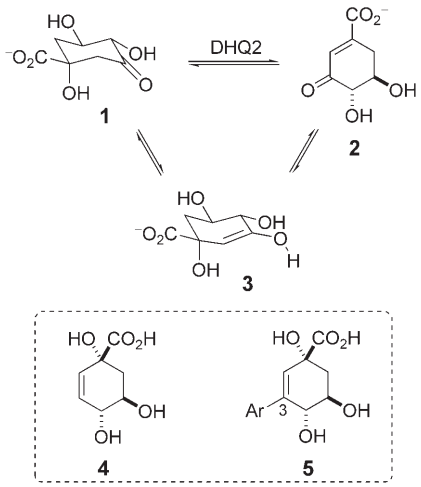
bined with its essential nature in certain microorganisms, makes the shikimic acid pathway enzymes attractive targets for the development of new antibiotics.[18]

Dehydroquinase (3-dehydroquinate dehydratase, DHQ, EC 4.2.1.10), the third enzyme of the shikimic acid pathway, catalyses the reversible dehydration of 3-dehydroquinic acid (**1**) to form 3-dehydroshikimic acid (**2**) (Scheme 1). Biochemical and



Scheme 1. Enzymatic conversion of 3-dehydroquinic acid (**1**) to 3-dehydroshikimic acid (**2**) catalysed by DHQ2. The reaction proceeds via an enol intermediate (**3**). Derivatives **4** and **5** are competitive inhibitors of DHQ2-Mt.

genetic studies have shown that there are two different dehydroquinases, known as type I and type II, and these utilize completely different mechanisms to catalyze the same overall reaction.[19] The type II dehydroquinase (DHQ2),[20,21] which is present in many bacterial species including *Mycobacterium tuberculosis*, *Streptomyces coelicolor*, and *Helicobacter pylori*, among other microorganisms, catalyzes this elimination reaction by a stepwise E_1CB mechanism involving the enol intermediate **3**.[22,23]

Compound **4**[24] (Scheme 1) is a known competitive inhibitor of DHQ2. We have recently shown that the incorporation of aryl groups bearing electron-withdrawing substituents at position C3 increases the potency of **4** against *M. tuberculosis* DHQ2 (DHQ2-Mt) by a factor of more than 3500, lowering the K_i from 200 μM for **4** to 54 nM for derivative **5** (Ar = 3-nitrophenyl).[25,26] We also showed that derivatives containing bulkier aryl groups at C3 (Ar = benzofuranyl, benzothienyl, naphthyl, etc.) are also good competitive inhibitors of DHQ2-Mt, with K_i values in the low or submicromolar range.[27] Moreover, analogues of **4** substituted at C3 with smaller heterocycles such as the 3-thienyl group (K_i of 590 nM) or 3-furanyl group (K_i of 830 nM) also proved to be potent.[25]

These promising results encouraged us to seek not only DHQ2-Mt inhibitors as potential antituberculostatic agents, but also to search for inhibitors of other pathogenic bacteria such as *Helicobacter pylori*. This bacterium causes gastric and duodenal ulcers and has been classified by the World Health Organization as a class I carcinogen.[28,29] The spiral shape and motion of this bacterium allows it to penetrate the protective mucous lining of the stomach, where it releases urease to neutralize stomach acids. As a result of its interference with stomach acid secretion, the stomach tissues become more susceptible to the damaging effects of acid and pepsin, and develop sores or ulcers in the stomach or duodenum. *H. pylori* infects approximately two-thirds of the world's population, with the highest prevalence among adults in developing countries. It has been estimated that 10–15% of individuals with *H. pylori* infection develop a duodenal ulcer at some point in their life.

Crystal structures of *H. pylori* DHQ2 (DHQ2-Hp) complexed with the substrate, 3-dehydroquinic acid (**1**),[30] and with the competitive inhibitors *N*-tetrazol-5-yl-9-oxo-9*H*-xanthene-2-sulfonamide[31] and derivative **4**[31] have recently been reported.[32] These structures show the enzyme to have the same folded structures as previously described for other DHQ2 systems.[23] In comparison to the situation observed in another DHQ2, DHQ2-Hp has a markedly longer C-terminal helix, which extends twelve residues beyond the main body of the monomer. Moreover, the active site of DHQ2-Hp is quite similar to that of DHQ2-Mt, both being smaller than that of *Streptomyces coelicolor* DHQ2.[23] For this reason, analogues containing small heterocyclic moieties, such as the DHQ2-Mt inhibitor **5** (Ar = 3-thienyl), may also be good competitive inhibitors of DHQ2-Hp. Accordingly we began our search for DHQ2-Hp inhibitors by examining the potency of the 3-aryl derivative **5** against this enzyme. The 3-thienyl derivative **5** (Ar = 3-thienyl) proved to be the most potent competitive inhibitor of the series, with a K_i value of 1.8 μM, which is more than 200 times more potent than the parent inhibitor **4** (K_i of 370 μM).[31] The potency of the 3-thienyl derivative **5** (Ar = 3-thienyl) against DHQ2-Hp encouraged us to examine more closely the importance of π-richness in the five-membered heterocycle, and to compare 3′-yl and 2′-yl substituents. To this end we prepared analogues containing a π-deficient five-membered ring (triazole **6**), a 3-thiophenyl ring with a strong electron-withdrawing group (nitrothiophene **7**), and the 2-furanyl and 2-thienyl derivatives **8a** and **8b** (Figure 1). We also decided to investigate whether the affinity of **8b** might be increased by the incorporation of elec-

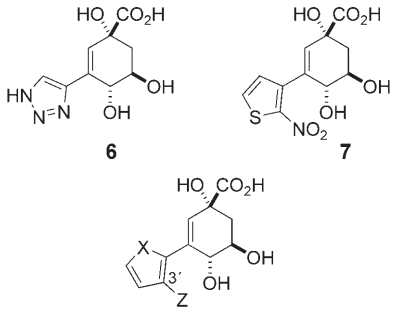


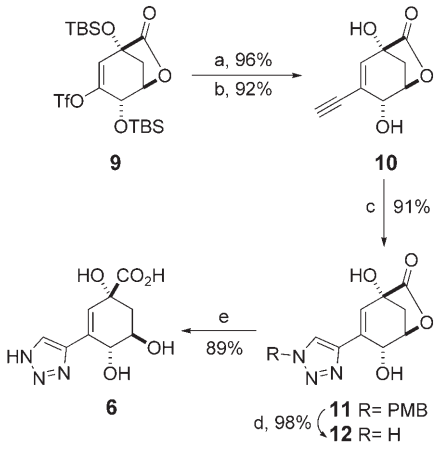
Figure 1. Newly designed 3-heteroaryl derivatives.

tron-withdrawing substituents in position 3′, to which end we prepared the bromo, nitro, and nitrile derivatives **8c–e** (Figure 1).

Results and Discussion

Synthesis of Compounds 6–8

*Synthesis of Triazole **6**.* The triazole moiety of **6** was introduced by a copper(I)-catalyzed 1,3-dipolar cycloaddition between *p*-methoxybenzylazide and vinyl alkyne **10**, which was synthesized from vinyl triflate **9** using our previously reported protocol[27] (Scheme 2). The cycloaddition reaction regioselectively

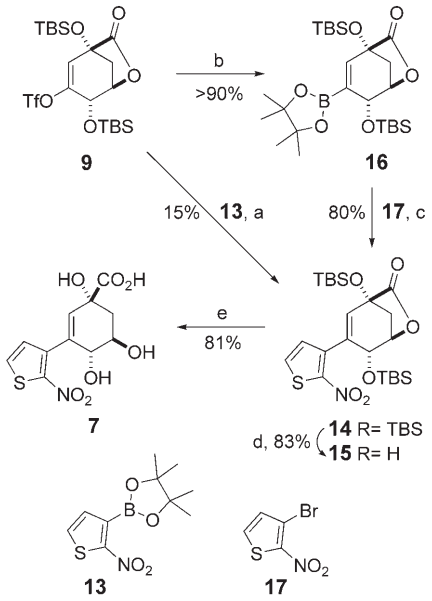


Scheme 2. Reagents and conditions: a) $Pd(PPh_3)_4$, CuI, TMSC≡CH, piperidine, THF, 40 °C. b) TBAF, THF, 0 °C→RT. c) $PMBN_3$, sodium ascorbate, *t*BuOH-H_2O, $CuSO_4$, RT. d) TFA, 65 °C. e) 1. LiOH, THF, RT; 2. Amberlite IR-120 (H^+).

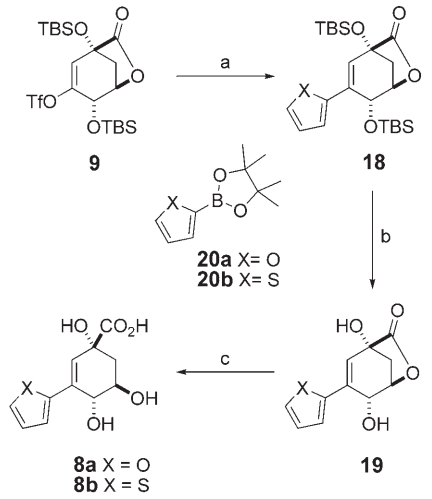
afforded *p*-methoxybenzyl triazole **11** in 91% yield. Acid removal of the PMB protecting group, followed by basic hydrolysis of the resulting carbolactone **12** and protonation with an ion-exchange resin, led to the desired acid **6** in excellent yield.

*Synthesis of Nitrothiophene **7**.* Attempts to synthesize nitro derivative **7** by Suzuki cross-coupling between 2-nitrothien-3-ylboronic acid pinacol ester **13**[33] and vinyl triflate **9**[25a] afforded only low yields of cross-coupling product **14**, possibly due to the instability of the strongly electron-withdrawing substituted boronic ester and its tendency to undergo deborylation (Scheme 3). We therefore tried cross-coupling between 3-bromo-2-nitrothiophene (**17**)[34] and the boronic ester of **9**, which was prepared by direct borylation of **9** with bis(pinacolato) diboron using $(PPh_3)_2PdCl_2$ as a catalyst in the presence of aqueous K_2CO_3 in dioxane. Coupling **16** and **17** afforded the cross-coupling product **14** in 80% yield. Removal of the TBS groups of **14** with TBAF (83%), followed by basic hydrolysis of the resulting carbolactone **15** and treatment with Amberlite IR-120 (H^+) ion-exchange resin, gave the desired nitroderivative **7** in 81% yield.

*Synthesis of Compounds **8**.* Derivatives **8a** and **8b** were synthesized from vinyl triflate **9** and commercially available pinacol boronate esters **20** by Suzuki cross-coupling (Scheme 4).



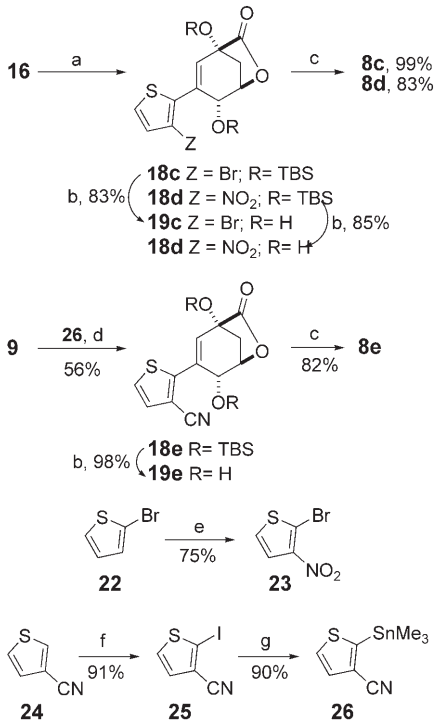
Scheme 3. Reagents and conditions: a) Pd_2dba_3, P(*t*Bu)$_3$, K_3PO_4, DMF, 100 °C. b) $(PPh_3)_2PdCl_2$, PPh_3, K_2CO_3, dioxane, 80 °C. c) $Pd(PPh_3)_4$, dioxane, K_3PO_4 (aq), Δ. d) TBAF, THF, 0 °C→RT. e) 1. LiOH, THF, RT; 2. Amberlite IR-120 (H^+).



Scheme 4. Reagents and conditions: a) **20**, $Pd(PPh_3)_4$, dioxane, K_3PO_4 (aq), Δ (**18a**, 86%; **18b**, quant.). b) TBAF, THF, 0 °C→RT (**19a**, 92%; **19b**, 80%). c) 1. LiOH, THF, RT; 2. Amberlite IR-120 (H^+) (**8a**, 98%; **8b**, 80%).

Conversion of the resulting cross-coupling products **18a–b** to the desired acids **8a** and **8b** was achieved by deprotection of the TBS groups followed by basic hydrolysis of the corresponding carbolactones **19a–b** and protonation with an ion-exchange resin.

Compounds **8c** and **8d** were synthesized by Suzuki cross-coupling between pinacol boronic ester **16** and 2,3-dibromothiophene (**21**) or 2-bromo-3-nitrothiophene (**23**), the latter compound having been prepared by nitration of commercially available 2-bromothiophene (**22**) (Scheme 5). Nitrile **8e** was synthesized by Stille cross-coupling between vinyl triflate **9** and trimethylstannane **26**, which was obtained from commercially available thiophene-3-carbonitrile (**24**) by iodination, lithi-



Scheme 5. Reagents and conditions: a) **21** or **23**, $Pd(PPh_3)_4$, dioxane, K_3PO_4 (aq.), Δ ((**18c**, 58%; **18d**, 41% from **9**). b) TBAF, THF, 0 °C→RT. c) 1. LiOH, THF, RT; 2. Amberlite IR-120 (H^+). d) $Pd(PPh_3)_4$, LiCl, THF, Δ. e) HNO_3, Ac_2O, −5 °C. f) 1. LDA, THF, −78 °C; 2. I_2, THF, −78 °C→RT. g) 1. *n*BuLi, Et_2O, −90 °C; 2. Me_3SnCl, −90 °C→RT.

um-iodo exchange with *n*BuLi, and final treatment with chlorotrimethylstannane. Finally, derivatives **18c–e** were transformed to the corresponding desired acids **8c-e** in the same way as for acids **8a–b** from **18a–b**.

Inhibition Assay Results

The K_i values of acids **5–8** against DHQ2-Hp, as obtained from Dixon plots (1/*v* versus [I]) of assay data, are summarized in Table 1. All of these compounds proved to be reversible competitive inhibitiors of DHQ2-Hp, with all—apart from nitrile **8e**—proving to be more potent than the parent inhibitor **4** (K_i = 370 μM).[31] DHQ2-Hp was inhibited more effectively by the *para*-trifluoromethylphenyl and *para*-nitrophenyl derivatives **5i** and **5j** than by the corresponding *meta*-substituted derivatives **5c** and **5d** (Table 1, entries 10 and 11 versus 4 and 5), but there were no significant differences in K_i among analogues with smaller phenyl substituents (Table 1, entries 2, 3, and 9). Bulky benzofuranyl, benzothienyl, and naphthyl derivatives **5o–s** also showed potency close to that of the phenyl derivative **5a**, with 5-benzothienyl derivative **5r** (K_i of 3.6 μM) and naphthyl derivative **5s** (K_i of 3.7 μM) being the most potent among the latter series.

Comparison among the heteroaryl series **5t–u** and **6–8** shows that replacement of the 3-thienyl or 3-furanyl group with a 1,2,3-triazolyl group (Table 1, entries 19 and 20 versus

Table 1. Inhibition of *H. pylori* type II dehydroquinase by compounds **4–8**.[a]

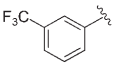
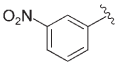
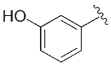
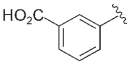
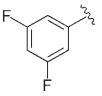
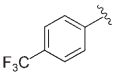
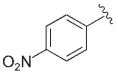
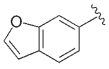
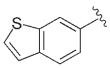
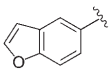
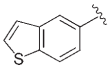
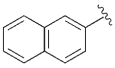
Entry	Compd.	Ar	Ki [μM]
1	**4**	H	370[b]
2	**5a**		7.4 ± 7
3	**5b**		9.4 ± 9
4	**5c**		139 ± 11
5	**5d**		38.4 ± 3
6	**5e**		128 ± 11
7	**5f**		150 ± 15
8	**5g**		20.5 ± 2
9	**5h**		11 ± 1
10	**5i**		8.4 ± 7
11	**5j**		6.8 ± 6
12	**5k**		141 ± 13
13	**5l**		56 ± 5
14	**5o**		7.1 ± 0.6
15	**5p**		8.1 ± 0.8
16	**5q**		14.2 ± 1.3
17	**5r**		3.6 ± 0.3
18	**5s**		3.7 ± 0.3
19	**5t**		4.85 ± 4
20	**5u**		1.8 ± 0.1
21	**6**		63 ± 5
22	**7**		110 ± 9
23	**8a**		1 ± 0.1

Table 1. (Continued)

Entry	Compd.	Ar	Ki [μM]
24	**8b**		0.54 ± 0.05
25	**8c**		4.6 ± 4
26	**8d**		1.0 ± 0.1
27	**8e**		> 400

[a] K_M under the assay conditions (50 mM Tris.HCl, pH 7.0, 25 °C) was 448 μM. [b] Data from ref. [31].

21) decreases the inhibitory potency, as does the incorporation of a nitro group in position 2 of the 3-thienyl moiety (Table 1, entry 22). By contrast, derivatives **8a** and **8b**, which have a 2-furanyl or 2-thienyl group, were between two and five times more potent than the 3-yl isomers **5t** and **5u** (Table 1, entries 19 and 20 versus 23 and 24). The 2-thienyl derivative **8b** was the most potent inhibitor, with a K_i of 540 nM, which is almost 700-times more potent than the parent inhibitor 2,3-anhydroquinic acid (**4**).[31] The 3-nitrothienyl derivative **8d** and 2-furanyl derivative **8a** also had a good affinity of 1 μM. However, incorporation of a bromide, nitro, or cyano group at position 3′ of the thienyl group did not increase the potency of **8b** against DHQ2-Hp (Table 1, entries 25–27).

Molecular Modeling and NMR Studies

*Binding mode of ligand **8b**.* The docking of **8b** into DHQ2-Hp was studied using GOLD 3.0.1[35] with the enzyme geometries found in crystals of DHQ2-Hp binding the natural substrate, that is, 3-dehydroquinic acid (**1**).[30] Ligand **8b** docked with its cyclohexene ring and carboxyl group occupying approximately the same locations as those of the substrate **1** in the crystal structure. These molecular modeling studies suggest two possible orientations for the thiophene ring. One binding mode has hydrogen H3′ of the heterocyclic ring oriented towards the cyclohexene double bond (Figure 2a) or, in the other putative binding mode (Figure 2b), the thiophene ring is rotated towards hydrogen H4. According to the docking studies, the latter orientation is predicted to be the more likely because it provides the highest score solution. To determine which of these possible orientations is actually adopted in DHQ2-Hp, we carried out NMR spectroscopy binding studies.

During the last few years, new NMR-based methods to characterize binding processes have been developed.[36] As a key example, transferred nuclear Overhauser enhancement (TR-NOE) may be employed to obtain conformational details on the three-dimensional structure of a ligand bound to the active site of a protein, as transfer nuclear Overhauser effect spectroscopy (TR-NOESY) experiments provide information about internuclear distances of the ligand/inhibitor when bound to the receptor.[37] Moreover, saturation transfer difference (STD) NMR experiments[38] have also been developed to detect the binding of ligands to receptor proteins and, in favorable cases, the STD method may also provide information on the binding epitope of the ligand.[39] The STD NMR technique relies on the transfer of saturation from the protein to

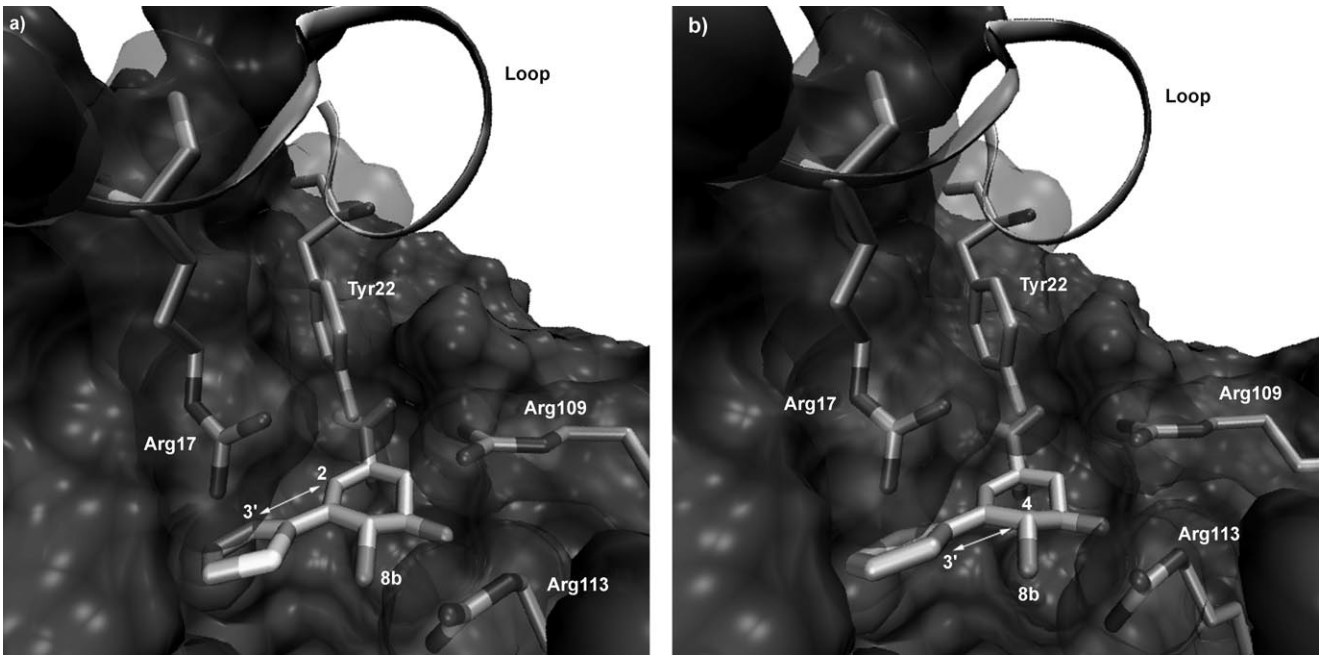


Figure 2. Two possible binding modes of ligand **8b** in the *H. pylori* DHQ2 active site (a and b).

the bound ligand that it exchanges into solution. The irradiation frequency for protein saturation is set to a value at which only protein (and no ligand) resonances occurs. Substracting a spectrum of the saturated protein (on-resonance) from that acquired in the absence of protein saturation (off-resonance) yields the final STD spectra in which only the signals of the ligand remain. The group epitope mapping analysis is carried out by integrating the signals of the ligand in the difference spectra (STD) and referencing these to the corresponding signals in the off-resonance spectra. It should be noted that for both the TR-NOE and STD techniques to be successful, the rate of ligand exchange must be faster than the magnetization life-time in the free state. Selective inversions, STD and TR-NOESY experiments were employed to establish the bound conformation of the most potent competitive inhibitor reported here (**8b**) against DHQ2-Hp.

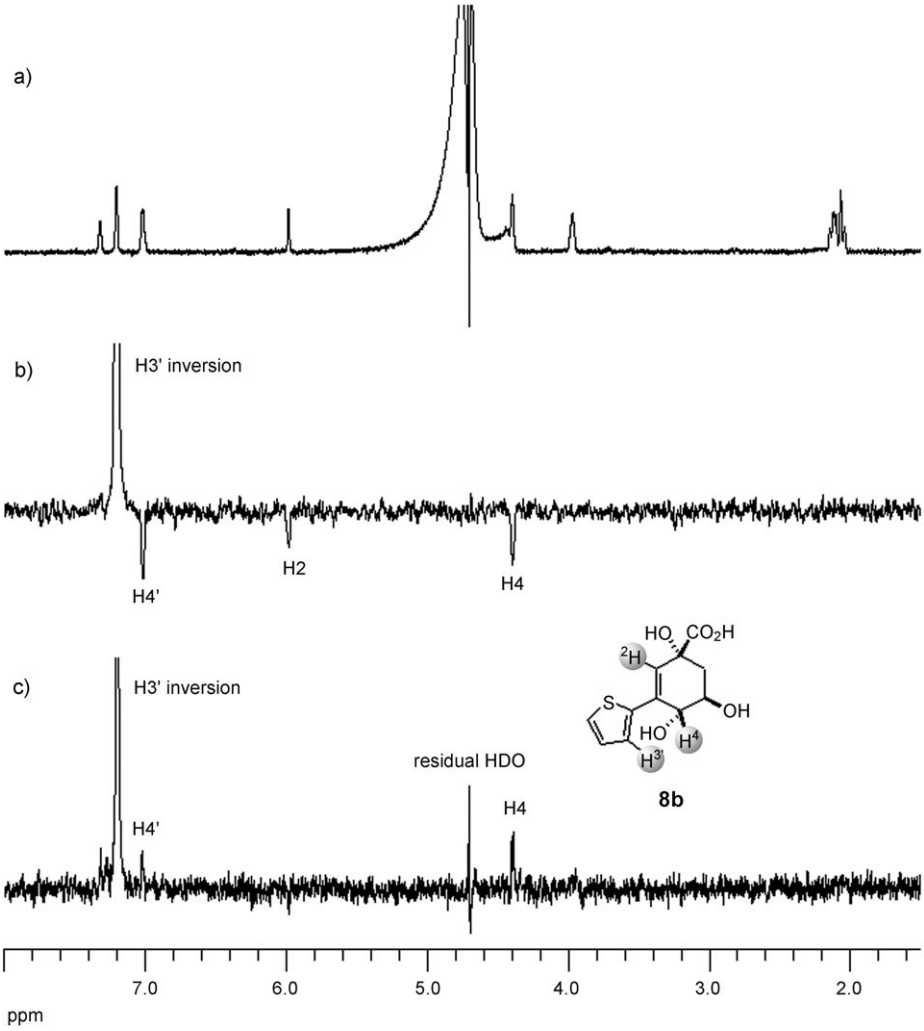


Figure 3. Identification of free and bound conformations of ligand **8b**. a) 500 MHz ^{1}H NMR spectrum of free ligand **8b**. b) 1D NOESY spectrum of free ligand **8b** obtained after selective inversion of H3′ (mixing time 700 ms). c) 1D TR-NOESY spectrum of a mixture of ligand **8b** and DHQ2-Hp (10:1 molar ratio) obtained after selective inversion of H3′ (mixing time 300 ms). All experiments were performed at 298 K in 50 mM deuterated potassium phosphate buffer of pD 7.2.

In a first step, the possible conformations of the free ligand **8b** in solution were elucidated by using 1D-selective inversion NOE experiments. As expected for a small molecule tumbling rapidly in solution, 1D selective inversion NOE experiments on **8b** in the absence of enzyme showed positive peaks (with a different sign to the inverted signal) (Figure 3b). Inversion of proton H3′ enhanced both H2 and H4 signals, thus showing free rotation around the C2′-C3 bond (Figure 3b). 1D TR-NOESY experiments on a mixture of **8b** and DHQ2-Hp were carried out in a variety of inhibitor/enzyme molar ratios, with the best results obtained when a 10:1 molar ratio was used. These experiments showed peaks with the same negative sign as the inverted signal, as expected for a ligand bound to a large macromolecule (Figure 3c). In addition, inversion of proton H3′ only enhanced proton H4 (2.3%), which indicates a conformational selection for the bound state (Figure 3c).

2D TR-NOESY experiments showing cross peaks with the same negative sign as the diagonal strongly support the situation in which the ligand had bound to the active site of the enzyme (Figure 4). The presence of cross peaks between protons H4 and H3′ but not between protons H2 and H3′ con-

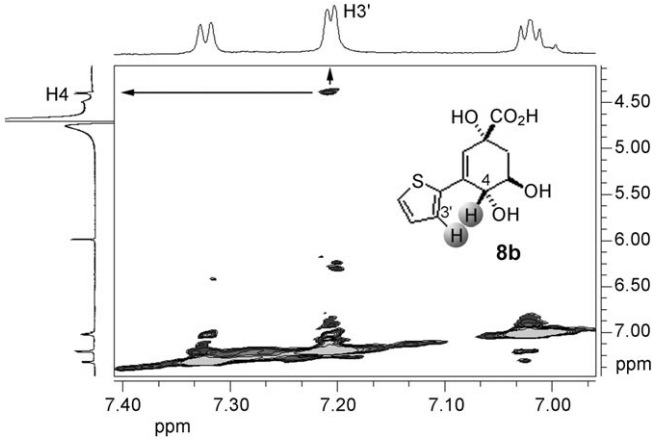
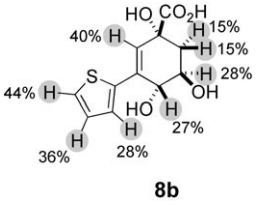


Figure 4. Section of the 2D TR-NOESY spectrum of a 10:1 mixture of ligand **8b** and DHQ2-Hp (mixing time 200 ms, 298 K). Relevant cross peaks are indicated.

firmed that it was the sulfur atom of the thienyl group that was oriented towards the double bond of the cyclohexene moiety.

Finally, STD NMR experiments[38,39] were also performed. The ^{1}H NMR spectrum of a 10:1 mixture of **8b** and DHQ2-Hp is shown in Figure 5a. The saturation effects on individual protons calculated from STD (Figure 5b) and the off-resonance spectra (Figure 5c) are shown in Figure 6. The largest STD effects were observed for protons H5′, H2, and H4′ (44%, 40%, and 36%, respectively) and the weakest for the cyclohexene methylene protons (15%), showing that it is the thiophene side of the ligand that makes closest contact with enzyme protons (Figure 7). Furthermore, the experimental and theoretical STD effects were compared for the eight nonexchangeable protons of 2-thienyl derivative **8b** by using the CORCEMA-ST protocol (complete relaxation and conformational exchange matrix procedure for the STD).[40,41] Calculations for the two possible orientations of the thiophene ring (see above and Figure 2a and b) were performed. Indeed, the best fit (R-factor) between the experimental and predicted data was also provided by the TR-NOESY-based solution, thus supporting the proposed binding mode.

Specifically, the GOLD studies described above suggest that in the conformation shown in Figure 2b, there are lipophilic interactions between the thiophene moiety of the ligand and side-chain atoms of Pro9, Asn10, and Ala79. In addition, hydrogen H2 is in close contact with the side chain of Ile11 and the backbone of Gly78 (Figure 7).



8b

Figure 6. Percentage of saturation transfer (after 2 s) for ligand **8b** bound to DHQ2-Hp in the STD spectrum (Figure 5b) relative to the off-resonance spectrum (Figure 5c).

*Binding mode of ligands **1b**, **1d**, and **1f**: competition studies.* Competition studies were performed to determine if the inhibitors reported herein bind at the same site of the *H. pylori* enzyme. Low-affinity ligand **5f** (K_i of 150 μM), medium-affinity ligand **5d** (K_i of 38.4 μM), and high-affinity ligand **8b** (K_i of 540 nM) were chosen for these studies.

Comparison of STD spectra of 10:1 mixtures of ligand **5d** with DHQ2-Hp that were recorded before (Figure 8a) and after addition of the same amount of high-affinity ligand **8b** (Figure 8b) shows that the magnitudes of the STD signals arising from the ligand **5d**–enzyme complex were dramatically reduced compared to those of the ligand **8b**–enzyme complex. These results clearly indicate that both inhibitors compete for the same site and that ligand **8b** has much higher affinity for binding to the *H. pylori* enzyme. Moreover, when ligand **8b** was added to a sample containing a much weaker competitive inhibitor, acid **5f**, bound to *H. pylori* dehydroquinase, the STD signals arising from the latter complex almost disappeared (Figure 8c and d). These experiments support the aforementioned inhibition data and clearly show that these ligands bind in the same site of the *H. pylori* dehydroquinase.

Interestingly, GOLD docking studies suggest that whereas the phenyl group of ligand **5d** would have its substituent (nitro) on the same side as the cyclohexene double bond (Figure 9b), the corresponding sub-

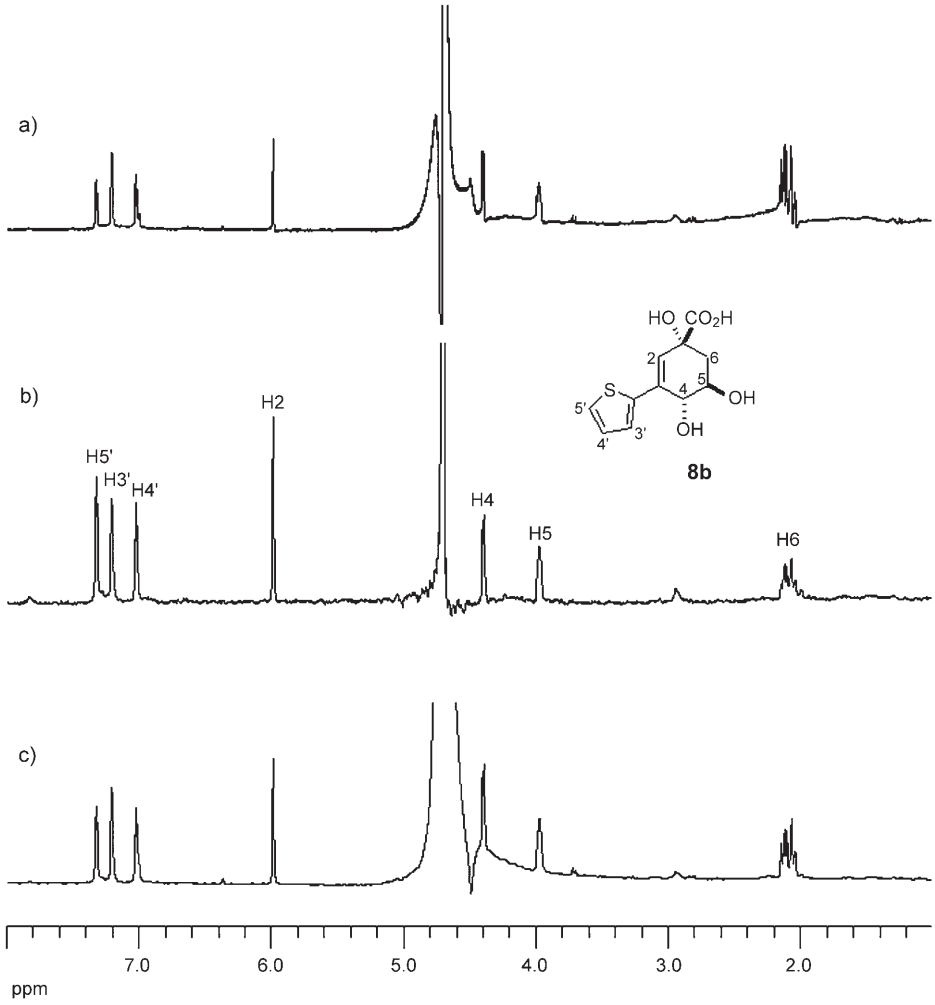


Figure 5. STD NMR binding studies. a) 500 MHz ^{1}H NMR spectrum at 298 K of a 10:1 mixture of **8b** and DHQ2-Hp. b) ^{1}H NMR STD spectrum after two seconds of irradiation at δ 0.5 ppm of a 10:1 mixture of **8b** and DHQ2-Hp. c) ^{1}H NMR off-resonance spectrum.

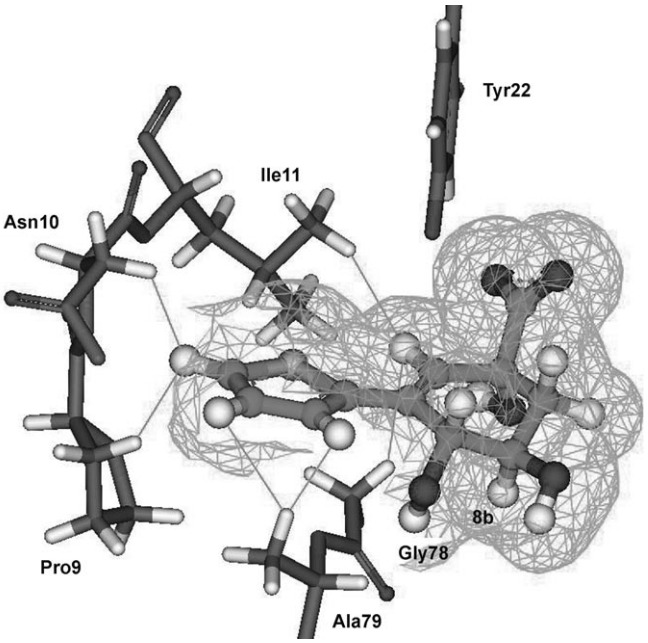


Figure 7. Binding mode of **8b** in the active site of DHQ2-Hp as inferred from GOLD studies and NMR data, with relevant binding interactions indicated.

stituent of ligand **5f** (carboxylate) would be rotated about 180° (Figure 9c). Both orientations would be possible for the smaller substituent of **5b** (fluoro, Figure 9a). These preferences were confirmed by 1D-NOESY and 1D- and 2D-TR-NOESY experiments (see supporting information), although a slight tendency for **5b** to behave similar to **5d** rather than **5f** was found (Figure 10).

Conclusions and Final Remarks

Several 3-heteroaryl analogs of the known dehydroquinase inhibitor **4** were synthesized and tested as inhibitors of DHQ2-Hp. All of these compounds proved to be reversible competitive inhibitors of this enzyme. The studies herein reported showed that derivatives containing electron-rich heterocycles, such as thienyl or furanyl groups, are more potent than π-deficient heterocycles, such as a 1,2,3-triazoyl group. In fact, the incorporation of electron-withdrawing groups in the aromatic ring caused a decrease in the inhibitory potency. These facts suggest that π-stacking interactions between the heterocyclic moiety and the essential Tyr22 do not occur in these cases. We and other authors have shown previously that the π–π interaction is the main one in the binding of this type of 3-heteroaryl derivatives with other DHQ2 (*S. coelicolor* and *M. tuberculosis*).[25a,27,32]

2′-Thienyl derivatives proved to be more potent than its 3′-yl isomers, probably because they mimicked more accurately the electron density of the enol intermediate at C3. The new 2′-thienyl derivative **8b** proved to be the most potent competitive inhibitor of the series. The K_i of **8b** against DHQ2-Hp is 540 nM, almost 700-times more potent than the parent DHQ2-Hp inhibitor, 2,3-anhydroquinic acid.[31] The results of STD and 1D and 2D TR-NOESY NMR experiments show that when

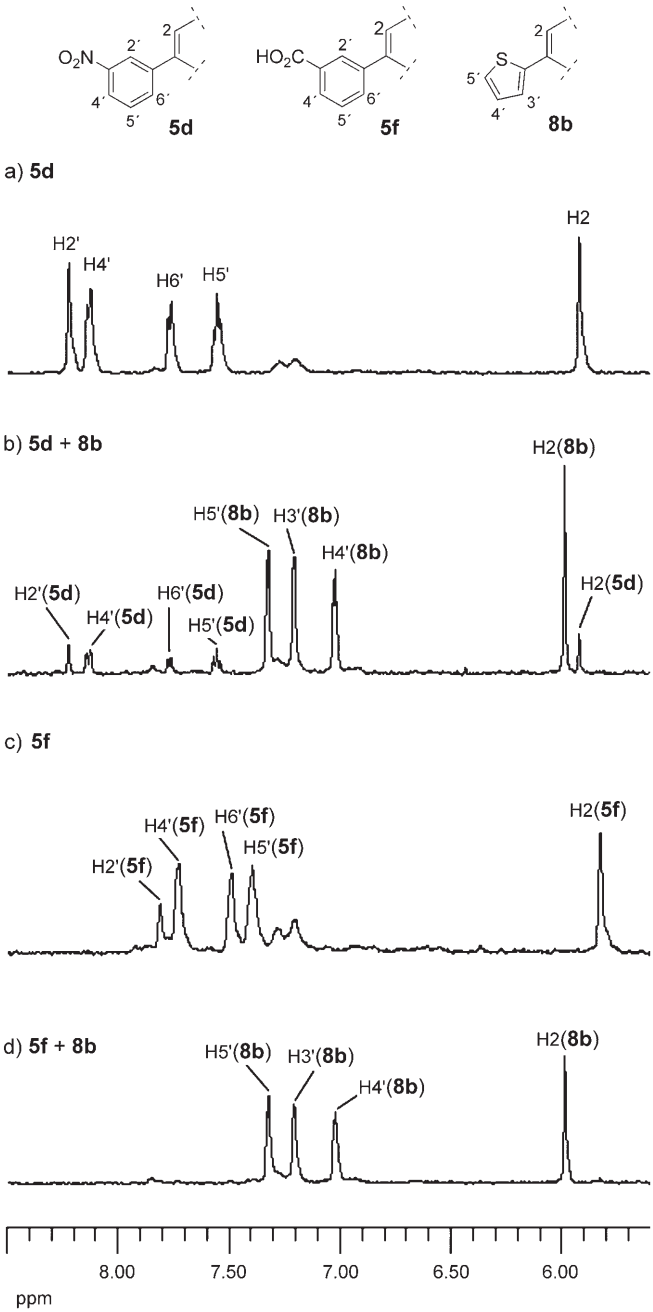


Figure 8. Section of the STD NMR spectra for the competitive binding of ligands **5d**/**8b** and **5f**/**8b** to DHQ2-Hp. a) ^{1}H NMR STD spectrum of medium-affinity ligand **5d** bound to DHQ2-Hp (10:1 molar ratio). b) ^{1}H NMR STD spectrum after addition of high-affinity ligand **8b** to "a" (ligands **5f** and **8b** in a 1:1 molar ratio). c) ^{1}H NMR STD spectrum of low-affinity ligand **5f** bound to DHQ2-Hp (10:1 molar ratio). d) ^{1}H NMR STD spectrum after addition of high-affinity ligand **8b** to "c" (ligands **5f** and **8b** in a 1:1 molar ratio). All experiments were recorded at 500 MHz and 298 K, and with 2 seconds irradiation at δ 0.5 ppm.

bound to DHQ2-Hp **8b** has its thiophenyl sulfur atom oriented towards the double bond side of its cyclohexene ring. Docking studies using GOLD suggest that this conformation allows strong lipophilic interactions with the enzyme residues Pro9, Asn10, Ile11, Gly78, and Ala79. Competitive STD experiments carried out with high-, medium-, and low-affinity ligands (**8b**,

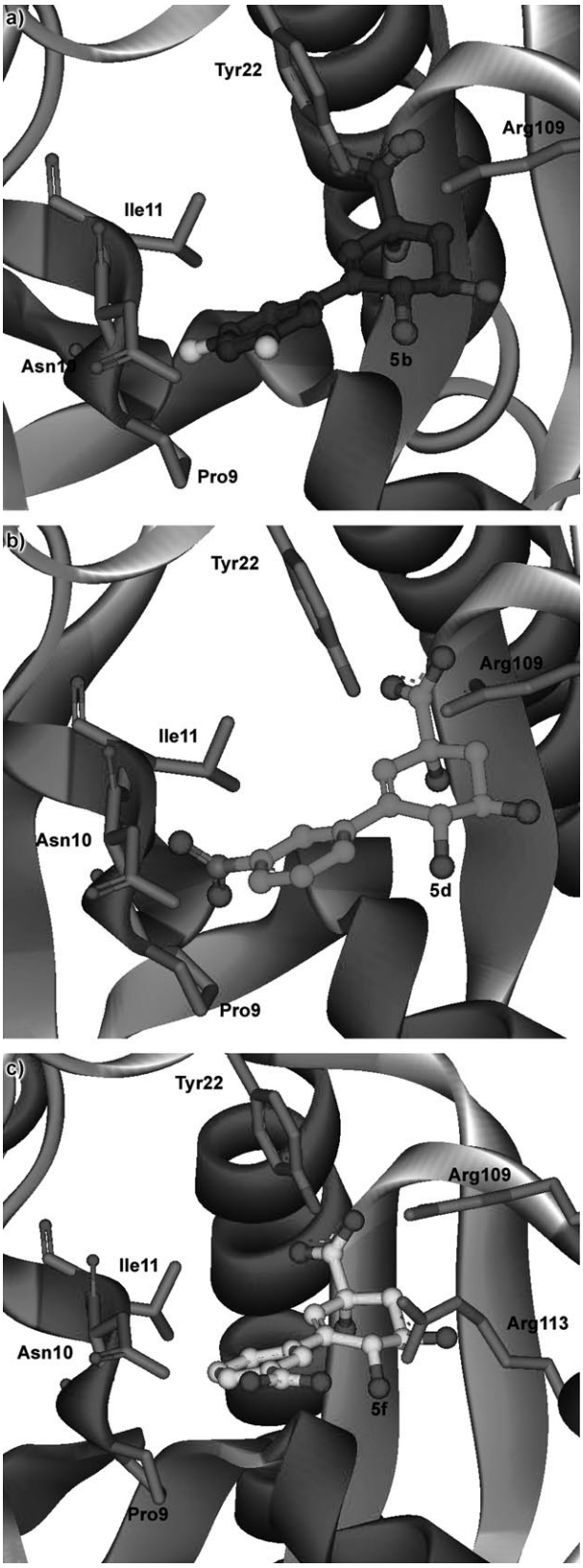


Figure 9. GOLD-predicted binding for ligands a) **5 b**, b) **5 d**, and c) **5 f** to the active site of DHQ2-Hp.

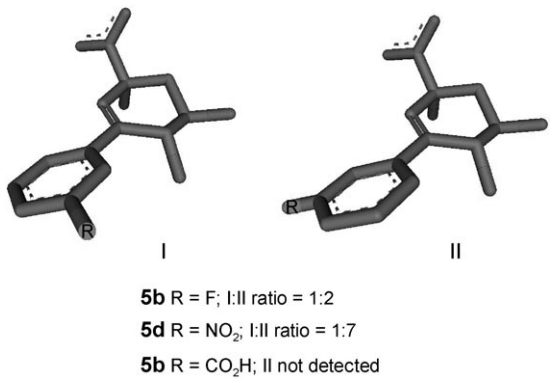


Figure 10. The major conformers of ligands **5 b**, **5 d**, and **5 f** when bound to DHQ2-Hp, as inferred from NMR data.

5 d, and **5 f**) from the series show that they all bind in the same site of DHQ2-Hp, which strongly suggests that the same is true of all the other putative competitors of the natural substrate for which K_i values against DHQ2-Hp are reported in this paper.

Experimental Section

General Procedures. All starting materials and reagents were commercially available and used without further purification. FT-IR spectra were recorded as NaCl plates, or KBr discs. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra (250, 300, and 500 MHz) and ^{13}C NMR spectra (63, 75, and 100 MHz) were measured in deuterated solvents. *J* values are given in hertz. NMR assignments made them by a combination of 1 D, COSY, and DEPT-135 experiments. All procedures involving the use of ion-exchange resins were carried out at room temperature and used Mili-Q deionized water. Amberlite IR-120 (H^+) (cation exchanger) was washed alternately with water, 10% NaOH, water, 10% HCl, and finally water before use. Purity of carboxylic acids were analyzed by HPLC and by NMR. HPLC was performed on a preparative (300×16 mm) Bio-Rad Aminex Ion exclusion HPX-87H organic acids column, Teknokroma Mediterranea Sea$_{18}$ reverse-phase (5 μm; 250×46 mm) and Merck LiChroCART RP-18 (10 μm, 250×10 mm). The eluent used for these columns was 100 mM aqueous formic acid and 0.1% aqueous TFA, at a flow rate of 0.6 and 1 mL min^{-1}, respectively.

Dehydroquinase Purification. The DHQ2-Hp in pET21a was transformed into *E. coli* strain BL21 DE3 and transformants selected on 100 μg mL^{-1} ampicillin. The transformed strain was grown in drug supplemented Luria Broth at 37 °C until an attenuance of 0.6 when it was made 0.2 mg mL^{-1} with IPTG, and incubation continued for a further 5 h. After harvesting by centrifugation, 50 g cells were disrupted by sonication in 50 mM potassium phosphate buffer pH 7.2, 1 mM DTT, 1 mM benzamidine (buffer 1) and centrifuged at 10000x***g*** for 40 mins. The supernatant was applied to a Q sepharose column (5×15 cm) and was washed with 500 mL of 50 mM potassium phosphate buffer pH 7.2, 1 mM DTT (buffer 2). The column was then eluted with a 1 L 0.0 to 1.0 M NaCl gradient in buffer 2 collecting 10 mL fractions. Dehydroquinase-encoding fractions were identified by direct assay and SDS PAGE and pooled appropriately. This pool was made 1.0 M with ammonium sulphate and applied to a phenyl sepharose (5×15 cm) column and washed with 500 mL of 50 mM potassium phosphate buffer pH 7.2, 1 mM DTT, 1.0 M ammonium sulphate (buffer 3). The column was then washed with a 1.0 L 1.0 to 0.0 M ammonium sulphate gradient

made by connecting 500 mL of buffer 3 to 500 mL of buffer 2 with a salt bridge. 10 mL fractions were collected and dehydroquinase-encoding fractions identified by direct assay and SDS PAGE and pooled appropriately. After dialysis into 2×5 L changes of 50 mM potassium phosphate buffer pH 6.6, 1 mM DTT (buffer 4), the dehydroquinase was loaded onto a hydroxapatite (5×7.5 cm) column and washed with 500 mL of buffer 4. The column was then eluted with a 1.0 L 50 to 400 mM potassium phosphate buffer gradient pH 6.6 containing 1 mM DTT. Dehydroquinase-encoding fractions were identified by direct assay and SDS PAGE and pooled appropriately. Following concentration using an Amicon pressure cell in conjunction with a 60 kDA cutoff Millipore filter, the dehydroquinase was applied to a Sephacryl S300 column (2.5×210 cm) equilibrated in 50 mM potassium phosphate buffer pH 7.2, 1 mM DTT, 150 mM NaCl collecting 10 mL fractions. Dehydroquinase-encoding fractions were identified by direct assay and SDS PAGE and pooled appropriately yielding approximately 220 mg of dehydroquinase of at least 95% purity.

Dehydroquinase Assays. A concentrated solution of DHQ2-Hp (6.4 mg mL^{-1}) in potassium phosphate buffer (50 mM, pH 7.2), DTT (1 mM), and NaCl (150 mM) was used. When required for assays, aliquots of the enzyme stocks were diluted into water and buffer and stored on ice. Dehydroquinase 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 (**2**) (ε/M^{-1}cm^{-1} 12000). Standard assay conditions for DHQ2-Hp were pH 7.0 at 25 °C in Tris/HCl (50 mM). Each assay was initiated by addition of the substrate. Solutions of 3-dehydroquinic acid (**1**) were calibrated by equilibration with DHQ2 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 (**2**).

Protein Preparation for NMR Analysis. A concentrated stock solution of *H. pylori* protein (6.4 mg mL^{-1}, 346 μM) stored in potassium phosphate buffer (50 mM, pH 7.2), DTT (1 mM) and NaCl (150 mM), was exchanged to deuterated buffer by successive concentration and dilution using deuterated potassium phosphate buffer (50 mM, pD 7.2) at 4 °C and in an Amicon Centricon-10 microconcentrator prior to NMR experiments.

Sample Preparation for NMR Analysis. NMR samples were prepared in a 1:10 molecular excess by adding a concentrated solution of the inhibitor (1 mM in NMR tube) to 500 μL of DHQ2-Hp (100 μM in NMR tube) both in deuterated potassium phosphate buffer (50 mM, pD 7.2).

1D Transient NOE, TR-NOESY, and STD Experiments. All protein–ligand NMR experiments were performed on a Bruker AVANCE 500 MHz spectrometer at 298 K. For regular 1D experiments solvent suppression of the residual HDO peak was achieved by continuous low power presaturation pulse during the relaxation delay. 1D selective NOESY experiments (mixing times between 500 ms and 1 s) were measured by using the double pulsed field gradient spin echo sequence proposed by Shaka and coworkers.[42] Selective pulses (gaussian types) were of 40 ms length. No water supression scheme was applied, as the buffer was prepared in D_2O.

For the experiments in the bound state, first, line broadening of the ligand protons was monitored in the presence and absence of protein.

1D TR-NOESY were performed using mixing times between 200 and 700 ms, for ligand:protein molar ratio 10:1.

2D-TR-NOESY experiments were performed with mixing times of 50, 100, 200, and 250 ms for a 10:1 molar ratio of ligand:protein. No purging spin lock period to remove the NMR signals of the macromolecule background was employed, as they were basically not observable because of the huge size of the receptor. First, line broadening of the ligand protons was monitored after the addition of the ligand. Strong negative NOE cross peaks were observed, in contrast to the free state, indicating binding. TR-ROESY experiments were also carried out to exclude spin-diffusion effects. A continuous wave spin lock pulse was used during the 250 ms mixing time. Key NOEs were shown to be direct cross peaks, as they showed different sign to diagonal peaks.

For STD experiments, two FIDs, one for off-resonance irradiation (at 50 ppm) and a second one for on-resonance saturation (at 0.5 ppm) were obtained, with scans acquired in interleaved fashion. A 15 ms purging spin lock period to remove the protein signals background was employed. A train of 20, 40, or 50 Gaussian-shaped pulses of 50 ms each was used, to run different STD experiments with a total saturation time of the protein envelope of 1, 2, or 2.5 s, respectively. Solvent suppression was achieved by using a Watergate module just before acquisition.

Two identical samples were used for the STD and TR-NOESY experiments with a 20:1 ligand/protein molar ratio and repeated twice, giving the same results.

Docking Studies. The ligands were docked in the active site of DHQ2-Hp using the program GOLD (version 3.0.1).[35] Receptor and ligand were used as MOL2 files. The ligand structure was prepared using Gaussian 03W,[43] and the energy was minimized using AM1. No energy minimization was performed on the enzyme. The crystal structure of the complex 3-dehydroquinic acid (**1**)–enzyme (PDB code: 1J2Y) was used. The ligands were docked using GOLD3.0.1 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 active site was introduced and the radius was set to 15 Å, with the automatic active-site detection on. The "flip ring corners" flag was switched on, while all the other flags were off. The GoldScore scoring function was used.

4-Methoxybenzyl azide. A solution of 4-methoxybenzyl chloride (173 μL, 1.28 mmol) and sodium azide (333 mg, 5.12 mmol) in dry THF (8.5 mL) was stirred at room temperature for 12 h. Diethyl ether and water were added and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2× 10 mL). All the combined organic extracts were dried (anh. Na_2SO_4), filtered, and evaporated. The obtained residue was purified by flash chromatography eluting with diethyl ether-hexanes (1:9) to afford 4-methoxybenzyl azide (207 mg, 99%) as a colorless light oil. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 3.80 (s, 3H; CH_3), 4.25 (s; 2H; CH_2), 6.91 (d, J = 8.5 Hz, 2H; ArH), 7.24 ppm (d, J = 8.5 Hz, 2H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): δ = 54.2 (CH_2), 55.1 (CH_3), 114.0 (2xCH), 127.2 (C), 129.6 (2xCH), 159.5 ppm (C); IR (film): $\tilde{\nu}$ = 2096 cm^{-1} (N≡N).

(1*R*, 4*R*, 5*R*)-3-[1-(4-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl]-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (11). Freshly prepared aqueous sodium ascorbate solution (32 μL, 1.0 M), followed by aqueous copper(II) sulfate solution (11 μL, 0.3 M) were added to a suspension of alkyne **10**[27] (57 mg, 0.32 mmol) and 4-methoxybenzylazide (52 mg, 0.32 mmol) in a 1:1 mixture of *t*BuOH-water (1.2 mL). The resultant heterogenous mixture was stirred vigorously during 16 h and then diluted with water (10 mL) and ethyl acetate (10 mL). The organic layer was separated and the aqueous phase was extracted twice with ethyl acetate (10 mL). All the combined organic extracts were dried (anh. Na_2SO_4), filtered, and evaporated. The obtained residue was purified by flash chromatography eluting with ethyl acetate-hexanes [1°) 4:1, 2°) 5:1] to afford triazole **11** (98 mg, 91%) as a yellow oil. $[\alpha]^{20}_D = -11.7°$ (*c*1.1, in CH_3OH);

^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.35$ (m, 2H; CH_2), 3.71 (s, 3H; CH_3), 4.39 (d, $J = 3.3$ Hz, 1H; CH), 4.64 (dd, $J = 6.1$ Hz, $J' = 3.3$ Hz, 1H; CH), 5.44 (s, 2H; CH_2), 6.62 (s, 1H; CH), 6.85 (d, $J = 8.8$ Hz, 2H; ArH), 7.22 (d, $J = 8.8$ Hz, 2H; ArH), 7.93 ppm (s, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 37.3$ (CH_2), 54.6 (CH_2), 55.8 (CH_3), 66.8 (CH), 74.2 (C), 77.8 (CH), 115.4 (2xCH), 123.2 (CH), 128.5 (C), 130.2 (C), 130.7 (2xCH), 131.9 (CH), 145.8 (C), 161.4 (C), 178.2 ppm (C); IR (KBr): $\tilde{\nu} =$ 1785 (C=O), 3390 cm^{-1} (O-H); MS (ESI) $m/z = 344$ [MH$^+$]; HRMS (ESI) m/z: calc for $C_{17}H_{18}O_5N_3$ [MH$^+$]: 344.1241, found 344.1244.

(1*R*, 4*R*, 5*R*)-1,4-dihydroxy-3-(1*H*-1,2,3-triazol-4-yl)cyclohex-2-en-1,5-carbolactone (12). A solution of the triazole **11** (26 mg, 0.076 mmol) in TFA (2.5 mL) was heated at 65 °C for 16 h. After cooling to room temperature the solvent was removed and the obtained residue was redisolved in ethyl acetate (10 mL) and water (10 mL). The organic layer was separated and the aqueous phase was extracted twice with ethyl acetate (2× 10 mL). All the combined organic extracts were dried (anh. Na_2SO_4), filtered, and evaporated. The obtained residue was purified by flash chromatography eluting with ethyl acetate-hexanes (4:1) to afford triazole **12** (18 mg, 98%) as a beige amorphous solid on cooling. $[\alpha]^{20}_D = -25.2°$ (*c*1.7, in CH_3OH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.36$ (m, 2H; CH_2), 4.47 (d, $J = 3.0$ Hz, 1H; CH), 4.66 (m, 1H; CH), 6.57 (s, 1H; CH), 7.86 ppm (s, 1H; ArH); ^{13}C NMR (75 MHz, CD_3OD, 25 °C): $\delta = 37.3$ (CH_2), 66.8 (CH), 74.2 (C), 77.8 (CH), 130.2 (C), 132.6 (2xCH), 144.5 (C), 178.3 ppm (C); IR (film): $\tilde{\nu} =$ 1783 (C=O), 3399 cm^{-1} (O-H); MS (ESI) $m/z = 224$ [MH$^+$]; HRMS (ESI) m/z: calc for $C_9H_{10}O_4N_3$ [MH$^+$]: 224.0666, found 224.0666.

(1*R*, 4*R*, 5*R*)-1,4,5-Trihydroxy-3-(1*H*-1,2,3-triazol-4-yl)cyclohex-2-en-1-carboxylic acid (6). A solution of the lactone **12** (19 mg, 0.084 mmol) in THF (0.8 mL) and aqueous lithium hydroxide (0.42 mL, 0.5 M) was stirred at room temperature for 30 min. Water was added and the THF was removed under reduced pressure and the resultant aqueous solution was washed with diethyl ether (x2). The aqueous extract was treated with Amberlite IR-120 until pH 6. The resin was filtered and washed with water. The filtrate and the washings were lyophilized. Purification by HPLC (column: Merck LiChroCART RP-18; gradient 0–5 min 0% B; 5–35 min 0–50% B; A: [TFA/H_2O (1:100)]; B: [TFA/CH_3CN (1:100)], with a flow of 5 mL min^{-1}) afforded triazole **6** (18 mg, 89%) as white amorphous solid. Mp 66–68 °C; $[\alpha]^{20}_D = -52°$ (*c*1.0, in H_2O); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta = 2.15$ (dd, $J = 13.5$ Hz, $J' = 4.0$ Hz, 1H; C*H*H); 2.23 (dd, $J = 13.5$ Hz, $J = 10.8$ Hz, 1H, CH*H*), 4.06 (ddd, $J = 10.8$ Hz, $J' = 7.0$ Hz, $J'' = 4.0$ Hz, 1H; CH), 4.45 (d, $J = 7.0$ Hz, 1H; CH), 6.39 (s, 1H; CH), 8.04 ppm (s, 1H; ArH); ^{13}C NMR (75 MHz, D_2O, 25 °C): $\delta = 37.9$ (CH_2), 69.4 (CH), 71.3 (CH), 72.8 (C), 126.5 (CH), 126.6 (CH), 132.2 (C), 142.1 (C), 177.4 ppm (C); IR (KBr): $\tilde{\nu} = 1724$ (C=O), 3348 (O-H) cm^{-1}; MS (ESI) $m/z = 242$ [MH$^+$]; HRMS (ESI) m/z: calc for $C_9H_{12}O_5N_3$ [MH$^+$]: 242.0771, found 242.0771.

(1*R*, 4*R*, 5*R*)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,4-di(*tert*-butyldimethylsilyloxy)cyclohex-2-en-1,5-carbolactone (16). A stirred solution of vinyl triflate **9**[25a] (300 mg, 0.56 mmol), $PdCl_2(PPh_3)_2$ (12 mg, 17 μmol), PPh_3 (9 mg, 34 μmol), bis(pinacolato) diboron (157 mg, 0.62 mmol), and anhydrous K_2CO_3 (116 mg, 0.84 mmol) in dry dioxane (3.3 mL) was heated at 80 °C for 24 h. After cooling to room temperature diethyl ether and water were added. The organic layer was separated and the aqueous phase was extracted with diethyl ether (2x). All the combined organic extracts were dried (anh. Na_2SO_4), filtered, and evaporated to afford a colorless oil which was used without further purification in the subsequent Suzuki coupling. Yield >90% by GC. A small amount was purified by flash chromatography eluting with DCM-hexanes (1:9) and characterized: $[\alpha]^{20}_D = -118°$ (*c*1.1, in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 0.12$ (s, 3H; CH_3), 0.13 (s, 3H; CH_3), 0.17 (s, 3H; CH_3), 0.18 (s, 3H; CH_3), 0.88 (s, 9H; $C(CH_3)_3$), 0.91 (s, 9H; $C(CH_3)_3$), 1.23 (s, 6H; 2xCH_3), 1.24 (s, 6H; 2xCH_3), 2.32 (ddd, $J = 10.3$ Hz, $J' = 5.5$ Hz, $J'' = 1.8$ Hz, 1H; C*H*H), 2.41 (d, $J = 10.3$ Hz, 1H; CH*H*), 4.31 (d, $J = 3.3$ Hz, 1H; CH), 4.50 (dd, $J = 5.5$ Hz, $J' = 3.3$ Hz, 1H; CH), 6.68 ppm (d, $J = 1.8$ Hz, 1H; CH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -4.7$ (CH_3), -4.6 (CH_3), -3.2 (CH_3), -3.2 (CH_3), 17.9 ($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), 24.5 (2xCH_3), 25.1 (2xCH_3), 25.6 ($C(CH_3)_3$), 25.8 ($C(CH_3)_3$), 36.4 (CH_2), 65.8 (CH), 75.8 (2xC), 76.5 (CH), 83.7 (C), 149.0 (C + CH), 175.8 ppm (C); IR (film): $\tilde{\nu} = 1799$ cm^{-1} (C=O); MS (CI) $m/z = 511$ [MH$^+$]; HRMS (CI) m/z: calc for $C_{25}H_{48}O_6Si_2B$ [MH$^+$]: 511.3083, found 511.3084.

(1*R*, 4*R*, 5*R*)-1,4-Di(tert-butyldimethylsilyloxy)-3-(2-nitrothien-3-yl)cyclohex-2-en-1,5-carbolactone (14). A stirred solution of 3-bromo-2-nitrothiophene (**17**)[32] (56 mg, 0.27 mmol), $Pd(PPh_3)_4$ (8 mg, 6.7 μmol) and boronic acid pinacol ester **16** (151 mg, 0.30 mmol) in dioxane (1.35 mL) and an aqueous solution of K_3PO_4 (0.45 mL, 0.9 M) was heated under reflux for 1.5 h. After cooling to room temperature diethyl ether and water were added and the organic layer was separated. The aqueous phase was extracted with diethyl ether (2x). The combined organic extracts were dried (anh. Na_2SO_4), filtered, and concentrated under reduced pressure. The crude reaction was purified by flash chromatography eluting with DCM-hexanes [1°) 7:20, 2°) 1:1] affording nitro derivative **14** (111 mg, 80%) as white foam. $[\alpha]^{20}_D = -123°$ (*c*1.1, in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = -0.38$ (s, 3H; CH_3), -0.03 (s, 3H; CH_3), 0.17 (s, 3H; CH_3), 0.20 (s, 3H; CH_3), 0.75 (s, 9H; $C(CH_3)_3$), 0.92 (s, 9H; $C(CH_3)_3$), 2.44 (ddd, $J = 10.7$ Hz, $J' = 5.7$ Hz, $J'' = 1.6$ Hz, 1H; C*H*H), 2.55 (d, $J = 10.7$ Hz, 1H; CH*H*), 4.54 (m, 2H; 2xCH), 6.02 (d, $J = 1.6$ Hz, 1H; CH), 6.90 (d, $J = 5.4$ Hz, 1H; ArH), 7.46 ppm (d, $J = 5.4$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.9$ (CH_3), -5.2 (CH_3), -3.1 (2xCH_3), 17.6 ($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), 25.4 ($C(CH_3)_3$), 25.6 ($C(CH_3)_3$), 36.9 (CH_2), 67.4 (CH), 74.8 (C), 75.9 (CH), 130.0 (CH), 131.0 (CH), 132.7 (2xC), 136.1 (CH), 139.3 (C), 174.7 ppm (C); IR (film): $\tilde{\nu} = 1799$ (C=O) cm^{-1}; MS (ESI) $m/z = 512$ [MH$^+$]; HRMS (ESI) m/z: calc for $C_{23}H_{38}O_6Si_2SN$ [MH$^+$]: 512.1952, found 512.1953.

(1*R*, 4*R*, 5*R*)-1,4-Dihydroxy-3-(2-nitrothien-3-yl)cyclohex-2-en-1,5-carbolactone (15). To a stirred solution of the silyl ether **14** (111 mg, 0.22 mmol) in dry THF (3.1 mL), under argon at 0 °C, was added tetrabutylammonium fluoride (0.5 mL, 0.50 mmol, *ca* 1.0 M in THF). After stirring for 30 min, dilute HCl was added and the organic layer was extracted with ethyl acetate (x3). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The crude reaction was purified by flash chromatography eluting with ethyl acetate-hexanes [1°) 3:1, 2°) 1:0] to give nitro derivative **15** (51 mg, 83%) as a yellow oil. $[\alpha]^{20}_D = -133°$ (*c*1.3, in CH_3OH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.46$ (m, 2H; CH_2), 4.52 (d, $J = 3.3$ Hz, 1H; CH), 4.72 (ddd, $J = 4.8$ Hz, $J' = 3.3$ Hz, $J'' = 1.3$ Hz, 1H; CH), 6.07 (d, $J = 1.3$ Hz, 1H; CH), 7.06 (d, $J = 5.4$ Hz, 1H; ArH), 7.74 ppm (d, $J = 5.4$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 37.2$ (CH_2), 67.4 (CH), 74.3 (C), 78.0 (CH), 132.2 (CH + C), 132.4 (CH), 135.2 (C), 136.1 (CH), 141.1 (C), 178.1 ppm (C); IR (KBr): $\tilde{\nu} = 1761$ (C=O), 3303 (O-H), 3409 cm^{-1} (O-H); MS (CI) $m/z = 284$ [MH$^+$]; HRMS (CI) m/z: calc for $C_{11}H_{10}O_6SN$ [MH$^+$]: 284.0229, found 284.0231.

(1*R*, 4*R*, 5*R*)-1,4,5-Trihydroxy-3-(2-nitrothien-3-yl)cyclohex-2-en-1-carboxylic acid (7). Procedure as described above for acid **6** was employed using lactone **15** (50 mg, 0.18 mmol), THF (1.6 mL) and LiOH (0.9 mL, 0.5 M). Brown oil, 43 mg (81%); Mp 80–82 °C; $[\alpha]^{20}_D = -5°$ (*c*1.2, in CH_3OH); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 2.15$ (m, 1H; C*H*H), 2.22 (d, $J = 11.7$ Hz, 1H; CH*H*), 3.96 (m, 1H; CH), 4.54 (dd, $J = 8.3$ Hz, $J' = 1.9$ Hz, 1H; CH), 5.81 (s, 1H; CH), 6.96 (d, $J = 5.4$ Hz, 1H; ArH), 7.68 ppm (d, $J = 5.4$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, D_2O, 25 °C): $\delta = 41.5$ (CH_2), 71.6 (CH), 75.1 (CH), 75.8 (C), 130.5 (CH), 133.8 (CH), 135.3 (CH), 141.7 (C), 143.2 (C), 149.3 (C), 179.6 ppm (C);

IR (KBr): $\tilde{\nu}=3417$ (O-H), 1739 cm^{-1} (C=O); MS (ESI) $m/z=324$ [MNa$^+$]; HRMS (ESI) m/z: calc for $C_{11}H_{11}O_7SNNa$ [MNa$^+$]: 324.0148, found 324.0141.

(1*R*, 4*R*, 5*R*)-1,4-Di(*tert*-butyldimethylsilyloxy)-3-(furan-2-yl)cyclohex-2-en-1,5-carbolactone (18a). To a stirred solution of the vinyl triflate **9** (200 mg, 0.38 mmol) in dioxane (1.9 mL) was added Pd(PPh$_3$)$_4$ (11 mg, 9.4 μmol), furan-2-boronic acid pinacol ester (**20a**, 174 mg, 0.83 mmol), and an aqueous solution of K_3PO_4 (0.6 mL, 0.9 M). The resultant reaction mixture was heated under reflux for 5 h. After cooling to room temperature diethyl ether and water were added and the organic layer was separated. The aqueous phase was extracted with diethyl ether (2x). The combined organic extracts were dried (anh. Na_2SO_4), filtered, and concentrated under reduced pressure. The crude reaction was purified by flash chromatography eluting with DCM-hexanes (7:20) to give furanyl derivative **18a** (145 mg, 86%) as white amorphous solid. Mp 90.5–92.5 °C; $[\alpha]^{20}_D=-182^\circ$ (*c*1.1, in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=0.09$ (s, 3H; CH_3), 0.18 (s, 6H; 2xCH_3), 0.22 (s, 3H; CH_3), 0.84 (s, 9H; C(CH_3)$_3$), 0.94 (s, 9H; C(CH_3)$_3$), 2.38 (m, 1H; C*H*H), 2.52 (d, $J=10.6$ Hz, 1H; CH*H*), 4.60 (m, 2H; 2xCH), 6.32 (br s, 1H; CH), 6.37 (br s, 2H; 2xArH), 7.36 ppm (br s, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=-4.7$ (CH_3), −4.3 (CH_3), −3.1 (2xCH_3), 17.9 (*C*-(CH_3)$_3$), 18.0 (*C*(CH_3)$_3$), 25.6 (2xC(*C*H$_3$)$_3$), 36.8 (CH_2), 65.7 (CH), 74.8 (C), 75.7 (CH), 107.4 (CH), 111.2 (CH), 128.8 (C), 129.7 (CH), 142.0 (CH), 151.3 (C), 175.2 ppm (C); IR (KBr): $\tilde{\nu}=1800$ cm^{-1} (C=O); MS (ESI) $m/z=473$ [MNa$^+$]; HRMS (ESI) m/z: calc for $C_{23}H_{38}O_5Si_2Na$ [MNa$^+$]: 473.2150, found 473.2138.

(1*R*, 4*R*, 5*R*)-1,4-Di(*tert*-butyldimethylsilyloxy)-3-(thien-2-yl)cyclohex-2-en-1,5-carbolactone (18b). Procedure as described above for furanyl derivative **18a**. White amorphous solid, 175 mg (quant.); Mp 90–92 °C; $[\alpha]^{20}_D=-163^\circ$ (*c*1.1, in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=0.02$ (s, 3H; CH_3), 0.15 (s, 3H; CH_3), 0.19 (s, 3H; CH_3), 0.22 (s, 3H; CH_3), 0.83 (s, 9H; C(CH_3)$_3$), 0.95 (s, 9H; C-(CH_3)$_3$), 2.40 (ddd, $J=10.7$ Hz, $J'=5.6$, $J''=1.8$ Hz, 1H; C*H*H), 2.51 (d, $J=10.7$ Hz, 1H; CH*H*), 4.56 (d, $J=3.4$ Hz, 1H; CH), 4.62 (dd, $J=5.6$ Hz, $J'=3.4$ Hz, 1H; CH), 6.20 (d, $J=1.8$ Hz, 1H; CH), 6.97 (dd, $J=5.0$ Hz, $J'=3.6$ Hz, 1H; ArH), 7.02 (dd, $J=3.6$ Hz, $J'=1.2$ Hz, 1H; ArH), 7.22 ppm (dd, $J=5.0$ Hz, $J'=1.2$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=-4.7$ (CH_3), −4.2 (CH_3), −3.0 (2xCH_3), 17.9 (*C*(CH_3)$_3$), 18.0 (*C*(CH_3)$_3$), 25.6 (2xC(*C*H$_3$)$_3$), 36.6 (CH_2), 67.9 (CH), 74.8 (C), 75.8 (CH), 124.9 (CH), 125.1 (CH), 127.1 (CH), 132.0 (CH), 132.8 (C), 140.7 (C), 175.2 ppm (C); IR (KBr): $\tilde{\nu}=1796$ cm^{-1} (C=O); MS (CI) $m/z=467$ [MH$^+$]; HRMS (CI) m/z: calc for $C_{23}H_{39}O_4Si_2S$ [MH$^+$]: 467.2108, found 467.2103.

(1*R*, 4*R*, 5*R*)-1,4-Di(tert-butyldimethylsilyloxy)-3-(3-bromothien-2-yl)cyclohex-2-en-1,5-carbolactone (18c). Procedure as described above for thiophene **14** was employed using 2,3-dibromothiophene (**21**, 12 μL, 0.11 mmol), Pd(PPh$_3$)$_4$ (3.1 mg, 2.7 μmol), dioxane (0.5 mL), boronic acid pinacol ester **16** (60 mg, 0.12 mmol), K_3PO_4 (0.2 mL). Reaction time: 10 h. Flash chromatography was carried out using dichloromethane-hexanes (7:20) as eluent. Yellow amorphous solid, 30 mg (58%); Mp 150–152 °C; $[\alpha]^{20}_D=-210^\circ$ (*c*1.4, in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=-0.24$ (s, 3H; CH_3), 0.04 (s, 3H; CH_3), 0.20 (s, 3H; CH_3), 0.21 (s, 3H; CH_3), 0.79 (s, 9H; C-(CH_3)$_3$), 0.93 (s, 9H; C(CH_3)$_3$), 2.39 (m, 1H; C*H*H), 2.51 (d, $J=10.7$ Hz; CH*H*), 4.56 (m, 2H; 2xCH), 6.23 (d, $J=1.8$ Hz, 1H; CH), 6.95 (d, $J=5.3$ Hz, 1H; ArH), 7.23 (d, $J=5.3$ Hz, 1H; ArH), ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=-5.5$ (CH_3), −4.9 (CH_3), −3.1 (2xCH_3), 17.7 (*C*-(CH_3)$_3$), 18.0 (*C*(CH_3)$_3$), 25.5 (2xC(*C*H$_3$)$_3$), 36.5 (CH_2), 68.0 (CH), 74.8 (C), 75.8 (CH), 109.7 (C), 125.3 (CH), 130.6 (CH), 131.7 (C), 134.1 (C), 138.5 (CH), 175.1 ppm (C); IR (film): $\tilde{\nu}=1807$ (C=O) cm^{-1}; MS (ESI) $m/z=567$ and 569 [MNa$^+$]; HRMS (ESI) m/z: calc for $C_{23}H_{37}O_4BrSi_2SNa$ [MNa$^+$]: 567.1027, found 567.1025.

2-Bromo-3-nitrothiophene (23). A solution of nitric acid (1.7 mL, 23.92 mmol, 65%) in acetic anhydride (3 mL) was added dropwise over 1 h to a solution of 2-bromothiophene (**22**) (1.5 g, 9.20 mmol) in acetic anhydride (46 mL) at −5 °C, keeping the temperature below 0 °C. The resultant reaction mixture was stirred at −5 °C for 12 h, and then water and diethyl ether were added. The organic layer was separated, and the aqueous phase was extracted twice with diethyl ether. All the combined organic extracts were dried (anh. Na_2SO_4), filtered, and evaporated. The crude residue was purified by flash chromatography eluting with diethyl ether-hexanes (1:9) to afford nitrothiophene **23** (1.44 g, 75%) as a yellow amorphous solid. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=7.10$ (d, $J=4.3$ Hz, 1H; ArH), 7.70 ppm ($J=4.3$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta=121.7$ (C), 128.6 (CH), 130.1 (CH), 151.6 ppm (C); MS (CI) $m/z=207$ and 209 [MH$^+$]; HRMS (CI) m/z: calc for $C_4H_2O_2SBrN$ [MH$^+$]: 207.9068, found 207.9074.

(1*R*, 4*R*, 5*R*)-1,4-Di(tert-butyldimethylsilyloxy)-3-(3-nitrothiophen-2-yl)cyclohex-2-en-1,5-carbolactone (18d). A stirred solution of 2-bromo-3-nitrothiophene (**23**, 129 mg, 0.62 mmol), Pd(PPh$_3$)$_4$ (16 mg, 14 μmol), and boronic acid pinacol ester **16** (crude of the above reaction) in dioxane (2.8 mL) and an aqueous solution of K_3PO_4 (1.1 mL, 0.9 M) was heated under reflux for 8 h. After cooling to room temperature diethyl ether and water were added and the organic layer was separated. The aqueous phase was extracted with diethyl ether (2x). The combined organic extracts were dried (anh. Na_2SO_4), filtered, and concentrated under reduced pressure. The crude reaction was purified by flash chromatography eluenting with diethyl ether-hexanes (1:9) affording nitro derivative **18d** (11 mg, 41% from triflate **9**) as a yellow oil. $[\alpha]^{20}_D=-103^\circ$ (*c*1.0, in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=0.11$ (s, 3H; CH_3), 0.18 (s, 3H; CH_3), 0.18 (s, 3H; CH_3), 0.22 (s, 3H; CH_3), 0.85 (s, 9H; C-(CH_3)$_3$), 0.94 (s, 9H; C(CH_3)$_3$), 2.46 (m, 2H; CH_2), 4.53 (d, $J=3.3$ Hz, 1H; CH), 4.67 (m, 1H; CH), 6.40 (s, 1H; CH), 6.98 (d, $J=4.3$ Hz, 1H; ArH), 7.81 ppm (d, $J=4.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=-4.6$ (CH_3), −4.0 (CH_3), −3.1 (2xCH_3), 17.9 (*C*(CH_3)$_3$), 18.0 (*C*(CH_3)$_3$), 25.5 (C(CH_3)$_3$), 25.6 (C(*C*H$_3$)$_3$), 36.2 (CH_2), 67.4 (CH), 74.9 (C), 75.3 (CH), 124.1 (CH), 128.5 (CH), 131.6 (C), 136.6 (CH), 147.7 (C), 150.6 (C), 174.3 ppm (C); IR (film): $\tilde{\nu}=1804$ cm^{-1} (C=O); MS (CI) $m/z=512$ [MH$^+$]; HRMS (CI) m/z: calc for $C_{23}H_{38}O_6Si_2SN$ [MH$^+$]: 512.1953, found 512.1956.

2-Iodothiophene-3-carbonitrile (25). Thiophene-3-carbonitrile (**24**) (167 μL, 1.83 mmol) was added dropwise to a solution of LDA (7.6 mL, 0.24 M) in dry THF at −78 °C under inert atmosphere. The resultant solution was stirred for 1 h at −78 °C and then a solution of iodine (510 mg, 2.01 mmol) in dry THF (0.8 mL) was added. After stirring for 2 h at −78 °C, the reaction mixture was allowed to warm up to room temperature. Ice-water was then added and the mixture was extracted with diethyl ether (3x). The combined ethereal extracts were successively washed with dilute HCl, water, and brine. The organic extract was dried (anh. Na_2SO_4), filtered, and evaporated. The obtained residue was purified by flash chromatography eluting with diethyl ether-hexanes (1:9) to afford 2-iodothiophene-3-carbonitrile (**25**)[44] (391 mg, 91%) as a yellow amorphous solid. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=7.09$ (d, $J=5.6$ Hz, 1H; ArH), 7.48 ppm (d, $J=5.6$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=86.7$ (C), 115.3 (C), 120.3 (C), 130.1 (CH), 132.9 ppm (CH); MS (ESI) $m/z=236$ [MH$^+$]; HRMS (ESI) m/z: calc for C_5H_3SNI [MH$^+$]: 235.9025, found 235.9022.

2-(Trimethylstannyl)thiophene-3-carbonitrile (26). *n*BuLi (0.3 mL, 2.14 M) was added dropwise to a solution of 2-iodothiophene-3-carbonitrile (**25**) (150 mg, 0.64 mmol) in dry diethyl ether at −90 °C under inert atmosphere. After 10 min, chlorotrimethylstannane (0.64 mL, approximately 1.0 M in THF) was added dropwise and the resultant reaction mixture was stirred at −90 °C for 1 h. Water was

then added and the reaction mixture was allowed to warm to room temperature. Extraction with diethyl ether (3x), drying (anh. Na_2SO_4), filtration, and evaporation afforded 2-(trimethylstannyl)thiophene-3-carbonitrile (**26**) as a colorless oil which was used without further purification. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 0.51$ (s, 9H; 3xCH_3), 7.40 (d, $J = 5.0$ Hz, 1H; ArH), 7.62 ppm (d, $J = 5.0$ Hz, 1H; ArH).

(1*R*, 4*R*, 5*R*)-1,4-Di(*tert*-butyldimethylsilyloxy)-3-(3-carbonitrilethien-2-yl)cyclohex-2-en-1,5-carbolactone (18e). A solution of vinyl triflate **9** (266 mg, 0.50 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), LiCl (64 mg, 1.5 mmol), and 2-(trimethylstannyl)thiophene-3-carbonitrile (**26**) (178 mg, 0.65 mmol) in dry THF under inert atmosphere was heated at reflux for 16 h. After cooling to room temperature, removal of THF afforded the crude product which was partioned between DCM and 10% aqueous ammonium hydroxide. The organic layer was separated and the aqueous phase was extracted with DCM. All the combined organic extracts were dried (anh. Na_2SO_4), filtered, and evaporated. The crude reaction was purified by flash chromatography eluting with diethyl ether-hexanes (15:85) to afford carbonitrile **18e** (136 mg, 56%) as a colorless oil. $[\alpha]^{20}_D = -179°$ (*c*1.1, in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = -0.12$ (s, 3H; CH_3), 0.11 (s, 3H; CH_3), 0.22 (s, 3H; CH_3), 0.23 (s, 3H; CH_3), 0.77 (s, 9H; $C(CH_3)_3$), 0.94 (s, 9H; $C(CH_3)_3$), 2.43 (m, 1H; C*H*H), 2.53 (d, $J = 10.8$ Hz, 1H; CH*H*), 4.61 (m, 2H; 2xCH), 6.46 (d, $J = 1.8$ Hz, 1H; CH), 7.20 (d, $J = 5.5$ Hz, 1H; ArH), 7.32 ppm (d, $J = 5.5$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.2$ (CH_3), -4.6 (CH_3), -3.1 (CH_3), -3.1 (CH_3), 17.8 ($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), 25.4 ($C(CH_3)_3$), 25.5 ($C(CH_3)_3$), 36.2 (CH_2), 68.4 (CH), 74.8 (C), 75.5 (CH), 108.7 (C), 114.8 (C), 125.9 (CH), 129.5 (CH), 130.7 (C), 138.2 (CH), 149.6 (C), 174.5 ppm (C); IR (film): $\tilde{\nu} = 1804$ (C=O), 2229 cm^{-1} (N≡N); MS (ESI) $m/z = 514$ [*M*Na^+]; HRMS (ESI) m/z: calc for $C_{24}H_{37}O_4SSi_2NNa$ [*M*Na^+]: 514.1874, found 514.1874.

(1*R*, 4*R*, 5*R*)-3-(Furan-2-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (19a). Procedure as described above for diol **15** was employed using silyl ether **18a** (166 mg, 0.37 mmol), tetrabutylammonium fluoride (0.8 mL, 0.81 mmol), and THF (5.3 mL). Flash chromatography was carried out using diethyl ether-hexanes [1°) (3:1), 2°) (1:0)] as eluent. White amorphous solid, 75 mg (92%); Mp 73–75 °C; $[\alpha]^{20}_D = -337°$ (*c*1.0, in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.34$ (m, 2H; CH_2), 4.42 (d, $J = 3.4$ Hz, 1H; CH), 4.68–4.64 (m, 1H; CH), 6.33 (br s, 1H; CH), 6.41 (dd, $J = 3.4$ Hz, $J' = 1.9$ Hz, 1H; ArH), 6.53 (d, $J = 3.4$ Hz, 1H; ArH), 7.43 ppm (d, $J = 1.9$ Hz, ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 37.4$ (CH_2), 65.9 (CH), 74.1 (C), 77.9 (CH), 109.5 (CH), 112.4 (CH), 128.4 (CH), 130.2 (C), 143.9 (CH), 152.6 (C), 178.4 ppm (C); IR (KBr): $\tilde{\nu} = 1783$ and 1752 (C=O), 3268, 3332, 3424 and 3515 cm^{-1} (O-H); MS (ESI) $m/z = 223$ [*M*H^+]; HRMS (ESI) m/z: calc for $C_{11}H_{11}O_5$ [*M*H^+]: 223.0594, found 223.0601.

(1*R*, 4*R*, 5*R*)-1,4-Dihydroxy-3-(thien-2-yl)cyclohex-2-en-1,5-carbolactone (19b). Procedure as described above for diol **15** was employed using silyl ether **18b** (233 mg, 0.50 mmol), tetrabutylammonium fluoride (1.1 mL, 1.1 mmol), and THF (7.1 mL). Flash chromatography was carried out using diethyl ether-hexanes [1°) (3:1), 2°) (1:0)] as eluent. White foam, 95 mg (80%); Mp 121–123 °C; $[\alpha]^{20}_D = -206°$ (*c*1.3, in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.43$ (m, 2H; CH_2), 4.53 (d, $J = 3.4$ Hz, 1H; CH), 4.72 (m, 1H; CH), 6.30 (d, $J = 1.4$ Hz, 1H; CH), 7.01 (ddd, $J = 5.1$ Hz, $J' = 3.7$ Hz, $J'' = 0.4$ Hz, 1H; ArH), 7.30 (dd, $J = 3.7$ Hz, $J' = 1.1$ Hz, 1H; ArH), 7.33 (dd, $J = 5.1$ Hz, $J' = 1.1$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 37.3$ (CH_2), 67.4 (CH), 74.2 (C), 78.0 (CH), 126.4 (CH), 126.5 (CH), 128.5 (CH), 129.9 (CH), 134.2 (C), 142.0 (C), 178.3 ppm (C); IR (KBr): $\tilde{\nu} = 1748$ and 1784 (C=O), 3288, 3268, 3417 and 3518 cm^{-1} (O-H); MS (ESI) $m/z = 261$ [*M*Na^+]; HRMS (ESI) m/z: calc for $C_{11}H_{10}O_4SNa$ [*M*Na^+]: 261.0197, found 261.0192.

(1*R*, 4*R*, 5*R*)-1,4-Dihydroxy-3-(3-bromothien-2-yl)cyclohex-2-en-1,5-carbolactone (19c). Procedure as described above for diol **15** was employed using silyl ether **18c** (120 mg, 0.22 mmol), tetrabutylammonium fluoride (0.5 mL, 0.5 mmol), and THF (3.2 mL). Flash chromatography was carried out using diethyl ether-hexanes (3:1) as eluent. White amorphous solid, 58 mg (83%); Mp 138–140 °C; $[\alpha]^{20}_D = -177°$ (*c*1.2, in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.38$ (m, 2H; CH_2), 4.44 (d, $J = 3.3$ Hz, 1H; CH), 4.65 (m, 1H; CH), 6.48 (s, 1H; CH), 6.96 (d, $J = 5.5$ Hz, 1H; ArH), 7.38 ppm (d, $J = 5.5$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 36.8$ (CH_2), 68.0 (CH), 74.2 (C), 77.9 (CH), 109.9 (C), 127.2 (CH), 132.2 (CH), 132.9 (C), 135.4 (C), 137.0 (CH), 178.2 ppm (C); IR (KBr): $\tilde{\nu} = 1769$ (C=O), 3399 cm^{-1} (O-H); MS (CI) $m/z = 317$ and 319 [*M*H^+]; HRMS (CI) m/z: calc for $C_{11}H_{10}O_4SBr$ [*M*H^+]: 318.9463, found 318.9450.

(1*R*, 4*R*, 5*R*)-1,4-Dihydroxy-3-(3-nitrothien-2-yl)cyclohex-2-en-1,5-carbolactone (19d). Procedure as described above for diol **15** was employed using silyl ether **18d** (60 mg, 0.12 mmol), tetrabutylammonium fluoride (0.31 mL, 0.31 mmol), and THF (1.7 mL). Flash chromatography was carried out using ethyl acetate-hexanes (3:2) as eluent. Yellow amorphous solid, 29 mg (85%); Mp 194–195 °C (dec.); $[\alpha]^{20}_D = -6°$ (*c*2.0, in CH_3OH); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 2.41$ (m, 2H; CH_2), 4.45 (d, $J = 3.3$ Hz, 1H; CH), 4.70 (dd, $J = 6.1$ Hz, $J' = 3.3$ Hz, 1H; CH), 6.58 (s, 1H; CH), 7.28 (d, $J = 4.3$ Hz, 1H; ArH), 7.86 ppm (d, $J = 4.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = 37.1$ (CH_2), 67.3 (CH), 74.4 (C), 77.7 (CH), 125.9 (CH), 130.1 (CH), 133.2 (C), 135.4 (CH), 149.2 (C), 152.0 (C), 177.5 ppm (C); IR (KBr): $\tilde{\nu} = 1770$ (C=O), 3306 and 3266 cm^{-1} (O-H); MS (ESI) $m/z = 266$ [*M*H^+-H_2O]; HRMS (ESI) m/z: calc for $C_{11}H_8O_5NS$ [*M*H^+-H_2O]: 266.0118, found 266.0111.

(1*R*, 4*R*, 5*R*)-5-(3-Carbonitrilethien-2-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (19e). Procedure as described above for diol **15** was employed using silyl ether **18e** (160 mg, 0.33 mmol), tetrabutylammonium fluoride (0.86 mL, 0.86 mmol), and THF (4.7 mL). Flash chromatography was carried out using ethyl acetate-hexanes (3:2) as eluent. Yellow-green oil, 85 mg (98%); $[\alpha]^{20}_D = -105°$ (*c*1.2, in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.43$ (m, 2H; CH_2), 4.51 (d, $J = 3.3$ Hz, 1H; CH), 4.72 (dd, $J = 6.4$ Hz, $J' = 3.3$ Hz, 1H; CH), 6.79 (s, 1H; CH), 7.27 (d, $J = 5.3$ Hz, 1H; ArH), 7.54 ppm (d, $J = 5.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 36.7$ (CH_2), 67.9 (CH), 74.3 (C), 77.7 (CH), 108.2 (C), 116.5 (C), 128.3 (CH), 130.8 (CH), 132.1 (C), 136.8 (CH), 150.7 (C), 177.6 ppm (C);); IR (film): $\tilde{\nu} = 1790$ (C=O), 2229 (C≡N) and 3418 cm^{-1} (O-H); MS (ESI) $m/z = 286$ [*M*Na^+]; HRMS (ESI) m/z: calc for $C_{12}H_9O_4SNNa$ [*M*Na^+]: 286.0144, found 286.0141.

(1*R*, 4*R*, 5*R*)-3-(Furan-2-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (8a). Procedure as described above for acid **6** was employed using lactone **19a** (34 mg, 0.15 mmol), THF (1.4 mL), and LiOH (aq) (0.77 mL). Orange amorphous solid, 36 mg (98%); Mp 82–84 °C; $[\alpha]^{20}_D = -15°$ (*c*0.5, in MeOH); ^{1}H NMR (400 MHz, D_2O, 25 °C): $\delta = 2.10$ (dd, $J = 3.0$ Hz, $J' = 13.5$ Hz, 1H; C*H*H), 2.22 (m, 1H; CH*H*), 4.02 (m, 1H; CH), 4.38 (d, $J = 6.2$ Hz, 1H; CH), 6.19 (s, 1H; CH), 6.47 (dt, $J = 1.7$ Hz, $J' = 3.4$ Hz, 1H; ArH), 6.62 (d, $J = 3.4$ Hz, 1H; ArH), 7.47 ppm (d, $J = 1.7$ Hz, 1H; ArH); ^{13}C NMR (100 MHz, D_2O, 25 °C): $\delta = 39.7$ (CH_2), 72.4 (CH), 72.5 (CH), 75.8 (C), 112.0 (CH), 114.5 (CH), 134.8 (CH), 140.6 (C), 146.0 (CH), 154.3 (C), 181.5 ppm (C); IR (KBr): $\tilde{\nu} = 1624$ and 1686 (C=O), 3434 cm^{-1} (O-H); MS (ESI) $m/z = 263$ [*M*Na^+]; HRMS (ESI) m/z: calc for $C_{11}H_{12}O_6Na$ [*M*Na^+]: 263.0526, found 263.0526.

(1*R*, 4*R*, 5*R*)-1,4,5-Trihydroxy-3-(thien-2-yl)cyclohex-2-en-1-carboxylic acid (8b). Procedure as described above for acid **6** was employed using lactone **19b** (95 mg, 0.40 mmol), THF (3.6 mL), and LiOH (aq) (2 mL). White amorphous solid, 82 mg (80%); Mp 135–137 °C; $[\alpha]^{20}_D = -61°$ (*c*1.0, in MeOH); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta = 2.27$ (dd, $J = 3.6$ Hz, $J' = 13.7$ Hz, 1H; C*H*H), 2.36 (dd, $J =$

13.7 Hz, $J'=9.2$ Hz, 1H; CH*H*), 4.22–4.15 (m, 1H; CH), 4.57 (d, $J=6.2$ Hz, 1H; CH), 6.22 (s, 1H; CH), 7.19 (dd, $J=5.1$ Hz, $J'=3.6$ Hz, 1H; ArH), 7.38 (d, $J=3.6$ Hz, 1H; ArH), 7.51 ppm (d, $J=5.1$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, D_2O, 25 °C): $\delta=39.6$ (CH_2), 72.4 (CH), 73.8 (CH), 75.7 (C), 127.9 (CH), 128.5 (CH), 128.7 (CH), 130.3 (CH), 138.7 (C), 143.7 (C), 181.3 ppm (C); IR (KBr): $\tilde{\nu}=1608$, 1721 (C=O), 3420 cm^{-1} (O-H); MS (CI) $m/z=221$ [MH$^+$-2xH_2O]; HRMS (CI) m/z: calc for $C_{11}H_9O_3S$ [MH$^+$-2xH_2O]: 221.0272, found 221.0273.

(1*R*, 4*R*, 5*R*)-1,4,5-Trihydroxy-3-(3-bromothien-2-yl)cyclohex-2-en-1-carboxylic acid (8c). Procedure as described above for acid **6** was employed using lactone **19c** (57 mg, 0.18 mmol), THF (1.6 mL), and LiOH (aq) (0.9 mL). White amorphous solid, 60 mg, (quant.); Mp 143–145 °C; $[\alpha]^{20}_D=-57°$ (*c*1.0, in MeOH); ^{1}H NMR (400 MHz, D_2O, 25 °C): $\delta=1.99$ (dd, $J=3.2$ Hz, $J'=13.6$ Hz, 1H; C*H*H), 2.10 (dd, $J=11.6$ Hz, $J'=13.6$ Hz, 1H; CH*H*), 3.86 (m, 1H; CH), 4.42 (dd, $J=7.2$ Hz, $J'=1.6$ Hz, 1H; CH), 5.89 (br s, 1H; CH), 6.88 (d, $J=5.6$ Hz, 1H; ArH), 7.25 ppm (d, $J=5.6$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, D_2O, 25 °C): $\delta=38.3$ (CH_2), 69.4 (CH), 72.5 (CH), 73.1 (C), 108.9 (C), 126.4 (CH), 130.4 (CH), 131.0 (CH), 134.6 (C), 136.5 (C), 177.6 ppm (C); IR (KBr): $\tilde{\nu}=3417$ and 1718 cm^{-1}; MS (ESI) $m/z=357$ and 359 [MNa$^+$]; HRMS (ESI) m/z: calc for $C_{11}H_{11}O_5SBrNa$ [MH$^+$]: 356.9404, found 356.9403.

(1*R*, 4*R*, 5*R*)-1,4,5-Trihydroxy-3-(3-nitrothien-2-yl)cyclohex-2-en-1-carboxylic acid (8d). Procedure as described above for acid **6** was employed using lactone **19d** (44 mg, 0.16 mmol), THF (1.5 mL), and LiOH (aq) (0.8 mL). Purification was carried out by HPLC (gradient 0→5 min 0% B; 5→35 min 0%→50% B; A: [TFA/H_2O (1:100)]; B: [TFA/CH_3CN (1:100)]), with a flow of 5 mL min^{-1}. Brown amorphous solid, 40 mg (83%); Mp 55–57 °C; $[\alpha]^{20}_D=-6°$ (*c*1.0, in H_2O); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.15$ (m, 2H; CH_2), 4.02 (m, 1H; CH), 4.36 (d, $J=6.8$ Hz, 1H; CH), 6.34 (s, 1H; CH), 7.22 (d, $J=4.0$ Hz, 1H; ArH), 7.89 ppm (d, $J=4.0$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, D_2O, 25 °C): $\delta=40.1$ (CH_2), 72.2 (CH), 74.0 (CH), 75.7 (C), 128.2 (CH), 132.4 (CH), 132.8 (CH), 137.7 (C), 151.9 (C), 152.9 (C), 180.8 ppm (C); IR (KBr): $\tilde{\nu}=1734$ (C=O) and 3399 cm^{-1} (O-H); MS (ESI) $m/z=324$ [MNa$^+$]; HRMS (ESI) m/z: calc for $C_{11}H_{11}O_7SNNa$ [MNa$^+$]: 324.0148, found 324.0140.

(1*R*, 4*R*, 5*R*)-3-(3-Carbonitrilethien-2-yl)-1,4,5-Trihydroxycyclohex-2-en-1-carboxylic acid (8e). Procedure as described above for acid **6** was employed using lactone **19e** (81 mg, 0.31 mmol), THF (2.8 mL), and LiOH (aq) (1.6 mL). Light orange amorphous solid, 71 mg (82%); Mp 110–112 °C; ; $[\alpha]^{20}_D=+95°$ (*c*1.0, in H_2O); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.19$ (m, 1H; C*H*H), 2.24 (d, $J=8.8$ Hz, 1H; CH*H*), 4.31 (m, 1H; CH), 4.97 (dd, $J=8.5$ Hz, $J'=2.0$ Hz, 1H; CH), 6.16 (s, 1H; CH), 7.23 (d, $J=5.0$ Hz, 1H; ArH), 7.36 ppm (d, $J=5.0$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, D_2O, 25 °C): $\delta=39.0$ (CH_2), 66.6 (CH), 73.7 (C), 82.5 (CH), 125.6 (CH), 127.0 (CH), 127.2 (C), 127.8 (C), 128.3 (CH), 147.4 (C), 162.8 (C), 177.1 ppm (C); IR (KBr): $\tilde{\nu}=1714$ (C=O), 2229 (C≡N), 3401 cm^{-1} (O-H); MS (ESI) $m/z=320$ [MK$^+$]; HRMS (ESI) m/z: calc for $C_{12}H_{11}O_5SNK$ [MK$^+$]: 320.9823, found 320.9801.

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