CHEMMEDCHEM

DOI: 10.1002/cmdc.200600208

Nanomolar Competitive Inhibitors of *Mycobacterium tuberculosis* and *Streptomyces coelicolor* Type II Dehydroquinase

Verónica F. V. Prazeres,[a] Cristina Sánchez-Sixto,[a] Luis Castedo,[a] Heather Lamb,[b] Alastair R. Hawkins,[b] Alan Riboldi-Tunnicliffe,[c] John R. Coggins,[c] Adrian J. Lapthorn,[c] and Concepción González-Bello*[a]

Dedicated to Prof. K. C. Nicolaou on the occasion of his 60th birthday

Isomeric nitrophenyl and heterocyclic analogues of the known inhibitor (1S,3R,4R)-1,3,4-trihydroxy-5-cyclohexene-1-carboxylic acid have been synthesized and tested as inhibitors of M. tuberculosis *and* S. coelicolor *type II dehydroquinase, the third enzyme of the shikimic acid pathway. The target compounds were synthesized by a combination of Suzuki and Sonogashira cross-coupling and copper(I)-catalyzed 2,3-dipolar cycloaddition reactions from a common vinyl triflate intermediate. These studies showed that a* para*-nitrophenyl derivative is almost 20-fold more potent as a competitive inhibitor against the* S. coelicolor *enzyme than that of* M. tuberculosis*. The opposite results were obtained with the* meta *isomer. Five of the bicyclic analogues reported herein proved to be potent competitive inhibitors of* S. coelicolor *dehydroquinase, with inhibition constants in the low nanomolar range (4–30 nM). These derivatives are also competitive inhibitors of the* M. tuberculosis *enzyme, but with lower affinities. The most potent inhibitor against the* S. coelicolor *enzyme, a 6-benzothiophenyl derivative, has a* K_i *value of 4 nM—over 2000-fold more potent than the best previously known inhibitor, (1*R*,4*R*,5*R*)-1,5-dihydroxy-4-(2-nitrophenyl)cyclohex-2-en-1-carboxylic acid (8 μM), making it the most potent known inhibitor against any dehydroquinase. The binding modes of the analogues in the active site of the* S. coelicolor *enzyme (GOLD 3.0.1), suggest a key π-stacking interaction between the aromatic rings and Tyr 28, a residue that has been identified as essential for enzyme activity.*

Introduction

The shikimate pathway, the biosynthetic route to the aromatic amino acids, folates, alkaloids, and vitamins is present in bacteria, fungi, and plants, and has been recently discovered in apicomplexan parasites.[1,2] The absence of the pathway in mammals combined with its essential nature in certain microorganisms makes the shikimic acid pathway enzymes attractive targets for the development of new antibiotics and herbicides. We have shown recently with derivatives **1**–**3** that the incorporation of aryl groups bearing electron-withdrawing substituents in position 3 of the known inhibitor **4a** markedly increases the inhibitory potency of this enol mimic ($K_i = 200$ μM, Figure 1) against *Mycobacterium tuberculosis* type II dehydroquinase, the third enzyme of the shikimic acid pathway.[3] In fact, these studies led to the discovery of the most potent known inhibitor against any type II dehydroquinase, the 3-nitrophenyl derivative **1d**, with a low K_i value of 54 nM. NMR studies (STD and 1D and 2D TR-NOESY experiments) have shown that only one of the conformations present in solution for the competitive inhibitor **1d** is selected when bound to the active site of the *M. tuberculosis* type II dehydroquinase.[4]

On the other hand, the crystal structures of the enzyme–inhibitor complexes reported for *M. tuberculosis* dehydroquinase and other dehydroquinases, such as that of *Streptomyces coelicolor*, suggest the presence of subtle differences in the active sites of type II dehydroquinases from various sources, in particular the larger available space in the active site of *S. coelicolor* dehydroquinase compared with that of other type II enzymes such as that of *M. tuberculosis*. These structural differences could explain the variation in potency of an inhibitor against type II dehydroquinases from various sources. For example, inhibitor **4a** (Figure 1) has been reported to have K_i values of 200 and 30 μM against *M. tuberculosis* and *S. coelicolor* dehy-

[a] *V. F. V. Prazeres, C. Sánchez-Sixto, Prof. Dr. L. Castedo, Prof. Dr. C. González-Bello*
Laboratorio de Química Orgánica, CSIC
and 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) 981-595012
E-mail: cgb1@lugo.usc.es

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

[c] *A. Riboldi-Tunnicliffe, Prof. Dr. J. R. Coggins, Dr. A. J. Lapthorn*
Department of Chemistry and Division of Biochemistry and Life Sciences
University of Glasgow, Glasgow G12 8QQ (UK)

Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author: ^{1}H NMR, ^{13}C NMR, and DEPT spectra of compounds **5**, **6**, and **10**–**14**.

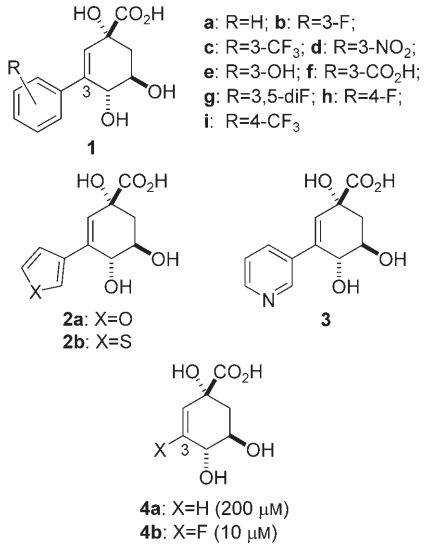


Figure 1. Competitive inhibitors of *M. tuberculosis* type II dehydroquinase.

droquinase, respectively.[5] With these facts in mind, we first studied the inhibitory potency of the 3-aryl derivatives **1**–**3** against *S. coelicolor* type II dehydroquinase.

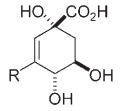
Results and Discussion

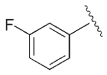
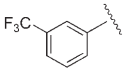
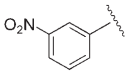
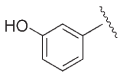
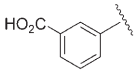
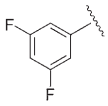
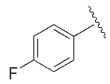
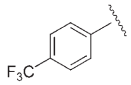
Inhibition data for 3-aryl derivatives 1–3 against *S. coelicolor* dehydroquinase

The K_i values obtained from Dixon plots ($1/v$ versus [I]) are summarised in Table 1. It was not surprising to find that their inhibitory potency against *S. coelicolor* dehydroquinase varies considerably compared with the *M. tuberculosis* enzyme. All the acids **1**–**3** except two (Entries 3 and 4, Table 1) were found to be more potent against *S. coelicolor* than *M. tuberculosis* dehydroquinase. Surprisingly, the most potent inhibitor against *M. tuberculosis* dehydroquinase, 3-nitrophenyl derivative **1d**, was over 30-fold less potent against the *S. coelicolor* enzyme than for the *M. tuberculosis* one (Entry 4, Table 1). *para*-Substituted acid **1i**, which contains a bulky trifluoromethyl group, was found to be the most potent compound against *S. coelicolor* dehydroquinase (Entry 9, $K_i = 130$ nM, Table 1).

Docking studies were carried out using GOLD 3.0.1[6,7] to gain some insight into how these competitive inhibitors bind the active site of *S. coelicolor* type II dehydroquinase. The crystal structure of the enzyme with the anhydro inhibitor **4a** bound in the active site was used as the starting point for this study (Figure 2, PDB ID: 1GU1[8]). In this crystal structure, a molecule of glycerol and tartrate originating from the enzyme storage buffer are also present in the active site of the enzyme (Figure 2a).[8] All acids **1**–**3** bind approximately to the same site as that of inhibitor **4a** in the crystal structure.[8] However, their cyclohexene rings are slightly twisted relative to the position of **4a**, leading to close proximity between the double bond in the ligands and Tyr 28, with hydrogen H2 over the plane of the aromatic ring, which occupies part of the glycerol pocket (see Supporting Information). The stronger affinity of the *para*-trifluoromethylphenyl derivative **1i** can be attributed to polar and electrostatic interactions of the *para*-CF_3 group with the Thr 96 methyl group of the neighbouring subunit and the essential Arg 23, respectively. Interactions of this type should be also strong with a nitro group and weaker for electron-withdrawing groups in the *meta* position, as might be the case for the 3-nitrophenyl and 3-trifluoromethylphenyl derivatives **1d** and **1c**, respectively, because substituents in this *meta* position should be more distal from these residues.

Table 1. Inhibition results for compounds **1**–**3** in assays with *S. coelicolor* type II dehydroquinase.



Entry	Compd	R	K_i [μM]
1	**1a**		0.75 ± 0.06
2	**1b**		0.67 ± 0.06
3	**1c**		6.8 ± 0.5
4	**1d**		1.95 ± 0.1
5	**1e**		2.4 ± 0.2
6	**1f**		33.5 ± 0.3
7	**1g**		0.82 ± 0.07
8	**1h**		0.55 ± 0.05
9	**1i**		0.13 ± 0.01
10	**2a**		0.60 ± 0.05
11	**2b**		0.49 ± 0.05
12	**3**		6.8 ± 0.5

Newly designed 3-aryl enol mimics

Taking into account these initial results, we decided to study the effect of the nitro group in the *para* and *ortho* positions on the affinity toward *M. tuberculosis* and *S. coelicolor* type II

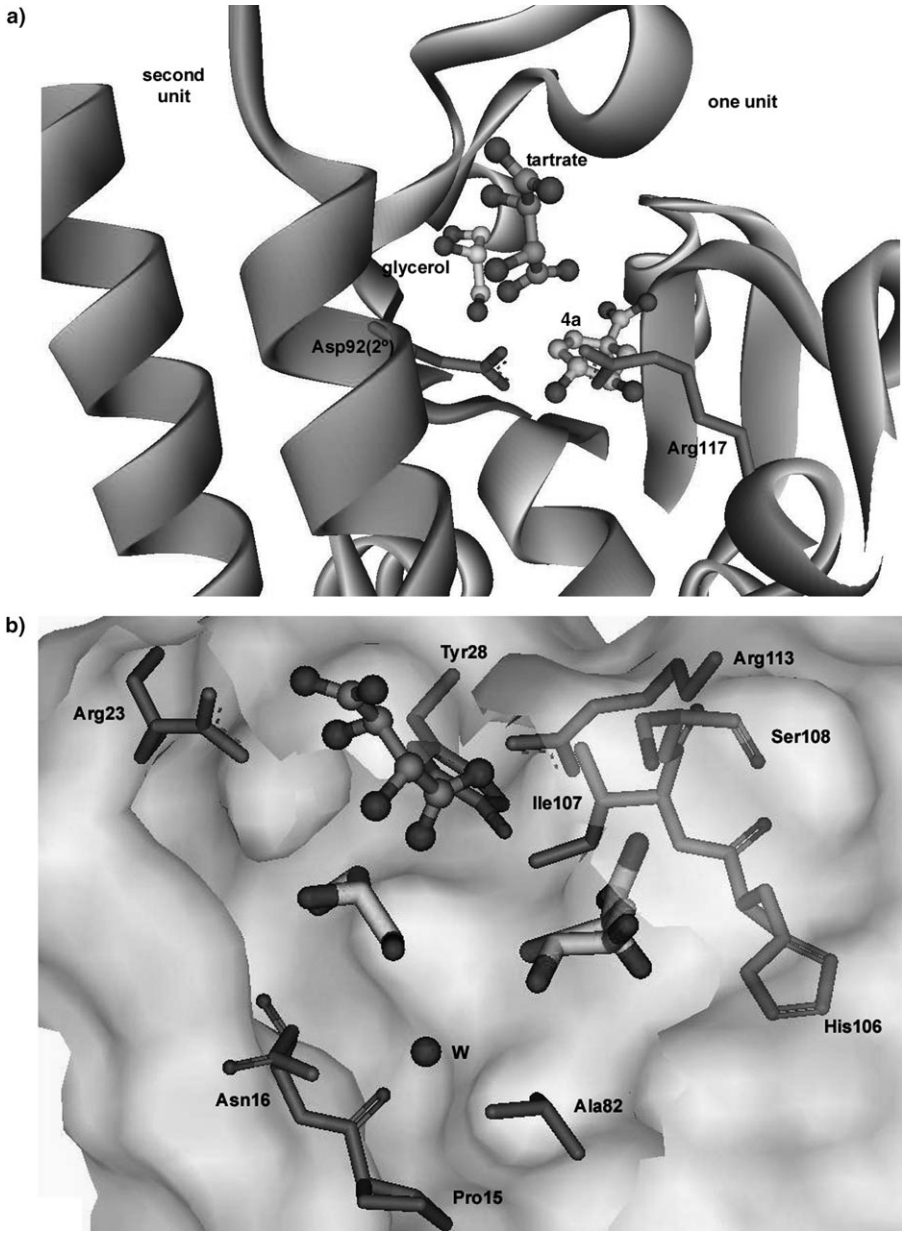


Figure 2. a) Active site of one subunit of *S. coelicolor* type II dehydroquinase (light grey ribbon) partially closed off by the interaction with the α3 helix of the neighbouring subunit (darker grey). The positions of inhibitor **4a** and the glycerol and tartrate molecules are shown. b) Selected view of *S. coelicolor* type II dehydroquinase active site. Relevant residues are indicated; hydrogen atoms are omitted; W = water molecule.

dehydroquinase with derivatives **5** (Figure 3). We also wanted to explore the effect of incorporating bulkier heterocycles at C3 of inhibitor **4a** on the inhibitory potency against both enzymes. In principal, it is expected that the latter type of derivatives should have stronger affinity for the *S. coelicolor* enzyme than for that of *M. tuberculosis*, owing to the greater available space in the *S. coelicolor* dehydroquinase. These bulky heterocyclic moieties should also be able to occupy the glycerol or tartrate binding pockets and stabilize new binding interactions, a situation that should help to increase affinity. These possibilities were initially tested with naphthyl enol mimic **6** (Figure 4), docking studies of which were carried out with the crystal structure of the *S. coelicolor* enzyme.[8] The results suggest that indeed the aromatic ring of **6** should be located between the glycerol and the tartrate pockets, a situation that brings the aromatic ring proximal to the essential Arg 23 residue as well as Thr 96 and Asn 95 of the neighbouring (2°) subunit (Figure 4).

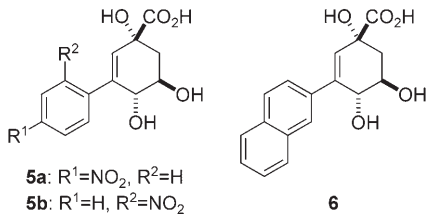


Figure 3. Newly designed 3-aryl enol mimics.

Synthesis of compounds 5 and 6

The target compounds **5a**, **5b**, and **6** were synthesized by Suzuki cross-coupling reactions between the appropriate commercially available arylboronic acids and vinyl triflate **7** (Scheme 1), which was prepared using our previously reported protocol.[3] Conversion of the cross-coupling products **8** to the desired acids **5a**, **5b**, and **6** was achieved by deprotection of the TBS groups followed by basic hydrolysis of the corresponding lactones **9** and protonation with an ion-exchange resin.

Inhibition assay results with acids 5 and 6

Acids **5** and **6** were assayed in the presence of 3-dehydroquinic acid for their inhibitory properties against *M. tuberculosis* and *S. coelicolor* type II dehydroquinase. The K_i values, obtained from Dixon plots (1/*v* versus [I]), are summarised in Table 2. As expected, the affinity of the nitrophenyl derivatives against the *S. coelicolor* enzyme increased with the nitro group placed at the *para* position (cf. Entry 1, Table 2 and Entry 4, Table 1). The

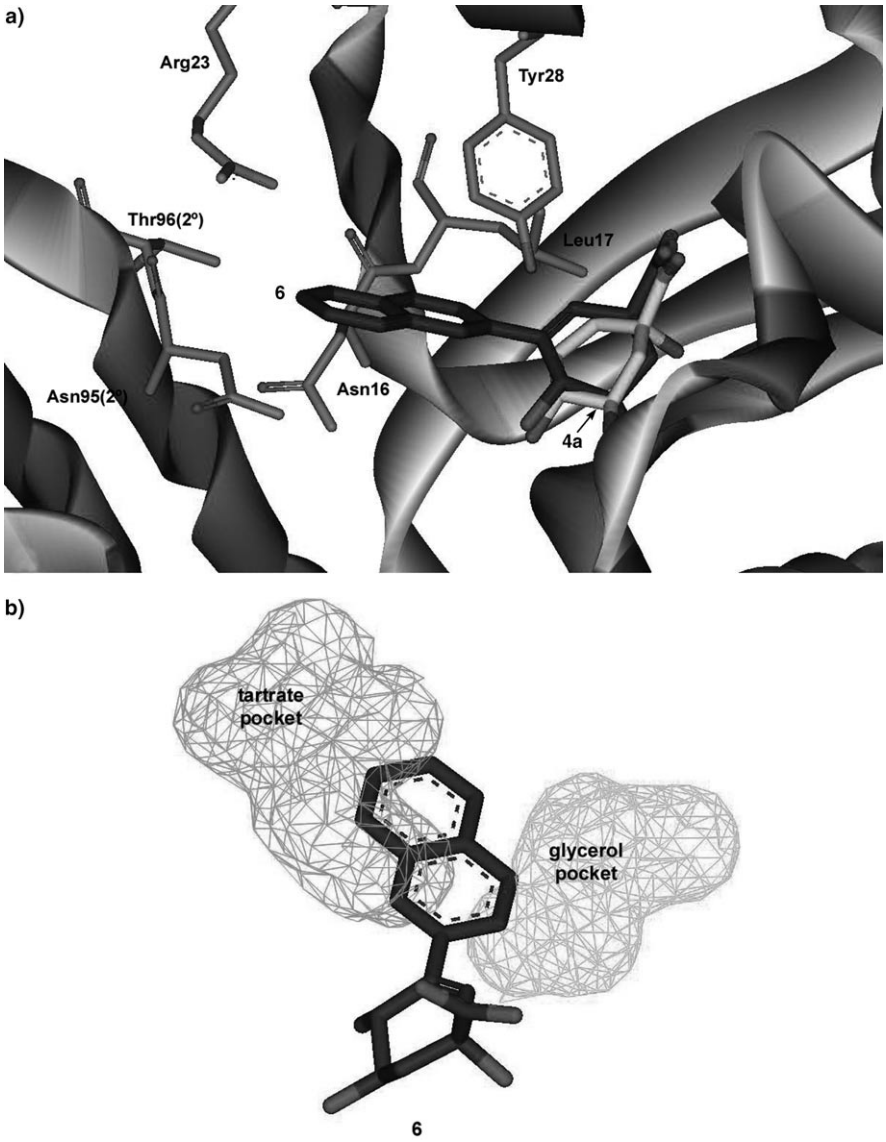
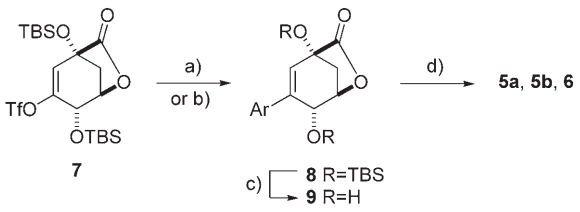


Figure 4. a) Docking results of ligand **6** (active site position of the inhibitor **4a** (indicated) is also shown). b) Relative position of ligand **6** to the glycerol and tartrate pockets.



Scheme 1. Reagents and conditions: a) $[Pd_2(dba)_3]\cdot CHCl_3$, CH_2Cl_2, THF, Et_3N, $ArB(OH)_2$, −78 °C→RT; b) $ArB(OR)_2$, $[Pd(PPh_3)_4]$, dioxane, K_3PO_4 (aq), Δ; c) TBAF, THF, 0 °C→RT; d) 1. LiOH, THF, RT, 2. Amberlite IR-120 (H^+).

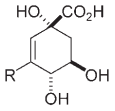
opposite effect was observed for the *M. tuberculosis* enzyme, in which the inhibition constant of **5a** increases sharply (Entry 1, Table 2). Surprisingly, *ortho*-nitrophenyl derivative **5b** proved to be a weak competitive inhibitor for both enzymes (Entry 2, Table 2). The naphthyl derivative **6** was shown to be a strong competitive inhibitor of *S. coelicolor* dehydroquinase, with a K_i value of 22 nM, which reflects a >300-fold enhancement in affinity relative to the best previously reported inhibitor for the *S. coelicolor* enzyme, (1*R*,4*R*,5*R*)-1,5-dihydroxy-4-(*o*-nitrobenzyloxy)cyclohex-2-en-1-carboxylic acid (K_i = 8 μM).[9] It was not surprising to find that naphthyl derivative **6** did not show such high affinity for *M. tuberculosis* dehydroquinase (K_i = 1.2 μM) as it does for the *S. coelicolor* enzyme (K_i = 22 nM).

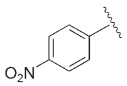
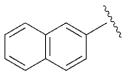
The good inhibition results obtained with naphthyl derivative **6** encouraged us to further study the effect of incorporating other bicyclic aromatic rings, including benzofuranyl, benzothiophenyl, indolyl, benzooxadiazolyl, and benzyltriazolyl groups. The newly designed enol mimics **10**–**14** are shown in Figure 5. Similarly to naphthyl derivative **6**, derivatives **10**–**14** were be expected to inhibit *S. coelicolor* dehydroquinase more strongly than the *M. tuberculosis* enzyme, as there is more available space in the *S. coelicolor* dehydroquinase.

Synthesis of compounds 10–13

Acids **10**, **12**, and **13** were synthesized in a manner similar to derivatives **5**–**6** from vinyl triflate **7** and the corresponding commercially available boronic acid (Scheme 1). However, the preparation of compounds **11** required the synthesis of the aryl boronic esters or acids **19**, **21**, and **23**, which were prepared as outlined in Scheme 2. The desired pinacolboronate ester **19** was synthesized by a four-step sequence. Firstly, reaction of the sodium phenoxide of 3-bromophenol (**15**) with bromoacetaldehyde dimethyl acetal afforded ether **16** in 93% yield. Treatment of **16** with polyphosphoric acid led to an intramolecular Friedel–Crafts cyclization with concomitant aromatization to give a chromatographically inseparable mixture of bromobenzofurans **17a** and **17b** (52% overall). Attempts at the direct boration of bromobenzofurans **17** by metal-catalyzed methods using bis(pinacolato) diboron or pinacolborane as the boron source gave low yields of the desired boronic esters. Finally, boration of bromobenzofurans **17** was achieved by lithium–bromine exchange with *n*BuLi followed by treat-

Table 2. Inhibition results for compounds **5** and **6** in assays with *M. tuberculosis* and *S. coelicolor* type II dehydroquinases.



Entry	Compd	R	K_i [μM] *M. tuberculosis*[a]	K_i [μM] *S. coelicolor*[b]
1	**5a**		6.5 ± 0.6	0.34 ± 0.03
2	**5b**		109 ± 9	26.4 ± 0.2
3	**6**		1.2 ± 0.1	0.022 ± 0.002

[a] Assay conditions: Tris-HOAc (50 mM, pH 8.2), 25 °C. [b] Assay conditions: Tris-HCl (50 mM, pH 7.0), 25 °C.

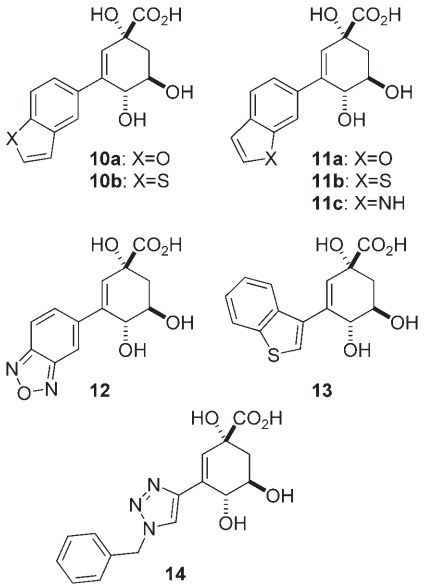
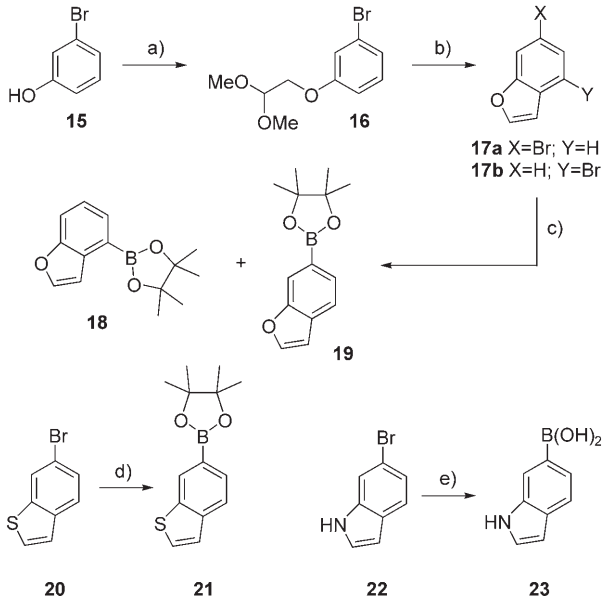


Figure 5. Newly designed inhibitors **10**–**14**.

ment with $B(OMe)_3$ and subsequent acid workup. The resulting benzofuran boronic acids could be used in the Suzuki cross-coupling reaction. However, product purification of both regioisomers proved to be difficult. In an effort to avoid this problem, conversion of crude boronic acids into the corresponding pinacolboronate esters **18** and **19** was carried out by treatment with pinacol at reflux to afford a chromatographically separable mixture of regioisomeric benzofuranyl pinacolboronate esters **18** (25%) and **19** (24%).

Benzothiophenyl boronate ester **21** was prepared from 6-bromobenzothiophene **20**[10] by direct boration using bis(pinacolato) diboron and $[Pd(PPh_3)_2Cl_2]$ catalyst in the presence of aqueous K_2CO_3 in dioxane. 1*H*-Indol-6-yl boronic acid (**23**) was synthesized by treatment of the potassium amide of 6-bromoindole (**22**) with *t*BuLi followed by treatment with $B(OMe)_3$ and subsequent acid hydrolysis.[11]



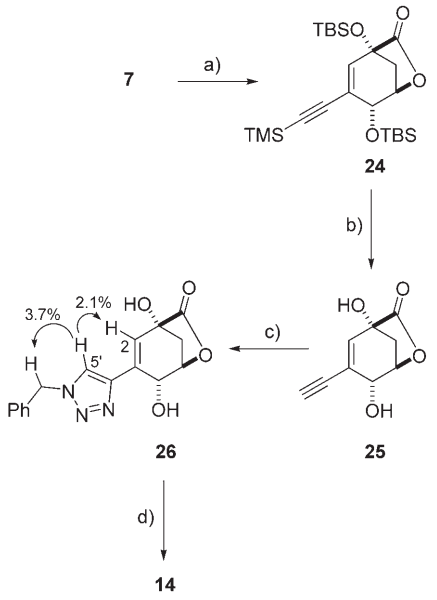
Scheme 2. Reagents and conditions: a) 1. NaH, 15-crown-5, DMF, 2. $BrCH_2CH(OMe)_2$, 130 °C (93%); b) polyphosphoric acid, toluene, Δ (52%); c) 1. *n*BuLi, Et_2O, −78 °C→−30 °C, 2. $B(OMe)_3$, −78 °C→RT, 3. HCl (dil), 4. pinacol, CH_2Cl_2, Δ (49%); d) $[Pd(PPh_3)_2Cl_2]$, PPh_3, bis(pinacolato) diboron, K_2CO_3, dioxane, 80 °C (45%); e) 1. KH, THF, 0 °C, 2. *t*BuLi, −78 °C→RT, 3. $B(OBu)_3$, −78 °C, 4. H^+ (56%).

Synthesis of compound 14

The benzyltriazole moiety in **14** was introduced by a copper(I)-catalyzed 1,3-dipolar cycloaddition between benzylazide and vinyl alkyne **25**, which was synthesized from vinyl triflate **7** by a Sonogashira cross-coupling reaction with trimethylsilylacetylene (Scheme 3). The latter reaction was performed in THF in the presence of piperidine, a catalytic amount of copper iodide, and a $[Pd(PPh_3)_4]$ catalyst to give an excellent yield of the protected alkyne **24**. Deprotection of the TBS and TMS groups with TBAF afforded terminal alkyne **25** in 92% yield. Copper(I)-catalyzed cycloaddition between benzyl azide and alkyne **25**, under typical click Sharpless conditions,[12,13] afforded benzyl triazole **26** in 88% yield regiospecifically. The regiochemistry of the 1,3-dipolar cycloaddition was confirmed by NOE experiments. It was found that irradiation of H5′ in **26** enhanced the methylene protons of the benzyl group (3.7%) and H2 (2.1%). Finally, basic hydrolysis of lactone **26** followed by treatment with Amberlite IR-120 (H^+) ion-exchange resin gave the desired acid **14** in excellent yield.

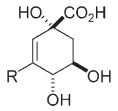
Inhibition assay results with acids 10–14

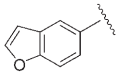
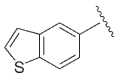
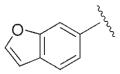
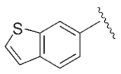
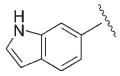
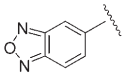
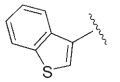
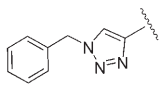
The new enol mimics **10**–**14** were tested for their inhibition activity against *M. tuberculosis* and *S. coelicolor* type II dehydroquinases. Acids **10**–**14** proved to be competitive inhibitors of both enzymes, all of which are more potent against the *S. coelicolor* than the *M. tuberculosis* enzyme (Table 3). Four of the



Scheme 3. Reagents and conditions: a) $[Pd(PPh_3)_4]$, CuI, TMSC≡CH, piperidine, THF, 40 °C (96%); b) TBAF, THF, 0 °C→RT (92%); c) BnN_3, sodium ascorbate, *t*BuOH/H_2O, $CuSO_4$, RT (88%); d) 1. LiOH, THF, RT, 2. Amberlite IR-120 (H^+) (97%).

Table 3. Inhibition results for compounds **10**–**14** in assays with *M. tuberculosis* and *S. coelicolor* type II dehydroquinases.



Entry	Compd	R	K_i [μM] *M. tuberculosis*[a]	K_i [μM] *S. coelicolor*[b]
1	**10a**		2.0 ± 0.1	0.094 ± 0.009
2	**10b**		2.85 ± 0.20	0.012 ± 0.001
3	**11a**		0.97 ± 0.10	0.026 ± 0.002
4	**11b**		0.85 ± 0.08	0.004 ± 0.0004
5	**11c**		> 300	0.30 ± 0.02
6	**12**		1.95 ± 0.19	2.2 ± 0.2
7	**13**		13.2 ± 1.3	0.13 ± 0.01
8	**14**		3.75 ± 0.36	0.030 ± 0.003

[a] Assay conditions: Tris-HOAc (50 mM, pH 8.2), 25 °C. [b] Assay conditions: Tris-HCl (50 mM, pH 7.0), 25 °C.

acids **10**–**14** have inhibition constants against *S. coelicolor* dehydroquinase in the low nanomolar range (5-benzothiophenyl **10b**, K_i = 12 nM; 6-benzofuranyl **11a**, K_i = 26 nM; 6-benzothiophenyl **11b**, K_i = 4 nM; and benzyltriazolyl **14**, K_i = 30 nM). The 6-benzothiophenyl derivative **11b** (Entry 4, Table 3) was found to be the most potent analogue with a low K_i value of 4 nM, which represents a 2000-fold enhancement in affinity for the *S. coelicolor* enzyme over the best previously reported inhibitor, (1*R*,4*R*,5*R*)-1,5-dihydroxy-4-(*o*-nitrobenzyloxy)cyclohex-2-en-1-carboxylic acid (K_i = 8 μM).[9] Benzothiophenyl derivatives were found to be about 7-fold more potent than the corresponding benzofuranyl compounds (Entries 2 and 4 versus 1 and 3, Table 3), with regioisomers at position 6 being over 3-fold more potent than the corresponding 5-position regioisomers (Entries 3 and 4 versus 1 and 2, Table 3). In contrast, the benzo-[1,2,5]oxadiazole derivative **12** showed a marked decrease in affinity among the bicyclic enol mimics (K_i = 2.2 μM).

As expected, bicyclic derivatives **10**–**14** do not show such high affinity for *M. tuberculosis* dehydroquinase as they do for the *S. coelicolor* enzyme. 6-Benzofuranyl **11a** and 6-benzothiophenyl **11b** derivatives proved to be the most potent inhibitors of the bicyclic series against the *M. tuberculosis* enzyme, with inhibition constants below 1 μM. Moreover, with the exception of 3-benzothiophenyl derivative **13** and 6-indolyl derivative **11c**, no significant differences were found among the other bicyclic derivatives, which showed affinities between 2 and 4 μM.

Antibacterial activity of acids **1**, **5**, **6**, and **10**–**14** against *M. tuberculosis* are currently being carried out through the Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF: NIH, NIAID 2056).

Molecular modelling studies with ligands 10–14

The GOLD-predicted binding of ligands **10**–**13** in the active site of *S. coelicolor* dehydroquinase shows, similarly to naphthyl derivative **6**, that the aromatic rings are located between glycerol and tartrate molecules, close to the essential Arg 23 and residues Thr 96 and Asn 95 of the neighbouring subunit (Figure 6). In this conformation, π-stacking interactions (edge-on or T-shaped geometry) with Tyr 28 can be established as well as lipophilic interactions between the ring B hydrogen atoms of bicyclic moieties and the side chain of Leu 17 and the methylene group of Asn 16 (Figure 6). These edge-on stacking interactions[14–16] with Tyr 28 should be stronger with π-electron-deficient rings such as benzothiophene or benzofuran, and this could explain the stronger inhibitory potency observed with benzothiophenyl derivative **11a** and benzofuranyl derivative **11b** in comparison with the indolyl derivative **11c**. In addition, certain differences in the position and orientation of the aromatic ring moieties of ligands **10**–**13** can be identified. Particularly, 5-benzo derivatives **10** docked with their heterocyclic heteroatom (O, S) pointing toward Arg 23 (see Supporting Information, Figure S2), whereas in the 6-regioisomers **11**, the corresponding heterocyclic heteroatom (O, S, N) is oriented toward the plane between arginines 113 and 117 (see Supporting Information, Figure S2), a situation that places both

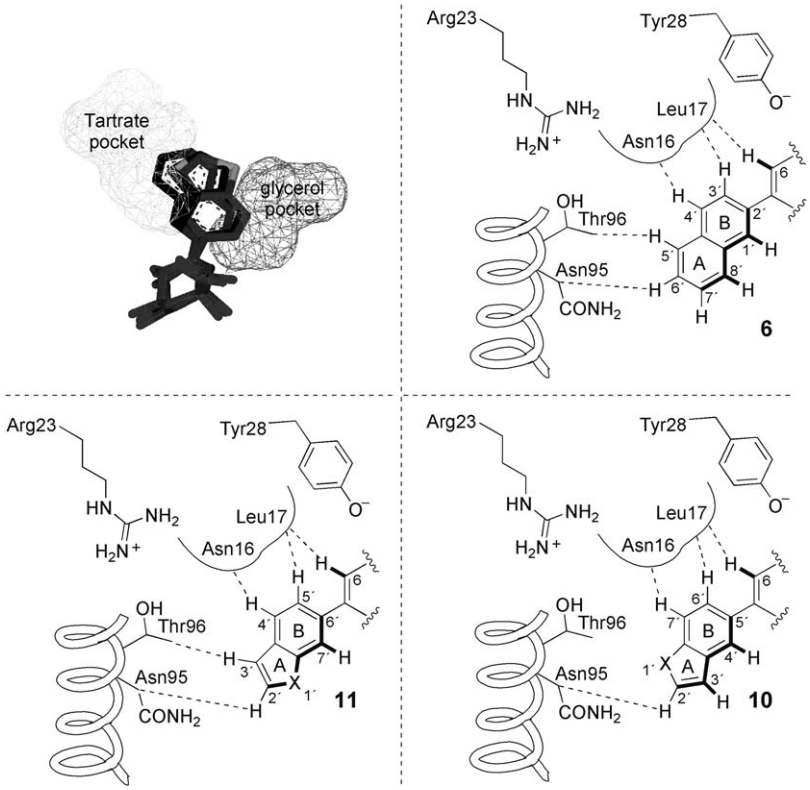


Figure 6. Relevant binding interactions of bicyclic moieties of ligands **6**, **11**, and **10**.

5-membered ring hydrogen atoms close to the Thr 96 methyl group of the neighbouring subunit (see Supporting Information, Figure S2). These opposite orientations might explain the $>$3-fold increase in affinity observed between 5- and 6-position regioisomers.

On the other hand, the predicted binding for the potent benzyltriazole analogue **14** puts the benzyl group in the pocket that had previously been occupied by the glycerol molecule in the crystal structure; in this conformation it is possible to have π-stacking interactions with Tyr 28, electrostatic interactions with Arg 23, and lipophilic interactions with the side chain of leucines 19 and 20 (Figure 7). Furthermore, the methylene unit of the benzyl group and hydrogen H5′ of the triazole group are also in close proximity to the methylene unit of Asn 16 and the Leu 17 side chain, respectively.

Docking studies were also carried out with *M. tuberculosis* dehydroquinase. However, important differences in the position of the aromatic ring were obtained depending on the enzyme–inhibitor co-crystal structure employed for docking. This fact is probably due to the lack of some important residues (Tyr 24 in 1H0S[17] and Arg 19 in 1H0R[18]) in the available crystal structures. Structural studies of the enzyme–inhibitor complexes with both enzymes are needed to identify, especially for the *M. tuberculosis* enzyme, the binding interactions of the aromatic rings incorporated into the core of the known inhibitor **4a**. Furthermore, preliminary fluorescence studies with *S. coelicolor* dehydroquinase indicate the presence of a fluorescence complex formed between the protein and the bicyclic derivatives reported herein.[19] These studies should provide some insight about the suggested key π-stacking binding interaction between the aromatic rings of the ligands and the essential tyrosine residue.

Conclusions

The relative inhibitory potency of the three positional isomers of nitrophenyl derivative **1** against *M. tuberculosis* and *S. coelicolor* dehydroquinase has been studied. These studies show that *para*-nitrophenyl derivative **5a** is almost 20-fold more potent a competitive inhibitor against the *S. coelicolor* enzyme

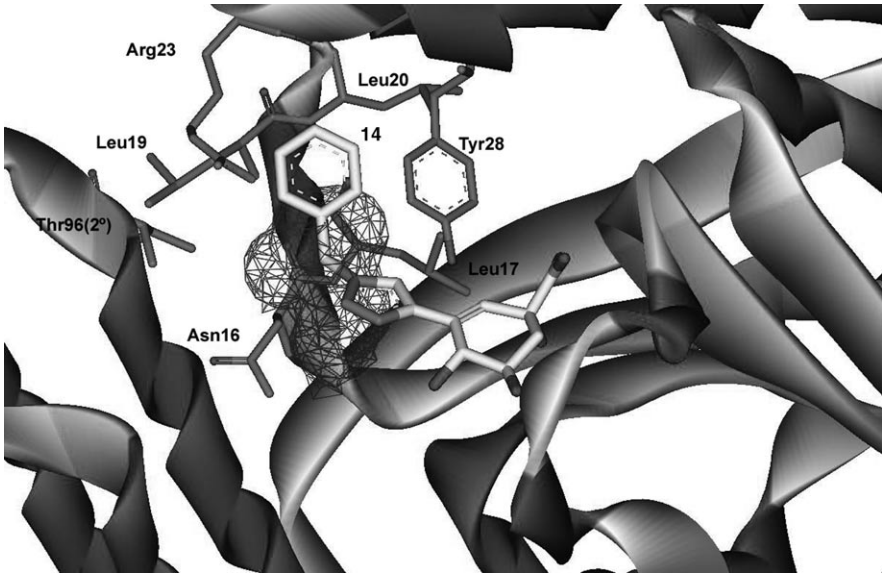


Figure 7. GOLD-predicted binding of ligand **14**; relevant residues and glycerol binding pocket (space-filling) are indicated.

than the *M. tuberculosis* isoform. Docking studies with *S. coelicolor* dehydroquinase using GOLD 3.0.1 suggest a key electrostatic binding interaction between Arg 23, a residue identified as essential for enzyme activity, and the aromatic rings and nitro group of compound **5a**. The opposite results were obtained with the *meta* isomer **1d** that is >120-fold more potent against the *M. tuberculosis* enzyme than the *S. coelicolor* isoform. The *ortho* isomer **5b** was found to be a weak competitive inhibitor against both dehydroquinases.

The incorporation of diverse bulky aryl groups in position 3 of the known inhibitor 2,3-anhydroquinic acid (**4a**) has resulted in the discovery of new potent competitive inhibitors against *S. coelicolor* dehydroquinase. Indeed, five of the analogues reported herein have affinities in the low nanomolar range (4–30 nM). The bicyclic derivatives **6** and **10**–**14** are also competitive inhibitors of the *M. tuberculosis* enzyme, but with lower binding affinities. With the exception of two of them, they all bind within the low micromolar range.

The most potent inhibitor of the *S. coelicolor* enzyme, 6-benzothiophenyl derivative **11b**, has a K_i value of 4 nM, which is over 2000-fold more potent than the best previously reported inhibitor, (1*R*,4*R*,5*R*)-1,5-dihydroxy-4-(2-nitrophenyl)cyclohex-2-en-1-carboxylic acid ($K_i = 8\ \mu M$),[9] making it so far the most potent inhibitor known against any dehydroquinase. Docking studies using GOLD 3.0.1 predict that the bicyclic moieties of the inhibitors occupy the glycerol or tartrate binding pockets present in the active site of the *S. coelicolor* enzyme crystal structure, and suggest a key π-stacking binding interaction between the aromatic rings of the ligands and the essential Tyr 28, a residue that has been identified to act as the base in the enzymatic mechanism.[8]

Experimental Section

General methods. All starting materials and reagents were commercially available and used without further purification. FTIR 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 1D, COSY, and DEPT-135 experiments. All procedures involving the use of ion-exchange resins were carried out at room temperature and used Milli-Q deionised water. Amberlite IR-120 (H^+) (cation-exchange resin) was washed alternately with water, 10% NaOH, water, 10% HCl, and finally water before use. The purity of carboxylic acids was analysed by HPLC and NMR. HPLC was performed on a preparative (300 mm × 16 mm) Bio-Rad Aminex ion-exclusion HPX-87H organic acids column and Teknokroma Mediterranea Sea18 reversed-phase (5 μm; 25 × 0.46 mm^2). The eluent used for these columns was aqueous formic acid (100 mM) and aqueous TFA (0.1%) at a flow rate of 0.6 and 1 mL min^{-1}, respectively.

Dehydroquinase assays. *S. coelicolor* and *M. tuberculosis* type II dehydroquinases were purified as described previously.[20,21] A concentrated solution of *S. coelicolor* enzyme (5 mg mL^{-1}) was stored in Tris-HCl (20 mM, pH 7.5) and DTT (0.5 mM); *M. tuberculosis* dehydroquinase (0.7 mg mL^{-1}) was stored in potassium phosphate buffer (50 mM, pH 7.2), DTT (1 mM), and NaCl (150 mM). When required for assays, aliquots of the enzyme stocks were diluted in water and buffer and stored on ice. Dehydroquinase was assayed in the forward direction by monitoring the increase in absorbance at $\lambda = 234$ nm that results from the absorbance of the enone–carboxylate chromophore of 3-dehydroshikimic acid ($\varepsilon = 12000\ M^{-1} cm^{-1}$). Standard assay conditions for type II dehydroquinase were Tris-HCl (50 mM, pH 7.0) at 25 °C for *S. coelicolor* dehydroquinase, and Tris-HOAc (50 mM, pH 8.2) at 25 °C for *M. tuberculosis* dehydroquinase. Each assay was initiated by the addition of substrate. Solutions of 3-dehydroquinic acid were calibrated by equilibration with type II dehydroquinase and measurement of the change in absorbance at $\lambda = 234$ nm that arises from the formation of the enone–carboxylate chromophore of 3-dehydroshikimic acid.

Docking. The receptor and ligands were used as MOL2 files. Energy minimisation was not performed on the enzymes. All ligands, as their carboxylate anions, were prepared using Gaussian 98W[22] and energy minimised using AM1. Each ligand was docked using GOLD 3.0.1[6,7] in 25 independent genetic algorithm (GA) runs, and for each of these a maximum of 100 000 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. The crystal structures of the anhydro inhibitor **4a** bound to the active site of the *M. tuberculosis* enzyme (PDB ID: 1H0R[11]) and *S. coelicolor* dehydroquinase (PDB ID: 1GU1[8]) were used.

General procedure of Suzuki coupling. *Procedure A*. Boronic acid (2 equiv, 0.15 M) in dry THF and dry triethylamine (3 equiv) were added to a stirred solution of the vinyl triflate **7**[3] (1 equiv) and $[Pd_2(dba)_3]\cdot CHCl_3$ (0.075 equiv) in dry CH_2Cl_2 under an inert atmosphere at −78 °C. The dry ice bath was removed, and after 1 h at room temperature, more boronic acid (1–1.5 equiv) in dry THF was added to the reaction mixture cooled at −78 °C. The reaction mixture was stirred at room temperature between 14–16 h. The solvents were removed under reduced pressure, and the crude residue was purified by flash chromatography.

Procedure B. $[Pd(PPh_3)_4]$ (0.025 equiv), the boronic ester (1.1–2.2 equiv), and an aqueous solution of K_3PO_4 (1.5 equiv, 0.9 M) were added to a stirred solution of the vinyl triflate **7**[3] (1 equiv) in dioxane. The resulting reaction mixture was heated at reflux for 2 h, unless otherwise indicated. 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 (2×). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude reaction was purified by flash chromatography.

*(1R,4R,5R)-1,4-Di(tert-butyldimethylsilyloxy)-3-(4-nitrophenyl)cyclohex-2-en-1,5-carbolactone (**8a**):* Procedure B. Vinyl triflate **7** (50 mg, 0.09 mmol), $[Pd(PPh_3)_4]$ (7 mg, 4.7 μmol), dioxane (0.7 mL), 4-nitrophenylboronic acid pinacol ester (70 mg, 0.28 mmol), K_3PO_4 (0.2 mL); 22 h. Chromatographic eluent: CH_2Cl_2/hexanes (30%). **8a** (37 mg, 78%). Yellow oil. $[\alpha]_D^{20} = -189^\circ$ ($c = 1.2$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = -0.20$ (s, 3H; CH_3), 0.08 (s, 3H; CH_3), 0.19 (s, 3H; CH_3), 0.22 (s, 3H; CH_3), 0.73 (s, 9H; $C(CH_3)_3$), 0.95 (s, 9H; $C(CH_3)_3$), 2.46 (m, 2H; CH_2), 4.64 (m, 2H; 2×CH), 6.20 (d, J = 1.3 Hz, 1H; CH), 7.46 (br d, J = 9.0 Hz, 2H; ArH), 8.21 ppm (br d, J = 9.0 Hz, 2H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.0$, −4.5, −3.1, −3.0, 17.8, 18.0, 25.4 (3×), 25.6 (3×), 36.2, 67.1, 74.9, 75.7,

123.6 (2×), 128.0 (2×), 136.0, 137.7, 144.4, 147.5, 174.9 ppm; IR (film): $\tilde{\nu}=1798\ cm^{-1}$ (C=O); MS (ESI) *m/z*: 528 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{25}H_{39}O_6Si_2NNa$ [*M*+Na$^+$]: 528.2208, found: 528.2215.

*(1R,4R,5R)-1,4-Di(tert-butyldimethylsilyloxy)-3-(2-nitrophenyl)cyclohex-2-en-1,5-carbolactone (**8b**):* Procedure A. Vinyl triflate **7** (250 mg, 0.47 mmol), $[Pd_2(dba)_3]\cdot CHCl_3$ (36 mg, 35 μmol), CH_2Cl_2 (2 mL), 2-nitrobenzeneboronic acid (197 mg, 1.18 mmol), THF (7.9 mL), triethylamine (0.2 mL, 1.41 mmol). After 1 h, a solution of 2-nitrobenzeneboronic acid (119 mg, 0.71 mmol) in dry THF (3.1 mL) was added. The resultant mixture was heated at 80 °C for 24 h. Chromatographic eluent: CH_2Cl_2/hexanes [1°) 30%, 2°) 40%]. **8b** (199 mg, 84%). Yellow oil. $[\alpha]_D^{20}=-171°$ ($c=1.2$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=-0.52$ (s, 3H; CH_3), −0.09 (s, 3H; CH_3), 0.17 (s, 3H; CH_3), 0.19 (s, 3H; CH_3), 0.75 (s, 9H; $C(CH_3)_3$), 0.91 (s, 9H; $C(CH_3)_3$), 2.43 (ddd, $J=10.8$ Hz, $J'=5.5$ Hz, $J''=1.8$ Hz, 1H; C*H*H), 2.53 (d, $J=10.8$ Hz, 1H; CH*H*), 4.41 (d, $J=3.5$ Hz, 1H; CH), 4.56 (dd, $J=5.5$ Hz, $J'=3.5$ Hz, 1H; CH), 5.89 (d, $J=1.8$ Hz, 1H; CH), 7.32 (dd, $J=7.5$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.49 (dt, $J=9.0$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.60 (dt, =9.0 Hz, $J'=1.3$ Hz, 1H; ArH), 8.02 ppm (dd, $J=8.3$ Hz, $J'=1.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=-5.8$, −5.3, −3.1 (2×), 17.7, 18.0, 25.5 (3×), 25.6 (3×), 36.9, 68.0, 74.8, 75.9, 124.4, 129.0, 132.7, 132.8, 133.1, 134.4, 136.7, 148.0, 174.8 ppm; IR (film): $\tilde{\nu}=1802\ cm^{-1}$ (C=O); MS (ESI) *m/z*: 506 [*M*+H$^+$]; HRMS (ESI) *m/z*: calcd for $C_{25}H_{40}O_6Si_2N$ [*M*+H$^+$]: 506.2389, found 506.2381.

*(1R,4R,5R)-1,4-Di(tert-butyldimethylsilyloxy)-3-(naphth-2-yl)cyclohex-2-en-1,5-carbolactone (**8c**):* Procedure A. Vinyl triflate **7** (50 mg, 0.09 mmol), $[Pd_2(dba)_3]\cdot CHCl_3$ (7.3 mg, 7.0 μmol), CH_2Cl_2 (0.4 mL), 2-naphthyl boronic acid (33 mg, 0.19 mmol), THF (1.3 mL), triethylamine (40 μL, 0.29 mmol). After 1 h, a solution of 2-naftaleneboronic acid (17 mg, 0.09 mmol) in dry THF (0.4 mL) was added; 16 h. Chromatographic eluent: CH_2Cl_2/hexanes [1°) 20%, 2°) 30%]. **8c** (47 mg, 98%). Yellow oil. $[\alpha]_D^{20}=-148°$ ($c=1.1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=-0.27$ (s, 3H; CH_3), 0.09 (s, 3H; CH_3), 0.23 (s, 3H; CH_3), 0.25 (s, 3H; CH_3), 0.73 (s, 9H; $C(CH_3)_3$), 0.98 (s, 9H; $C(CH_3)_3$), 2.45 (ddd, $J=10.5$ Hz, $J'=5.6$ Hz, $J''=1.8$ Hz, 1H; C*H*H), 2.56 (d, $J=10.5$ Hz, 1H; CH*H*), 4.67 (dd, $J=3.5$ Hz, $J'=5.6$ Hz, 1H; CH), 4.77 (d, $J=3.5$ Hz, 1H; CH), 6.21 (d, $J=1.8$ Hz, 1H; CH), 7.45 (m, 3H; 3×ArH), 7.79 ppm (m, 4H; 4×ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=-4.9$, −4.6, −3.0, −2.9, 17.8, 18.1, 25.5 (3×), 25.6 (3×), 36.6, 67.3, 75.0, 76.0, 124.9, 126.1, 126.1, 126.3, 127.6, 127.9, 128.0, 132.9, 133.0, 133.4, 135.1, 139.1, 175.6 ppm; IR (film): $\tilde{\nu}=1802\ cm^{-1}$ (C=O); MS (ESI) *m/z*: 533 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{29}H_{42}O_4Si_2Na$ [*M*+Na$^+$]: 533.2514, found 533.2519.

General procedure of TBS deprotection: Tetrabutylammonium fluoride (TBAF, 2.2–2.6 equiv, ≈1.0 M in THF) was added to a stirred solution of the silylether **8** (1 equiv) in dry THF (75 mM) under argon at 0 °C. After stirring for 30 min, dilute HCl was added, and the organic layer was extracted with ethyl acetate (3×). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude reaction was purified by flash chromatography.

*(1R,4R,5R)-1,4-Dihydroxy-3-(4-nitrophenyl)cyclohex-2-en-1,5-carbolactone (**9a**):* Silylether **8a** (141 mg, 0.28 mmol), tetrabutylammonium fluoride (0.73 mL, 0.73 mmol), and THF (4 mL). Chromatographic eluent: ethyl acetate/hexanes (60%). **9a** (71 mg, 93%). Beige amorphous solid; mp: 161–165 °C; $[\alpha]_D^{20}=-259°$ ($c=1.1$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.42$ (m, 2H; CH_2), 4.71 (d, $J=3.3$ Hz, 1H; CH), 4.75 (dd, $J=5.9$ Hz, $J'=3.3$ Hz, 1H; CH), 6.53 (s, 1H; CH), 7.74 (d, $J=9.0$ Hz, 2H; 2×ArH), 8.17 ppm (d, $J=9.0$ Hz, 2H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=36.7$, 66.3, 74.5, 77.9, 124.7 (2×), 128.5 (2×), 135.6, 138.2, 144.8, 148.8, 178.0 ppm; IR (KBr): $\tilde{\nu}=3508$ (O–H), 3479 (O–H), 1779 cm^{-1} (C=O); MS (ESI) *m/z*: 300 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{13}H_{11}O_6NNa$ [*M*+Na$^+$]: 300.0479, found 300.0481.

*(1R,4R,5R)-1,4-dihydroxy-3-(2-nitrophenyl)cyclohex-2-en-1,3-carbolactone (**9b**):* Silylether **8b** (198 mg, 0.39 mmol), tetrabutylammonium fluoride (1 mL, 1.01 mmol), and THF (5.6 mL). Chromatographic eluent: ethyl acetate/hexanes (60%). **9b** (105 mg, 97%). Yellow oil. $[\alpha]_D^{20}=-167°$ ($c=1.0$ in CH_3OH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.46$ (m, 2H; CH_2), 4.35 (d, $J=3.3$ Hz, 1H; CH), 4.72 (ddd, $J=4.8$ Hz, $J'=3.3$ Hz, $J''=1.3$ Hz, 1H; CH), 5.91 (d, $J=1.3$ Hz, 1H; CH), 7.48 (dd, $J=7.5$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.56 (dt, $J=8.8$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.69 (dt, $J=8.8$ Hz, $J'=1.3$ Hz, 1H; ArH), 7.99 ppm (dd, $J=8.3$ Hz, $J'=1.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=37.2$, 67.9, 74.2, 78.0, 125.3, 130.3, 133.7, 134.0, 134.3, 134.3, 138.9, 149.7, 178.2 ppm; IR (film): $\tilde{\nu}=3426$ (O–H), 1779 cm^{-1} (C=O); MS (ESI) *m/z*: 300 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{13}H_{11}O_6NNa$ [*M*+Na$^+$]: 300.0479, found 300.0477.

*(1R,4R,5R)-1,4-Dihydroxy-3-(naphth-2-yl)cyclohex-2-en-1,5-carbolactone (**9c**):* Silylether **8c** (210 mg, 0.41 mmol), tetrabutylammonium fluoride (1.1 mL, 1.07 mmol), and THF (5.9 mL). Chromatographic eluent: ethyl acetate/hexanes (60%). **9c** (111 mg, 95%). Beige amorphous solid; mp: 175–176 °C; $[\alpha]_D^{20}=-231°$ ($c=1.2$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.74$ (m, 2H; CH_2), 5.09 (m, 2H; 2×CH), 6.76 (s, 1H; CH), 7.70 (m, 2H, 2×ArH), 7.88 (d, $J=8.5$ Hz, 1H; ArH), 8.06 (m, 3H; 3×ArH), 8.26 ppm (s, 1H; ArH); ^{1}H NMR (63 MHz, CD_3OD, 25 °C): $\delta=37.0$, 66.4, 74.5, 78.1, 124.9, 126.6, 127.4 (2×), 128.6, 129.1, 129.4, 132.3, 134.6, 134.8, 135.3, 139.5, 178.6 ppm; IR (KBr): $\tilde{\nu}=3432$ (O–H), 3305 (O–H), 1754 cm^{-1} (C=O); MS (ESI) *m/z*: 305 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{17}H_{14}O_4Na$ [*M*+Na$^+$]: 305.0784, found 305.0795.

General procedure of lactone hydrolysis: A solution of the lactone **9** (1 equiv, 0.1 M) in THF and aqueous lithium hydroxide (2.5 equiv, 0.5 M) was stirred at room temperature for 30 min. Water was added, and the THF was removed under reduced pressure. The resultant aqueous solution was washed with diethyl ether (2×). The aqueous extract was treated with Amberlite IR-120 until pH 6 was reached. The resin was filtered and washed with water. The filtrate and the washings were lyophilised.

*(1R,4R,5R)-1,4,5-Trihydroxy-3-(4-nitrophenyl)cyclohex-2-en-1-carboxylic acid (**5a**):* Lactone **9a** (71 mg, 0.26 mmol), THF (2.4 mL) and LiOH (aq, 1.3 mL). **5a** (76 mg, 99%). Orange amorphous solid; mp: 110–115 °C; $[\alpha]_D^{20}=-116°$ ($c=1.0$, in MeOH); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.18$ (m, 2H; CH_2), 4.02 (m, 1H; CH), 4.57 (dd, $J=7.5$ Hz, $J'=1.3$ Hz, 1H; CH), 5.96 (s, 1H; CH), 7.45 (d, $J=8.8$ Hz, 2H; 2×ArH), 8.06 ppm (d, $J=8.8$ Hz, 2H; 2×ArH); ^{13}C NMR (63 MHz, D_2O, 25 °C): $\delta=38.3$, 69.6, 71.8, 73.0, 123.7 (2×), 128.2 (3×), 142.7, 145.2, 147.0, 177.5 ppm; IR (KBr): $\tilde{\nu}=3389$ (O–H), 1727 cm^{-1} (C=O); MS (ESI) *m/z*=318 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{13}H_{13}O_7NNa$ [*M*+Na$^+$]: 318.0584, found 318.0577; Anal. for $C_{13}H_{13}O_7N\cdot 1/2\,H_2O$: calcd C 51.32, H 4.64, N 4.60; found C 51.54, H 4.48, N 4.24.

*(1R,4R,5R)-1,3,4-Trihydroxy-3-(2-nitrophenyl)cyclohex-2-en-1-carboxylic acid (**5b**):* Lactone **9b** (101 mg, 0.36 mmol), THF (3.3 mL) and LiOH (aq, 1.8 mL). **5b** (105 mg, 99%). Orange amorphous solid. $[\alpha]_D^{20}=+7.7°$ ($c=1.1$, in H_2O); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.21$ (m, 2H; CH_2), 4.00 (ddd, $J=8.3$ Hz, $J'=12.6$ Hz, $J''=4.5$ Hz, 1H; CH), 4.36 (dd, $J=8.3$ Hz, $J'=1.8$ Hz, 1H; CH), 5.61 (s, 1H; CH), 7.35 (d, $J=7.3$ Hz, 1H; ArH), 7.49 (t, $J=7.3$ Hz, 1H; ArH), 7.64 (t, $J=7.3$ Hz, 1H; ArH), 7.94 ppm (d, 1H, $J=8.0$; ArH); ^{13}C NMR (63 MHz, D_2O, 25 °C): $\delta=39.3$, 69.3, 73.4, 73.6, 124.7, 126.6, 129.5, 132.3, 133.4,

134.2, 143.1, 148.2, 177.6 ppm; IR (KBr): $\tilde{\nu}=3399$ (O–H), 1726 cm^{-1} (C=O); MS (ESI) *m/z*: 318 [*M*+Na^+]; HRMS (ESI) *m/z*: calcd for $C_{13}H_{13}O_7NNa$ [*M*+Na^+]: 318.0584, found 318.0599.

*(1R,4R,5R)-1,4,5-Trihydroxy-3-(naphth-2-yl)cyclohex-2-en-1-carboxylic acid (**6**):* Lactone **9c** (102 mg, 0.36 mmol), THF (3.3 mL) and LiOH (aq, 1.8 mL). **6** (95 mg, 89%). White amorphous solid; mp: 138–139 °C; $[\alpha]_D^{20}=-30°$ ($c=1.1$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD, 25 °C): $\delta=2.18$ (m, 2H; CH_2), 4.05 (m, 1H; CH), 4.35 (d, $J=6.3$ Hz, 1H; CH), 5.98 (s, 1H; CH), 7.36 (m, 2H; 2×ArH), 7.51 (dd, $J=8.4$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.74 (m, 3H; 3×ArH), 7.85 ppm (s, 1H; ArH); ^{13}C NMR (75 MHz, CD_3OD, 25 °C): $\delta=39.4$, 71.4, 73.2, 74.0, 126.4, 127.0, 127.1 (2×), 128.2, 128.5, 128.6, 129.2, 134.4, 134.8, 137.8, 144.3, 178.2 ppm; IR (KBr): $\tilde{\nu}=3418$ (O–H), 3365 (O–H), 1716 cm^{-1} (C=O); MS (CI) *m/z*: 283 [*M*+H^+-H_2O]; HRMS (CI) *m/z*: calcd for $C_{17}H_{15}O_4$ [*M*+H^+-H_2O]: 283.0965, found 283.0960; Anal. for $C_{17}H_{16}O_5\cdot 3/4H_2O$: calcd C 65.06, H 5.62; found C 65.43, H 5.32.

*1-(2,2-Dimethoxyethoxy)-3-bromobenzene (**16**):* 3-Bromophenol (**15**) (10 g, 57.80 mmol) was added to a suspension of NaH (3.47 g, 86.70 mmol, ≈60% in mineral oil) in anhydrous DMF (45.0 mL) at 0 °C. After hydrogen evolution had ceased, crown ether 15-crown-5 (1.0 mL, 5.03 mmol) and bromoacetaldehyde dimethyl acetal (12.3 mL, 0.10 mol) were added. The reaction mixture was heated at 130 °C for 16 h. The mixture was allowed to warm to room temperature, and water and diethyl ether were added. The organic layer was separated, and the aqueous layer was extracted twice with diethyl ether. All the combined organic layers were dried (Na_2SO_4), filtered, and evaporated. The residue was purified by flash chromatography, eluting with 10% diethyl ether/hexanes to afford acetal **15** (14 g, 93%) as a colourless oil. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=3.44$ (s, 6H; 2×CH_3), 3.98 (d, $J=5$ Hz, 2H; CH_2), 4.69 (m, 1H; CH), 6.85 (m, 1H; ArH), 7.17–7.08 ppm (m, 3H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=54.1$ (2×), 67.7, 101.9, 113.5, 117.9, 122.7, 124.2, 130.5, 159.2 ppm; MS (CI) *m/z*: 261, 263 [*M*+H^+]; HRMS (CI) *m/z*: calcd for $C_{10}H_{14}O_3{}^{79}Br$ [*M*+H^+]: 261.0126, found 261.0130.

*Cyclization of acetal **16**:* Polyphosphoric acid (8.6 g, 38.30 mmol) was added to a solution of acetal **16** (10 g, 38.30 mmol) in toluene (96.0 mL). The reaction mixture was stirred vigorously while being heated at reflux for 3 h. The reaction mixture was cooled to room temperature and decanted from the polyphosphoric acid. The solvent was evaporated, and the crude mixture was purified by flash chromatography, eluting with 100% hexanes to afford a mixture of 6-bromobenzofuran (**17a**) and 4-bromobenzofuran (**17b**) (3.5 g, 52%) as a yellow oils. A small amount of the mixture was separated and characterised.

*6-bromobenzofuran (**17a**):* ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=6.75$ (dd, $J=2.0$ Hz, $J'=0.9$ Hz, 1H; ArH), 7.36 (dd, $J=8.3$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.46 (d, $J=8.3$ Hz, 1H; ArH), 7.60 (d, $J=2.0$ Hz, 1H; ArH), 7.69 ppm (br s, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=106.4$, 114.8, 117.5, 122.0, 126.1, 126.4, 145.4, 155.1 ppm; MS (CI) *m/z*: 196, 198 [*M*+H^+]; HRMS (CI) *m/z*: calcd for $C_8H_6O^{81}Br$ [*M*+H^+]: 198.9582, found 198.9580.

*4-bromobenzofuran (**17b**):* ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=6.82$ (dd, $J=2.3$ Hz, $J'=0.8$ Hz, 1H; ArH), 7.17 (t, $J=8.0$ Hz, 1H; ArH), 7.40 (d, $J=7.5$ Hz, 1H; ArH), 7.46 (br d, $J=8.3$ Hz, 1H; ArH), 7.67 (d, $J=2.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=106.8$, 110.5, 114.2, 125.1, 125.7, 129.0, 145.3, 154.6 ppm; MS (CI) *m/z*: 196, 198 [*M*+H^+]; HRMS (CI) *m/z*: calcd for $C_8H_6O^{81}Br$ [*M*+H^+]: 198.9582, found 198.9585.

*2-(benzofuran-4-yl)- (**18**) and 2-(benzofuran-6-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**19**):* *n*BuLi (2.6 mL, 2.22 M) at −78 °C under inert atmosphere was added to a solution of bromobenzofurans **17** (1.0 g, 5.08 mmol) in dry diethyl ether (101.0 mL). The reaction was kept at −78 °C for 1 h, slowly warmed to −30 °C, and then cooled to −78 °C again. $B(OMe)_3$ (1.1 mL, 10.16 mmol) was added at −78 °C, and the reaction was stirred overnight and warmed to room temperature gradually. Aqueous hydrochloric acid was added, the organic layer was separated, and the aqueous layer was extracted twice with diethyl ether. All the combined organic layers were dried (Na_2SO_4), filtered, and evaporated. Pinacol (0.6 g, 5.08 mmol) was added to the residue of boronic acids in dry dichloromethane (51.0 mL), under inert atmosphere and heated at reflux for 4 h. After removal of the solvent, the crude product was purified by flash chromatography eluting with dichloromethane/hexanes [1) 10%, 2) 15%] to afford 310 mg of 4-benzofuranyl boronic acid pinacol ester **18** (25%) and 300 mg of 6-benzofuranyl boronic acid pinacol ester **19** (24%), both as a beige solids.

18: mp: 63–65 °C; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=1.23$ (s, 12H; 4×CH_3), 7.08 (m, 1H; ArH), 7.15 (t, $J=7.5$ Hz, 1H; ArH), 7.44 (dd, $J=8.3$ Hz, $J'=0.8$ Hz, 1H; ArH), 7.51 (d, $J=2.0$ Hz, 1H; ArH), 7.57 ppm (d, $J=7.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=24.9$ (4×), 83.6 (2×), 108.4, 114.0, 123.6, 130.1, 132.6, 145.2 (×2), 154.2 ppm; MS (ESI) *m/z*: 245 [*M*+H^+]; HRMS (ESI) *m/z*: calcd for $C_{14}H_{18}O_3B$ [*M*+H^+]: 245.1349, found 245.1346.

19: mp: 53–55 °C; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=1.37$ (s, 12H; 4×CH_3), 6.78 (dd, $J=2.3$ Hz, $J'=1.0$ Hz, 1H; ArH), 7.61 (dd, $J=7.8$ Hz, $J'=0.8$ Hz, 1H; ArH), 7.67 (d, $J=2.3$ Hz, 1H; ArH), 7.70 (dd, $J=7.8$ Hz, $J'=0.8$ Hz, 1H; ArH), 7.98 ppm (d, $J=0.8$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=24.8$ (4×), 83.8 (2×), 106.6, 117.6, 120.6, 128.7, 130.2, 146.0 (×2), 154.7 ppm; MS (ESI) *m/z*: 245 [*M*+H^+]; HRMS (ESI) *m/z*: calcd for $C_{14}H_{18}O_3B$ [*M*+H^+]: 245.1349, found 245.1346.

*2-(Benzo[b]thiophene-6-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**21**):* A stirred solution of 6-bromobenzo[*b*]thiophene (**20**)[12] (1.0 g, 4.69 mmol), $[Pd(PPh_3)_2Cl_2]$ (99 mg, 0.14 mmol), PPh_3 (74 mg, 0.28 mmol), bispinacolato diboron (1.3 g, 5.16 mmol), and anhydrous K_2CO_3 (973 mg, 7.04 mmol) in dry dioxane (28 mL) was heated at 80 °C for 20 h. After cooling to room temperature the solvent was removed, and the crude residue was redissolved in diethyl ether. The resulting suspension was filtered over Celite, and the residue was washed with diethyl ether. The filtrate and washings were evaporated under reduced pressure to yield a brown solid which was purified by flash chromatography eluting with 35% dichloromethane/hexanes to yield 6-benzothiophenyl boronic ester **21** (544 mg, 45%) as a yellow solid. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=1.37$ (s, 12H; 4×CH_3), 7.35 (dd, $J=5.5$ Hz, $J'=1.0$ Hz, 1H; ArH), 7.52 (d, $J=5.5$ Hz, 1H; ArH), 7.77 (dd, $J=8.0$ Hz, $J'=1.0$ Hz, 1H; ArH), 7.83 (dd, $J=8.0$ Hz, $J'=0.75$ Hz, 1H; ArH), 8.37 ppm (br d, $J=0.75$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=24.9$ (4×), 83.9 (2×), 122.9, 123.8, 128.2, 129.5, 129.7, 134.7, 139.3, 141.7 ppm; MS (CI) *m/z*: 501 [*M*+H^+].

*1H-indol-6-yl boronic acid (**23**):* A solution of 6-bromo-1*H*-indole (**22**) (880 mg, 4.49 mmol) in dry THF (9 mL) was added to a suspension of potassium hydride (600 mg, 4.49 mmol, ≈30% suspension in mineral oil) in dry THF (9 mL) at 0 °C. After 15 min the resultant mixture was cooled to −78 °C, and *t*BuLi (5.3 mL, 1.7 M in pentane), also at −78 °C, was added. A yellow precipitate was immediately formed, and after 10 min a solution of $B(OBu)_3$ (2.4 mL, 9.0 mmol) in dry THF (2.3 mL) was added. The reaction mixture was allowed to slowly warm to room temperature, and the suspension was

poured into ice-cold phosphoric acid (34 mL, 1 M). The organic phase was separated, and the aqueous layer was extracted twice with diethyl ether. The organic extracts were extracted with NaOH (1 M, 3×) and all the combined aqueous layers were acidified with 1 M HCl and then extracted with diethyl ether (3×). All the combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to afford a beige amorphous solid which was recrystallised from water to afford 6-indolyl boronic acid **23** (402 mg, 56%) as a beige solid.

*(1R,4R,5R)-3-(Benzofuran-5-yl)-1,4-di(tert-butyldimethylsilyloxy)cyclohex-2-en-1,5-carbolactone (**8d**):* Procedure B. Vinyl triflate **7** (250 mg, 0.47 mmol), [$Pd(PPh_3)_4$] (16 mg, 14.1 μmol), dioxane (2.4 mL), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-benzofuran (173 mg, 0.71 mmol), K_3PO_4 (aq, 0.9 mL, 0.8 M). 3 h. Chromatographic eluent: CH_2Cl_2/hexanes [1) 30%, 2) 35%]. **8d** (152 mg, 64%). Pale-yellow oil. $[\alpha]_D^{20} = -146^\circ$ ($c = 1.8$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = -0.33$ (s, 3H; CH_3), 0.04 (s, 3H; CH_3), 0.21 (s, 3H; CH_3), 0.23 (s, 3H; CH_3), 0.72 (s, 9H; $C(CH_3)_3$), 0.96 (s, 9H; $C(CH_3)_3$), 2.42 (ddd, $J = 10.8$ Hz, $J' = 5.4$ Hz, $J'' = 1.8$ Hz, 1H; C*H*H), 2.51 (d, $J = 10.8$ Hz, 1H; CH*H*), 4.63 (m, 2H; 2×CH), 6.06 (d, $J = 1.8$ Hz, 1H; CH), 6.75 (dd, $J = 2.0$ Hz, $J' = 0.8$ Hz, 1H; ArH), 7.20 (dd, $J = 8.5$ Hz, $J' = 1.8$ Hz, 1H; ArH), 7.45 (d, $J = 8.5$ Hz, 1H; ArH), 7.50 (d, $J = 1.5$ Hz, 1H; ArH), 7.63 ppm (d, $J = 2.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.1$, −4.7, −3.0, −2.9, 17.8, 18.0, 25.5 (3×), 25.6 (3×), 36.6, 67.8, 74.9, 76.1, 106.5, 111.1, 119.9, 123.7, 127.4, 132.9, 133.0, 139.6, 145.6, 154.7, 175.7 ppm; IR (film): $\tilde{\nu} = 1804$ cm^{-1} (C=O); MS (ESI) $m/z = 523$ [M+Na^+]; HRMS (ESI) m/z: calcd for $C_{27}H_{40}O_5Si_2Na$ [M+Na^+]: 523.2306, found 523.2305.

*(1R,3R,4R)-3-(Benzo[b]thiophen-5-yl)-1,4-di(tert-butyldimethylsilyloxy)-cyclohex-2-en-1,5-carbolactone (**8e**):* Procedure B. Vinyl triflate **7** (50 mg, 0.09 mmol), [$Pd(PPh_3)_4$] (2.7 mg, 2.35 μmol), dioxane (0.5 mL), 2-(1-benzothiophen-5-yl)-4,4,5,5-tetramethyl)-1,3,2-dioxaborolane (27 mg, 0.10 mmol), K_3PO_4 (0.15 mL). After 1 h, 2-(1-benzothiophen-5-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (10 mg, 0.04 mmol) was added. 2.5 h. Chromatographic eluent: CH_2Cl_2/hexanes (35%). **8e** (19 mg, 39%). Colourless oil. $[\alpha]_D^{20} = -150^\circ$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = -0.44$ (s, 3H; CH_3), −0.08 (s, 3H; CH_3), 0.08 (s, 3H; CH_3), 0.09 (s, 3H; CH_3), 0.59 (s, 9H; $C(CH_3)_3$), 0.83 (s, 9H; $C(CH_3)_3$), 2.29 (ddd, $J = 10.6$ Hz, $J' = 5.5$ Hz, $J'' = 1.7$ Hz, 1H; CH), 2.39 (d, $J = 10.6$ Hz, 1H; CH), 4.50 (td, $J = 3.3$ Hz, $J' = 1.9$ Hz, 1H; CH), 4.55 (d, $J = 3.3$ Hz, 1H; CH), 5.99 (d, $J = 1.7$ Hz, 1H; CH), 7.12 (dd, $J = 8.4$ Hz, $J' = 1.7$ Hz, 1H; ArH), 7.16 (d, $J = 5.4$ Hz, 1H; ArH), 7.31 (d, $J = 5.4$ Hz, 1H; ArH), 7.59 (d, $J = 1.2$ Hz, 1H; ArH), 7.69 ppm (d, $J = 8.4$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.0$, −4.6, −3.0, −2.9, 17.8, 18.0, 25.5 (3×), 25.6 (3×), 36.5, 67.6, 74.9, 76.0, 122.1, 122.2, 123.4, 123.7, 127.1, 133.1, 134.2, 139.3 (2×), 139.5, 175.6 ppm; IR (film): $\tilde{\nu} = 1799$ cm^{-1} (C=O); MS (ESI) m/z: 539 [M+Na^+]; HRMS (ESI) m/z: calcd for $C_{27}H_{40}O_4SSi_2Na$ [M+Na^+]: 539.2083, found 539.2078.

*(1R,4R,5R)-3-(Benzofuran-6-yl)-1,4-di(tert-butyldimethylsilyloxy)cyclohex-2-en-1,5-carbolactone (**8f**):* Procedure B. Vinyl triflate **7** (100 mg, 0.19 mmol), [$Pd(PPh_3)_4$] (6 mg, 4.8 μmol), dioxane (1.0 mL), 6-benzofuranyl boronic acid pinacol ester (**19**) (71 mg, 0.29 mmol), K_3PO_4 (0.4 mL). 3 h. Chromatographic eluent: CH_2Cl_2/hexanes [1) 20%, 2) 25%, 3) 35%]. **8f** (46 mg, 49%). Yellow oil. $[\alpha]_D^{20} = -161^\circ$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = -0.28$ (s, 3H, CH_3), 0.07 (s, 3H, CH_3), 0.22 (s, 3H, CH_3), 0.23 (s, 3H, CH_3), 0.72 (s, 9H, $C(CH_3)_3$), 0.96 (s, 9H, $C(CH_3)_3$), 2.42 (ddd, $J = 10.8$ Hz, $J' = 5.5$ Hz, $J'' = 1.8$ Hz, 1H; CH), 2.52 (d, $J = 10.8$ Hz, 1H; CH), 4.63 (dd, $J = 5.5$ Hz, $J' = 3.3$ Hz, 1H; CH), 4.70 (d, $J = 3.3$ Hz, 1H; CH), 6.11 (d, $J = 1.8$ Hz, 1H; CH), 6.75 (dd, $J = 2.3$ Hz, $J' = 1.0$ Hz, 1H; ArH), 7.17 (dd, $J = 8.3$ Hz, $J' = 1.5$ Hz, 1H; ArH), 7.44 (s, 1H; ArH), 7.54 (d, $J = 8.3$ Hz, 1H; ArH), 7.63 ppm (d, $J = 2.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.0$, −4.6, −3.0, −2.9, 17.8, 18.0, 25.5 (3×), 25.6 (3×), 36.5, 67.5, 74.9, 76.0, 106.4, 110.1, 120.8, 122.0, 127.2, 133.0, 134.5, 139.4, 145.7, 154.9, 175.6 ppm; IR (film): $\tilde{\nu} = 1805$ cm^{-1} (C=O); MS (CI) m/z: 501 [M+H^+]; HRMS (CI) m/z: calcd for $C_{27}H_{41}O_5Si_2$ [M+H^+]: 501.2493, found 501.2490.

*(1R,4R,5R)-3-(Benzo[b]thiophen-6-yl)-1,4-di(tert-butyldimethylsilyloxy)-cyclohex-2-en-1,5-carbolactone (**8g**):* Procedure B. Vinyl triflate **7** (122 mg, 0.23 mmol), [$Pd(PPh_3)_4$] (7 mg, 5.7 μmol), dioxane (1.2 mL), 6-benzothiopheneboronic acid pinacol ester (**21**) (89 mg, 0.34 mmol), K_3PO_4 (0.4 mL). Room temperature. 11 h. Chromatographic eluent: CH_2Cl_2/hexanes (35%). **8g** (50 mg, 42%). Orange oil. $[\alpha]_D^{20} = -147^\circ$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = -0.28$ (s, 3H, CH_3), 0.07 (s, 3H, CH_3), 0.22 (s, 3H, CH_3), 0.24 (s, 3H, CH_3), 0.73 (s, 9H, $C(CH_3)_3$), 0.97 (s, 9H, $C(CH_3)_3$), 2.43 (ddd, $J = 10.6$ Hz, $J' = 5.7$ Hz, $J'' = 1.8$ Hz, 1H; C*H*H), 2.53 (d, $J = 10.6$ Hz, 1H; CH*H*), 4.64 (dd, $J = 3.4$ Hz, $J' = 5.7$ Hz, 1H; CH), 4.70 (d, $J = 3.4$ Hz, 1H; CH), 6.15 (d, $J = 1.8$ Hz, 1H; CH), 7.31 (m, 2H; 2×ArH), 7.45 (d, $J = 5.4$ Hz, 1H; ArH), 7.77 (d, $J = 8.3$ Hz, 1H; ArH), 7.80 ppm (m, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -4.7$, −4.3, −2.7, −2.7, 18.1, 18.3, 25.7 (×3), 25.9 (×3), 36.8, 67.8, 75.2, 76.2, 121.2, 123.6, 123.7, 123.9, 127.3, 133.5, 134.4, 139.4, 139.5, 140.0, 175.8 ppm; IR (film): $\tilde{\nu} = 1801$ cm^{-1} (C=O); MS (ESI) m/z: 517 [M+H^+]; HRMS (ESI) m/z: calcd for $C_{27}H_{41}O_4SSi_2$ [M+H^+]: 517.2259, found 517.2259.

*(1R,4R,5R)-1,4-Di(tert-butyldimethylsilyloxy)-3-(1H-indol-6-yl)cyclohex-2-en-1,5-carbolactone (**8h**):* Procedure A. Vinyl triflate **7** (270 mg, 0.51 mmol), [$Pd_2(dba)_3$]·$CHCl_3$ (39 mg, 38 μmol), CH_2Cl_2 (2.2 mL), 1*H*-indol-6-yl boronic acid (**23**) (205 mg, 1.28 mmol), THF (8.5 mL), triethylamine (217 μL, 1.55 mmol). 24 h. Chromatographic eluent: CH_2Cl_2/hexanes [1) 30%, 2) 40%]. **8h** (122 mg, 63%). Yellow oil. $[\alpha]_D^{20} = -161^\circ$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = -0.33$ (s, 3H), 0.05 (s, 3H; CH_3), 0.21 (s, 3H; CH_3), 0.22 (s, 3H; CH_3), 0.72 (s, 9H, $C(CH_3)_3$), 0.95 (s, 9H, $C(CH_3)_3$), 2.41 (ddd, $J = 10.5$ Hz, $J' = 5.6$ Hz, $J'' = 2.0$ Hz, 1H; C*H*H), 2.52 (d, $J = 10.5$ Hz, 1H; CH*H*), 4.63 (dd, $J = 5.6$ Hz, $J' = 3.5$ Hz, 1H; CH), 4.69 (d, $J = 3.5$ Hz, 1H; CH), 6.07 (d, $J = 2.0$ Hz, 1H; CH), 6.53 (m, 1H; ArH), 7.05 (dd, $J = 8.3$ Hz, $J' = 1.5$ Hz, 1H; ArH), 7.23 (dd, $J = 3.3$ Hz, $J' = 2.5$ Hz, 1H; ArH), 7.31 (m, 1H; ArH), 7.58 (d, $J = 8.3$ Hz, 1H; ArH), 8.19 ppm (br s, 1H; NH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.0$, −4.8, −3.0, −2.9, 17.8, 18.0, 25.5 (3×), 25.6 (3×), 36.6, 67.4, 75.0, 76.2, 102.3, 109.7, 119.0, 120.3, 125.1, 127.7, 131.7, 131.8, 135.7, 140.3, 176.1 ppm; IR (film): $\tilde{\nu} = 3396$ (O–H), 1792 cm^{-1} (C=O); MS (CI) m/z: 500 [M+H^+]; HRMS (CI) m/z: calcd for $C_{27}H_{42}O_4NSi_2$ [M+H^+]: 500.2652, found 500.2656.

*(1R,4R,5R)-3-(Benzo[b]thiophen-3-yl)-1,4-di(tert-butyldimethylsilyloxy)-cyclohex-2-en-1,5-carbolactone (**8j**):* Procedure A. Vinyl triflate **7** (50 mg, 0.09 mmol), [$Pd_2(dba)_3$]·$CHCl_3$ (7.3 mg, 7.0 μmol), CH_2Cl_2 (0.5 mL), 3-benzothiopheneboronic acid (34 mg, 0.19 mmol), THF (1.25 mL), triethylamine (40 μL, 0.29 mmol). 6 h. Chromatographic eluent: CH_2Cl_2/hexanes (35%). **8j** (47 mg, 97%). White amorphous solid; mp: 99–101 °C; $[\alpha]_D^{20} = -170^\circ$ ($c = 1.1$ in $CHCl_3$, 25 °C); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = -0.55$ (s, 3H; CH_3), −0.09 (s, 3H; CH_3), 0.21 (s, 3H; CH_3), 0.23 (s, 3H; CH_3), 0.71 (s, 9H, $C(CH_3)_3$), 0.94 (s, 9H, $C(CH_3)_3$), 2.48 (ddd, $J = 10.7$ Hz, $J' = 5.8$ Hz, $J'' = 1.8$ Hz, 1H; C*H*H), 2.60 (d, $J = 10.7$ Hz, 1H; CH*H*), 4.50 (d, $J = 3.3$ Hz, 1H; CH), 4.62 (dd, $J = 5.8$ Hz, $J' = 3.3$ Hz, 1H; CH), 6.16 (d, $J = 1.8$ Hz, 1H; CH), 7.26 (s, 1H; ArH), 7.39–7.31 (m, 2H; 2×ArH), 7.72–7.67 (m, 1H; ArH), 7.88–7.80 ppm (m, 1H; ArH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta = -5.6$, −5.2, −3.0, −3.0, 17.6, 18.0, 25.4 (3×), 25.6 (3×), 36.8, 68.4, 74.8, 76.0, 122.5, 122.7, 124.3 (2×), 124.5, 133.7, 134.1, 135.9, 138.4, 139.8, 175.7 ppm; IR (KBr): $\tilde{\nu} = 1800$ cm^{-1} (C=O); MS (ESI) m/z: 539

[M+Na^+]; HRMS (ESI) m/z: calcd for $C_{27}H_{40}O_4SSi_2Na$ [M+Na^+]: 539.2083, found 539.2100.

*(1R,4R,5R)-3-(Benzofuran-5-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**9d**):* Silylether **8d** (170 mg, 0.34 mmol), tetrabutylammonium fluoride (0.88 mL, 0.88 mmol), and THF (4.9 mL). Chromatographic eluent: ethyl acetate/hexanes (60%). **9d** (87 mg, 94%). White solid; mp: 155–158 °C; $[\alpha]_D^{20}=-236°$ ($c=1.1$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.38$ (m, 2H; CH_2), 4.58 (br s, 1H; OH), 4.69 (d, $J=3.3$ Hz, 1H; CH), 4.72 (m, 1H; CH), 6.25 (s, 1H; CH), 6.75 (d, $J=2.0$ Hz, 1H; ArH), 7.39 (m, 2H; 2×ArH), 7.66 (d, $J=2.3$ Hz, 1H; ArH), 7.70 ppm (t, $J=1.1$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=36.9$, 66.8, 74.4, 78.1, 107.8, 112.1, 120.3, 124.0, 129.1, 131.3, 133.2, 140.0, 147.1, 156.3, 178.8 ppm; IR (KBr): $\tilde{\nu}=3492$ (O–H), 3376 (O–H), 1764 cm^{-1} (C=O); MS (ESI) m/z: 295 [M+Na^+]; HRMS (ESI) m/z: calcd for $C_{15}H_{12}O_5Na$ [M+Na^+]: 295.0577, found 295.0572.

*(1R,4R,5R)-3-(Benzo[b]thiophen-5-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**9e**):* Silylether **8e** (109 mg, 0.21 mmol), tetrabutylammonium fluoride (0.5 mL, 0.46 mmol), and THF (3 mL). Chromatographic eluent: Et_2O/hexanes 1) 75%; 2) 100%. **9e** (42 mg, 70%). White amorphous solid; mp: 157–159 °C; $[\alpha]_D^{20}=-254°$ ($c=1.0$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.40$ (m, 2H; CH_2), 4.73 (m, 2H; CH_2), 6.35 (d, $J=1.1$ Hz, 1H; CH), 7.30 (dd, $J=5.5$ Hz, $J'=0.6$ Hz, 1H; ArH), 7.46 (dd, $J=1.7$ Hz, $J'=8.5$ Hz, 1H; ArH), 7.50 (d, $J=5.5$ Hz, 1H; ArH), 7.80 (d, $J=8.5$ Hz, 1H; ArH), 7.96 ppm (d, $J=1.7$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=37.0$, 66.7, 74.4, 78.1, 122.4, 123.4, 123.7, 125.2, 128.2, 131.8, 134.5, 139.8, 141.0, 141.4, 178.6 ppm; IR (KBr): $\tilde{\nu}=3484$ (O–H), 1754 cm^{-1} (C=O); MS (ESI) m/z: 289 [M+H^+]; HRMS (ESI) m/z: calcd for $C_{15}H_{13}O_4S$ [M+H^+]: 289.0529, found 289.0538.

*(1R,4R,5R)-3-(Benzofuran-6-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**9f**):* Silylether **8f** (119 mg, 0.24 mmol), tetrabutylammonium fluoride (0.6 mL, 0.62 mmol), and THF (3.4 mL). Chromatographic eluent: Chromatographic eluent: ethyl acetate/hexanes (60%). **9f** (57 mg, 88%). Yellow oil. $[\alpha]_D^{20}=-21°$ ($c=1.0$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.37$ (m, 2H), 4.69 (m, 2H; CH_2), 6.31 (d, $J=0.8$ Hz, 1H; CH), 6.74 (dd, $J=2.3$ Hz, $J'=1.0$ Hz, 1H; ArH), 7.36 (dd, $J=8.3$ Hz, $J'=1.8$ Hz, 1H; ArH), 7.50 (d, $J=8.3$ Hz, 1H; ArH), 7.63 (m, 1H; ArH), 7.68 ppm (d, $J=2.3$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=37.0$, 66.7, 74.4, 78.0, 107.6, 110.2, 122.1, 122.4, 129.0, 131.7, 134.8, 139.8, 147.5, 156.7, 178.6 ppm; IR (film): $\tilde{\nu}=3446$ (O–H), 1771 cm^{-1} (C=O); MS (CI) m/z: 273 [M+H^+]; HRMS (CI) m/z: calcd for $C_{15}H_{13}O_5$ [M+H^+]: 273.0763, found 273.0762.

*(1R,4R,5R)-3-(Benzo[b]thiophen-6-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**9g**):* Silylether **8g** (113 mg, 0.22 mmol), tetrabutylammonium fluoride (0.5 mL, 0.48 mmol), and THF (3.1 mL). Chromatographic eluent: diethyl ether/hexanes [1) 75%, 2) 100%]. **9g** (53 mg, 84%). White foam. $[\alpha]_D^{20}=-184°$ ($c=1.1$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.44$ (m, 2H; CH_2), 4.78 (m, 2H; 2× CH), 6.42 (d, $J=0.9$ Hz, 1H; CH), 7.33 (dd, $J=5.4$ Hz, $J'=0.6$ Hz, 1H; ArH), 7.54 (m, 2H; 2×ArH), 7.79 (d, $J=8.4$ Hz, 1H; ArH), 8.07 ppm (br s, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=37.0$, 66.6, 74.4, 78.1, 121.2, 123.7, 124.5, 124.7, 128.5, 131.9, 134.4, 139.6, 141.0, 141.5, 178.6 ppm; IR (film): $\tilde{\nu}=3417$ (O–H), 1791 cm^{-1} (C=O); MS (ESI) m/z: 311 [M+Na^+]; HRMS (ESI) m/z: calcd for $C_{15}H_{12}O_4SNa$ [M+Na^+]: 311.0349, found 311.0340.

*(1R,4R,5R)-1,4-Dihydroxy-3-(1H-indol-6-yl)cyclohex-2-en-1,5-carbolactone (**9h**):* Silylether **8h** (160 mg, 0.32 mmol), tetrabutylammonium fluoride (0.83 mL, 0.83 mmol), and THF (4.6 mL). Chromatographic eluent: ethyl acetate/hexanes 1) 60%; 2) 80%. **9h** (86 mg, 99%). Beige amorphous solid. $[\alpha]_D^{20}=-15°$ ($c=1.1$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.39$ (m, 2H; CH_2), 4.72 (m, 2H; 2× CH), 6.26 (d, $J=1.3$ Hz, 1H; CH), 6.37 (dd, $J=3.0$ Hz, $J'=0.8$ Hz, 1H; ArH), 7.18 (m, 2H; 2×ArH), 7.47 (d, $J=8.3$ Hz, 1H, ArH), 7.56 ppm (m, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=37.1$, 66.9, 74.4, 78.2, 102.4, 110.3, 118.7, 121.2, 126.8, 129.7, 129.9, 131.3, 137.8, 140.8, 178.9 ppm; IR (KBr): $\tilde{\nu}=3490$ (O–H), 3390 (O–H), 1754 cm^{-1} (C=O); MS (CI) m/z: 272 [M+H^+]; HRMS (CI) m/z: calcd for $C_{15}H_{14}O_4N$ [M+H^+]: 272.0923, found 272.0918.

*(1R,4R,5R)-3-(5-Benzo[c][1,2,5]oxadiazol-5-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**9i**):* Procedure A. Vinyl triflate **7** (300 mg, 0.56 mmol), $[Pd_2(dba)_3]\cdot CHCl_3$ (43 mg, 42 μmol), CH_2Cl_2 (2.4 mL), benzo[*c*][1,2,5]oxadiazol-5-yl-5-boronic acid (184 mg, 1.12 mmol), THF (7.5 mL), triethylamine (240 μL, 1.70 mmol). After 1 h a solution of benzo[*c*][1,2,5]oxadiazol-5-yl-5-boronic acid (134 mg, 0.84 mmol) in dry THF (4.2 mL) was added. 24 h. The crude residue was used in the deprotection step using tetrabutylammonium fluoride (0.9 mL, 0.88 mmol) and THF (4.9 mL). Chromatographic eluent: ethyl acetate/hexanes (60%). **9i** (83 mg, 54% overall). Beige amorphous solid; mp: 178–181 °C; $[\alpha]_D^{20}=-251°$ ($c=1.2$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.43$ (m, 2H; CH_2), 4.75 (m, 2H; 2×CH), 6.62 (s, 1H; CH), 7.69 (dd, $J=9.6$ Hz, $J'=1.4$ Hz, 1H; ArH), 7.81 (dd, $J=9.6$ Hz, $J'=0.9$, 1H; ArH), 8.00 ppm (m, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=36.9$, 66.2, 74.6, 77.8, 113.9, 117.2, 132.3, 136.2, 137.9, 141.3, 150.0, 150.9, 177.9 ppm; IR (KBr): $\tilde{\nu}=3350$ (O–H), 3257 (O–H), 1773 cm^{-1} (C=O); MS (CI) m/z: 275 [M+H^+]; HRMS (CI) m/z: calcd for $C_{13}H_{11}O_5N_2$ [M+H^+]: 275.0668, found 275.0664.

*(1R,4R,5R)-3-(Benzo[b]thiophen-3-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**9j**):* Silylether **8j** (125 mg, 0.24 mmol), tetrabutylammonium fluoride (0.53 mL, 0.53 mmol), and THF (3.5 mL). Chromatographic eluent: diethyl ether/hexanes (75%). **9j** (68 mg, 97%. White amorphous solid; mp: 171–173 °C; $[\alpha]_D^{20}=-138°$ ($c=1.1$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.44$ (ddd, $J=10.7$ Hz, $J'=5.1$ Hz, $J''=1.6$ Hz, 1H; C*H*H), 2.51 (d, $J=10.7$ Hz, 1H; CH*H*), 4.57 (d, $J=3.2$ Hz, 1H; CH), 4.73 (dd, $J=5.1$ Hz, $J'=3.2$ Hz, 1H; CH), 6.30 (d, $J=1.6$ Hz, 1H; CH), 7.36–7.25 (m, 2H; 2×ArH), 7.62 (s, 1H; ArH), 7.87–7.79 ppm (m, 2H; 2×ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=37.0$, 68.1, 74.3, 78.1, 123.7, 123.9, 125.5, 125.6, 126.0, 134.4 (2×), 135.3, 139.0, 141.8, 178.8 ppm; IR (KBr): $\tilde{\nu}=3436$ (O–H), 1752 cm^{-1} (C=O); MS (ESI) m/z: 289 [M+H^+]; HRMS (ESI) m/z: calcd for $C_{15}H_{13}O_4S$ [M+H^+]: 289.0529, found 289.0526.

*(1R,4R,5R)-3-(Benzofuran-5-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (**10a**):* Lactone **9d** (84 mg, 0.31 mmol), THF (2.8 mL) and LiOH (aq, 1.6 mL). **10a** (80 mg, 90%). Beige amorphous solid; mp: 96–100 °C; $[\alpha]_D^{20}=-65°$ ($c=1.0$ in MeOH); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta=2.19$ (dd, $J=10.2$ Hz, $J'=2.6$ Hz, 1H; C*H*H), 2.29 (dd, $J=10.2$ Hz, $J'=8.7$ Hz, 1H, CH*H*), 4.09 (m, 1H; CH), 4.61 (d, $J=5.4$ Hz, 1H; CH), 5.83 (s, 1H; CH), 6.83 (s, 1H; ArH), 7.26 (dd, $J=6.0$ Hz, $J'=1.2$ Hz, 1H; ArH), 7.46 (d, $J=6.0$ Hz, 1H; ArH), 7.55 (s, 1H; ArH), 7.72 ppm (d, $J=1.2$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, D_2O, 25 °C): $\delta=41.3$, 72.5, 75.1, 76.0, 109.7, 114.0, 122.9, 126.5, 128.1, 130.3, 135.8, 147.5, 149.1, 157.2, 180.6 ppm; IR (KBr): $\tilde{\nu}=3367$ (O–H), 1719 cm^{-1} (C=O); MS (ESI) m/z: 313 [M+Na^+]; HRMS (ESI) m/z: calcd for $C_{15}H_{14}O_6Na$ [M+Na^+]: 313.0683, found 313.0683; Anal. for $C_{15}H_{14}O_6\cdot 1/2\,H_2O$: calcd C 60.20, H 5.05; found C 60.19, H 4.68.

*(1R,4R,5R)-3-(Benzo[b]thiophen-5-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (**10b**):* Lactone **9e** (37 mg, 0.13 mmol), THF (1.2 mL) and LiOH (aq, 0.64 mL). **10b** (37 mg, 94%). White amorphous solid; mp: 122–124 °C; $[\alpha]_D^{20}=-85°$ ($c=1.1$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): $\delta=2.26$ (m, 2H; CH_2), 4.08 (m, 1H; CH), 4.53 (d, $J=$

5.2 Hz, 1H; CH), 5.98 (s, 1H; CH), 7.35 (m, 1H; ArH), 7.47 (m, 1H; ArH), 7.53 (d, $J=5.4$ Hz, 1H; ArH), 7.86 (m, 1H; ArH), 7.95 ppm (br s, 1H; ArH); ^{13}C NMR (100 MHz, CD_3OD, 25 °C): $\delta=38.2$, 71.6, 72.6, 74.2, 122.9 (2×), 124.6, 125.1, 127.8, 129.0, 137.1, 140.3, 141.2, 143.1, 186.7 ppm; IR (KBr): $\tilde{\nu}=3389$ (O–H), 1714 cm^{-1} (C=O); MS (ESI) *m/z*: 329 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{15}H_{14}O_5NaS$ [*M*+Na$^+$]: 329.0454, found 329.0455.

*(1R,4R,5R)-3-(Benzofuran-6-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (**11a**):* Lactone **9f** (51 mg, 0.19 mmol), THF (1.7 mL) and LiOH (aq, 1.0 mL). **11a** (55 mg, 99%). Beige amorphous solid; mp: 90–92 °C; ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.19$ (dd, $J=13.5$ Hz, $J'=3.5$ Hz, 1H; C*H*H), 2.28 (dd, $J=13.5$ Hz, $J'=11.0$ Hz, 1H; CH*H*), 4.10 (m, 1H; CH), 4.66 (dd, $J=7.4$ Hz, $J'=1.0$ Hz, 1H; CH), 5.96 (s, 1H; CH), 6.90 (d, $J=2.0$ Hz, 1H; ArH), 7.31 (dd, $J=8.0$ Hz, $J'=1.0$ Hz, 1H; ArH), 7.57 (s, 1H; ArH), 7.66 (d, $J=8.0$ Hz, 1H; ArH), 7.78 ppm (d, $J=2.0$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, D_2O, 25 °C): $\delta=41.2$, 72.6, 74.9, 76.0, 109.5, 112.9, 124.1, 125.1, 128.6, 130.2, 137.6, 147.2, 149.4, 157.6, 180.8 ppm; IR (KBr): $\tilde{\nu}=3379$ (O–H), 1717 cm^{-1} (C=O); MS (ESI) *m/z*: 313 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{15}H_{14}O_6Na$ [*M*+Na$^+$]: 313.0683, found 313.0675; Anal. for $C_{15}H_{14}O_6\cdot 1/4H_2O$: calcd C 61.12, H 4.96; found C 60.83, H 4.76.

*(1R,4R,5R)-5-(Benzo[b]thiophen-6-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (**11b**):* Lactone **9g** (50 mg, 0.17 mmol), THF (1.6 mL) and LiOH (aq, 0.9 mL). **11b** (46 mg, 87%). Beige amorphous solid; mp: 118–120 °C; $[\alpha]_D^{20}=-81°$ ($c=1.1$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD, 25 °C): $\delta=2.24$ (m, 2H; CH_2), 4.11 (m, 1H; CH), 4.54 (d, $J=6.7$ Hz, 1H; CH), 5.99 (s, 1H; CH), 7.33 (d, $J=5.4$ Hz, 1H; ArH), 7.46 (dd, $J=8.3$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.53 (d, $J=5.4$ Hz, 1H; ArH), 7.79 (d, $J=8.3$ Hz, 1H; ArH), 7.98 ppm (s, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=39.5$, 71.3, 73.4, 73.9, 122.0, 124.1, 124.7, 124.9, 127.8, 127.9, 136.7, 140.6, 141.2, 144.5, 178.1 ppm; IR (KBr): $\tilde{\nu}=3436$ (O–H), 1717 cm^{-1} (C=O); MS (ESI) *m/z*: 329 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{15}H_{14}O_5NaS$ [*M*+Na$^+$]: 329.0454, found 329.0458.

*Sodium (1R,4R,5R)-1,4,5-Trihydroxy-3-(1H-indol-6-yl)cyclohex-2-en-1-carboxylate (**11c**):* A solution of the lactone **9h** (11 mg, 0.04 mmol) in THF (0.4 mL) and aqueous sodium hydroxide (84 μL, 0.5 M) was stirred at room temperature for 30 min. Water was added, the THF was removed under reduced pressure, and the resultant aqueous solution was washed with diethyl ether (×2). The aqueous extract was lyophilised to afford sodium salt **11c** (13 mg, 99%) as a beige amorphous solid. $[\alpha]_D^{20}=-54°$ ($c=1.4$ in H_2O); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.14$ (m, 2H; CH_2), 4.06 (m, 1H; CH), 4.65 (dd, $J=6.9$ Hz, $J'=1.1$ Hz, 1H; CH), 5.85 (s, 1H; CH), 6.54 (d, $J=3.0$ Hz, 1H; ArH), 7.18 (dd, $J=8.5$ Hz, $J'=1.5$ Hz, 1H; ArH), 7.37 (d, $J=3.0$ Hz, 1H; ArH), 7.54 (s, 1H; ArH), 7.65 ppm (d, $J=8.5$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, D_2O, 25 °C): $\delta=38.2$, 70.3, 71.9, 74.1, 101.2, 110.0, 110.2, 118.9, 120.4, 126.7, 127.4, 132.5, 136.0, 142.9, 181.2 ppm; IR (KBr): $\tilde{\nu}=3401$ (O-H, N-H), 3128 (O–H), 1578 cm^{-1} (C=O); MS (ESI) *m/z*: 312 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{15}H_{15}O_5NNa$ [*M*+Na$^+$]: 312.0842, found 312.0842.

*(1R,4R,5R)-3-(Benzo[c][1,2,5]oxadiazol-6-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (**12**):* Lactone **9i** (76 mg, 0.28 mmol), THF (2.5 mL) and LiOH (aq, 1.4 mL). **12** (80 mg, 96%) as an orange amorphous solid; mp: 92–95 °C; ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.22$ (m, 2H; CH_2), 4.04 (m, 1H; CH), 4.60 (dd, $J=7.6$ Hz, $J'=1.5$ Hz, 1H; CH), 6.10 (s, 1H; CH), 7.47 (dd, $J=9.0$ Hz, $J=1.5$ Hz, 1H; ArH), 7.72 (s, 1H; ArH), 7.73 ppm (br d, $J=9.0$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, D_2O, 25 °C): $\delta=41.2$, 72.3, 74.6, 75.8, 116.0, 118.5, 131.2, 135.8, 145.5, 145.5, 151.5, 152.1, 180.2 ppm; IR (KBr): $\tilde{\nu}=3399$ (O–H), 1723 cm^{-1} (C=O); MS (ESI) *m/z*: 293 [*M*+H$^+$]; HRMS (ESI) *m/z*: calcd for $C_{13}H_{13}O_6N_2$ [*M*+H$^+$]: 293.0768, found 293.0757.

*(1R,4R,5R)-3-(Benzo[b]thiophen-3-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (**13**):* Lactone **9j** (69 mg, 0.24 mmol), THF (2.2 mL) and LiOH (aq, 1.2 mL). **13** (71 mg, 97%) as white amorphous solid; mp: 132–134 °C; $[\alpha]_D^{20}=-48°$ ($c=1.0$ in MeOH); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta=2.37$–2.18 (m, 2H; CH_2), 4.08 (m, 1H; CH), 4.51 (d, $J=6.2$ Hz, 1H; CH), 5.83 (s, 1H; CH), 7.13 (m, 2H; 2×ArH), 7.29 (s, 1H; ArH), 7.54 (d, $J=7.5$ Hz, 1H; ArH), 7.75 ppm (d, $J=7.1$ Hz, 1H; ArH); ^{13}C NMR (63 MHz, D_2O, 25 °C): $\delta=39.1$, 70.0, 73.6 (2×), 123.2, 123.4, 125.0 (2×), 125.7, 127.8, 134.1, 138.2, 139.4, 140.4, 178.3 ppm; IR (KBr): $\tilde{\nu}=3377$ (O–H), 1717 cm^{-1} (C=O); MS (ESI) *m/z*: 329 [*M*+Na$^+$]; HRMS (ESI) *m/z*: calcd for $C_{15}H_{14}O_5SNa$ [*M*+Na$^+$]: 329.0454, found 329.0450.

*(1R,4R,5R)-1,4-Di(tert-butyldimethylsilyloxy)-3-[2-(trimethylsilyl)ethynyl]cyclohex-2-en-1,5-carbolactone (**24**):* Trimethylsilylacetylene (0.24 mL, 1.67 mmol) and piperidine (0.49 mL, 4.94 mmol) were added to a suspension of vinyl triflate **7** (200 mg, 0.38 mmol), [Pd(PPh_3)$_4$] (66 mg, 57 μmol), and CuI (12 mg, 65 μmol) in dry THF (19 mL). The resultant reaction mixture was heated at 40 °C for 3 h. After the mixture was cooled to room temperature, saturated aqueous $NaHCO_3$ solution was added, and the organic layer was separated. The aqueous phase was extracted with Et_2O (2×). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The obtained residue was purified by flash chromatography, eluting with CH_2Cl_2/hexanes (25%) to give alkyne **24** (180 mg, 99%) as a beige amorphous solid; mp: 72–73 °C; $[\alpha]_D^{20}=-176°$ ($c=1.3$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta=0.14$ (s, 3H; CH_3), 0.15 (s, 3H; CH_3), 0.18 (s, 12H; 4×CH_3), 0.20 (s, 3H; CH_3), 0.91 (s, 9H; $C(CH_3)_3$), 0.92 (s, 9H; $C(CH_3)_3$), 2.32 (ddd, $J=11.0$ Hz, $J'=5.3$ Hz, $J''=1.3$ Hz, 1H; C*H*H), 2.39 (d, $J=11.0$ Hz, 1H; CH*H*), 4.14 (d, $J=3.3$ Hz, 1H; CH), 4.47 (m, 1H; CH), 6.30 ppm (d, $J=1.3$ Hz, 1H; CH); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C): $\delta=-4.8$, −4.4, −3.2 (2×), −0.3 (3×), 18.0, 18.0, 25.5 (3×), 25.7 (3×), 36.7, 68.0, 74.9, 75.7, 96.5, 102.3, 122.6, 142.3, 174.7 ppm; IR (KBr): $\tilde{\nu}=2148$ (C≡C), 1797 cm^{-1} (C=O); MS (ESI) *m/z*: 481 [*M*+H$^+$]; HRMS (ESI) *m/z*: calcd for $C_{24}H_{45}O_4Si_3$ [*M*+H$^+$]: 481.2620, found 481.2630.

*(1R,4R,5R)-3-Ethynyl-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**25**):* Silylether **24** (171 mg, 0.36 mmol), tetrabutylammonium fluoride (1.4 mL, 1.4 mmol), and THF (5.1 mL). Chromatographic eluent: ethyl acetate/hexanes (60%). **25** (60 mg, 92%) as a yellow oil. $[\alpha]_D^{20}=-13°$ ($c=1.0$ in CH_3OH); ^{1}H NMR (250 MHz, CD_3OD, 25 °C): $\delta=2.23$ (d, $J=11.0$ Hz, 1H; C*H*H), 2.31 (ddd, $J=11.0$ Hz, $J'=5.4$ Hz, $J''=1.8$ Hz, 1H; CH*H*), 3.40 (s, 1H; CH), 4.00 (d, $J=3.3$ Hz, 1H; CH), 4.57 (dd, $J=5.4$ Hz, $J'=3.3$ Hz, 1H; CH), 6.25 ppm (s, 1H; CH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta=36.7$, 68.3, 74.1, 77.4, 80.9, 81.7, 124.0, 142.4, 177.7 ppm; IR (film): $\tilde{\nu}=3399$ (O–H), 3284 (O–H), 1783 cm^{-1} (C=O); MS (CI) *m/z*: 181 [*M*+H$^+$]; HRMS (CI) *m/z*: calcd for $C_9H_9O_4$ [*M*+H$^+$]: 181.0501, found 181.0507.

*(1R,4R,5R)-3-(1-Benzyl-1H-1,2,3-triazol-4-yl)-1,4-dihydroxycyclohex-2-en-1,5-carbolactone (**26**):* A freshly prepared solution of aqueous sodium ascorbate (42 μL, 1.0 M) followed by aqueous copper(II) sulfate (13 μL, 0.32 M) were added to a suspension of alkyne **25** (75 mg, 0.42 mmol) and benzylazide (53 μL, 0.42 mmol) in a mixture of *t*BuOH/water (1:1, 1.8 mL). The resultant heterogeneous mixture was stirred vigorously over 16 h and then diluted with water and ethyl acetate. The organic layer was separated, and the aqueous phase was extracted twice with ethyl acetate. All the combined organic extracts were dried (Na_2SO_4), filtered, and evaporated. The obtained residue was purified by flash chromatography

eluting with ethyl acetate/hexanes [1°) 80%, 2°) 90%] to afford triazol **26** (115 mg, 88%) as a white amorphous solid; mp: 147–149 °C; $[\alpha]_D^{20} = -122°$ ($c = 1.2$ in CH_3OH); ^1H NMR (250 MHz, CD_3OD, 25 °C): $\delta = 2.39$ (m, 2H; CH_2), 4.42 (d, $J = 3.5$ Hz, 1H; CH), 4.67 (dd, $J = 6.8$ Hz, $J' = 3.5$ Hz, 1H; CH), 5.51 (s, 2H; CH_2), 6.67 (s, 1H; CH), 7.25 (m, 5H; 5×ArH), 7.99 ppm (s, 1H; ArH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 37.3$, 54.9, 66.7, 74.2, 77.8, 123.5, 129.1 (2×), 129.6, 130.0 (2×), 130.2, 131.9, 136.6, 145.8, 178.2 ppm; IR (film): $\tilde{\nu} = 3403$ (O–H), 1790 cm^{-1}(C=O); MS (CI) *m/z*: 314 [*M*+H$^+$]; HRMS (CI) *m/z*: calcd for $C_{16}H_{16}O_4N_3$ [*M*+H$^+$]: 314.1141, found 314.1133.

*(1R,4R,5R)-3-(1-Benzyl-1H-1,2,3-triazol-4-yl)-1,4,5-trihydroxycyclohex-2-en-1-carboxylic acid (**14**):* Lactone **26** (109 mg, 0.35 mmol), THF (3.2 mL), and LiOH (aq, 1.8 mL). **14** (111 mg, 97%). Beige amorphous solid; mp: 76–78 °C; $[\alpha]_D^{20} = -16°$ ($c = 0.5$ in CH_3OH); ^1H NMR (250 MHz, D_2O, 25 °C): $\delta = 2.10$ (m, 2H; CH_2), 3.97 (m, 1H; CH), 4.22 (d, $J = 3.5$ Hz, 1H; CH), 5.30 (s, 2H; CH_2), 6.24 (s, 1H; CH) 7.10 (m, 5H; 5×ArH), 7.87 ppm (s, 1H; CH); ^{13}C NMR (63 MHz, CD_3OD, 25 °C): $\delta = 38.0$, 54.1, 69.7, 71.4, 73.0, 124.1, 126.1, 128.2 (2×), 129.0, 129.3 (2×), 132.6, 134.9, 144.7, 177.8 ppm; IR (KBr): $\tilde{\nu} = 3379$ (O–H), 1719 cm^{-1} (C=O); MS (ESI) *m/z*: 332 [*M*+H$^+$]; HRMS (ESI) *m/z*: calcd for $C_{16}H_{18}O_5N_3$ [*M*+H$^+$]: 332.1241, found 332.1249; Anal. for $C_{16}H_{17}O_5N_3 \cdot 1/2\,H_2O$: calcd C 56.47, H 5.33; found C 56.46, H 5.09.

Acknowledgements

Financial support from the Xunta de Galicia (PGIDIT05RAG20901PR) and the Spanish Ministry of Education and Culture (CTQ2004-04238) is gratefully acknowledged. V.F.V.P. thanks Fundação para a Ciência e a Tecnologia for an FCT scholarship. C.S.S. thanks the Spanish Ministry of Education and Culture for an FPU scholarship.

Keywords: antibiotics · competitive inhibitors · dehydroquinase · enzymes · tuberculosis

[1] E. Haslam, *The Shikimate Pathway*; Wiley, New York, **1974**.
[2] a) F. Roberts, C. W. Roberts, J. J. Johnson, D. E. Kyle, T. Krell, J. R. Coggins, G. H. Coombs, W. K. Milhous, S. Tzipori, D. J. P. Ferguson, D. Chakrabarti, R. McLeod, *Nature* **1998**, *393*, 801–805; b) P. J. Keeling, J. D. Palmer, R. G. Donald, D. S. Roos, R. F. Waller, G. I. McFadden, *Nature* **1999**, *397*, 219–220; c) S. A. Campbell, T. A. Richards, E. J. Mui, B. U. Samuel, J. R. Coggins, R. McLeod, C. W. Roberts, *Int. J. Parasitol.* **2004**, *34*, 5–13; d) C. W. Roberts, F. Roberts, R. E. Lyons, M. J. Kirisits, E. J. Mui, J. Finnerty, J. J. Johnson, D. J. P. Ferguson, J. R. Coggins, T. Krell, G. H. Coombs, W. K. Milhous, D. E. Kyle, S. Tzipori, J. Barnwell, J. B. Dame, J. Carlton, R. McLeod, *J. Infect. Dis.* **2002**, *185* (suppl. 1), S25–S36; e) G. A. McConkey, J. W. Pinney, D. R. Westhead, K. Plueckhahn, T. B. Fitzpatrick, P. Macheroux, B. Kappes, *Trends Parasitol.* **2004**, *20*, 60–65.
[3] C. Sánchez-Sixto, V. F. V. Prazeres, L. Castedo, H. Hamb, A. R. Hawkins, C. González-Bello, *J. Med. Chem.* **2005**, *48*, 4871–4881.
[4] V. F. V. Prazeres, C. Sánchez-Sixto, L. Castedo, A. Canales, F. J. Cañada, J. Jiménez-Barbero, H. Hamb, A. R. Hawkins, C. González-Bello, *ChemMedChem* **2006**, *1*, 990–999.
[5] M. Frederickson, E. J. Parker, A. R. Hawkins, J. R. Coggins, C. Abell, *J. Org. Chem.* **1999**, *64*, 2612–2613.
[6] a) G. Jones, P. Willett, R. C. Glen, *J. Mol. Biol.* **1995**, *245*, 43–53; b) G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, *267*, 727–748.
[7] http://www.ccdc.cam.ac.uk/products/life_sciences/gold/.
[8] D. G Gourley, A. K. Shrive, I. Polikarpov, T. Krell, J. R. Coggins, A. R. Hawkins, N. W. Isaacs, L. Sawyer, *Nat. Struct. Biol.* **1999**, *6*, 521–525.
[9] C. González-Bello, E. Lence, M. D. Toscano, L. Castedo, J. R. Coggins, C. Abell, *J. Med. Chem.* **2003**, *46*, 5735–5744.
[10] K. Takeuchi, T. J. Kohn, D. J. Sall, M. L. Denney, J. R. McCowan, G. F. Smith, D. S. Gifford-Moore, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 759–764.
[11] M. Prieto, E. Zurita, E. Rosa, L. Muñoz, P. Lloyd-Williams, E. Giralt, *J. Org. Chem.* **2004**, *69*, 6812–6820.
[12] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
[13] H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128–1137.
[14] C. A. Hunter, J. K. M. Sanders, *J. Am. Chem. Soc.* **1990**, *112*, 5525–5534.
[15] S. M. Malathy, M. N. Ponnuswamy, *Cryst. Growth Des.* **2006**, *6*, 736–742.
[16] C. A. Hunter, K. R. Lawson, J. Perkins, C. J. Urch, *J. Chem. Soc. Perkin Trans. 2* **2001**, 651–669.
[17] X-ray crystal structure (PDB ID: 1H0S) of type II dehydroquinase from *M. tuberculosis* complexed with 3-hydroxyiminoquinic acid (1.7 Å resolution) by A. W. Roszak, M. Frederickson, C. Abell, J. R. Coggins, A. J. Lapthorn, *submitted*. Residues 20–25 are not visible including essential Tyr 24.
[18] X-ray crystal structure (PDB ID: 1H0R) of type II dehydroquinase from *M. tuberculosis* complexed with 2,3-anhydroquinic acid (2.1 Å resolution) by A. W. Roszak, D. A. Robinson, M. Frederickson, C. Abell, J. R. Coggins, A. J. Lapthorn, *submitted*. Residues 19–22 are not visible including essential Arg 19.
[19] These studies are being carried out in collaboration with Professor José Manuel Gaspar Martinho from Universidade Técnica de Lisboa, Lisboa, Portugal.
[20] D. G. Gourley, J. R. Coggins, N. W. Isaacs, J. D. Moore, I. G. Charles, A. R. Hawkins, *J. Mol. Biol.* **1994**, *241*, 488–491.
[21] P. J. White, J. Young, I. S. Hunter, H. G. Nimmo, J. R. Coggins, *Biochem. J.* **1990**, *265*, 735–738.
[22] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. González, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle, J. A. Pople, *Gaussian 98W (Revision A.9)*, *Gaussian, Inc.*, *Pittsburgh PA (USA)*, **1998**.

Received: August 29, 2006
Published online on January 24, 2007

