Article

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Molecular hybridization of potent and selective γ-Hydroxybutyric Acid (GHB) ligands; Design, synthesis, binding studies, and molecular modelling of novel 3-hydroxycyclopent-1-enecarboxylic Acid (HOCPCA) and *trans*-γ-hydroxycrotonic acid (T-HCA) Analogs

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Keywords γ-hydroxybutyric acid (GHB), 3-hydroxycyclopent-1-enecarboxylic acid (HOCPCA), *trans*-4-hydroxycrotonic acid (T-HCA), high-affinity GHB binding site, structure-affinity studies, pharmacophore model

Abstract γ-hydroxybutyric acid (GHB) is a neuroactive substance with specific high-affinity binding sites. To facilitate target identification and ligand optimization, we herein report a comprehensive structure-affinity relationship study for novel ligands targeting these binding sites. A molecular hybridization strategy was used based on the conformationally restricted 3-hydroxycyclopent-1-enecarboxylic acid (HOCPCA) and the linear GHB analog, *trans*-4-hydroxycrotonic acid (T-HCA). In general, all structural modifications performed on HOCPCA led to reduced affinity. In contrast, introduction of diaromatic substituents into the 4-position of T-HCA led to high-affinity analogs (medium nanomolar K_i) for the GHB high-affinity binding sites as the most high-affinity analogs reported to date. The SAR data formed the basis for a 3-dimensional pharmacophore model for GHB ligands, which identified molecular features important for high-affinity binding, with high predictive validity. These findings will be valuable in the further processes of both target characterization and ligand identification for the high-affinity GHB binding sites.

Introduction

γ-Hydroxybutyric acid (GHB, Figure 1) is an endogenous neuroactive substance and a proposed neurotransmitter found in micromolar concentrations in mammalian brains and metabolically derived from the more potent inhibitory neurotransmitter γ-aminobutyric acid (GABA, Figure 1).[1] GHB is clinically prescribed for treatment of alcohol dependence[2] and narcolepsy.[3] Additionally, GHB is a drug of abuse (Fantasy or liquid ecstasy), producing mild euphoria, sedation, and coma at high dosage.[4] In the central nervous system, GHB displays a complex neuropharmacology involving both low- and high-affinity targets. The *in vivo* pharmacological effects of GHB are largely mediated by weak agonism of the $GABA_B$ receptors.[5] In addition, GHB binds with nanomolar to micromolar affinity to another unknown target preserved in $GABA_{B(1)}$ knockout mice and with distinct spatial distribution and ontogenesis in the brain.[6, 7]

This emphasizes the existence of several high-affinity GHB binding sites in distinct proteins, all of which may be therapeutic targets.[1] With the use of photoaffinity labelling, proteomic analysis, and molecular pharmacology studies, we recently reported the $\alpha_4\beta_{1\text{-}3}\delta$ ionotropic $GABA_A$ receptors as high-affinity GHB targets.[8] However, with a ~40% reduction in the high-affinity GHB binding sites in α_4 knockout mice, the remaining ~60% of the high-affinity binding sites remain elusive.[8] In fact, no direct agonist effect of GHB at extrasynaptic $GABA_A$ receptors could be demonstrated in rat brain slices in relevant regions.[9] Hence, in order to guide the search for the identity and localization of the high-affinity GHB binding sites, and facilitate future drug development in the field, more structure-affinity information of ligands targeting these specific sites is desirable.

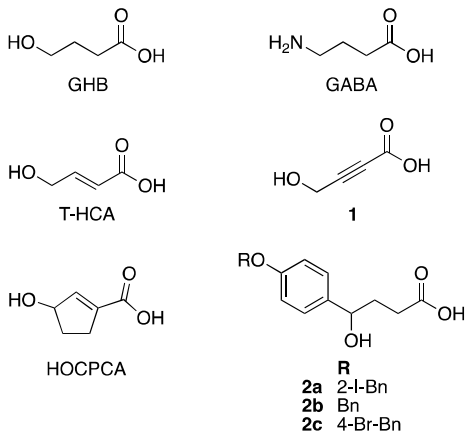


Figure 1. Chemical structures of GHB, GABA, T-HCA, compound **1**, HOCPCA, and compounds **2a**–**c**.

As one of the first GHB analogs reported, the semi-rigid *trans*-4-hydroxycrotonic acid (T-HCA) was found to displace [^{3}H]GHB binding with a small (four-fold) improved affinity compared to GHB.[10] In contrast, replacing the double bond with a triple bond to give the more rigid and extended analog of GHB, compound **1**, was reported to result in loss of affinity.[10] We have published two series of high-affine and selective GHB analogs that showed markedly improved binding compared to previously reported GHB analogs.[10-13] With the conformationally restricted GHB analog 3-hydroxycyclopent-1-enecarboxylic acid (HOCPCA, Figure 1, K_i 0.16 μM), displaying a 27-fold higher affinity compared to that of GHB (K_i 4.3 μM), we hypothesized that the structure of HOCPCA closely mimics the bioactive conformation of GHB at the high-affinity binding site.[13] Additionally, 4-substituted bi-aromatic GHB analogs, represented by 4-(4-(2-iodobenzyloxy)phenyl)-4-hydroxybutanoic acid (Figure 1, **2a**), displayed a 71-fold improved affinity compared to GHB, which indicated the presence of a hydrophobic cavity near the GHB scaffold able to accommodate large aromatic substituents.[12] Furthermore, it was shown that the GHB binding site interacts stereoselectively with substituted GHB analogs, *e.g.* **2a**, as well as the

conformational restricted HOCPCA, as the (*R*)-isomers displayed a 10- and 13-fold, respectively, improved affinity compared to the (*S*)-isomer.[12, 13] The high selectivity of HOCPCA for the high-affinity GHB binding sites was later established, as HOCPCA did not show any affinity for the $GABA_B$ receptors and 45 other neurotargets.[13, 14] Previous structure-affinity relationship (SAR) studies of GHB have shown that introduction of even small substituents into the 2-position is highly detrimental for affinity and that only minor alkyl substituents are tolerated in the 3-position of GHB.[11] Conversely, several studies have reported improved binding affinity by introduction of substituents in the 4-position of GHB.[10-12] A similar structural trend has been reported for smaller substituents in the corresponding position in T-HCA.[10, 11] In addition to conformationally constraining the main pharmacophore elements, the scaffold of HOCPCA allows for introduction of substituents in well-defined positions suitable for addressing the reported SAR of GHB. Thus, the high selectivity, conformational restriction, and stereoselective interaction provides HOCPCA as a unique scaffold for further exploration of the high-affinity GHB binding sites.

In the present study, we merge the structural features of three classes of high-affinity GHB ligands and study the conformational determinants of the high-affinity GHB binding sites. The synthesis and binding affinity determination of this series of compounds are described along with a pharmacophore model rationalizing the SAR data observed in the present study relative to previously reported data. A reliable and well-validated pharmacophore model has not previously been published and will be valuable for future progress in the field.

Results

Design strategy

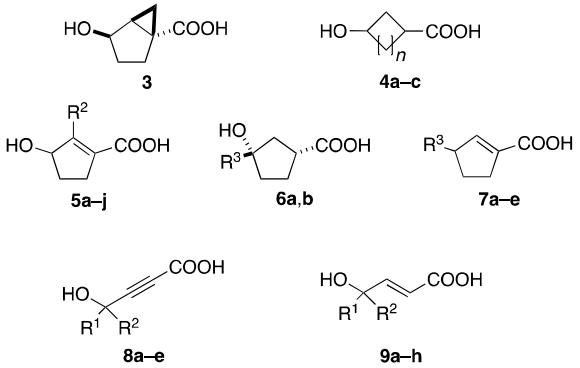


Figure 2. General structures of target compounds.

Comparison of HOCPCA and compounds **2a**–**c** by simply overlaying the hydroxyl and carboxyl functionalities, indicates an area in the binding site in close vicinity to the hydroxyl group of GHB and HOCPCA, which could be favorable for large hydrophobic and/or aromatic moieties. To investigate and validate this hypothesis we chose to introduce substituents in the 2- or 3-position of HOCPCA and the corresponding 4-position of T-HCA and compound **1** thereby probing the suggested hydrophobic area. A set of substituents was chosen inspired by previously reported 4-substituted GHB analogs such as **2a**. Due to challenges in the synthesis we were not able to obtain the direct 3-substituted HOCPCA analogs. However, since previous reports demonstrate that the hydroxyl group of many GHB ligands is favorable, but not vital for obtaining high-affinity ligands,[15, 16] we decided to test if hydrophobic substituents in the 3-position of HOCPCA could compensate for the absence of the hydroxyl group. Finally, by varying the size and flexibility of the cyclic scaffold, the impact of the conformationally restricted structure of the cyclopentene ring on the binding affinity of HOCPCA was addressed.

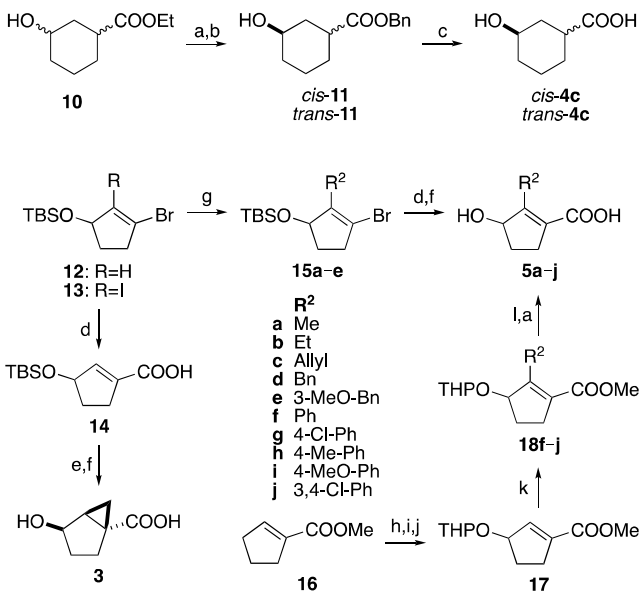
Chemistry

The HOCPCA and T-HCA analogs **1**, **4a**,**b**, and **7a** were obtained from commercial suppliers, while the developed HOCPCA and T-HCA analogs were prepared according to Schemes 1–4.

The cyclohexane analogs of HOCPCA (*cis*-**4c** and *trans*-**4c**, Scheme 1) were obtained from compound **10**, which was converted into compound **11** by changing the ethyl ester to a benzyl ester. Diastereomeric separation of racemic mixture into the *cis*- and *trans*-stereoisomers (*cis*-**11** and *trans*-**11**) followed by catalytic hydrogenation afforded compounds *cis*-**4c** and *trans*-**4c**.

Replacement of the α,β-double bond of HOCPCA with a cyclopropane ring was performed by a Simmons-Smith cyclopropanation using the Furukawa zinc reagent[17] on the alkene **14**, prepared as previously described (Scheme 1).[14] Due to coordination between the zinc reagent and the allylic alcohol,[18] mainly the *cis*-stereoisomer with respect to the hydroxyl group and cyclopropane ring was formed, [19] thus only the *cis*-stereoisomer was isolated. Deprotection with aqueous hexafluorosilicic acid furnished the cyclopropane analog **3**.

Scheme 1[a]

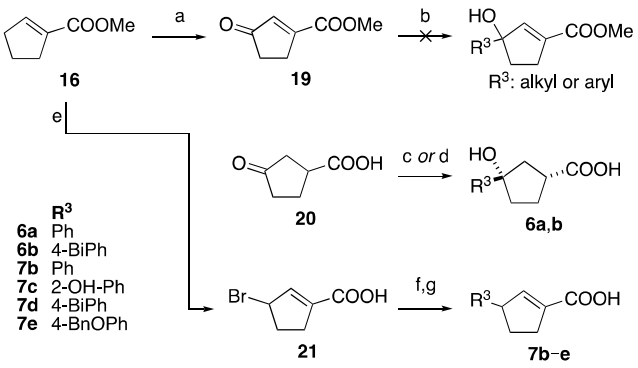


[a] *Reagents and conditions:* (a) LiOH, H_2O/THF, rt., (b) Cs_2CO_3, BnBr, DMF, rt., (c) H_2, 10% Pd/C, MeOH, rt., (d) *i)* **12**, ^{t}BuLi, THF, –78 °C, *ii)* CO_2, THF, –50 °C, (e) Et_2Zn, CH_2I_2, CH_2Cl_2, molecular sieves, 0 °C to rt., (f) H_2SiF_6, MeCN, rt., (g) **13**, ^{n}BuLi, R^2-X, THF, –78 °C, (h) CrO_3, Ac_2O, AcOH, CH_2Cl_2, rt., (i) $NaBH_4$, $CeCl_3$, MeOH, 0 °C, (j) 3,4-dihydro-2*H*-pyran, HCl, rt., (k) $Pd(OAc)_2$, $N(Bu)_4Cl$, $NaHCO_3$, R^2-I, DMF, 4Å molecular sieves, DMF, 60 °C, (l) Pyridinium *p*-toluenesulfonate, MeOH, 50 °C.

Inspired by a procedure reported by Luparia *et al.,*[20] a series of 2-alkyl substituted analogs (**5a**–**e**) were synthesized from compound **13**[20] (Scheme 1). Regioselective iodine-lithium exchange performed with ^{n}BuLi followed by subsequently quenching with an appropriate alkyl halide provided the 2-substituted bromocyclopentenes intermediates **15a**–**e**. Carboxylation of the bromides **15a**–**e** with ^{t}BuLi and carbon dioxide followed by cleavage of the *tert*-butyldimethylsilyl (TBS) group with aqueous hexafluorosilicic acid gave the 2-alkyl substituted analogs **5a**–**e**.

The 2-aryl substituted analogs of HOCPCA (compound **5f**–**j**) were synthesized via allylic oxidation of the methyl ester **16**. Reduction of the ketone by a Luche reduction and tetrahydropyranyl (THP) protection of the formed alcohol gave the intermediate **17**. The aryl substituents were introduced *via* a Heck cross coupling procedure with palladium acetate and tetrabutylammonium chloride to give compounds **18f**–**j**.[21] Subsequently cleavage of the THP-group and hydrolysis of the methyl ester using pyridinium *p*-toluenesulfonate and lithium hydroxide, successively, provided 2-aryl substituted HOCPCA analogs **5f**–**j**.

Scheme 2[a]

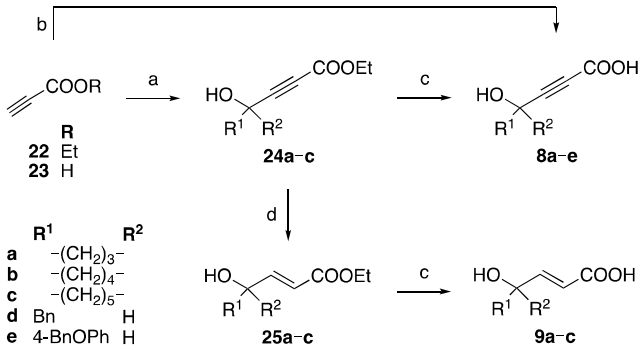


[a] *Reagents and conditions:* (a) CrO_3, Ac_2O, AcOH, CH_2Cl_2, rt., (b) R^3-MgX or R^3-Li, THF, 0 °C, –20 °, or –78 °C, (c) PhMgBr, THF, –78 °C, (d) *i)* 4-Br-BiPh, Mg, I_2 (cat), THF, reflux, *ii)* **20**, THF, –78 °C, (e) NBS, AIBN, CH_2Cl_2, reflux, (f) $Pd(OAc)_2$, KF, R^3-$B(OH)_2$, 1,4-dioxane, rt., (g) LiOH, H_2O/THF, rt.

It was initially envisaged that a series of 3-substituted analogs of HOCPCA could be synthesized directly by organometallic addition to ketone **19**, obtained from allylic oxidation of methyl ester **16** with chromium trioxide as previously reported (Scheme 2).[14] However, all attempts to perform a nucleophilic addition to the α,β-unsaturated ketone **19** with either organolithium or Grignard reagents (PhLi, PhMgBr or BnMgCl), with or without addition of hexamethylphosphoramide (HMPA) or 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU), and at varying temperatures (0 °C, –20 °C or –78 °C) resulted in rapid elimination of the formed alcohol or decomposition of the molecule. Therefore, no further efforts were put into the synthesis of the 3-substituted analogs of HOCPCA. Instead, two saturated 3-aryl-substituted analogs (**6a**,**b**) of HOCPCA were synthesized from the corresponding commercially available ketone **20**. NMR analysis of the crude mixtures revealed a 9:1 ratio between the *cis*- and *trans*-stereoisomers, with respect to the hydroxyl and carboxyl groups, of both **6a** and **6b**. Due to the minor formation of the *trans*-stereoismer, only the *cis*-stereoisomers could be obtained pure after purification.

To probe the mutual importance of the hydroxyl group and the presence of lipohilic groups in the 3-position, the dehydroxylated analogs of HOCPCA were pursued (compounds **7b**–**e**, Scheme 2). Wolf-Ziegler bromination of the α,β-unsaturated carboxylic acid ester **16**, followed by Suzuki cross coupling and hydrolysis of the methyl ester provided the dehydroxylated 3-aryl-substituted series of HOCPCA analogs **7b**–**e**. Due to instability of the allyl bromide **21**, the Suzuki cross coupling was performed immediately upon isolation of compound **21**.

Scheme 3[a]

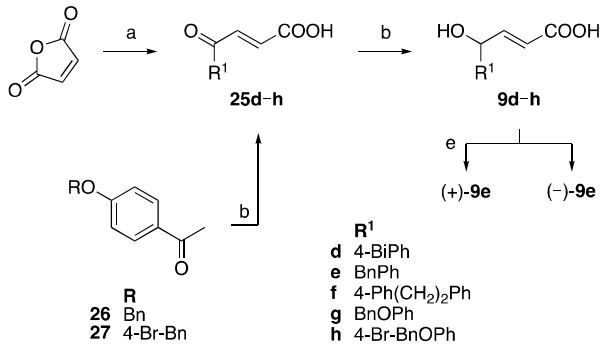


[a] *Reagents and conditions:* (a) **22**, ^{n}BuLi, appropriate ketone, THF, –78 °C to –40°C, (b) **23**, ^{n}BuLi, appropriate ketone, THF, –78 °C to –40°C, (c) LiOH, H_2O/THF, rt., (d) Red-Al, THF, –78 °C.

The 4-spirocyclic- and 4-aryl-substituted alkynyl and 4-spirocyclic-substituted T-HCA analogs (**8a**–**c**, **8d**,**e**, and **9a**–**c**, respectively) were synthesized as described in Scheme 3 starting from either ethyl propiolate (**22**) or propiolic acid (**23**). Formation of the acetylide of **22** or **23** followed by subsequently quenching with appropriate ketones[22, 23] provided compounds **24a**–**c** or **8a**–**e**, respectively, and **24a**–**c** were then converted into **8a**–**c** under basic conditions. The 4-spirocyclic-substituted T-HCA analogs **9a**–**c** were obtained from **24a**–**c** by a selective reduction

of the alkynes into the *trans*-alkenes **25a–c** following a procedure described by Meta and Koiide[24] using Red-Al followed by basic hydrolysis of the ethyl ester.

Scheme 4[a]



[a] *Reagents and conditions:* (a) 1,1'-BiPh, $PhCH_2Ph$ *or* $Ph(CH_2)_2Ph$, $AlCl_3$, CH_2Cl_2, rt., (b) **26** *or* **27**, Glyoxylic acid, NaOH, H_2O/EtOH, reflux, (d) $CeCl_3$, $NaBH_4$, MeOH, 0 °C to rt., (e) Chiral HPLC separation: Colomn: ChiralPak IF, mobile phase: ^{n}heptane/2-PrOH/TFA (95/5/0.1)

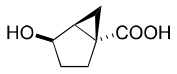
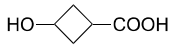
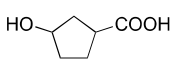
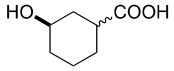
The 4-aryl-substituted T-HCA analogs **9d–h** were synthesized as described in Scheme 4. The α,β-unsaturated ketone intermediates **25d–h** were synthesized by either a Friedel-Craft acylation of 1,1'-biphenyl, diphenylmethane or bibenzyl with maleic acid anhydride (**25d–f**) following a procedure described by Kameo *et al.*[22] or an aldol condensation between glyoxylic acid and either 1-(4-(benzyloxy)phenyl)ethanone (**26**)[25] or 1-(4-((4-bromobenzyl)oxy)phenyl)ethanone (**27**)[25] (**25g** and **25h**, respectively). A Luche reduction of the ketone intermediates (**25d–h**) provided the 4-aryl substituted T-HCA analogs **9d–h**. The enantiomers **9e**, (+)-**9e** (98% *ee*) and (–)-**9e** (98% *ee*), were obtained in excellent *ee*'s by chiral preparative HPLC using a Chiralpak IF column.

Structure-affinity relationship studies

The binding affinities of the compounds for the high-affinity GHB binding sites were measured by displacement of [^{3}H]NCS-382 in rat brain synaptosomal membranes. At the applied concentration (16 nM), NCS-382 is selective for the high-affinity GHB binding sites.[26] The obtained data are summarized in Tables 1–4.

The affinity data for the conformationally restricted cyclic analogs of GHB (compounds **3** and **4a**–**c**) are presented in Table 1 alongside with data for GHB and HOCPCA. In general, the reduced planarity and increased structural flexibility compared to HOCPCA led to lower affinity. Installation of a cyclopropyl-ring as an isostere for the double bond in HOCPCA (**3**) led to loss of affinity in concentrations up to 100 μM. Likewise, the cyclobutane analog **4a** showed a markedly reduced binding affinity. A similar trend was shown for the less planar conformations of GHB represented in the cyclopentane and cyclohexane analogs, **4b** and **4c**, respectively, which showed affinity in the mid-micromolar range. Interestingly, the *cis*- and *trans*-stereoisomers of **4c** were shown to be equally efficient in binding to the GHB binding site indicating some flexibility of the site around the six-membered ring.

Table 1. Pharmacological data for GHB, HOCPCA, compound **3**, and compounds **4a**–**c**: Binding affinities at the high-affinity GHB binding site at rat brain cortical homogenate in the [^{3}H]NCS-382 binding assay.

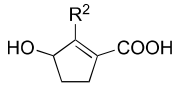
 **3**  **4a**  **4b**  *cis*-**4c**, *trans*-**4c**	
	[^{3}H]NCS-382 binding K_i (μM)[a] [pK_i ± SEM]
GHB	3.6 [5.44 ± 0.07]

HOCPCA	0.13 [6.89 ± 0.04]
(*S*)-HOCPCA	1.4[b]
(*R*)-HOCPCA	0.11[b]
3	>100
4a	70 [4.16 ± 0.04]
4b	20 [4.71± 0.05]
cis-**4c**	35 [4.45 ± 0.05]
trans-**4c**	28 [4.55 ± 0.01]

[a] IC_{50} values were calculated from inhibition curves and converted to K_i values. Data are given as mean [mean pK_i ± SEM] of three to four independent experiments. [b] From Wellendorph *et al.*, 2005.[13]

Introduction of substituents in the 2-position of HOCPCA was in general detrimental for binding affinity (Table 2). Unbranched alkyl groups, such as an ethyl or allyl led to loss of affinity at concentrations up to 300 μM, whereas larger aromatic substituents in the 2-position of HOCPCA showed binding affinities in the mid-micromolar range.

Table 2. Pharmacological data for GHB, HOCPCA, and compounds **5a**–**j**: Binding affinities at the high-affinity GHB binding site at rat brain cortical homogenate in the [^{3}H]NCS-382 binding assay.



5a–j

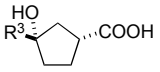
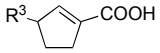
	R^2	[^{3}H]NCS-382 binding K_i (μM)[a] [pK_i ± SEM]
GHB		3.6 [5.44 ± 0.07]
HOCPCA		0.13 [6.89 ± 0.04]
5a	Me	80 [4.10 ± 0.02]
5b	Et	>300
5c	Allyl	>300
5d	Bn	38 [4.43 ± 0.03]

5e	3-MeO-Bn	46 [4.43 ± 0.02]
5f	Ph	48 [4.32 ± 0.02]
5g	4-Cl-Ph	70 [4.16 ± 0.04]
5h	4-Me-Ph	157 [3.81 ± 0.03]
5i	4-MeO-Ph	>300
5j	3,4-Cl-Ph	62 [4.21 ± 0.02]

[a] IC_{50} values were calculated from inhibition curves and converted to K_i values. Data are given as mean [mean pK_i ± SEM] of three to four independent experiments.

The binding affinities for the 3-substituted analogs (**6a**,**b** and **7a**–**e**) are presented in Table 3. As indicated previously, absence of the hydroxyl group is detrimental for affinity as shown by a ~200-fold affinity decrease of **7a** *versus* HOCPCA. The addition of a phenyl ring (**7b**) in the 3-position did not compensate for the removal of the hydroxyl group with only an approximately two-fold increased affinity relative to **7a**. Further introduction of a hydroxyl group in the 2-position of the phenyl ring (**7c**) led to a slight enhancement in affinity as compared to **7b**. Introduction of an additional 4-phenyl (**7d**) or 4-benzyloxy ring (**7e**) displayed a five- and ten-fold increase, respectively, in binding affinities compared to that of **7b**. A similar trend was observed for the saturated 3-substituted analogs (**6a**,**b**), with the phenyl substituted analog (**6a**) displaying an approximately three-fold decrease in binding affinity relative to **4b**. Interestingly, an additional 4-phenyl ring (**6b**) led to an affinity increase by a factor of 100 compared to that of **6a**, thus displaying a binding affinity only three-fold lower than that of HOCPCA.

Table 3. Pharmacological data for GHB, HOCPCA, and compounds **6a**,**b** and **7a**–**e**: Binding affinities at the high-affinity GHB binding site at rat brain cortical homogenate in the [^{3}H]NCS-382 binding assay.

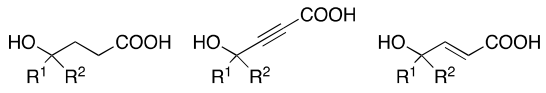
	 **6a**,**b**	 **7a–e**
	R_3	[^{3}H]NCS-382 binding K_i (μM)[a] [pK_i ± SEM]
GHB		3.6 [5.44 ± 0.07]
HOCPCA		0.13 [6.89 ± 0.04]
6a	Ph	53 [4.28 ± 0.01]
6b	4-biPh	0.51 [6.29 ± 0.10]
7a	H	31 [4.51 ± 0.04]
7b	Ph	16 [4.80 ± 0.10]
7c	2-OH-Ph	4.6 [5.34 ± 0.03]
7d	4-biPh	3.2 [5.49 ± 0.10]
7e	4-BnOPh	1.6 [5.77 ± 0.05]

[a] IC_{50} values were calculated from inhibition curves and converted to K_i values. Data are given as mean [mean pK_i ± SEM] of three to four independent experiments.

In contrast to previous reports,[10] we found that the triple bond analog of GHB, **1**, displayed affinity for the high-affinity GHB binding sites equivalent to GHB (Table 4). Only slight or no enhancement in affinity was achieved by introduction of spirocylic aliphatic substituents in the 4-position of **1** (**8a**–**c**). A similar effect was observed for the corresponding analogs of T-HCA (**9a**–**c**), the double bond analog of GHB. Likewise, introduction of larger aromatic substituents in the 4-position in **1** did not affect the binding affinity (**8d**,**e**). However, introduction of larger aromatic substituents in the 4-position of T-HCA provided a series of very high-affinity T-HCA analogs (**9d**–**h**) that were able to displace [3H]NCS-382 binding with K_i values up till six times lower than that of the corresponding GHB analogs in the study and previously reported.[12] The 4-bromobenxyloxyphenyl substituted T-HCA analog **9h**, together with the previously reported (*R*)-**2a**, thus represents the most high-affinity ligand reported to date (Figure 3). Interestingly, a 36-fold preference for the (+)-isomer of the 4-benzylphenyl T-HCA analog **9e** was shown, which is a markedly higher stereochemical preference than the ten-fold difference reported for the

stereoisomers of 2-iodobenzyloxyphenyl substituted analog **2a** of GHB.[12] Functional studies on the compounds are awaiting.

Table 4. Pharmacological data for GHB, HOCPCA, T-HCA, and compounds **1**, **2a**–**c**, **8a**–**e**, and **9a**–**h**: Binding affinities at the high-affinity GHB binding site at rat brain cortical homogenate in the [^{3}H]NCS-382 binding assay.



	R_1	R_2	[^{3}H]NCS-382 binding K_i (μM)[a] [pK_i ± SEM]
GHB			3.6 [5.44 ± 0.07]
HOCPCA			0.13 [6.89 ± 0.04]
T-HCA	H	H	1.1[b]
1	H	H	4.3 [5.50 ± 0.04]
(*S*)-**2a**	2-I-BnOPh	H	0.22[c]
(*R*)-**2a**	2-I-BnOPh	H	0.022[c]
2b	4-BnOPh	H	0.11[c]
2c	4-Br-BnOPh	H	0.034[c]
8a	–$(CH_2)_3$–		1.6 [5.78 ± 0.04]
8b	–$(CH_2)_4$–		2.7 [5.57 ± 0.05]
8c	–$(CH_2)_5$–		7.7 [5.12 ± 0.06]
8d	Bn	H	3.5 [5.45 ± 0.04]
8e	4-BnOPh	H	1.6 [5.80 ±0.03]
9a	–$(CH_2)_3$–		3.5 [5.47 ± 0.06]
9b	–$(CH_2)_4$–		3.1 [5.51 ± 0.03]
9c	–$(CH_2)_5$–		4.3 [5.37 ± 0.01]
9d	4-biPh	H	0.075 [7.14 ± 0.08]
9e	4-BnPh	H	0.042 [7.38 ± 0.02]
(+)-**9e**	4-BnPh	H	0.030 [7.47 ± 0.06]
(–)-**9e**	4-BnPh	H	1.16 [5.97 ± 0.02]
9f	4-Ph$(CH_2)_2$Ph	H	0.042 [7.39 ± 0.02]
9g	4-BnOPh	H	0.043 [7.43 ± 0.03]

9h	4-Br-BnOPh	H	0.023 [7.64 ± 0.08]

[a] IC_{50} values were calculated from inhibition curves and converted to K_i values. Data are given as mean [mean pK_i ± SEM] of three to four independent experiments. [b] From Wellendorph *et al.*, 2005.[13] [c] From Høg *et al.*, 2008.[12]

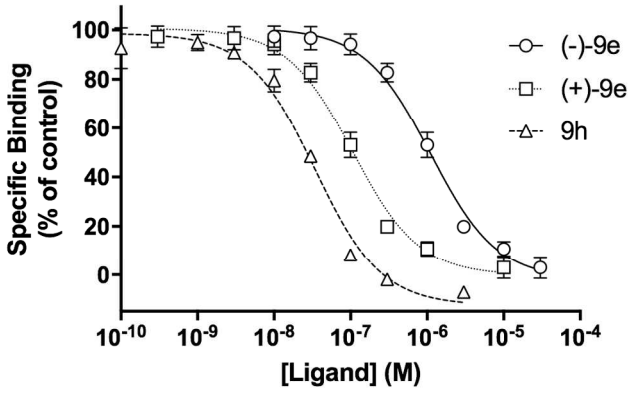


Figure 3. Concentration-dependent inhibition of [^{3}H]NCS-382 binding to rat brain cortical homogenate by T-HCA analogs (+)-**9e**, (–)-**9e**, and **9h**. The affinities were determined as described under Experimental Section. Results are given as mean ± SD of a single representative experiment performed in triplicate. Two additional experiments gave similar results (data summarized in Table 4).

Pharmacophore modeling

To improve understanding of the SAR of the developed GHB ligands we constructed a pharmacophore model, which will aid in future design of new ligands targeting the high-affinity GHB binding site. The pharmacophore model (Figure 4) was based on HOCPCA and compounds **2b**, **3**, **5b**, **5i**, **6a**, **8e**, **9d**, and **9e** divided into high affinity ligands (pK_i > 6), intermediate binders (4 < pK_i < 6), and compounds with weak or no affinity (pK_i < 4).

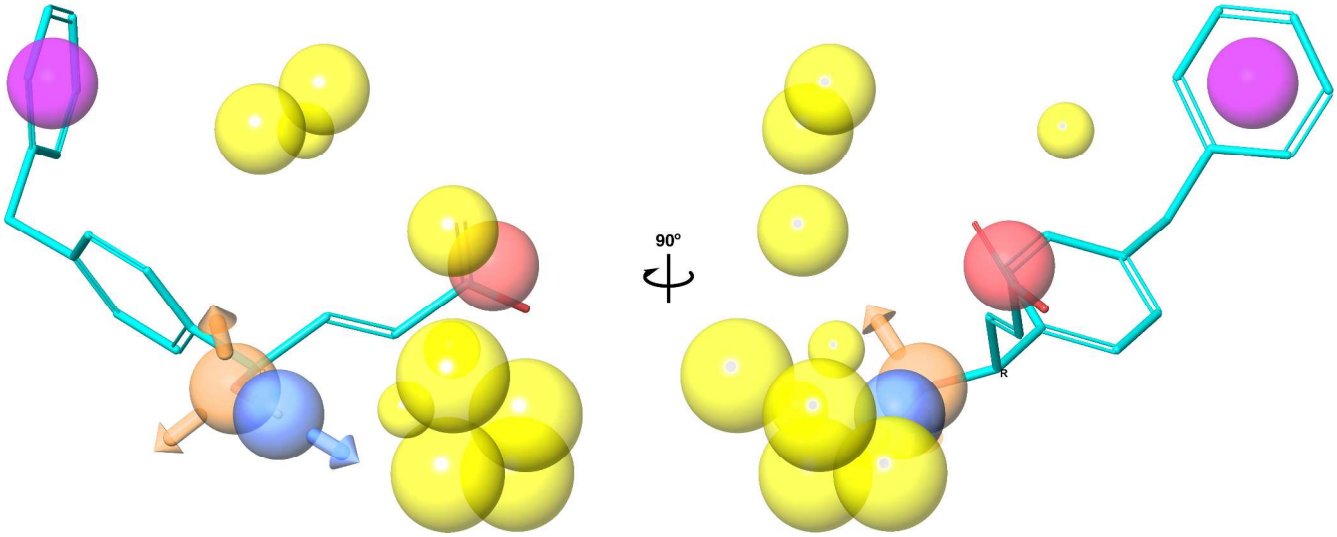


Figure 4. Pharmacophore model for the GHB high-affinity binding site displaying the proposed binding mode of compound (*R*)-**9e** (cyan carbon atoms). The yellow spheres indicate unfavorable positions of substituents (exclusion volumes) in the high-affinity binding sites while the other spheres indicate positions favorable for: aromatic ring systems (purple), hydrogen bond acceptor (orange), hydrogen bond donor (blue), and negatively charged group (red). The arrows show the direction of hydrogen bond acceptor lone pairs and donor hydrogen.

The best scoring pharmacophore model, comprised of the hydroxyl group as both a hydrogen bond acceptor and donor, the negatively charged carboxylic acid and an aromatic ring, was refined by adding exclusion volumes based on the inactive compounds **3** and **5b**. Requiring a match of three out of the four pharmacophore elements identified both enantiomers of all ten high-affinity ligands, 18 out of the 27 intermediate affinity compounds, and discarded both enantiomers of the five inactive compounds (see Supplementary Table 1 for the complete performance of the pharmacophore model). The model ranked the compounds with four matching pharmacophore elements as "best fits" and thus, qualitatively matching the fact that

compounds with the distal aromatic moiety in the 4-position of GHB display the highest affinity. All 2-substituted HOCPCA analogs (**5a**–**j**), with the exception of **5a** having the smallest 2-substituent, were discarded due to the exclusion volumes based on **5b**. HOCPCA analogs **7a**–**e**, which lack the hydroxyl group, were only recognized if asking for two out of four matching pharmacophore elements (not shown). With the exception of *trans*-**4a**, (*S*)-*cis*-**4c** and (*S*)-**5a**, the model recognized both stereoisomer (all four for **4b**) of the identified high and intermediate affinity compounds and, with the exception of *trans*-**4c**, **7c**, **8d**, **8e**, **9h** and **9f**, the (*R*)-enantiomer were ranked higher than the (*S*)-enantiomer. For **8d**,**e** the (*S*)-enantiomer corresponds to the (*R*)-enantiomer of the other analogs due to the adjacent triple bond. These observations are consistent with the experimental data for HOCPCA, **2a** and **9e**.[12, 13]

Discussion

Despite notable interest in the field especially to the enigmatic GHB high-affinity binding sites and their possible implication in neurological disorders, the physiological function of the high-affinity GHB binding sites distinct from the $GABA_B$ receptors are still elusive.[1] Most of the observed pharmacological effects of GHB have been demonstrated to involve $GABA_B$ receptors,[6, 27, 28] yet several GHB ligands that show no affinity for the $GABA_B$ receptors display pharmacological effects *in vitro*, putatively mediated by the high-affinity GHB binding sites.[29-31] Interestingly, in the clinic, GHB is preferably used over the $GABA_B$ agonist baclofen in both narcolepsy and alcoholism.[2, 3] To achieve a more detailed understanding of the molecular basis for GHB actions and to provide potential future drug candidates, new selective ligands for the specific GHB high-affinity binding sites with a broad structural diversity are needed.

In the absence of knowledge about the molecular nature of the high-affinity GHB binding site, most molecular research in the field has been focused on developing tool compounds, primarily based on the structure of GHB. However, since GHB is a small and flexible molecule it is not ideal for establishing a binding mode and thereby providing more precise information on the topography of the binding site. In the present study, we have expanded the investigation of the SAR for ligands targeting the high-affinity GHB binding sites and comprised a pharmacophore model highlighting the molecular features important for high affinity binding. We have been using HOCPCA and the conformationally less flexible linear double or triple bond containing GHB analogs, T-HCA, and **1** as lead structures in combination with structural fragments previously described for a series of linear GHB analogs displaying high affinity.

Being a small low molecular weight compound displaying high binding efficiency, HOCPCA constitutes an ideal lead structure for further development. The replacement of the cyclopentene ring of HOCPCA with saturated cycloalkanes (**4a**–**c**) was detrimental for the binding affinity supporting the hypothesis that the cyclopentene ring of HOCPCA represents the bioactive conformation of GHB (Figure 5A). The pharmacophore model confirms that the relative orientation/location of the carboxylic acid and hydroxyl group of HOCPCA is important for binding and explains the favoring of the almost planar cyclopentene ring of HOCPCA with dis-allowed areas. The poor tolerance of out-of-plane bulk is best exemplified by the lack of binding for **3** (K_i >100 μM) but is likely also part of the explanation for the 175-fold lower affinity of *trans*-**4c** relative to HOCPCA (Figure 5B). Additionally, saturation of the cyclopentene ring increase the entropic penalty of binding with the conformational flexibility.[8] The reverse structural modification is reported for GHB and the corresponding unsaturated analog T-HCA, a naturally occurring compound in the human brain[32] that binds to the high-affinity GHB site,

increasing the planarity and decreasing the flexibility while retaining a perfect fit to the pharmacophore model (Figure 5A). Interestingly, replacement of the double bond in T-HCA by a triple bond to give **1**, representing a rigid and slightly extended conformation of GHB, was shown to display an affinity similar to GHB itself. Altogether this reflects limited steric and directional tolerability for the high-affinity GHB binding.

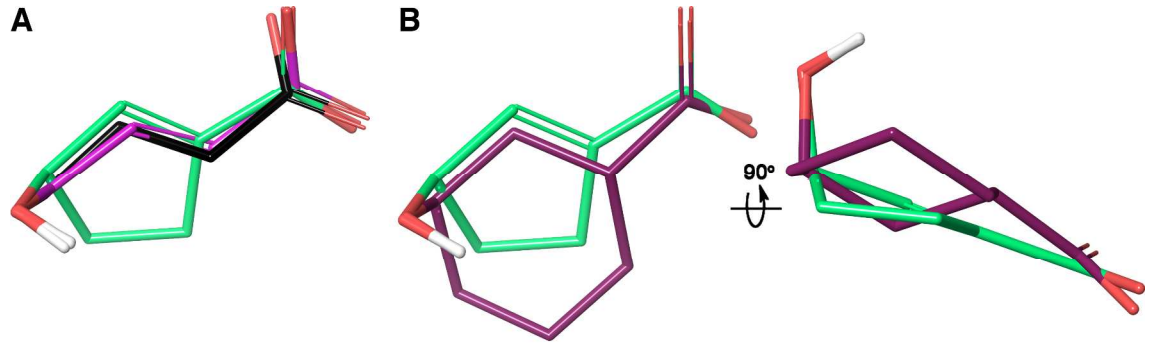


Figure 5. ***A.*** The pharmacophore model predicts an almost perfect overlay of the functional groups of the conformationally restricted T-HCA (purple stick) and (*R*)-HOCPCA (green stick) with the believed active conformation of the flexible GHB (black stick). ***B.*** Overlay of (*R*)-HOCPCA (green stick) and (*1R,3R*)-*trans*-**4c** (violet-purple stick) from the pharmacophore model showing the poor tolerance for ligands deviating from the near-planar conformation of HOCPCA.

Bourguignon *et al.*,[11] have demonstrated that no substitutions are allowed in the 2-position of GHB and only small substituents are allowed in the 3-position. The pharmacophore model presented here shows that substituents in the 2-position of GHB, like it is the case for **4**, will violate the steric requirements for planarity close to the carboxylic acid. Additionally, we find that the 2-position of HOCPCA is analogous to the 3-position in GHB (Figure 5) and we have demonstrated a comparable trend, where alkyl and aromatic substituents led to significant

decrease or absence of affinity in general (Table 2) represented by dis-allowed areas in the pharmacophore model.

The most high-affinity GHB analogs reported so far, including HOCPCA, all possess a hydroxyl group in the equivalent position and we prove the importance of this functional group by the 193-fold drop in affinity for **7a** relative to HOCPCA. There is no clear evidence from the included or previously reported compounds to whether the hydroxyl group acts as a hydrogen bond donor, an acceptor or both. In our pharmacophore model, the dual role, as both hydrogen bond donor and acceptor, has been included with good performance, indicating the presence of suitable positioned interaction partners for both in the binding pocket. Another structural characteristic for high-affinity GHB ligands is an aromatic substituent in a location equivalent to the 4-position of GHB.[12] With the aim of investigating this in an equivalent position of **2a**–**c**, we synthesized a series of compounds addressing the aromatic feature.

For both the saturated and unsaturated analog of HOCPCA, **4b** and **7a**, respectively, (Table 3) a single aromatic substituent in the 3-position, as for **6a** and **7b**, is tolerated with a slight drop in affinity (two- to three-fold) compared to the parent compounds. However, the affinity improved by adding an additional aromatic moiety (Table 3) and especially the 100-fold affinity increase of **6b** *versus* **6a** proves a very favorable additional interaction between the distal aromatic moiety and the high-affinity binding site. This corresponds to what has been previously reported for the corresponding GHB analogs,[12] where the 4-biphenyl-GHB analog shows equivalent affinity to that of **6b**, indicating an area near the 4-position of GHB and the equivalent position of HOCPCA that is favorable for aromatic moieties. Furthermore, analogs of GHB, T-HCA, and less so compound **1** (Table 4) display a similar effect upon introduction of aromatic 4-sustituents of varying length and flexibility (e.g. **9e**–**g**). As for compounds **6a**,**b** and **7a**–**e**, the

pharmacophore model shows that the first aromatic ring of the 4-substituent in **9e**–**g** is not fitting into a well defined pocket. In contrast, the model suggests the distal aromatic ring to be positioned in a very well defined position (Figure 6, purple sphere) where favorable interactions between the distal aromatic ring and the high-affinity bindings can take place.

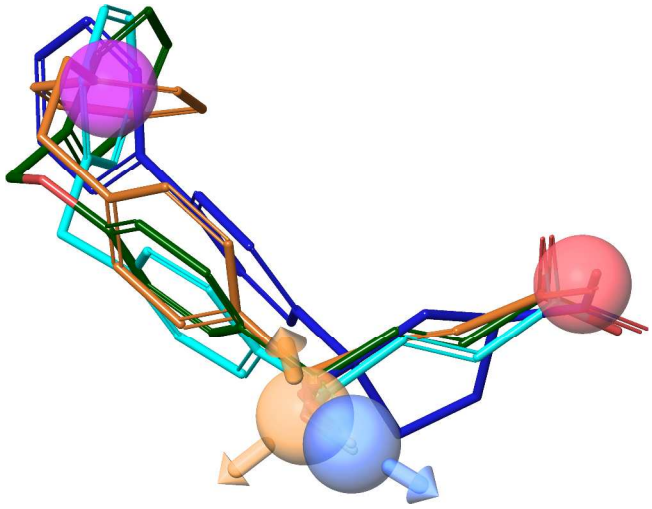


Figure 6. The proposed binding modes from the pharmacophore model of compounds (*R*)-**9e** (cyan stick), (*R*)-**9g** (dark green stick), (*R*)-**9f** (bright orange stick), and **(***R***)-6b** (dark blue stick). The spheres indicate positions favorable for: aromatic ring systems (purple), hydrogen bond acceptor (orange), hydrogen bond donor (blue), and negative charged group (red). The arrows show the direction of hydrogen bond acceptor lone pairs and donor hydrogen. The pharmacophore model shows a well-defined pocket for the distal phenyl ring system while the first phenyl ring is unimportant for the binding affinity of the developed GHB and HOCPCA analogs.

Introducing substituents in the 4-position of GHB, T-HCA, and compound **1** introduces chirality with a 39-fold difference in affinity between the enantiomers of **9e** (Table 4). In contrast, the enantiomers of HOCPCA only display a 13-fold difference in affinity with (*R*)-

HOCPCA having the highest affinity (Table 1, K_i 0.11 μM). Interestingly, our pharmacophore model can accommodate both enantiomers of HOCPCA and **9e** (Figure 7) and, in accordance with the affinity, ranks (*R*)-HOCPCA higher than (*S*)-HOCPCA. Additionally, the (*R*)-enantiomer of **9e** and similar ligands were ranked higher than the (*S)*-enantiomer, i.e. predicted as the higher affinity configuration.

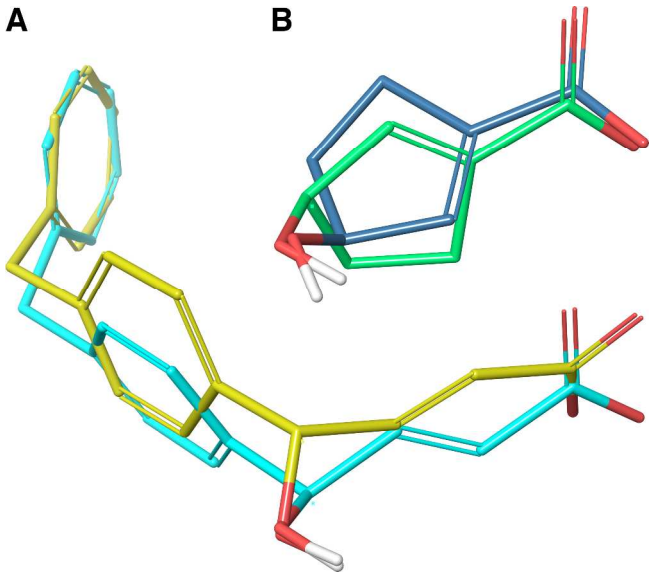


Figure 7. The pharmacophore model generally identifies both enantiomers of the high-affinity ligands and intermediate binders as hits illustrated by the overlays of, ***A.*** (*R*)-**9e** (cyan stick) and (*S*)-**9e** (yellow stick) and ***B.*** (*R*)-HOCPCA (green stick) and (*S*)-**9e** (skyblue stick).

In conclusion, by use of a molecular hybridization strategy based on structural scaffolds and fragments from three classes of potent and selective ligands for the high-affinity GHB binding sites, we have generated a series of ligands exploring the core scaffold and substitutions in the 2- and 3-position of HOCPCA as well as the 4-position of GHB, T-HCA, and compound **1**. Importantly, these compounds guided the development of the first reported 3D-pharmacophore model for the high-affinity GHB binding site. This model identified the acid, the hydroxyl, and

the distal aromatic moiety in the 4-position of GHB as the structural elements important for affinity, and which can rationalize the obtained SAR. In general, all structural modifications performed in this study for the HOCPCA core scaffold led to reduced affinity indicating that for this specific structural scaffold, HOCPCA represents the optimal conformation and fit for the specific binding sites. In contrast, using T-HCA as a core scaffold led to some of the most potent ligands for the GHB high-affinity binding sites reported to date. However, some general trends for limited steric and directional tolerability of the scaffold, accommodation of larger aromatic substituents in the 4-position of GHB and conformational preference were exposed. In the absence of knowledge about the binding site and thus the structural details of the binding sites in the form of a model of the protein, further insights into the structural determinants for binding are valuable. The pharmacophore model presented here, will aid in the continued efforts to design and synthesize even better GHB ligands for the molecular dissection and potentially future drug development.

Experimental Section

Chemistry

General procedures: Compounds **1**, **3a**,**b**, and **7a** were obtained from the commercial suppliers: **1**, Combi Blocks, Inc. (USA); **3a**, Combi Blocks, Inc. (USA); **3b**, Enamine Ltd. (Latvia); **7a**, Combi Blocks, Inc. (USA). Compounds **12**,[14] **13**,[20] **14**,[14] **15a**,**c**,[20] **19**,[33] **24b**,[23] **25d–f**,[22] **26**,[25] and **27**[25] were synthesized as described in the literature, while compounds **24a**,**c**[23] and **25c**[24] were synthesized after modified literature procedures. The spectroscopic data for compounds **24a**,[34] **24c**,[35] and **25c**[36] is in agreement with data previously reported in the literature. All reagents and solvents (reagent or chromatography grade) were obtained from commercial

suppliers and used without further purification. Air- and/or moisture-sensitive reactions were performed under a nitrogen or argon atmosphere using syringe-septum cap techniques and with the use of flame or oven dried glassware. Anhydrous solvents were obtained by using a solvent purification system (THF, CH_2Cl_2) or by storage over 3 or 4 Å molecular sieves. Thin layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates, and compounds were visualized using UV (254 and 366 nm) and $KMnO_4$, Ninhydrin or Anisaldehyde spray reagent. Flash Chromatography was carried out according to standard procedures using Merck silica gel 60 (0.040–0.063 mm). Melting points were recorded on a SRS OptiMelt apparatus in open capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR data were recorded on a Bruker Avance 400 MHz spectrometer equipped with a 5 mm PABBO BB(^{1}H, ^{19}F) Z-GRD probe or a Bruker Avance 600 MHz spectrometer equipped with a cryogenically cooled 5 mm CPDCH ^{13}C(^{1}H) Z-GRD probe, at 300 K. Data are tabulated in the following order: chemical shift (δ) [multiplicity (b, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant(s) *J* (Hz), number of protons]. The solvent residual peak was used as internal reference.[37] Analytical High performance liquid chromatography (HPLC) was performed on a Merck-Hitachi HPLC system consisting of an L-7100 pump, an L-7200 autosampler, and an L-7400 UV detector (210 or 254 nm), using a Chromolith SpeedROD RP-18 column (4.6 × 50 mm). A linear gradient elution was performed with eluent A (H_2O/TFA 100:0.1) containing 0% of solvent B (MeCN/H_2O/TFA, 90:10:0.01) rising to 100% of B during 5 minutes with a flow rate of 4.0 $mLmin^{-1}$. Data were acquired and processed using the EZChrom Elite Software version 3.1.7 by Hitachi. The purity of the analyzed compounds are ≥95%, unless otherwise stated. Determination of enantiomeric excess (*ee*) and purity for compounds (+)-**9e** and **(**–)-**9e** were performed using an Ultimate 3000 HPLC system with a PG-3200 pump, a Rheodyne

injector 9725i, a 10 mL loop, and an MWD-3000SD detector (254 nm) using a Chiralpak IF (5 µm, 4.6 × 250 mm) column and a mobile phase consisting of *n*-heptane/2-PrOH/TFA (95:5:0.1) at a flow rate of 1 $mLmin^{-1}$. For HPLC control, data collection, and data handling Chromeleon software ver. 6.80 was used. The *ee* and purity of the analyzed compounds are ≥95%, unless otherwise stated. Preparative reversed-phase HPLC was carried out on an Ultimate 3000 HPLC system (Dionex) with a multi-wavelength UV detector (210 or 254 nm) and 10 mL loop using a preparative RP Phenomenex Gemini NX-C_{18} column (5 mm, 21.2 × 250 mm) using eluent A (H_2O/TFA, 100:0.1) and eluent B (MeCN/H_2O/TFA, 90:10:0.1) at a flow rate of 20 $mLmin^{-1}$ For HPLC control, data collection, and data handling, Chromeleon Software ver. 6.80 was used. Chiral preparative HPLC was carried out on an Ultimate 3000 HPLC system with a PG-3200 pump, a Rheodyne injector 9725i, a 10 mL loop, and an MWD-3000SD detector (254 nm) using a Chiralpak IF (5 µm, 10 × 250 mm) column and a mobile phase consisting of *n*-heptane/2-PrOH/TFA (95:5:0.1) at a flow rate of 6 $mLmin^{-1}$. For HPLC control, data collection, and data handling Chromeleon software ver. 6.80 was used. Optical rotation was recorded on an Anton Paar Modular Circular Polarimeter MCP300 with the temperature set to 25 °C. The calculated specific optical rotations were the average of three separate readings. Elemental analyses were performed by Mr. J. Theiner, Department of Physical Chemistry, University of Vienna, Austria, and are within ±0.4% of the calculated values, unless otherwise stated.

***cis*-4-hydroxybicyclo[3.1.0]hexane-1-carboxylic acid (3).** Under a nitrogen atmosphere, a mixture of 3Å molecular sieves and Et_2Zn (1.1M in toluene, 24.7 mL, 27.2 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C was added CH_2I_2 (2.19 mL, 27.2 mmol) drop wise. The mixture was stirred for 10 min before a solution of compound **14**[14] (1.1 g, 4.5 mmol) in anhydrous CH_2Cl_2 (40

mL) with 3 Å molecular sieves was added rapidly at 0 °C. The reaction mixture was allowed to reach rt. and stirred for 20 h. H_2O (50 mL) and brine (20 mL) were added and the mixture was acidified with HCl (aq. 1M) before it was extracted with EtOAc (4 × 100 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 3:1 + 1% AcOH) afforded 4-((*tert*-butyldimethylsilyl)oxy)bicyclo[3.1.0]hexane-1-carboxylic acid (295 g, 25%) as white solid: mp 97 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.56–4.48 (m, 1H), 2.15–2.01 (m, 2H), 1.92–1.83 (m, 2H), 1.42–1.36 (m, 1H), 1.31–1.26 (m, 1H), 1.26–1.17 (m, 1H), 0.90 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 180.7, 72.8, 34.4, 29.7, 29.4, 25.9, 24.3, 18.2, 14.7, –4.6, –4.8. In a polypropylene vial, 4-((*tert*-butyldimethylsilyl)oxy)bicyclo[3.1.0]hexane-1-carboxylic acid (286 mg, 1.1 mmol) was dissolved in MeCN (16 mL) and aq. H_2SiF_6 (20–25% w/w, 0.66 mL) was added. The solution was stirred for 1 h at rt. before H_2O (20 mL) was added and the aqueous phase was extracted with Et_2O (3 × 20 mL) and EtOAc (2 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated. Purification by column chromatography (Heptane/EtOAc 1:1 + 1% AcOH) afforded **3** (112 g, 71%) as clear colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.99 (s, 1H), 4.61 (s, 1H), 4.35–4.27 (m, 1H), 2.01–1.90 (m, 1H), 1.82–1.66 (m, 3H), 1.12–0.98 (m, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 175.2, 70.8, 32.5, 29.0, 28.7, 24.8, 13.0. The free acid of **3** (112 mg, 0.8 mmol) was converted into the sodium salt by dissolving in EtOH (5 mL) and addition of NaOH (aq. 0.4935M, 1.598 mL, 0.8 mmol). The mixture was stirred for 15 min at rt. before it was evaporated *in vacuo* to afford the sodium salt of **3** (126 mg, 97%) as white solid: mp > 240 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 4.47–4.40 (m, 1H), 2.15–2.06 (m, 1H), 1.90–1.78 (m, 3H),

1.20–1.07 (m, 2H), 0.99–0.94 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 183.2, 73.9, 33.6, 32.5, 30.6, 27.8, 13.4. Purity by anal. HPLC: 99% (210 nm).

***cis*-Hydroxycyclohexane-1-carboxylic acid (*cis*-4c).** A solution of compound *cis*-**11** (33 mg, 0.1 mmol) in MeOH (1 mL) was added Pd/C (10% w/w, 10 mg). H_2 was bubbled through the mixture for 10 min and the resulting reaction mixture was stirred under a H_2 atmosphere at rt. for 2 h. The mixture was filtered and the filter was washed with MeOH (5 mL). The filtrate was evaporated *in vacuo* to afford compound *cis*-**4c** (20 mg, 99%) as white solid: mp 122–124 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 3.53 (tt, J = 4.2, 11.0 Hz, 1H), 2.32 (tt, J = 3.5, 12.1 Hz, 1H), 2.10–2.03 (m, 1H), 1.99–1.72 (m, 3H), 1.46–1.03 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ 177.2, 69.2, 41.8, 37.6, 34.4, 28.1, 23.2. Purity by anal. HPLC, >99% (210 nm).

***trans*-3-Hydroxycyclohexane-1-carboxylic acid (*trans*-4c).** A solution of compound *trans*-**11** (30 mg, 0.1 mmol) in MeOH (1 mL) was added (10% w/w, 11 mg). H_2 was bubbled through the mixture for 10 min and the resulting reaction mixture was stirred under a H_2 atmosphere at rt. for 2 h. The mixture was filtered and the filter was washed with MeOH (5 mL). The filtrate was evaporated *in vacuo* to afford compound *trans*-**4c** (18 mg, 97%) as white solid: mp 104–108 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 3.97 (q, J = 4.4 Hz, 1H), 2.77–2.67 (m, 1H), 1.88–1.63 (m, 4H), 1.63–1.44 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ 179.7, 66.8, 39.1, 36.5, 33.7, 29.2, 21.0. Purity by anal. HPLC: >99% (210 nm).

3-Hydroxy-2-methylcyclopent-1-enecarboxylic acid (5a). Compound **15a**[20] (0.54 g, 1.8 mmol) was dissolved in anhydrous THF (18 mL) and the solution was cooled to –78 °C. ^{t}BuLi (1.7M, 2.3 mL, 3.9 mmol) was added drop wise. The solution was allowed to warm to –50 °C over 2 h before cooled again to –78 °C. CO_2 (g) was bubbled through the solution for 20 min and the reaction mixture was stirred for another 15 min before sat. aq. NH_4Cl (20 mL) and Et_2O (30

mL) were added slowly. The mixture was allowed to warm to rt. before the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The resulting residue was redissolved in Et_2O and added HCl (4M in 1,4-dioxane). The solution was stirred at rt. for 2 h. before H_2O (10 mL) was added. The aqueous phase was extracted with Et_2O (3 × 50 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **5a** (98 mg, 37%) as white solid: mp 134–138 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 4.69 (t, J = 7.1 Hz, 1H), 2.58–2.67 (m, 1H), 2.35–2.45 (m, 1H), 2.22–2.29 (m, 1H), 2.08–2.09 (m, 3H), 1.57–1.66 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 169.7, 156.3, 130.2, 81.7, 32.7, 31.3, 13.6. Anal. calcd. ($C_7H_{10}O_3$): C, 59.14; H, 7.09. Found: C, 59.17; H, 6.92.

2-Ethyl-3-hydroxycyclopent-1-enecarboxylic acid (5b). Compound **15b** (0.51 g, 1.7 mmol) was dissolved in anhydrous THF (16 mL) and the solution was cooled to –78 °C. ^{t}BuLi (1.7M, 2.1 mL, 3.6 mmol) was added drop wise. The solution was allowed to warm to –50 °C over 2 h before cooled again to –78 °C. CO_2 (g) was bubbled through the solution for 20 min and the reaction mixture was stirred for 15 min before sat. aq. NH_4Cl (20 mL) and Et_2O (30 mL) were added slowly. The mixture was allowed to warm rt. before the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 4:1 + 2% AcOH) afforded 3-((*tert*-butyldimethylsilyl)oxy)-2-ethylcyclopent-1-ene-1-carboxylic acid (412 mg, 90%) as white solid: mp 92–102 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.22 (b s, 1H), 4.73 (t, J = 7.3 Hz, 1H), 2.71–2.66 (m, 1H), 2.60–2.52 (m, 1H), 2.40–2.26 (m, 2H), 2.16–2.09 (m, 1H), 1.60–1.50 (m, 1H), 0.97 (t, J = 7.5 Hz, 3H), 0.82 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H). ^{13}C NMR

(100 MHz, $CDCl_3$): δ 172.0, 164.4, 126.7, 79.5, 32.9, 29.8, 25.8, 20.3, 18.0, 12.6, –4.4, –5.0. In a polypropylene vial, 3-((*tert*-butyldimethylsilyl)oxy)-2-ethylcyclopent-1-ene-1-carboxylic acid (396 mg, 1.5 mmol) was dissolved in MeCN (15 mL) and aq. H_2SiF_6 (20–25% w/w, 226 μL, 0.4 mmol) was added. The solution was stirred for 1 h at rt. before sat. aq. Na_2CO_3 (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 20 mL). The aqueous phase was acidified (HCl, aq. 4M) and extracted with Et_2O (3 × 40 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 4:1 + 2% AcOH) followed by recrystallization from MeCN afforded compound **5b** (25 mg, 11%) as white solid: mp 122–128 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 4.75 (t, J = 6.0 Hz, 1H), 2.90–2.81 (m, 1H), 2.68–2.60 (m, 1H), 2.44–2.34 (m, 2H), 2.27–2.19 (m, 1H), 1.67–1.58 (m, 1H), 1.07 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 169.6, 161.6, 129.8, 79.5, 32.8, 31.4, 21.1, 12.9. Anal. calcd. ($C_8H_{12}O_3$): C, 61.52; H, 7.74. Found: C, 61.48; H, 7.69.

2-Allyl-3-hydroxycyclopent-1-enecarboxylic acid (5c). Compound **15c**[20] (333 mg, 1.1 mmol) was dissolved in anhydrous THF (10 mL) and the solution was cooled to –78 °C. ^{t}BuLi (1.7M, 1.3 mL, 2.2 mmol) was added drop wise. The solution was allowed to warm to –50 °C over 2 h before cooled again to –78 °C. CO_2 (g) was bubbled through the solution for 20 min and the reaction mixture was stirred for 15 min before sat. aq. NH_4Cl (20 mL) and Et_2O (30 mL) were added slowly. The mixture was allowed to warm to rt. before the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 6:1 + 2% AcOH) afforded 2-allyl-3-((*tert*-butyldimethylsilyl)oxy)cyclopent-1-ene-1-carboxylic acid (166 mg, 56%) as white solid: mp 50–52 °C. ^{1}H NMR (400 MHz, $CDCl_3$):

δ 11.64 (b s, 1H), 5.77–5.67 (m, 1H), 5.01–4.87 (m, 2H), 4.75–4.68 (m, 1H), 3.61–3.51 (m, 1H), 3.06–2.97 (m, 1H), 2.67–2.54 (m, 1H), 2.40–2.28 (m, 1H), 2.19–2.09 (m, 1H), 1.66–1.49 (m, 1H), 0.82 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 158.5, 133.5, 127.2, 115.1, 78.2, 31.7, 30.1, 28.9, 24.8, 17.0, –5.3, –5.9. In a polypropylene vial, 2-allyl-3-((*tert*-butyldimethylsilyl)oxy)cyclopent-1-ene-1-carboxylic acid (161 mg, 0.6 mmol) was dissolved in MeCN (6 mL) and aq. H_2SiF_6 (20–25% w/w, 88 μL, 0.2 mmol) was added. The solution was stirred for 1 h at rt. before sat. Na_2CO_3 (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 20 mL). The aqueous phase was acidified (HCl, aq. 4M) and extracted with Et_2O (3 × 40 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 4:1 + 2% AcOH) followed by recrystallization from MeCN afforded compound **5c** (47 mg, 49%) as white solid: mp 80–86 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 5.91–5.78 (m, 1H), 5.12–5.04 (m, 1H), 5.02–4.96 (m, 1H), 4.74–4.67 (m, 1H), 3.69 (dd, *J* = 5.8, 14.0 Hz, 1H), 3.07 (dd, *J* = 7.5, 14.0 Hz, 1H), 2.72–2.61 (m, 1H), 2.48–2.37 (m, 1H), 2.28–2.17 (m, 1H), 1.70–1.59 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 169.5, 156.8, 136.1, 131.1, 116.6, 79.5, 32.8, 32.3, 31.6. Anal. calcd. ($C_9H_{12}O_3$): C, 64.27; H, 7.19. Found: C, 63.89; H, 7.25.

2-Benzyl-3-hydroxycyclopent-1-enecarboxylic acid (5d). Compound **13**[20] (0.41 g, 1.0 mmol) was dissolved in anhydrous THF (10 mL) and cooled to –78 °C. ^{n}BuLi (1.57M, 0.75 mL, 1.2 mmol) was added drop wise and the solution was stirred for 15 min. Benzyl bromide (133 μL, 1.1 mmol) was added and the solution was allowed to warm to –30 °C over 4 h. Sat. NH_4Cl (30 mL) and Et_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic phases were washed with brine (2 × 10 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo* to afford the crude of compound **15d** (0.32 g) (the

presence was confirmed by ^{1}H NMR analysis). The crude **15d** was dissolved in anhydrous THF (9 mL) and the solution was cooled to –78 °C. ^{t}BuLi (1.7M, 1.1 mL, 1.9 mmol) was added drop wise. The solution was allowed to warm to –50 °C over 2 h before cooled again to –78 °C. CO_2 (g) was bubbled through the solution for 10 min. The solution was stirred for another 15 min before sat. aq. NH_4Cl (20 mL) and Et_2O (30 mL) were added slowly. The mixture was allowed to warm to rt. before the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 4:1 + 2% AcOH) afforded 2-benzyl-3-((*tert*-butyldimethylsilyl)oxy)cyclopent-1-ene-1-carboxylic acid (184 mg, 55% over 2 steps) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.53 (b s, 1H), 7.34–7.16 (m, 5H), 4.73 (t, J = 6.8 Hz, 1H), 4.55 (d, J = 13.7 Hz, 1H), 3.61 (d, J = 13.7 Hz, 1H), 2.85–2.74 (m, 1H), 2.59–2.46 (m, 1H), 2.29–2.18 (m, 1H), 1.80–1.66 (m, 1H), 0.93 (s, 9H), 0.03 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 171.9, 160.2, 138.4, 128.9, 128.9, 128.4, 126.2, 78.7, 32.8, 32.3, 30.2, 25.9, 18.0, –4.2, –4.9. In a polypropylene vial, 2-Benzyl-3-((*tert*-butyldimethylsilyl)oxy)cyclopent-1-ene-1-carboxylic acid (176 mg, 0.5 mmol) was dissolved in MeCN (5 mL) and aq. H_2SiF_6 (20–25% w/w, 82 µL, 0.2 mmol) was added. The solution was stirred for 1 h at rt. before sat. aq. Na_2CO_3 (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 20 mL). The aqueous phase was acidified (HCl, aq. 4M) and extracted with Et_2O (3 × 40 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN provided compound **5d** (66 mg, 57%) as white solid: mp 127–134 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.30–7.11 (m, 5H), 4.55–4.46 (m, 2H), 3.54–3.46 (m, 1H), 2.78–2.65 (m, 1H), 2.20–2.09 (m, 1H), 1.72–1.59 (m, 1H). ^{13}C

NMR (100 MHz, CD_3OD): δ 169.5, 157.7, 140.3, 131.4, 130.1, 129.5, 127.3, 78.9, 33.3, 32.8, 31.8. Anal. calcd. ($C_{13}H_{14}O_3 \cdot 0.1H_2O$): C, 70.96; H, 6.50. Found: C, 71.04; H, 6.47.

3-Hydroxy-2-(3-methoxybenzyl)cyclopent-1-enecarboxylic acid (5e). Compound **15e** (0.45 g, 1.1 mmol) was dissolved in anhydrous THF (10 mL) and the solution was cooled to –78 °C. ^{t}BuLi (1.7M, 1.38 mL, 2.4 mmol) was added drop wise. The solution was allowed to warm to –50 °C over 2 h before cooled again to –78 °C. CO_2 (g) was bubbled through the solution for 10 min and the solution was stirred for another 15 min before sat. aq. NH_4Cl (20 mL) was added slowly. The reaction was allowed to warm to rt. before the aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 6:1 + 2% AcOH) afforded 3-((*tert*-butyldimethylsilyl)oxy)-2-(2-methoxybenzyl)cyclopent-1-ene-1-carboxylic acid (332 mg, 82%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.05 (b s, 1H), 7.18 (t, J = 7.9 Hz, 1H), 6.85–6.70 (m, 3H), 4.73 (t, J = 6.9 Hz, 1H), 4.51 (d, J = 13.8 Hz, 1H), 3.76 (s, 3H), 3.56 (d, J = 13.8 Hz, 1H), 2.83–2.72 (m, 1H), 2.56–2.44 (m, 1H), 2.26–2.15 (m, 1H), 1.76–1.64 (m, 1H), 0.93 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 159.8, 159.6, 139.8, 129.1, 128.8, 121.3, 114.7, 111.3, 78.6, 54.9, 32.6, 32.1, 30.1, 25.8, 17.9, –4.3, –5.1. In a polypropylene vial, 3-((*tert*-butyldimethylsilyl)oxy)-2-(2-methoxybenzyl)cyclopent-1-ene-1-carboxylic acid (0.33 g, 0.9 mmol) was dissolved in MeCN (9 mL) and aq. H_2SiF_6 (20–25% w/w, 140 μL, 0.3 mmol) was added. The solution was stirred for 1 h at rt. before sat. aq. Na_2CO_3 (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 20 mL). The aqueous phase was acidified (HCl, aq. 4M) and extracted with Et_2O (3 × 40 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **5e** (92 mg, 28%) as white solid: mp 111–116

°C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.18–7.12 (m, 1H), 6.87–6.71 (m, 3H), 4.56–4.51 (m, 1H), 4.47 (d, *J* = 13.6 Hz, 1H), 3.75 (s, 3H), 3.47 (d, *J* = 13.6 Hz, 1H), 2.77–2.66 (m, 1H), 2.50–2.40 (m, 1H), 2.21–2.10 (m, 1H), 1.70–1.59 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 169.6, 161.4, 157.6, 141.8, 131.5, 130.4, 122.5, 115.8, 112.8, 78.9, 55.7, 33.3, 32.8, 31.8. Anal. calcd. ($C_{14}H_{16}O_3 \cdot 0.3H_2O$): C, 66.28; H, 6.60. Found: C, 66.31; H, 6.57.

3-Hydroxy-2-phenylcyclopent-1-ene-1-carboxylic acid (5f). Pyridinium *p*-toluenesulfonate (13 mg, 0.05 mmol) was added to a solution of compound **18f** (0.48 g, 1.6 mmol) in MeOH (6 mL). The resulting mixture was left at 50 °C for 24 h before aq. sat. $NaHCO_3$ (40 mL) and H_2O (20 mL) were added. The aqueous phase was extracted with Et_2O (3 × 100 mL) and the combined organic phases were washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/Et_2O 1:1 + 1% AcOH) afforded methyl 3-hydroxy-2-phenylcyclopent-1-ene-1-carboxylate (280 mg, 80%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17–6.94 (m, 5H), 6.86–6.80 (m, 1H), 4.15–4.08 (m, 1H), 3.93–3.88 (m, 1H), 3.45 (s, 3H), 2.82–2.72 (m, 1H), 2.35–2.26 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 142.4, 140.6, 136.7, 128.3, 126.8, 126.4, 79.5, 60.1, 51.2, 41.2. Methyl 3-hydroxy-2-phenylcyclopent-1-ene-1-carboxylate (280 mg, 1.3 mmol) was dissolved in THF (2 mL) and LiOH (aq. 2M, 1.7 mL) was added. The resulting mixture was stirred at rt. for 24 h before H_2O (15 mL) was added and the mixture was washed with Et_2O (3 × 2 mL). The pH of the aqueous phase was adjusted to 1 with HCl (aq. 12M) and the aqueous phase was extracted with EtOAc (4 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **5f** (174 mg, 65%) as white solid: mp 136–140 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.30–7.24 (m, 2H), 7.21–7.15 (m, 1H), 7.15–7.11 (m, 2H), 6.98–6.95 (m, 1H), 4.21–4.17 (m, 1H), 3.99–3.96 (m, 1H),

3.00–2.91 (m, 1H), 2.48–2.40 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 168.1, 143.8, 142.9, 139.2, 129.7, 128.3, 127.7, 81.0, 61.9, 42.5. Anal. calcd. ($C_{12}H_{12}O_3$): C, 70.57; H, 5.92. Found: C, 70.33; H, 5.77.

2-(4-Chlorophenyl)-3-hydroxycyclopent-1-ene-1-carboxylic acid (5g). Pyridinium *p*-toluenesulfonate (20 mg, 0.08 mmol) was added to a solution of Compound **18g** (0.84 g, 2.5 mmol) in MeOH (10 mL). The resulting mixture was left at 50 °C for 24 h before aq. sat. $NaHCO_3$ (40 mL) and H_2O (20 mL) were added. The aqueous phase was extracted with Et_2O (3 × 100 mL) and the combined organic phases were washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/Et_2O 1:1 + 1% AcOH) provided methyl 2-(4-chlorophenyl)-3-hydroxycyclopent-1-ene-1-carboxylate (0.59 g, 93%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (d, J = 8.5 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.29–7.25 (m, 1H), 4.54–4.48 (m, 1H), 4.32–4.28 (m, 1H), 3.91 (s, 3H), 3.25–3.16 (m, 1H), 2.80–2.70 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.7, 142.7, 139.2, 136.2, 132.0, 128.3, 128.1, 79.2, 59.2, 51.2, 41.1. Methyl 2-(4-chlorophenyl)-3-hydroxycyclopent-1-ene-1-carboxylate (0.59 g, 2.4 mmol) was dissolved in THF (4 mL) and LiOH (aq. 2M, 3 mL) was added. The resulting mixture was stirred at rt. for 24 h before H_2O (15 mL) was added and the mixture was washed with Et_2O (3 × 2 mL). The pH of the aqueous phase was adjusted to 1 with HCl (aq. 12M) and the aqueous phase was extracted with EtOAc (4 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **5g** (300 mg, 53%) as white solid: mp 184–187 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.30–7.25 (m, 2H), 7.14–7.09 (m, 2H), 6.99–6.95 (m, 1H), 4.20–4.15 (m, 1H), 3.96–3.93 (m, 1H), 3.01–2.91 (m, 1H), 2.49–2.41 (m, 1H). ^{13}C

NMR (100 MHz, CD_3OD): δ 167.9, 144.2, 141.8, 138.9, 133.4, 130.0, 129.7, 80.9, 61.3, 42.4. Anal. calcd. ($C_{12}H_{11}ClO_3 \cdot 0.25H_2O$): C, 59.27; H, 4.77. Found: C, 59.07; H, 4.47.

3-Hydroxy-2-(*p*-tolyl)cyclopent-1-ene-1-carboxylic acid (5h). Pyridinium *p*-toluenesulfonate (20 mg, 0.08 mmol) was added to a solution of compound **18h** (0.85 g, 2.7 mmol) in MeOH (10 mL). The resulting mixture was left at 50 °C for 24 h before aq. sat. $NaHCO_3$ (40 mL) and H_2O (20 mL) were added. The aqueous phase was extracted with Et_2O (3 × 100 mL) and the combined organic phases were washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/Et_2O 1:1 + 1% AcOH) provided methyl 3-hydroxy-2-(*p*-tolyl)cyclopent-1-ene-1-carboxylate (0.52 g, 82%) as yellow oil. ^1H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.29–7.24 (m, 1H), 4.58–4.53 (m, 1H), 4.35–4.30 (m, 1H), 3.93 (s, 3H), 3.26–3.17 (m, 1H), 2.80–2.71 (m, 1H), 2.63 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 142.2, 137.5, 136.9, 135.8, 129.0, 126.6, 79.5, 59.7, 51.2, 41.2, 20.7. Methyl 3-hydroxy-2-(*p*-tolyl)cyclopent-1-ene-1-carboxylate (0.52 g, 2.3 mmol) was dissolved in THF (4 mL) and LiOH (aq. 2M, 3 mL) was added. The resulting mixture was stirred at rt. for 24 h before H_2O (15 mL) was added and the mixture was washed with Et_2O (3 × 2 mL). The pH of the aqueous phase was adjusted to 1 with HCl (aq. 12M) and aqueous phase was extracted with EtOAc (4 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN provided the compound **5h** (360 mg, 72%) as white solid: mp 184–188 °C. ^1H NMR (400 MHz, CD_3OD): δ 7.10–7.06 (m, 2H), 7.03–6.99 (m, 2H), 6.96–6.93 (m, 1H), 4.18–4.15 (m, 1H), 3.95–3.92 (m, 1H), 2.97–2.88 (m, 1H), 2.46–2.39 (m, 1H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 168.2, 143.6, 139.7, 139.3, 137.3, 130.3, 128.2, 81.0, 61.5, 42.4, 21.2. Anal. calcd. ($C_{13}H_{14}O_3$): C, 71.54; H, 6.47. Found: C, 71.49; H, 6.30.

3-Hydroxy-2-(4-methoxyphenyl)cyclopent-1-ene-1-carboxylic acid (5i). Pyridinium *p*-toluenesulfonate (14 mg, 0.06 mmol) was added to a solution of compound **18i** (0.63 g, 1.9 mmol) in MeOH (7 mL). The resulting mixture was left at 50 °C for 24 h before aq. sat. $NaHCO_3$ (40 mL) and H_2O (20 mL) were added. The aqueous phase was extracted with Et_2O (3 × 100 mL) and the combined organic phases were washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/Et_2O 1:1 + 1% AcOH) provided methyl 3-hydroxy-2-(4-methoxyphenyl)cyclopent-1-ene-1-carboxylate (0.34 g, 72%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38–7.33 (m, 2H), 7.29–7.25 (m, 1H), 7.17–7.12 (m, 2H), 4.59–4.55 (m, 1H), 4.33–4.30 (m, 1H), 4.09 (s, 3H), 3.95 (s, 3H), 3.29–3.19 (m, 1H), 2.82–2.73 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 158.1, 142.0, 137.0, 132.7, 127.8, 113.8, 79.6, 59.4, 54.9, 51.2, 41.2. Methyl 3-hydroxy-2-(4-methoxyphenyl)cyclopent-1-ene-1-carboxylate (0.34 g, 1.4 mmol) was dissolved in THF (2 mL) and LiOH (aq. 2M, 2 mL) was added. The resulting mixture was stirred at rt. for 24 h before H_2O (15 mL) was added and the mixture was washed with Et_2O (3 × 2 mL). The pH of the aqueous phase was adjusted to 1 with HCl (aq. 12M) and the aqueous phase was extracted with EtOAc (4 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Et_2O + 1% AcOH) followed by recrystallization from MeCN provided compound **5i** (230 mg, 69%) as white solid: mp 189–193 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.06–7.02 (m, 2H), 6.95–6.92 (m, 1H), 6.86–6.81 (m, 2H), 4.18–4.14 (m, 1H), 3.93–3.91 (m, 1H), 3.75 (s, 3H), 2.98–2.89 (m, 1H), 2.46–2.38 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 168.2, 160.1, 143.4, 139.5, 134.8, 129.3, 115.1, 81.0, 61.1, 55.8, 43.4. Anal. calcd. ($C_{13}H_{14}O_4$): C, 66.66; H, 6.02. Found: C, 66.42; H, 5.83.

2-(3,4-Dichlorophenyl)-3-hydroxycyclopent-1-ene-1-carboxylic acid (5j). Pyridinium *p*-toluenesulfonate (16 mg, 0.06 mmol) was added to a solution of compound **18j** (0.68 g, 1.8 mmol) in MeOH (8 mL). The resulting mixture was left at 50 °C for 24 h before aq. sat. $NaHCO_3$ (40 mL) and H_2O (20 mL) were added. The aqueous phase was extracted with Et_2O (3 × 100 mL) and the combined organic phases were washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/Et_2O 1:1 + 1% AcOH) provided methyl 2-(3,4-dichlorophenyl)-3-hydroxycyclopent-1-ene-1-carboxylate (0.47 g, 88%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J* = 8.3 Hz, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.29–7.25 (m, 1H), 7.23 (dd, *J* = 2.0, 8.3 Hz, 1H), 4.53–4.46 (m, 1H), 4.28–4.21 (m, 1H), 3.92 (s, 3H), 3.26–3.15 (m, 1H), 2.81–2.71 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.5, 143.3, 141.2, 135.8, 132.2, 130.3, 130.2, 128.7, 126.2, 79.1, 59.2, 51.4, 41.2. Methyl 2-(3,4-dichlorophenyl)-3-hydroxycyclopent-1-ene-1-carboxylate (0.47 g, 1.7 mmol) was dissolved in THF (4 mL) and LiOH (aq. 2M, 2 mL) was added. The resulting mixture was stirred at rt. for 24 h before H_2O (15 mL) was added and the mixture was washed with Et_2O (3 × 2 mL). The pH of the aqueous phase was adjusted to 1 with HCl (aq. 12M) and the aqueous phase was extracted with EtOAc (4 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Et_2O + 1% AcOH) followed by recrystallization from MeCN provided compound **5j** (270 mg, 60%) as white solid: mp 162–165 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.42 (d, *J* = 8.3 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 7.06 (dd, *J* = 2.0, 8.3 Hz, 1H), 7.01–6.97 (m, 1H), 4.22–4.17 (m, 1H), 3.95–3.91 (m, 1H), 3.02–2.92 (m, 1H), 2.50–2.42 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 167.7, 144.8, 144.1, 138.3, 133.4, 131.8, 131.4, 130.5, 128.5, 80.7, 61.1, 42.4. Purity by anal. HPLC: 99% (254 nm). Anal. calcd. ($C_{12}H_{10}Cl_2O_4$): C, 52.77; H, 3.69. Found: C, 52.83; H, 3.64.

3-Hydroxy-3-phenylcyclopentanecarboxylic acid (6a). Under a nitrogen atmosphere, a solution of compound **20** (300 mg, 2.3 mmol) in anhydrous THF (8 mL) was cooled to –78 °C whereupon PhMgBr (1M, 11.72 mL, 11.7 mmol,) was added drop wise. The resulting solution was stirred at –78 °C for 2 h before it was allowed to reach rt. and stirred at this temperature overnight. The reaction was quenched with H_2O (3 mL) and evaporated *in vacuo*. Purification by preparative TLC (Heptane/EtOAc 1:1 + 1% AcOH) provided compound **6a** (129 mg, 27%) as yellow solid: mp 67–71 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.52–7.44 (m, 2H), 7.35–7.27 (m, 2H), 7.25–7.17 (m, 1H), 3.13–3.02 (m, 1H), 2.38–2.20 (m, 3H), 2.19–2.09 (m, 1H), 2.08–2.02 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 180.3, 148.0, 129.3, 127.9, 126.4, 83.8, 45.8, 44.2, 43.0, 29.2. Purity by anal. HPLC: 96% (254 nm).

3-([1,1'-Biphenyl]-4-yl)-3-hydroxycyclopentanecarboxylic acid (6b). A solution of 4-bromo-1,1'-biphenyl (2.19 g, 9.4 mmol) in anhydrous THF (5 mL) was added to a mixture of Mg turnings (0.45 g, 18.8 mmol) and a catalytic iodine crystal anhydrous THF (5 mL). The resulting solution was gently heated with a heating gun until the mixture turned clear and colorless. Then an additional solution of 4-bromo-1,1'-biphenyl (2.19 g, 9.4 mmol) in anhydrous THF (5 mL) was added and the reaction mixture was refluxed for 2 h. Upon cooling to rt., the Grignard reagent was added drop wise to a solution of compound **20** (0.30 g, 2.3 mmol) in THF (5 mL) at –78 °C. The resulting reaction mixture was allowed to reach rt. overnight before quenching with H_2O (6 mL). The solvents were evaporated *in vacuo* and purification by column chromatography (Hexane/EtOAc 3:1 + 1% AcOH) afforded compound **6b** (115 mg, 17%) as white solid: mp 137–139 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.65–7.55 (m, 6H), 7.48–7.41 (m, 2H), 7.37–7.31 (m, 1H), 3.18–3.07 (m, 1H), 2.44–2.28 (m, 3H), 2.25–2.04 (m, 3H). ^{13}C NMR (150 MHz,

DMSO-d_6): δ 176.7, 147.1, 138.0, 128.9, 127.2, 126.5, 126.1, 125.8, 125.7, 81.5, 44.6, 42.4, 41.9, 27.5. Purity by anal. HPLC: 95% (254 nm).

3-Phenylcyclopent-1-enecarboxylic acid (7b). Under a nitrogen atmosphere, a mixture of compound **21** (87 mg, 0.4 mmol), $Pd(OAc)_2$ (4.5 mg, 0.02 mmol), phenylboronic acid (98 mg, 0.8 mmol), and KF (106 mg, 1.8 mmol) in 1,4-dioxane (5 mL) was stirred at rt. After 24 h, the solvent was evaporated *in vacuo* and purification by column chromatography (Petroleum ether 40–65 °C/Et_2O 9:1) afforded methyl 3-phenylcyclopent-1-ene-1-carboxylate (51 mg, 60%) as a colorless oil. ^{1}H NMR (600 MHz, CD_3OD): δ 7.35–7.28 (m, 2H), 7.25–7.20 (m, 1H), 7.19–7.15 (m, 2H), 6.79 (q, J = 2.1 Hz, 1H), 4.10–4.05 (m, 1H), 3.77 (s, 3H), 2.81–2.72 (m, 1H), 2.71–2.64 (m, 1H), 2.58–2.51 (m, 1H), 1.96–1.88 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.9, 146.0, 144.3, 137.3, 128.8, 127.4, 126.7, 52.0, 51.7, 33.9, 31.6. Methyl 3-phenylcyclopent-1-ene-1-carboxylate (51 mg, 0.3 mmol) was dissolved in THF (2 mL) and H_2O (1.5 ml) and added LiOH (aq. 2M, 3.5 mL). The resulting mixture was stirred at rt. for 72 h before the pH was adjusted to 1 with HCl (aq. 1M). The aqueous phase was extracted with Et_2O (2 × 100 mL) and the combined organic phases were evaporated *in vacuo.* The resulting solid was washed with ice-cold MeCN to afford compound **7b** (6.3 mg, 13%) as yellow solid: mp 86–88 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.33–7.29 (m, 2H), 7.22–7.15 (m, 3H), 6.76–6.73 (m, 1H), 4.12–4.07 (m, 1H), 2.77–2.69 (m, 1H), 2.66–2.58 (m, 1H), 2.58–2.51 (m, 1H), 1.91–1.85 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 169.3, 147.5, 146.1, 139.5, 130.1, 128.6, 128.0, 53.6, 35.4, 32.8. Purity by anal. HPLC: 99% (254 nm).

3-(2-Hydroxyphenyl)cyclopent-1-enecarboxylic acid (7c). Under a nitrogen atmosphere, a mixture of compound **21** (79 mg, 0.4 mmol), $Pd(OAc)_2$ (11 mg, 0.05 mmol), (2-hydroxy-phenyl)boronic acid (82 mg, 0.6 mmol), and KF (206 mg, 3.5 mmol) in 1,4-dioxane (5 mL) was

stirred at rt. After 72 h, the solvent was evaporated *in vacuo* and purification by column chromatography (Petroleum ether 40–65 °C/EtOAc 3:1) provided methyl 3-(2-hydroxyphenyl)cyclopent-1-ene-1-carboxylate (25 mg, 30%) as colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.14–7.01 (m, 2H), 6.88 (td, J = 1.2, 7.5 Hz, 1H), 6.84 (q, J = 2.1 Hz, 1H), 6.76 (dd, J = 1.2, 8.0 Hz, 1H), 4.39–4.34 (m, 1H), 3.78 (s, 3H), 2.82–2.76 (m, 1H), 2.71–2.61 (m, 1H), 2.60–2.54 (m, 1H), 1.94–1.87 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.9, 153.1, 145.8, 137.5, 130.2, 128.2, 127.8, 121.2, 115.6, 51.7, 45.8, 32.1, 31.5. Methyl 3-(2-hydroxyphenyl)cyclopent-1-ene-1-carboxylate (25 mg, 0.1 mmol) was dissolved in THF (4 mL) and added LiOH (aq. 2M, 4 mL). The resulting mixture was stirred at rt. for 24 h before the alkaline solution was washed with Et_2O (10 mL) and the pH was adjusted to 1 with HCl (aq. 1M). The aqueous phase was extracted with EtOAc (2 × 100 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo* to afford compound **7c** as yellow oil (21 mg, 86%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.06–6.95 (m, 2H), 6.80–6.69 (m, 3H), 4.43–4.37 (m, 1H), 2.72–2.65 (m, 1H), 2.62–2.55 (m, 1H), 2.54–2.46 (m, 1H), 1.85–1.78 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 167.5, 154.4, 146.5, 136.9, 130.2, 127.0, 126.9, 119.3, 114.6, 45.1, 31.8, 30.7. Purity by anal. HPLC (254 nm), 98%.

3-([1,1'-Biphenyl]-4-yl)cyclopent-1-enecarboxylic acid (7d). Under a nitrogen atmosphere, a mixture of compound **21** (90 mg, 0.4 mmol), $Pd(OAc)_2$ (7 mg, 0.03 mmol), (4-(benzyloxy)phenyl)boronic acid (137 mg, 0.7 mmol), and KF (172 mg, 3.0 mmol) in 1.4-dioxane (4 mL) was stirred at rt. After 24 h, the solvent was evaporated *in vacuo* and purification by column chromatography (Petroleum ether 40–65 °C/Et_2O 9:1) afforded methyl 3-([1,1'-biphenyl]-4-yl)cyclopent-1-ene-1-carboxylate as colorless oil (65 mg, 53%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.61–7.50 (m, 4H), 7.48–7.39 (m, 2H), 7.38–7.34 (m, 1H), 7.28–7.21 (m, 2H),

6.81 (q, *J* = 2.1 Hz, 1H), 4.14–4.09 (m, 1H), 3.78 (s, 3H), 2.86–2.75 (m, 1H), 2.73–2.66 (m, 1H), 2.61–2.54 (m, 1H), 2.00–1.92 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 166.1, 146.1, 143.6, 141.3, 140.0, 137.6, 129.1, 128.0, 127.8, 127.5, 127.4, 51.9, 51.8, 34.1, 31.8. Methyl 3-([1,1'-biphenyl]-4-yl)cyclopent-1-ene-1-carboxylate (65 mg, 0.2 mmol) was dissolved in THF (2 mL) and H_2O (1.5 mL) and added LiOH (aq. 2M, 3.5 mL). The resulting mixture was was stirred at rt. overnight before the pH was adjusted to 1 with HCl (aq. 1M). The aqueous phase was extracted with Et_2O (3 × 100 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo* to afford compound **7d** (63 mg, 98%) as yellow solid: mp 135−138 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.62–7.54 (m, 4H), 7.44–7.38 (m, 2H), 7.33–7.29 (m, 1H), 7.29–7.23 (m, 2H), 6.77 (q, *J* = 2.1 Hz, 1H), 4.15–4.10 (m, 1H), 2.82–2.71 (m, 1H), 2.69–2.60 (m, 1H), 2.60–2.52 (m, 1H), 1.95–1.87 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 167.4, 145.6, 143.4, 140.8, 139.4, 137.7, 128.4, 127.3, 126.8, 126.8, 126.4, 51.4, 33.5, 31.0. Purity by anal. HPLC: 98% (254 nm).

3-(4-(Benzyloxy)phenyl)cyclopent-1-enecarboxylic acid (7e). Under a nitrogen atmosphere, a mixture of compound **21** (56 mg, 0.3 mmol), $Pd(OAc)_2$ (4.5 mg, 0.02 mmol), (4-(benzyloxy)-phenyl)boronic acid (98 mg, 0.4 mmol), and KF (111 mg, 1.9 mmol) in 1,4-dioxane (5 mL) was stirred at rt. After 24 h, the solvent was evaporated *in vacuo* and purification by column chromatography (Petroleum ether 40–65 °C/Et_2O 9:1) afforded methyl 3-(4-(benzyloxy)phenyl)cyclopent-1-ene-1-carboxylate (61 mg, 73%) as colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.43 (d, *J* = 7.4 Hz, 2H), 7.38 (t, *J* = 7.4, 2H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.08 (d, *J* = 8.3 Hz, 2H), 6.94 (d, *J* = 8.3 Hz, 2H), 6.79–6.77 (m, 1H), 5.05 (s, 2H), 4.04–4.00 (m, 1H), 3.76 (s, 3H), 2.83–2.70 (m, 1H), 2.69–2.58 (m, 1H), 2.54–2.48 (m, 1H), 1.93–1.74 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 166.2, 157.9, 146.6, 137.5, 137.2, 137.0, 128.9, 128.6, 128.3, 127.8, 115.4,

70.5, 51.9, 51.5, 34.2, 31.7. Methyl 3-(4-(benzyloxy)phenyl)cyclopent-1-ene-1-carboxylate (47 mg, 0.2 mmol) was dissolved in THF (3 mL) and LiOH (aq. 2M, 4 mL) was added. The resulting mixture was stirred at rt. for 3 days before the pH was adjusted to 1 with HCl (aq. 1M). The aqueous phase was extracted with Et_2O (2 × 100 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo.* The resulting solid washed with ice-cold MeCN to afford compound **7e** (25 mg, 56%) as white solid: mp 147–148 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.44 (d, J = 7.2 Hz, 2H), 7.39–7.31 (m, 2H), 7.32–7.26 (m, 1H), 7.11–7.04 (m, 2H), 6.96–6.90 (m, 2H), 6.70 (q, J = 2.1 Hz, 1H), 5.05 (s, 2H), 4.06–4.01 (m, 1H), 2.76–2.69 (m, 1H), 2.65–2.57 (m, 1H), 2.54–2.47 (m, 1H), 1.88–1.80 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 168.8, 159.0, 147.7, 138.9, 138.5, 138.0, 129.5, 129.2, 128.8, 128.5, 116.1, 71.0, 52.5, 35.0, 32.3. Purity by anal. HPLC (254 nm), 95%.

3-(1-Hydroxycyclobutyl)propiolic acid (8a). A solution of **24a** (0.32 g, 1.9 mmol) in THF (2 mL) was added aqueous LiOH (2N, 4 mL). The resulting solution was stirred at rt. for 4 h before water (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 10 mL). The aqueous phase was acidified with aqueous HCl (4N) and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **8a** (115 mg, 43%) as white crystals: mp 88–90 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 2.50–2.43 (m, 2H), 2.34–2.25 (m, 2H), 1.94–1.84 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 155.0, 90.2, 74.7, 66.6, 37.4, 12.3. Anal. calcd. ($C_7H_8O_3$): C, 60.00; H, 5.75. Found: C, 59.91; H, 5.70.

3-(1-Hydroxycyclopentyl)propiolic acid (8b). A solution of **24b**[23] (0.50 g, 2.7 mmol) in THF (6 mL) was added aqueous LiOH (2N, 6 mL). The resulting mixture was stirred at rt. for 4 h before water (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 10 mL). The

aqueous phase was acidified with aqueous HCl (4N) and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **8b** (0.26 g, 61%) as white crystals: mp 107–110 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 2.00–1.91 (m, 4H), 1.88–1.71 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ 156.5, 92.0, 75.9, 74.5, 42.8, 24.4. Anal. calcd. ($C_8H_{10}O_3$): C, 62.33; H, 6.54. Found: C, 62.23; H, 6.35.

3-(1-Hydroxycyclohexyl)propiolic acid (8c). A solution of **24c** (0.46 g, 2.3 mmol) in THF (4 mL) was added aqueous LiOH (2N, 6 mL). The resulting solution was stirred at rt. for 2 h before water (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 10 mL). The aqueous phase was acidified with aqueous HCl (4N) and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **8c** (0.20 g, 57%) as white crystals: mp 123–124 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 1.94–1.87 (m, 2H), 1.75–1.68 (m, 2H), 1.64–1.50 (m, 5H), 1.36–1.23 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 156.4, 91.9, 77.1, 68.8, 40.1, 26.2, 23.9. Anal. calcd. ($C_9H_{12}O_3 \cdot 0.15\ H_2O$): C, 63.52; H, 7.24. Found: C, 63.10; H, 6.80.

(±)-4-Hydroxy-5-phenylpen-2-ynoic acid (8d). Under a nitrogen atmosphere, a solution of propiolic acid (0.8 mL, 13.0 mmol) in anhydrous THF (50 mL) was cooled to –78 °C and added ^{n}BuLi (1.6 M, 16.3 mL, 26.0 mmol) dropwise. The mixture was to reach –40 °C before a solution of phenylacetaldehyde (1.37 g, 11.4 mmol) in anhydrous THF (10 mL) was added dropwise. The resulting reaction mixture was stirred for 3 h before it was poured into aqueous HCl (0.5N, 80 mL). The aqueous phase was extracted with EtOAc (3 × 40 mL) and the combined organic phases were washed with brine (60 mL), dried over anhydrous $MgSO_4$,

filtered, and evaporated *in vacuo*. Recrystallization from toluene afforded compound **8d** (0.64 g, 30%) as white crystals: mp 117–118 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.35–7.15 (m, 5H), 4.63 (t, J = 6.9 Hz, 1H), 3.06–2.96 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 154.7, 136.6, 129.3, 127.9, 126.4, 87.4, 76.5, 62.3, 43.1. Purity by anal. HPLC: 97% (254 nm).

(±)-4-(4-(Benzyloxy)phenyl)-4-hydroxybut-2-ynoic acid (8e). Under a nitrogen atmosphere, a solution of propiolic acid (0.8 mL, 13.0 mmol) in anhydrous THF (50 mL) was cooled to –78 °C and added ^{n}BuLi (1.6 M, 16.3 mL, 26.0 mmol) dropwise. The mixture was allowed to reach –40 °C before a solution of 4-(benzyloxy)benzaldehyde (2.49 g, 11.7 mmol) in anhydrous THF (10 mL) was added dropwise. The resulting reaction mixture was stirred for 2 h before it was poured into aqueous HCl (0.5N, 80 mL). The aqueous phase was extracted with EtOAc (3 × 40 mL) and the combined organic phases were washed with brine (60 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification first by column chromatography (Heptane/ EtOAc 1:1 + 1% AcOH) followed by recrystallization from MeCN followed by further recrystallization from toluene/heptane afforded compound **8e** (23 mg, 1 %) as white crystals: mp 154–155 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.46–7.41 (m, 4H), 7.39–7.35 (m, 2H), 7.32–7.28 (m, 1H), 7.05–7.00 (m, 2H), 5.63 (s, 1H), 5.10 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD): δ 161.0, 160.5, 138.7, 130.4, 129.7, 129.4, 129.1, 128.7, 116.2, 71.2, 71.2, 70.5, 64.3. Purity by anal. HPLC: 96% (254 nm).

(*E*)-3-(1-Hydroxycyclobutyl)acrylicacid (9a). A solution of **25a** (0.24 g, 1.4 mmol) in THF (2 mL) was added aqueous LiOH (2N, 4 mL). The resulting solution was stirred at rt. for 4 h before water (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 10 mL). The aqueous phase was acidified with aqueous HCl (4N) and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over $MgSO_4$, filtered, and

evaporated *in vacuo*. Recrystallization from MeCN afforded compound **9a** (92 mg, 45%) as white crystals: mp 108–110 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.23 (d, J = 15.6 Hz, 1H), 6.01 (d, J = 15.6 Hz, 1H), 2.35–2.19 (m, 4H), 1.93–1.82 (m, 1H), 1.81–1.70 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 169.0, 152.4, 117.0, 73.5, 35.4, 11.5. Anal. calcd. ($C_7H_{10}O_3$): C, 59.14; H, 7.09. Found: C, 59.11; H, 6.84.

(*E*)-3-(1-Hydroxycyclopentyl)acrylicacid (9b). A solution of **25b** (0.73 g, 4.0 mmol) in THF (4 mL) was added aqueous LiOH (2N, 6 mL). The resulting mixture was stirred at rt. for 4 h before water (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 10 mL). The aqueous phase was acidified with aqueous HCl (4N) and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded compound **9b** (0.32 g, 52%) as white crystals: mp 90–95 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.03 (d, J = 15.6 Hz, 1H), 6.02 (d, J = 15.6 Hz, 1H), 1.95–1.85 (m, 2H), 1.79–1.67 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 170.4, 156.3, 119.2, 82.2, 41.0, 24.9. Anal. calcd. ($C_8H_{12}O_3$): C, 61.52; H, 7.74. Found: C, 61.59; H, 7.61.

(*E*)-3-(1-Hydroxycyclohexyl)acrylicacid (9c). A solution of **25c** (0.19 g, 1.0 mmol) in THF (1 mL) was added aqueous LiOH (2N, 2 mL). The resulting mixture was stirred at rt. for 4 h before water (20 mL) was added and the aqueous phase was washed with Et_2O (3 × 10 mL). The aqueous phase was acidified with aqueous HCl (4N) and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Recrystallization from MeCN afforded **9c** (145 mg, 89%) as white crystals: mp 105–109 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.01 (d, J = 15.6 Hz, 1H), 5.97 (d, J = 15.6 Hz, 1H), 1.75–1.48 (m, 9H), 1.39–1.28 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 170.5, 157.3,

119.2, 72.2, 37.7, 26.5, 22.6. Anal. calcd. ($C_9H_{14}O_3$): C, 63.51; H, 8.29. Found: C, 63.58; H, 8.16.

(±)-(*E*)-4-([1,1'-Biphenyl]-4-yl)-4-hydroxybut-2-enoic acid (9d). A solution of **25d**[22] (0.21 g, 0.8 mmol) and $CeCl_3 \cdot 7H_2O$ (0.33 g, 0.9 mmol) in MeOH (6 mL) was cooled to 0 °C and slowly added $NaBH_4$ (32 mg, 0.9 mmol). The mixture was allowed to reach rt. and after 4 h, water 12 mL was added. The pH of the aqueous phased was adjusted to pH 1 using aqueous HCl (1N). The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 1:1 + 1% AcOH) afforded **9d** (0.18 g, 84%) as white solid: mp 132–133 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.67–7.61 (m, 4H), 7.50–7.42 (m, 4H), 7.38–7.32 (m, 1H), 7.06 (dd, *J* = 5.1, 15.6 Hz, 1H), 6.13 (dd, *J* = 1.7, 15.6 Hz, 1H) , 5.39 (dd, *J* = 1.7, 5.1 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 170.3, 151.2, 142.4, 142.3, 142.2, 130.0, 128.5, 128.4, 128.3, 128.1, 121.5, 74.0. Purity by anal. HPLC: 99% (254 nm).

(±)-(*E*)-4-(4-Benzylphenyl)-4-hydroxybut-2-enoic acid (9e). A solution of **25e**[22] (0.17 g, 0.6 mmol) and $CeCl_3 \cdot 7H_2O$ (0.25 g, 0.7 mmol) in MeOH (5 mL) was cooled to 0 °C and slowly added $NaBH_4$ (25 mg, 0.7 mmol). The mixture was allowed to reach rt. and after 4 h, water (10 mL) was added. The pH of the aqueous phase was adjusted to pH 1 using aqueous HCl (1N). The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 1:1 + 1% AcOH) afforded compound **9e** (72 mg, 42%) as clear oil. ^{1}H NMR (600 MHz, CD_3OD): δ 7.31–7.13 (m, 9H), 7.02 (dd, *J* = 5.2, 15.4 Hz, 1H), 6.09 (dd, *J* = 1.6, 15.4 Hz, 1H), 5.29 (dd, *J* = 1.6, 5.2 Hz, 1H), 3.96 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD): δ 170.3, 151.6, 142.7, 142.7, 140.9, 130.3, 130.0, 129.6, 128.0, 127.2, 121.1, 74.0,

42.6. The free acid **9e** (72 mg) was converted in to the sodium salt by dissolving the oil in EtOH (1 mL) where upon aqueous NaOH (0.4935N, 0.550 mL, 0.3 mmol) was added. The solvent was removed *in vacuo* to afford the sodium salt of **9e** (78 mg, >99%) as brown solid: mp >220 °C. ^{1}H NMR (600 MHz, D_2O): δ 7.27–7.22 (m, 4H), 7.21–7.14 (m, 5H), 6.61 (dd, *J* = 6.0, 15.7 Hz, 1H), 5.98 (dd, *J* = 1.5, 15.7 Hz, 1H), 5.24 (dd, *J* = 1.5, 6.0 Hz, 1H), 3.87 (s, 2H). ^{13}C NMR (150 MHz, D_2O): δ 175.1, 143.6, 141.7, 141.6, 139.0, 129.1, 128.8, 128.8, 127.0, 126.3, 126.2, 72.8, 40.8. Anal. calcd. ($C_{17}H_{15}O_3 \cdot 1Na \cdot 1H_2O$): C, 66.23; H, 5.55. Found: C, 66.42; H, 5.56.

(–)-(*E*)-4-(4-Benzylphenyl)-4-hydroxybut-2-enoic acid ((–)-9e) and **(+)-(*E*)-4-(4-benzylphenyl)-4-hydroxybut-2-enoic acid ((+)-9e).** (±)-**9e** (111 mg, 0.41 mmol, as protonated specie) was dissolved in the eluent (*n*-heptane/2-PrOH/TFA, 95:5:0.1), filtered (45 μm, Milipore), and separated using a Chiralpak IF column. Fractions containing the first eluting enantiomer were pooled and evaporated *in vacuo* to give (–)-**9e** (55 mg, 50%) as white solid: mp 116–118 °C. 98% *ee*. t_R = 26 minutes. $[\alpha]^{25}_D$ = –36.69° (*c* = 0.278, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.29–7.23 (m, 4H), 7.21–7.14 (m, 5H), 7.00 (dd, *J* = 5.1, 15.4 Hz, 1H), 6.05 (dd, *J* = 1.9, 15.4 Hz, 1H), 5.28 (dd, *J* = 1.9, 5.1 Hz, 1H), 3.96 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD): δ 170.1, 151.6, 142.8, 142.7, 140.9, 130.3, 130.0, 129.6, 128.0, 127.2, 121.0, 74.0, 42.6. Purity by anal. HPLC: 97% (254 nm). Fractions containing the second eluting enantiomer were pooled and evaporated to give (+)-**9e** (49 mg, 44%) as white solid: mp 116–118 °C. 98% *ee*. t_R = 38 minutes. $[\alpha]^{25}_D$ = +46.83° (*c* = 0.261, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ 7.29–7.23 (m, 4H), 7.22–7.14 (m, 5H), 6.99 (dd, *J* = 5.0, 15.4 Hz, 1H), 6.05 (dd, *J* = 1.5, 15.4 Hz, 1H), 5.28 (dd, *J* = 1.5, 5.0 Hz, 1H), 3.96 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD): δ 170.3, 151.5, 142.8, 142.7, 141.0, 130.3, 130.0, 129.6, 128.0, 127.2, 121.1, 74.0, 42.6. Purity by anal. HPLC: 98% (254 nm).

(±)-(*E*)-4-Hydroxy-4-(4-phenethylphenyl)but-2-enoic acid (9f). A solution of **25f**[22] (0.25 g, 0.9 mmol) and $CeCl_3 \cdot 7H_2O$ (0.35 g, 1.0 mmol) in MeOH (6 mL) was cooled to 0 °C and slowly added $NaBH_4$ (36 mg, 1.0 mmol. The mixture was allowed to reach rt. and after 3 h, water (10 mL) was added. The pH of the aqueous phase was adjusted to pH 1 using aqueous HCl (1N). The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 1:1 + 1% AcOH) afforded **9f** (0.21 g, 83%) as white solid: mp 95–96 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.31–7.13 (m, 9H), 7.09–6.98 (m, 1H), 6.10 (d, *J* = 15.3 Hz, 1H), 5.30 (d, *J* = 4.9 Hz, 1H), 2.92 (s, 4H). ^{13}C NMR (150 MHz, CD_3OD): δ 170.4, 151.6, 143.1, 143.0, 140.8, 130.0, 129.7, 129.4, 127,8, 127.0, 121.2, 74.1, 39.2, 38.9. Purity by anal. HPLC: 99% (254 nm).

(*E*)-4-(4-(Benzyloxy)phenyl)-4-hydroxybut-2-enoic acid (9g). A solution of **25g** (0.21 g, 0.8 mmol) and $CeCl_3 \cdot 7H_2O$ (0.31 g, 0.8 mmol) in MeOH (6 mL) was cooled to 0 ºC and slowly added $NaBH_4$ (32 mg, 0.8). The mixture was allowed to rt. and after 2 h, water (12 mL) was added. The pH of the aqueous phase was adjusted to pH 1 with aqueous HCl (3M). The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by preparative HPLC (Gradient 40–84% B over 11 min) afforded compound **9g** (61 mg, 28%) as white solid: mp 115 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.42 (d, *J* = 7.5 Hz, 2H), 7.36 (t, *J* = 7.4Hz, 2H), 7.31–7.27 (m, 3H), 7.01–6.98 (m, 3H), 6.05 (dd, *J* = 1.6, 15.5 Hz, 1H), 5.26 (dd, *J* = 1.9, 5.2 Hz, 1H), 5.08 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD): δ 170.0, 160.0, 151.6, 138.7, 135.4, 129.5, 129.0, 128.8, 128.5, 120.7, 116.1, 73.7, 71.0. Purity by anal. HPLC: 96% (254 nm).

(*E*)-4-(4-((4-Bromobenzyl)oxy)phenyl)-4-hydroxybut-2-enoic acid (9h). A solution of **25h** (0.23 g, 0.7 mmol) and $CeCl_3 \cdot 7H_2O$ (0.27 g, 0.7 mmol) in MeOH (6 mL) was cooled to 0 ºC and slowly added $NaBH_4$ (27 mg, 0.7). The mixture was allowed to rt. and after 2 h, water (12 mL) was added. The pH of the aqueous phase was adjusted to pH 1 with aqueous HCl (3M). The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by preparative HPLC (Gradient 40–84% B over 11 min) afforded compound **9h** (127 mg, 54%) as white solid: mp 131 °C. ^{1}H NMR (600 MHz, CD_3OD): δ 7.52 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.28 (d, *J* = 8.7 Hz, 2H), 7.01–6.97 (m, 3H), 6.05 (dd, *J* = 1.6, 15.6 Hz, 1H), 5.26 (dd, *J* = 1.7, 5.1 Hz, 1H), 5.05 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD): δ 170.1, 159.7, 151.5, 138.0, 135.6, 132.6, 130.4, 129.1, 122.6, 120.8, 116.1, 73.6, 70.2. Purity by anal. HPLC: 97% (254 nm).

***cis*-Benzyl-3-hydroxycyclohexanecarboxylate (*cis*-11)** and ***trans*-benzyl-3-hydroxycyclohexanecarboxylate (*trans*-11).** A solution of ethyl 3-hydroxycyclohexane-1-carboxylate (**10**, 0.55 g, 3.2 mmol) and LiOH (aq. 2M, 4 mL) in THF (4 mL) was stirred at rt. for 4 h before it was washed with Et_2O (2 × 30 mL). The aqueous phase was acidified to pH 1 with H_2SO_4 (aq. 5M) and extracted with EtOAc (3 × 50 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo* to afford a mixture of the two diastereomers of 3-hydroxycyclohexane-1-carboxylic acid (421 mg, 92%) as white solid: mp 133–134 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 3.97 (q, *J* = 4.5 Hz, 0.4H), 3.53 (tt, *J* = 4.2, 11.2 Hz, 0.6H), 2.78–2.69 (m, 0.4H), 2.33 (tt, *J* = 3.5, 12.2 Hz, 0.6H), 2.21–2.13 (m, 0.6H), 1.97–1.48 (m, 5H), 1.42–1.10 (m, 2.4H). ^{13}C NMR (150 MHz, CD_3OD): δ 178.2, 177.5, 69.1, 69.0, 65.5, 65.4, 41.8, 41.7, 37.8, 37.7, 37.5, 37.4, 35.1, 35.0, 34.4, 34.4, 32.4, 32.3, 28.0, 27.9, 27.9, 27.8, 23.2, 23.1, 19.7, 19.6. A suspension of 3-hydroxycyclohexane-1-carboxylic acid (103 mg, 0.7

mmol) and Cs_2CO_3 (153 mg, 0.5 mmol) in DMF (10 mL) was added benzyl bromide (0.19 mL, 1.6 mmol). The resulting mixture was stirred overnight before quenched with brine (3 mL) and H_2O (25 mL). The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by preparative TLC (Heptane/EtOAc, 1:1) provided *cis*-**11** (33 mg, 20%) and *trans*-**11** (30 mg, 18%) as colorless oils. *cis*-**11**: ^{1}H NMR (150 MHz, $CDCl_3$): δ 7.34–7.23 (m, 5H), 5.05 (s, 2H), 3.55 (tt, *J* = 4.2, 10.5 Hz, 1H), 2.35 (tt, *J* = 3.8, 11.6 Hz, 1H), 2.20–2.12 (m, 1H), 1.91–1.71 (m, 3H), 1.42–1.08 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.2, 136.4, 128.9, 128.5, 128.4, 70.1, 66.6, 42.2, 37.9, 35.3, 28.3, 23.5. *trans*-**11**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40–7.29 (m, 5H), 5.11 (s, 2H), 4.04–3.99 (m, 1H), 2.81–2.73 (m, 1H), 1.85–1.73 (m, 3H), 1.76–1.65 (m, 1H), 1.64–1.48 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.8, 136.4, 128.7, 128.3, 128.2, 66.3, 66.3, 38.1, 35.7, 33.0, 28.3, 20.0.

((3-Bromo-2-ethylcyclopent-2-en-1-yl)oxy)(*tert*-butyl)dimethylsilane (15b). Compound **13**[20] (0.41 g, 1.0 mmol) was dissolved in anhydrous THF (10 mL) and cooled to –78 °C. ^{n}BuLi (1.57M, 0.74 mL, 1.2 mmol) was added drop wise and the solution was stirred for 15 min. Ethyl iodide (104 µL, 1.3 mmol) was added and the solution was allowed to warm to –30 °C over 4 h. Sat. aq. NH_4Cl (40 mL) and Et_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic phases were washed with brine (2 × 10 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane) afforded compound **15b** (208 mg, 67%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.66–4.62 (m, 1H), 2.65–2.56 (m, 1H), 2.46–2.37 (m, 1H), 2.24–2.01 (m, 3H), 1.70–1.62 (m, 1H), 9.92 (t, *J* = 7.9 Hz, 3H), 0.82 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.7, 119.5, 76.6, 37.5, 33.5, 25.8, 20.4, 18.1, 11.6, –4.4, –4.9.

((3-Bromo-2-(3-methoxybenzyl)cyclopent-2-en-1-yl)oxy)(*tert*-butyl)dimethylsilane (15e). Compound **13**[20] (0.82 g, 2.0 mmol) was dissolved in anhydrous THF (20 mL) and cooled to –78 °C. ^{n}BuLi (1.57M, 1.48 mL, 2.3 mmol) was added drop wise and the solution was stirred for 15 min. 3-Methoxybenzyl bromide (368 µL, 2.6 mmol) was added and the solution was allowed to warm to –30 °C over 4 h. Sat. aq. NH_4Cl (30 mL) and Et_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic phases were washed with brine (2 × 10 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Petroleum ether 40–65 °C/EtOAc 100:1) afforded compound **15e** as colorless oil (450 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (t, *J* = 7.6 Hz, 1H), 6.86–6.74 (m, 1H), 4.65–4.58 (m, 1H), 3.82 (s, 3H), 3.74 (d, *J* = 14.4 Hz, 1H), 3.37 (d, *J* = 14.4 Hz, 1H), 2.86–2.75 (m, 1H), 2.66–2.55 (m, 1H), 2.36–2.25 (m, 1H), 1.86–1.75 (m, 1H), 0.91 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 142.2, 139.8, 129.2, 122.0, 121.1, 114.5, 111.4, 79.0, 55.1, 37.6, 33.4, 32.9, 25.8, 18.0, –4.4, –4.9.

Methyl 3-(tetrahydro-2H-pyran-2-yloxy)cyclopent-1-enecarboxylate (17). A solution of methyl 3-hydroxycyclopent-1-ene-1-carboxylate (Obtained from compound **16** as described by Aye *et al.*[33]) (0.94 g, 6.6 mmol) and 3,4-dihydro-2*H*-pyran (0.6 mL, 6.6 mmol) with HCl (35%, 10 µL) was stirred for 22 h at rt. Et_2O (150 mL) was added and the organic phase was washed with aq. sat. $NaHCO_3$ (2 × 10 mL) and H_2O (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Heptane/EtOAc 8:1) provided compound **17** (1.27 g, 84%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.77–6.73 (m, 1H), 4.95–4.84 (m, 1H), 4.73–4.67 (m, 1H), 3.92–3.82 (m, 1H), 3.73 (s, 3H), 3.56–3.46 (m, 1H), 2.75–2.64 (m, 1H), 2.50–2.24 (m, 2H), 2.00–1.45 (m, 7H). ^{13}C NMR

(150 MHz, $CDCl_3$): δ 165.6, 142.7, 141.0, 139.0, 138.5, 98.3, 98.2, 82.0, 81.3, 62.7, 62.5, 51.6, 31.4, 30.9, 30.7, 30.5, 30.0, 29.7, 25.4, 19.6.

Methyl 2-phenyl-3-((tetrahydro-2*H*-pyran-2-yl)oxy)cyclopent-1-ene-1-carboxylate (18f). Under a nitrogen atmosphere, a suspension of $NaHCO_3$ (0.39 g, 4.6 mmol), $N(Bu)_4Cl$ (0.62 g, 2.2 mmol), and 4Å molecular sieves in anhydrous DMF (2.3 mL) was stirred for 15 min. Iodobenzene (0.25 mL, 2.2 mmol) and compound **17** (0.60 g, 2.7 mmol) were then added and the suspension was stirred for another 15 min before $Pd(OAc)_2$ (25 mg, 0.1 mmol) was added. The reaction mixture was stirred at 60 °C for 4 days. Upon cooling to rt., H_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (6 × 100 mL). The combined organic phases were washed with H_2O (3 × 25 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Toluene/EtOAc 20:1) provided compound **18f** (0.48 g, 71%) as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–6.85 (m, 5H), 4.84–4.57 (m, 1H), 4.33–4.18 (m, 1H), 3.80–3.70 (m, 1H), 3.54 (s, 3H), 3.42–3.31 (m, 1H), 2.96–2.76 (m, 1H), 2.62–2.39 (m, 1H), 1.82–1.36 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8, 164.8, 142.7, 142.0, 141.3, 141.2, 137.5, 136.9, 128.6, 128.5, 127.1, 127.0, 126.5, 126.5, 97.5, 97.5, 83.7, 83.4, 62.8, 62.2, 58.1, 57.4, 51.4, 51.3, 40.0, 38.9, 30.9, 30.9, 25.4, 19.7, 19.4.

Methyl 2-(4-chlorophenyl)-3-((tetrahydro-2*H*-pyran-2-yl)oxy)cyclopent-1-ene-1-carboxylate (18g). Under a nitrogen atmosphere, a suspension of $NaHCO_3$ (0.65 g, 7.7 mmol), $N(Bu)_4Cl$ (1.02 g, 3.7 mmol), and 4Å molecular sieves in anhydrous DMF (3.7 mL) was stirred for 15 min. 1-Chloro-4-iodobenzene (0.88 g, 3.7 mmol) and compound **17** (1.0 g, 4.4 mmol) were then added and the suspension was stirred for another 15 min before $Pd(OAc)_2$ (41 mg, 0.2 mmol) was added. The reaction mixture was stirred at 60 °C for 3 days. Upon cooling to rt., H_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (6 × 100 mL). The combined

organic phases were washed with H_2O (3 × 25 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Toluene/EtOAc 20:1) afforded compound **18g** (0.84 g, 67%) as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20–7.14 (m, 2H), 7.03–6.96 (m, 2H), 4.69–4.55 (m, 1H), 4.28–4.15 (m, 1H), 3.78–3.69 (m, 1H), 3.55 (s, 3H), 3.41–3.34 (m, 1H), 2.95–2.77 (m, 1H), 2.63–2.41 (m, 1H), 1.87–1.36 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6, 164.6, 143.0, 142.3, 140.1, 139.9, 137.2, 136.6, 132.3, 132.2, 128.8, 128.6, 128.5, 128.5, 97.8, 97.6, 83.8, 83.3, 62.8, 62.3, 57.6, 56.8, 51.5, 51.4, 40.0, 39.0, 30.9, 25.3, 19.6, 19.4.

Methyl 3-(tetrahydro-2H-pyran-2-yloxy)-2-p-tolylcyclopent-1-enecarboxylate (18h). Under a nitrogen atmosphere, a suspension of $NaHCO_3$ (0.65 g, 7.7 mmol), $N(Bu)_4Cl$ (1.02 g, 3.7 mmol), and 4Å molecular in dry DMF (3.7 mL) was stirred for 15 min. 1-Iodo-4-methylbenzene (0.80 g, 3.7 mmol) and compound **17** (1.0 g, 4.4 mmol) were then added and the suspension was stirred for another 15 min before $Pd(OAc)_2$ (41 mg, 0.2 mmol) was added. The reaction mixture was stirred at 60 °C for 2 days. Upon cooling to rt., H_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (6 × 100 mL). The combined organic phases were washed with H_2O (3 × 25 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Toluene/EtOAc 20:1) afforded compound **18h** (0.85 g, 72%) as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19–6.94 (m, 5H), 4.79–4.54 (m, 1H), 4.31–4.13 (m, 2H), 3.84–3.70 (m, 1H), 3.55 (s, 3H), 3.48–3.32 (m, 1H), 3.00–2.36 (m, 2H), 2.23 (s, 3H), 1.85–1.35 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 142.6, 141.9, 138.4, 138.2, 137.8, 137.1, 136.2, 136.0, 129.4, 129.3, 127.0, 127.0, 97.6, 83.9, 83.5, 62.9, 62.3, 57.8, 57.1, 51.4, 40.1, 39.0, 31.0, 31.0, 25.4, 21.1, 19.8, 19.5.

Methyl 2-(4-methoxyphenyl)-3-((tetrahydro-2*H*-pyran-2-yl)oxy)cyclopent-1-ene-1-carboxylate (18i). Under a nitrogen atmosphere, a suspension of $NaHCO_3$ (0.39 g, 4.6 mmol), $N(Bu)_4Cl$ (0.62 g, 2.2 mmol), and 4Å molecular sieves in anhydrous DMF (2.3 mL) was stirred for 15 min. 1-Iodo-4-methoxybenzene (0.52 g, 2.2 mmol) and compound **17** (0.60 g, 2.7 mmol) were then added and the suspension was stirred for another 15 min before $Pd(OAc)_2$ (25 mg, 0.1 mmol) was added. The reaction mixture was stirred at 60 °C for 3 days. Upon cooling to rt., H_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (6 × 100 mL). The combined organic phases were washed with H_2O (3 × 25 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatography (Toluene/EtOAc 20:1) afforded compound **18i** (550 mg, 74%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10–6.92 (m, 2H), 6.89–6.76 (m, 2H), 4.99–4.65 (m, 1H), 4.37–4.24 (m, 1H), 3.96–3.81 (m, 1H), 3.78 (s, 3H), 3.64 (s, 3H), 3.58–3.41 (m, 1H), 3.03 (m, 1H), 2.68–2.46 (m, 1H), 1.92–1.44 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2, 173.1, 164.8, 164.8, 158.2, 158.2, 142.4, 141.7, 137.7, 137.1, 133.5, 133.3, 128.0, 128.0, 114.0, 113.9, 97.5, 97.5, 83.8, 83.3, 62.8, 62.8, 55.1, 55.1, 51.3, 51.3, 39.9, 38.8, 30.9, 30.9, 25.4, 25.3, 19.7, 19.4.

Methyl 2-(3,4-dichlorophenyl)-3-((tetrahydro-2*H*-pyran-2-yl)oxy)cyclopent-1-ene-1-carboxylate (18j). Under a nitrogen atmosphere, a suspension of $NaHCO_3$ (0.65 g, 7.7 mmol), $N(Bu)_4Cl$ (1.02 g, 3.7 mmol), and 4Å molecular sieves in dry DMF (3.7 mL) was stirred for 15 min. 1,2-Dichloro-4-iodobenzene (1.0 g, 3.7 mmol) and compound **17** (1.0 g, 4.4 mmol) were then added and the suspension was stirred for another 15 min before $Pd(OAc)_2$ (41 mg, 0.2 mmol) was added. The reaction mixture was stirred at 60 °C for 3 days. Upon cooling to rt., H_2O (50 mL) was added and the aqueous phase was extracted with Et_2O (6 × 100 mL). The combined organic phases were washed with H_2O (3 × 25 mL), dried over anhydrous $MgSO_4$, filtered, and

evaporated *in vacuo*. Purification by column chromatography (Toluene/EtOAc 20:1) afforded compound **18j** (620 mg, 44%) as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29–7.24 (m, 1H), 7.20–7.13 (m, 1H), 7.12–7.07 (m, 1H), 4.67–4.53 (m, 1H), 4.27–4.13 (m, 1H), 3.78–3.69 (m, 1H), 3.57 (s, 3H), 3.42–3.34 (m, 1H), 2.96–2.79 (m, 1H), 2.65–2.43 (m, 1H), 1.83–1.36 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.4, 164.4, 143.5, 142.8, 142.0, 141.9, 136.7, 136.1, 132.6, 132.5, 130.6, 130.4, 129.1, 129.0, 128.2, 126.7, 126.6, 125.2, 98.2, 97.6, 83.8, 83.2, 62.8, 62.6, 57.4, 56.4, 51.5, 51.5, 40.1, 39.1, 30.9, 30.8, 25.3, 25.3, 19.6, 19.5.

Methyl 3-bromocyclopent-1-enecarboxylate (21). Compound **16** (210 mg, 1.7 mmol), NBS (297 mg, 1.7 mmol), and AIBN (6.8 mg, 0.04 mmol) were mixed in CH_2Cl_2 (25 mL) and the resulting solution was heated at reflux for 1 h. Upon cooling to rt., the solvent was evaporated *in vacuo*. Purification by column chromatography (Petroleum ether 40–65 °C/Et_2O 9:1) afforded compound **21** (186 mg, 55%) as colorless oil. ^{1}H NMR (600 MHz, CD_3OD): δ 6.85–6.81 (m, 1H), 5.26–5.22 (m, 1H), 3.78 (s, 3H), 2.83–2.73 (m, 1H), 2.65–2.54 (m, 2H), 2.44–2.36 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.7, 142.7, 139.4, 57.6, 52.3, 35.3, 30.3.

(*E*)-Ethyl 3-(1-hydroxycyclobutyl)acrylate (25a). Under a nitrogen atmosphere, a solution of Red-Al (3.5M, 1.42 mL, 5.4 mmol) in anhydrous THF (12 mL) was cooled to –78 °C and added a solution of compound **24a** (0.50 g, 3.0 mmol) in anhydrous THF (15 mL) dropwise. The mixture was stirred for 30 minutes at –78 °C before aqueous HCl (0.1N, 200 mL) was added. The aqueous phase was extracted with EtOAc (3 × 150 mL) and combined organic phases were dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatograph (Heptane/EtOAc 3:1) afforded **25a** (0.29 g, 57%) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13 (d, J = 15.7 Hz, 1H), 5.96 (d, J = 15.7 Hz, 1H), 4.12 (q, J = 7.0 Hz, 2H), 3.65 (b s, 1H),

2.23–2.12 (m, 4H), 1.82–1.73 (m, 1H), 1.69–1.57 (m, 1H), 1.21 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.2, 151.9, 117.3, 74.2, 60.4, 36.1, 14.1, 12.0.

(*E*)-Ethyl 3-(1-hydroxycyclopentyl)acrylate (25b). Under a nitrogen atmosphere, a solution of Red-Al (3.5M, 2.0 mL, 7.0 mmol) in anhydrous THF (24 mL) was cooled to –78 °C and added a solution of compound **24b** (1.09 g, 6.0 mmol) in anhydrous THF (30 mL) drop wise. The mixture was stirred for 30 min at –78 °C before aqueous HCl (0.1N, 200 mL) was added. The aqueous phase was extracted with EtOAc (3 × 200 mL) and the combined organic phases were dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Purification by column chromatograph (Heptane/EtOAc 4:1) afforded compound **25b** (0.81 g, 73%) as clear colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97 (d, J = 15.6 Hz, 1H), 6.02 (d, J = 15.6 Hz, 1H), 4.13 (q, J = 7.3 Hz, 2H), 2.02–1.81 (m, 3H), 1.74–1.62 (m, 6H), 1.22 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0, 154.0, 118.2, 81.7, 60.4, 40.5, 24.0, 14.2.

(*E*)-4-(4-(Benzyloxy)phenyl)-4-oxobut-2-enoic acid (25g). A solution of compound **26** (2.57 g, 11.3 mmol) and glyoxylic acid monohydrate (4.18 g, 45.4 mmol) in water (18 mL) was added slowly added a solution of NaOH (2.8 g, 69 mmol) in water (35 mL) and EtOH (18 mL) at rt. The mixture was stirred at rt. for 1 h followed by 2 h at reflux. Upon cooling to rt., the solvents was evaporated *in vacuo*. The resulting residue was acidified with aq. HCl (10M, 30 mL) and the aqueous phase was extracted with EtOAc (3 × 50 mL). Purification by column chromatography (EtOAc/Heptane 2:1 + 1% AcOH) afforded compound **25g** product (414 mg, 13%) as yellow sticky solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.98 (b s, 1H), 8.04 (d, J = 8.9 Hz, 2H), 7.89 (d, J = 15.6 Hz, 1H), 7.48–7.33 (m, 5H), 7.17 (d, J = 8.9 Hz, 2H), 6.65 (d, J = 15.6 Hz, 1H), 5.24 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6), δ: 187.5, 166.4, 163.0, 136.3, 136.2, 132.3, 131.3, 129.3, 128.5, 128.0, 127.8, 115.1, 69.6.

(*E*)-4-(4-((4-Bromobenzyl)oxy)phenyl)-4-oxobut-2-enoic acid (25h). A solution of compound **27** (1.53 g, 5.0 mmol) and glyoxylic acid monohydrate (1.85 g, 20.1 mmol) in water (8 mL) was added slowly added a solution of NaOH (1.2 g, 31 mmol) in water (15 mL) and EtOH (8 mL) at rt. The mixture was stirred at rt. for 1 h followed by 2 h at reflux. Upon cooling to rt., the solvents was evaporated *in vacuo*. The resulting residue was acidified with aq. HCl (10M, 10 mL) and the aqueous phase was extracted with EtOAc (3 × 15 mL). Purification by column chromatography (EtOAc/Heptane 2:1 + 1% AcOH) afforded compound **25h** product (342 mg, 19%) as yellow sticky solid. ^{1}H NMR (600 MHz, CD_3OD): δ 8.02 (d, J = 8.9 Hz, 2H), 7.93 (d, *J* = 15.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.12 (d, *J* = 8.9 Hz, 2H), 6.76 (d, *J* = 15.6 Hz, 1H), 5.15 (s, 2H). ^{13}C NMR (100MHz, CD_3OD): δ 189.5, 168.5, 164.8, 137.8, 137.2, 133.2, 132.7, 132.4, 131.2, 130.5, 122.9, 116.2, 70.5.

Radioligand binding studies

Membrane Preparations: All the binding assays were performed with the use of rat brain synaptosomal membranes (homogenate) from the cortex of Sprague-Dawley adult male rats. Tissue preparation was performed as described earlier.[38] On the day of the assay, the membrane preparation was quickly thawed and suspended in 40 volumes of ice-cold binding buffer (50 mM KH_2PO_4 buffer; pH 6.0) with the use of an UltraTurrax homogenizer before centrifugation at 48.000 × *g* for 10 min at 4 °C. The washing procedure was repeated four times. The final pellet was suspended in binding buffer.

[^{3}H]NCS-382 Binding Assays: [^{3}H]NCS-382 (20 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). For the [^{3}H]NCS-382 binding assay, 96

well filtration was adopted as previously reported.[13] Aliquots of membrane preparation (50–70 µg/aliquot) in triplicates were incubated with [^{3}H]NCS-382 (16 nM) in the absence or presence of test compound at 0 °C for 1 h in a total volume of 200 µl binding buffer. Compound stock solutions were typically made in 100 mM in DMSO and tested in concentrations of maximum 1000 µM. Nonspecific binding was determined with the use of GHB (1 mM). The binding reaction was terminated by rapid filtration through GF/C unifilters (PerkinElmer), using a 96-well Packard FilterMate cell-harvester, followed by washing with 3 × 250 µL of ice-cold binding buffer. Microscintillation fluid (PerkinElmer) was added to the dried filters, and the amount of filter-bound radioactivity was quantified in a Packard TopCount microplate scintillation counter. Typical radioactivity counts were in the range 100–2000 cpm.

Data Analysis: The binding data were analysed with the use of GraphPad Prism 7.0b (GraphPad Software Inc., San Diego, CA, USA). Specific binding (% control) was calculated for each data point (obtained in triplicate). Curve data were fitted by nonlinear regression using the formula for one-site competition. K_i were calculated from IC_{50} values by means of the Cheng-Prusoff equation[39] using the earlier determined K_d value of 430 nM for [^{3}H]NCS-382.[13] Data from each individual experiment were analyzed separately to give K_i values, and the means ± SEM of at least three independent experiments were then calculated from the pK_i values.

Computational modeling

All Molecular modelling software modules mentioned below (Maestro, LigPrep, MacroModel and Phase) are part of the Small-Molecule Drug Discovery Suite 2017-2 (Schrödinger, LLC, New York, NY, 2017).

Ligand preparation: All compounds were built in both enantiomeric forms with the 2D sketch editor in Maestro and assigned ionization state with LigPrep using default settings. Conformations were generated with MacroModel using the MCMM search method with the default energy window of 5.02 kcal/mol as cut-off for the conformational energy with few exceptions. For GHB and for compounds **2a**, **2b**, **2c**, **4b**, *cis*-**4c**, **6a,** and **6b**, the lowest energy conformations suffered from electrostatic collapse[40, 41] and therefore the energy window was doubled to 10.4 kcal/mol and collapsed conformations were discarded.

Pharmacophore model generation: The pharmacophore model of the GHB high-affinity binding site was generated using Phase. The high affinity ligands ($pK_i \geq 6$), (*R*)-HOCPCA, **2b**, **9d** and (*R*)-**9e**, the intermediate affinity ligands ($4 < pK_i < 6$) (*S*)-HOCPCA, **6a**, **8e** and (*S*)-**9e**, and the inactive/poor binding compounds **3**, **5b** and **5i** were selected to constitute the training set. All the calculated conformations (see above) were used as input and for chiral compounds where affinities are only available for the racemic mixture the pK_i were assigned to both enantiomers. Based on the similarity to **2a**, where (*R*)-**2a** is the higher affinity stereoisomer, (*R*)-**9e** was assigned to the higher affinity (+)-enantiomer and (*S*)-**9e** to the lower affinity (–)-enantiomer.

The compounds reported here, contain four pharmacophore elements important for affinity: *1.* The negatively charged carboxylate (N), *2.* a distal aromatic ring (R) connected to the γ-position, *3.* a hydrogen bond donor (D) and *4.* a hydrogen bond acceptor (A) functionality of the γ-hydroxyl group. The inclusion of both the donor and acceptor functionality further holds the advantage that compounds like HOCPCA, which contain only three pharmacophore elements can still be included in the model, *i.e.* three features are needed for superimposition. Thus, when

generating the initial pharmacophore model, four pharmacophore elements were requested with a minimum inter-site distance of 0.8 Å.

The best scoring pharmacophore hypothesis from Phase that identified the above-mentioned pharmacophore elements, ADNR, and ranked the active compounds of the training set according to their affinity values was selected and further refined. The aromatic ring element, R, was substituted with a custom feature, Z, representing an aromatic surface without vector characteristics and exclusion volumes were introduced based on the two inactive compounds, **3** and **5b** (Figure 4).

Pharmacophore screening: All the pre-generated conformations of all the reported compounds were screened using the pharmacophore model, with the following criteria: *1.* tolerances were setto 1.5 Å for the custom feature, Z, and 1 Å for the other three, *2.* the negative pharmacophore element, N, was always required, *3.* the custom feature, Z, was permitted to be matched by any aromatic ring, *4.* at least three out of four pharmacophore elements were required and *5.* the atom type was considered when computing volume scores.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: **UNKNOWN**; Molecular formular Strings (.CSV) and Performance of the Developed Pharmacophore Model (.PDF).

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡ JK, CHJ, and FB contributed equally to this work.

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The Authors declare no financial interest.

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ABBREVIATIONS

GABA, γ-aminobutyric acid; GHB, γ-hydroxybutyric acid; HOCPCA, 3-hydroxycyclopent-1-enecarboxylic acid; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; Red-Al, Sodium bis(2-methoxyethoxy)aluminumhydride; SAR, structure-affinity relationship; TBS, *tert*-butyldimethylsilyl; T-HCA, *trans*-4-hydroxycrotonic acid; THP, tetrahydropyranyl.

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