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Novel easily accessible glucosidase inhibitors: 4-hydroxy-5-alkoxy-1,2-cyclohexanedicarboxylic acids

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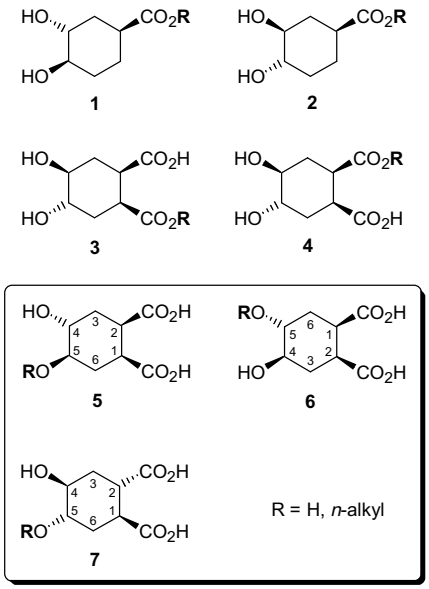
ABSTRACT

Glycosidases are very important enzymes involved in a variety of biochemical processes with a special importance to biotechnology, food industry, and pharmacology. Novel structurally simple inhibitors derived from cyclohexane-1,2-dicarboxylic acids were synthesized and tested against several fungal glycosidases from *Aspergillus oryzae* and *Penicillium canescens*. The presence of at least two carboxylic groups and one hydroxy group was essential for efficient inhibition. Significant selective inhibition was observed for α- and β-glucosidases, the magnitude of which depended on the configuration of substituents; inhibition increased for β-glucosidase by lengthening the alkoxy group of the inhibitor.

1. Introduction

Glycosidases (glycoside hydrolases) are a very important class of enzymes that catalyze a hydrolytic cleavage of glycosidic bonds. [1] They are widespread in microorganisms, plants, and animals, and are widely used in biotechnology, food industry, and pharmacology.[1–3] α/β-Glucosidases are able to cleave, respectively, the α- or β-linkages at anomeric center of a glucose fragment, and are involved in a variety of biochemical processes related to metabolic disorders and diseases, such as diabetes, viral or bacterial infections, lysosomal storage disorders, and cancer.[1–3] Therefore, much effort has been focused on the design of efficient inhibitors of glucosidase activity for many potential applications, for example, as tools for the understanding of biochemical processes and as prospective therapeutic agents.[1–3]

The structure of inhibitors usually resembles a substrate, a transition state, or the product of an enzyme-catalyzed reaction.[1–3] Various polyhydroxylated five- and six-membered cyclic compounds may be considered as monosaccharide structural analogs, and thus potential inhibitors of glycosidases. In the search for simple, readily accessible, but efficient glycosidase inhibitors, we designed, prepared, and assayed a series of cyclohexanecarboxylic acids of types **1**–**7** with different substitution patterns, configuration of substituents, and different lengths of *n*-alkyl groups R, to reveal the effects of these parameters upon the inhibitory activity. The proposed structures contained a six-membered ring and several hydrophilic groups, which is typical for many carbohydrate mimetics. Alkyl groups were introduced to enhance the inhibition. A similar effect of lipophilic groups was observed previously[1–3] for derivatives of 1-deoxynojirimycin and other iminosugars, alkyl glycosides, and some other glycosidase inhibitors.



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The preliminary tests against fungal glycosidases[4] revealed only a weak to moderate inhibition of β-D-glucosidase by compounds **1** (R = H) and **3 + 4** (R = CH_3, *n*-C_6H_{13}), and a weak inhibition of α-L-fucosidase by compounds **1 + 2**, and **3 + 4** (R = CH_3, *n*-C_6H_{13}). However, very promising preliminary results were obtained for cyclohexanedicarboxylic acids of types **5**–**7**. In this paper, we demonstrate that some of these simple racemic compounds can be potent inhibitors of α- and β-D-glucosidases.

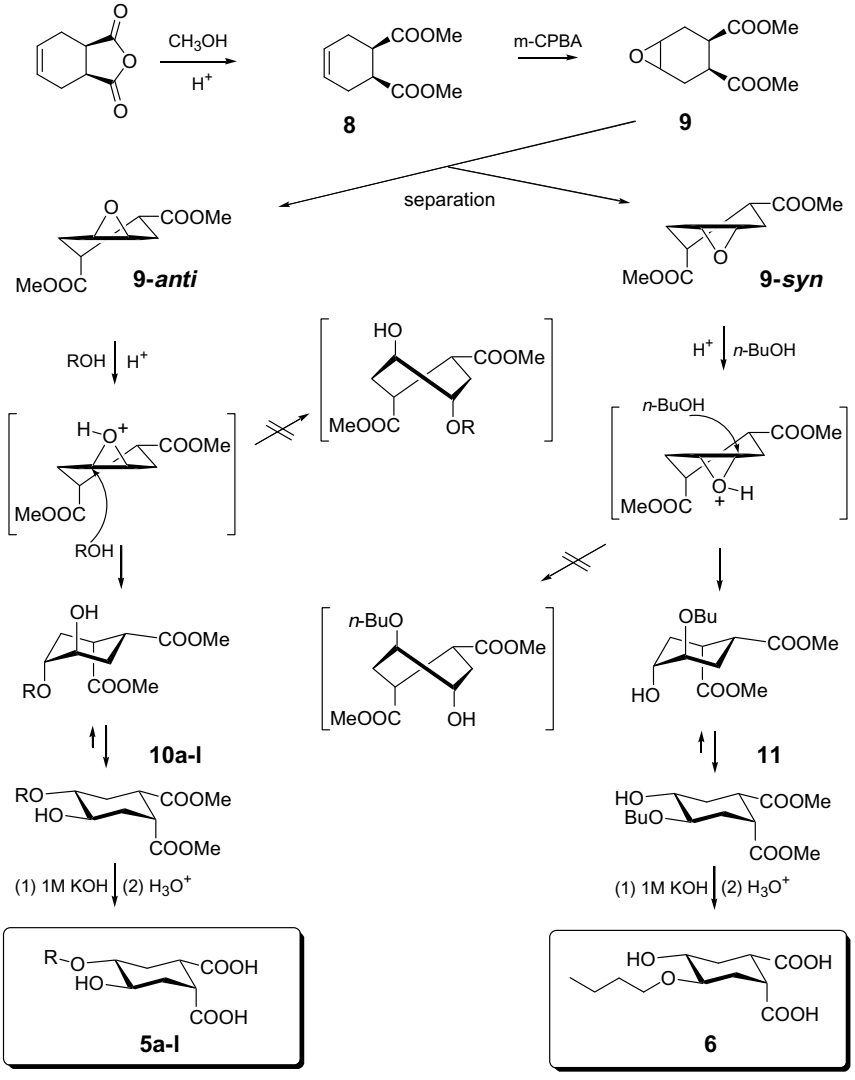
2. Results and discussion

2.1. Synthesis of 4-hydroxy-5-alkoxycyclohexane-1,2-dicarboxylic acids

Stereoisomeric 4-hydroxy-5-alkoxycyclohexane-1,2-dicarboxylic acids **5a**–**l**, **6**, and **7** were synthesized according to Schemes 1 and 2 using previously developed[5] procedures. The epoxides **9** were obtained as a mixture of stereoisomers, and were separated by column chromatography. The well-known tendency of epoxides to open trans-diaxially[6] and regioselectively[7] (via a chair-like transition state rather than a strained twist-boat transition state, Scheme 1), allowed for the diastereoselective synthesis of diesters **10a**–**l**, **11**, and **13**, which were further hydrolyzed into the corresponding diacids **5a**–**l**, **6**, and **7**, respectively. All products were racemic mixtures.

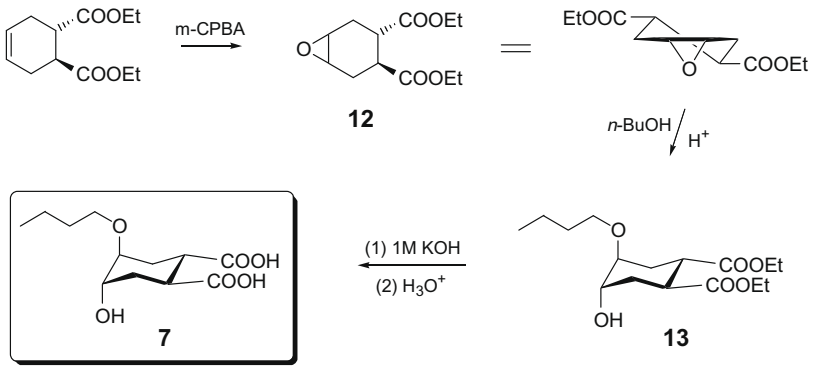
The structures and predominant conformations of all products and intermediates were determined from the data of ^{1}H NMR and ^{13}C NMR, including COSY, HETCOR, and homonuclear decoupling techniques. Thanks to a bias of the conformational equilibria, the structural assignments were rather straightforward: large spin–spin coupling constants (9–11 Hz) indicated a trans-diaxial orientation of the corresponding vicinal protons, while all other relative positions (axial–equatorial, equatorial–equatorial) resulted in small couplings between them (2–4 Hz). Fragments of ^{1}H NMR spectra are shown in Figures 1 and 2 for illustration.

We estimated the position of conformational chair–chair equilibrium using the measured averaged values of coupling constants, and their values for individual conformers of structurally similar



R = H (**a**), CH_3 (**b**), Et (**c**), *n*-Pr (**d**), *n*-Bu (**e**),
n-C_5H_{11} (**f**), *n*-C_6H_{13} (**g**), *n*-C_7H_{15} (**h**), *n*-C_8H_{17} (**i**),
n-C_9H_{19} (**j**), *n*-$C_{10}H_{21}$ (**k**), *n*-$C_{12}H_{25}$ (**l**)

Scheme 1. Preparation of 4-hydroxy-5-alkoxy-*cis*-1,2-cyclohexanedicarboxylic acids **5** and **6**.



Scheme 2. Preparation of 4-hydroxy-5-alkoxy-*trans*-1,2-cyclohexanedicarboxylic acid **7**.

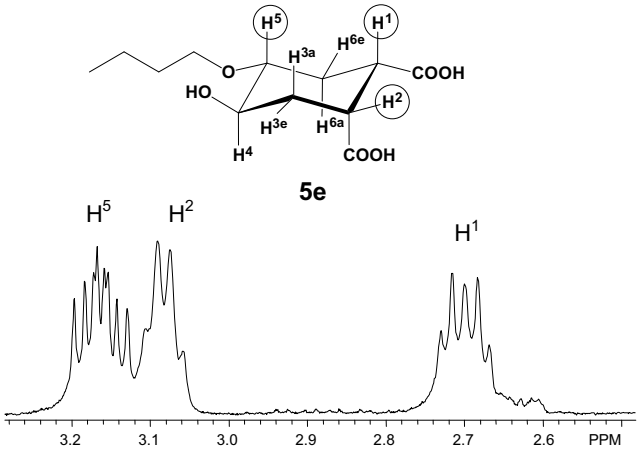


Figure 1. ^{1}H NMR signals for (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-butoxycyclohexane-1,2-dicarboxylic acid **5e** (CD_3OD, 300 MHz).

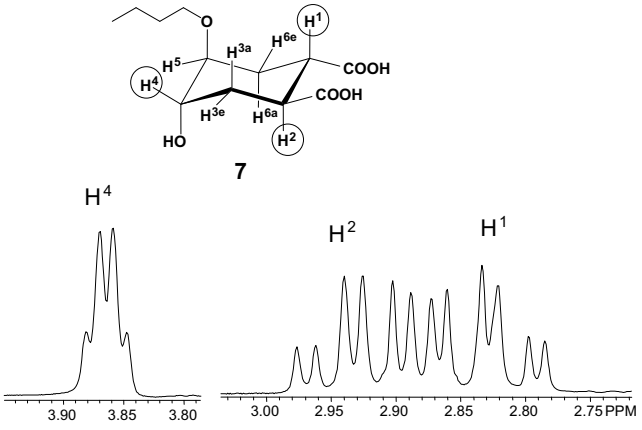


Figure 2. ^{1}H NMR signals for (1*S**, 2*S**, 4*S**, 5*S**)-4-hydroxy-5-butoxycyclohexane-1,2-dicarboxylic acid **7** (CD_3OD, 300 MHz).

compounds studied before.[5] The length of alkoxy groups at C4 did not affect the conformational properties of the six-membered ring. As expected, all derivatives of *cis*-1,2-dicarboxylic acid (**5**, **6**, **10**, **11**) preferred the conformation with three of four substituents in the favorable equatorial position as shown in Scheme 1 and Figure 1. The population of this form was approximately 90% for **10** and 80% for **11** in $CDCl_3$, and 70% for **5e** and >95% for **6** in CD_3OD (diacids were insoluble in $CDCl_3$). The difference between **10** and **11**, and between **5e** and **6** may indicate some difference in intra- and intermolecular interactions (e.g., hydrogen bonding between substituents and/or with a solvent). The derivatives of *trans*-1,2-dicarboxylic acid (**7**, **13**) may have only two groups in equatorial position in any of two possible chair conformers. However, the equilibrium between these chairs was also biased. The form shown in Scheme 2 and Figure 2 comprised 65% of molecules in $CDCl_3$, and 90% in CD_3OD for diester **13**, and >95% for the butoxy-diacid **7** in CD_3OD. The latter result is similar to 90% of this conformer in CD_3OD for the analogous methoxy-diacid described before.[5a]

2.2. Glycosidase inhibitory activity

The synthesized compounds have been assayed for enzyme inhibitory activity against several glycosidases in multi-enzyme complexes isolated from fungi *Penicillium canescens* and *Aspergillus oryzae*.[8] The multi-enzyme complex from *P. canescens* contains α-D- and β-D-galactosidase, β-D-glucosidase, and α-L- and β-D-fucosidase.[8a–d] The multi-enzyme complex from *A. oryzae* contains α-D- and β-D-galactosidase, α-D- and β-D-glucosidase, and α-L- and β-D-fucosidase.[8e] All assays have been performed in a standard way[9] by spectrophotometric monitoring, at 400 nm, the release of *p*-nitrophenol from the corresponding *p*-nitrophenyl glycosides. The inhibitory activity of the studied compounds is specific with regard to the configuration of inhibitor, the type of enzyme, and even the particular source of enzyme.

2.2.1. Effect of configuration

We compared three stereoisomeric butoxy-substituted diacids **5e**, **6**, and **7** to elucidate the effect of configuration upon the inhibitory activity. Most noticeably, these compounds inhibit α- and β-D-glucosidases (Table 1). In addition, α-L-fucosidase from *A. oryzae* is slightly inhibited by compound **6**, and the analogous enzyme from *P. canescens*, by compound **7**. Other activities are not affected. It is worth mentioning that compound **5e** produces the same inhibition

Table 1
Loss of α/β-D-glucosidase activity in presence of inhibitors (in %)

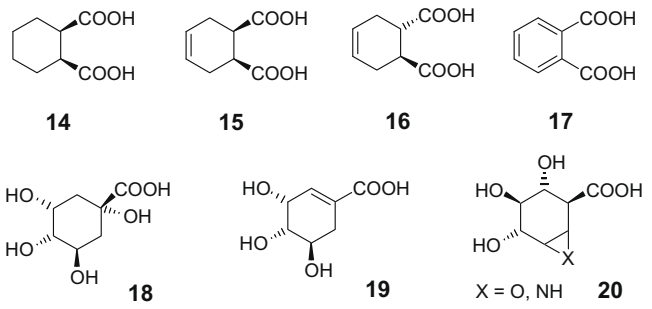
Enzyme	β-D-Glucosidase *P. canescens*			β-D-Glucosidase *A. oryzae*		α-D-Glucosidase *A. oryzae*
Inhibitor, mM	1.42	0.36	0.036	1.42	0.36	0.11
5e	94	88	60	82	80	65
6	80	49	0	55	10	65
7	46	26	9	39	12	55
14	40	16	0	33	14	26
15	49	23	0	48	23	29
16	0	0	0	0	0	28
17	21	8	0	10	0	42
18	0	0	0	0	0	12
19	0	0	0	0	0	18

effect upon β-D-glucosidase from *A. oryzae*, as approximately a 10-fold higher concentration of methyldeoxynojirimycin.[10]

The isomers **6** and **7** appear to be more selective inhibitors. At low concentration they are active against α-D-glucosidase, but much less against β-D-glucosidase, even from the same origin. The isomer **5e** is a much stronger inhibitor toward β-D-glucosidase, especially from *P. canescens*, but it inhibits α-D-glucosidase as well.

The inhibitory activity toward β-D-glucosidases is overall higher for compounds **5e** and **6** with *cis* configuration of carboxylic groups as compared to the corresponding *trans*-diacid **7**. α-D-Glucosidase seems to be roughly indifferent to the configuration of inhibitor. The difference observed for inhibition by stereoisomers **5e** and **6**, which look so much alike, indicates that inhibitory abilities may be very structure-sensitive.

We also checked simple carbocyclic dicarboxylic acids **14**–**17** for the inhibitory activity toward the same glycosidases (Table 1). Their effect is much weaker than the one of structural analogs described above: compare **5e**, **6** versus **14**, **15**, and **7** versus **16**. The presence of other substituents, for example, hydroxy and alkoxy group(s), is apparently essential for efficient inhibition of glycosidases. However, the polyhydroxylated quinic (**18**) and shikimic (**19**) acids had a negligible effect (Table 1). Likewise, strong β-glucuronidase inhibitors **20** were reported to be inactive toward α- and β-glucosidases, mannosidases, galactosidases, and *N*-acetyl-galactosaminidases.[11] Together with our data on cyclohexanecarboxylic acids **1**–**4**,[4a] these facts apparently point to the importance of a second carboxylic group for inhibition.



Similar to the situation for **5e** and **6** versus **7**, *cis*-diacids **14** and **15** are stronger inhibitors of β-D-glucosidases than *trans*-diacid **16**, while all three compounds **14**–**16** show equal inhibitory activity toward α-D-glucosidase (Table 1).

We estimated kinetic parameters for inhibition of β-D-glucosidase by the most potent stereoisomer **5e**: IC_{50} = 7.08 μM, K_i = 0.43 μM (β-D-glucosidase from *P. canescens*, K_M = 40 μM), and IC_{50} = 20.0 μM, K_i = 8.58 μM (*A. oryzae*, K_M = 500 μM). It is interesting to compare our results to the data for some other *n*-butyl-substituted inhibitors of β-D-glucosidases: *N*-butyl-1-deoxynojirimycin (Zavesca®), K_i = 850 μM; butylglucoside, K_i = 280 μM; *N*-butylglucosylamine, K_i = 0.74 μM (all toward calf liver enzyme);[2c] *N*-butyl-D-gluconamidine, K_i = 4.2 μM (sweet almond), K_i = 0.13 μM (bovine, cytosolic), K_i = 0.003 μM (*A. wentii*).[2d] This comparison attests to compound **5**e being a strong β-D-glucosidase inhibitor.

2.2.2. Effect of lipophilic substituent

The presence of alkyl and other lipophilic group(s) often increases the efficiency of glycosidase inhibitors.[1–3] This effect was observed for derivatives of 1-deoxynojirimycin and other iminosugars, alkyl glycosides, carbasugars, and some other inhibitors, mostly toward β-glucosidases. For a set of primary amines, the affinity to almond β-glucosidase was found to be proportional to substituent hydrophobicity.[3c] When the series of *n*-alkyl-substituted compounds was studied, their inhibitory activity typically increased with lengthening of the hydrocarbon tail.[1–3] This result was explained by hydrophobic interactions of the alkyl chain at the active site of enzyme. Thus, Legler et al. found that K_i values for interaction of the calf liver β-glucosidase with alkyl β-glucosides, *N*-alkyl β-glucosylamines, and *N*-alkyl derivatives of 1-deoxynojirimycin (with two exceptions) gradually decreased with increasing chain length.[2c] From the plots of the standard free energy of binding as a function of the number of CH_2 groups, they estimated the contribution of each methylene unit to the binding energy as approximately 3.1 kJ/mol.[2c] The aglycon site of the enzyme was postulated to have the form of an extended hydrophobic cleft able to accommodate up to 10 methylene groups. Additional CH_2 units contributed significantly less to the binding than the first ten. A comparison of the inhibition by *n*-butyl, phenyl, and cyclohexyl β-glucosides suggested that the aglycon hydrophobic cleft is rather narrow, with high affinity for straight chain aliphatic and aromatic residues.[2c] Other researchers also noticed that branching of the alkyl chain usually decreased inhibition,[3d,g,h] although an opposite effect was observed as well.[3g,h] Noteworthy, similar linear correlations of binding energy with the length of hydrocarbon chains were observed for some other guest–host interactions, for example, between *n*-alkanols and cyclodextrins or proteins.[12]

Direct evidence has been obtained recently by X-ray crystal structure analysis for the binding of alkyl groups via hydrophobic interactions in complexes of *N*-butyl- and *N*-nonyl-deoxynojirimycin with a recombinant human acid β-glucosidase (GlcCerase).[13] The alkyl chains are oriented toward the entrance of the active site, which has a hydrophobic surface favoring the more hydrophobic ligand.[13] It is worth mentioning that earlier the finding from an X-ray crystallographic study of a large hydrophobic pocket in the enzyme binding site helped to design a new class of influenza neuraminidase inhibitors,[3g,h] including oseltamivir, which was further developed into Tamiflu.[1m]

We hypothesized that the activity of our new inhibitors may also be enhanced by additional alkyl group(s). However, while the concept of a hydrophobic cleft looks quite evident for enzymes that have glycosides with long hydrocarbon chains as natural substrates (e.g., β-glucosyl ceramide[3a,k,13]), it is not so obvious for enzymes whose natural substrates are di- and oligosaccharides.[2e] The desirable size of such a tail was also unclear. In many cases [2,3a,b,e,f,l,m,t–v], the dependence of inhibition on the length of hydrophobic chain was not as smooth as described above, and a positive effect of a small alkyl group could even change to a negative one with its extension.[2,3i,u] The largest series of *n*-alkyl-substituted glycosidase inhibitors described so far included up to 7 homologues.[1n,2c,3a,f,l] Ten *n*-alkyl homologues were studied in a search for a potent influenza neuraminidase inhibitor.[3h] We synthesized and studied a series of alkoxy derivatives **5a**–**l** with a number of carbon atoms in the linear alkyl group varying from 0 to 12 (Scheme 1). The effect of this variation was different for α- and β-D-glucosidases. Inhibition parameters for these new inhibitors toward β-D-glucosidases are presented in Table 2. The analysis of Lineweaver–Burk plot and of the Cornish-Bowden–Eisenthal plot indicated that compounds **5g** and **5i** inhibited the β-D-glucosidase from *P. canescens* uncompetitively.

For β-D-glucosidases, the K_i values decrease with elongation of the hydrocarbon chain (Fig. 3), in a good agreement with the concept described above. Accordingly, the observed regularity may be rationalized in terms of additional stabilization of the alkyl group by the hydrophobic pocket or cleft within or in vicinity of the enzyme binding site. The increase of the alkyl length brings about an increase of this additional binding, but up to a certain point, after which the further elongation does not increase inhibition. In case

Table 2
Inhibition of β-D-glucosidases at pH 4.2 and 30 °C

Enzyme	β-D-glucosidase *P. canescens*		β-D-glucosidase *A. oryzae*	
Inhibitor (n)[a]	IC_{50}, μM	K_i, μM	IC_{50}, μM	K_i, μM
5a (0)	56.2	3.41	355	153
5b (1)	24.5	1.49	209	89.9
5c (2)	19.1	1.16	200	85.9
5d (3)	11.7	0.71	14.1	6.08
5e (4)	7.08	0.429	20	8.58
5f (5)	3.98	0.243	12	5.16
5g (6)	3.16	0.192	10	4.30
5h (7)	2.51	0.152	7.24	3.11
5i (8)	2.51	0.152	4.79	2.06
5j (9)	2.00	0.121	5.62	2.43
5k (10)	1.58	0.096	7.42	3.19
5l (12)	7.08	0.43	50.1	21.6

[a] n—The number of carbon atoms in n-alkyl chain R.

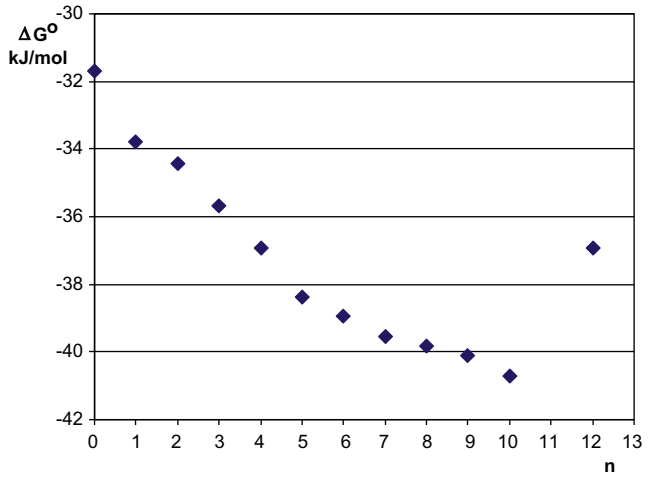


Figure 3. Free energy of binding between inhibitors **5a–l** and β-D-glucosidase from *P. canescens* versus the length of n-alkyl chain. Estimated from the K_i values of Table 2.

of β-D-glucosidase from *P. canescens* and inhibitors **5a–l**, a limit is practically achieved for octyl or nonyl derivatives (Fig. 3). Similar results were obtained previously for different inhibitors.[1n,2c,3a,b,f,h,r] With further addition of the methylene units, the effect fades out. The last point for the dodecyl derivative **5l** (Fig. 3, n = 12) deviated dramatically from the general trend. It was verified additionally several times. This strong deviation is not an accident, but most probably a systematic error caused by low solubility in water (it formed a cloudy solution). Noteworthy, it was suggested recently that longer alkyl chains may result in the formation of amphiphilic micelles in aqueous medium.[3t]

The first six to seven entries in Figure 3 represent a fairly good straight line with approximately equal differences in ΔG° values between the neighboring points. Using an average magnitude of this difference, we estimated the contribution of hydrophobic interactions with each of the first six CH_2 groups to the free binding energy of inhibitor as approximately 1.3 kJ/mol. This parameter is commensurable with 3.1 kJ/mol estimated for the interaction of a calf liver β-D-glucosidase with alkyl groups of various inhibitors.[2c]

The data for α-D-glucosidase are very different. Regardless of the length of lipophilic tails, the inhibitors **5a–l** deactivate the enzyme from *A. oryzae* to approximately the same extent. The K_i values slightly increase with elongation of the alkyl chains (from 25 μM for **5a** to 30 μM for **5l**), which is in contrast with the steep decrease observed for β-D-glucosidases (Table 2). Apparently, this α-D-glucosidase does not possess a hydrophobic aglycon-binding site able to strongly bind alkyl groups. Similarly, the inhibitory potency against α-glucosidase remained the same upon N-alkylation of 1-*N*-iminosugars,[3i,k] or even decreased nonsystematically for 2-N-alkylated 1-azafagomine.[3u] However, other researchers mentioned in some cases a noticeable increase of α-glucosidase inhibition upon extension of an alkyl group of inhibitor.[3l,m,r–t]

Thus, using a novel structural type of inhibitors we confirmed the existence of a systematic increase of inhibitory activity with an increase of alkyl chain length for β-D-glucosidases, and found the absence of such an effect for α-D-glucosidase.

3. Conclusions

The results of our studies prove that simple and readily available racemic derivatives of 1,2-cyclohexanedicarboxylic acid can be potent inhibitors for α- and β-D-glucosidases. The presence of at least two carboxylic groups and one hydroxy group is apparently essential for efficient inhibition. Its magnitude depends on configuration of substituents and increases for β-glucosidase with lengthening of the alkoxy group on the inhibitor. Further studies toward the enhancement of inhibitory activity will be focused on separation of enantiomers, on variation of the substituent configuration, of the length and structure of the lipophilic groups, and of the nature of heteroatoms.

4. Experimental

The reactions were monitored by TLC (silica gel, 8 × 2 cm plates with UV-indicator (254 nm), manufactured by Analtech Inc.). The yields were not optimized. Column chromatography was performed on silica gel (Sorbent Technologies, 40–75 μm). ^{1}H NMR and ^{13}C NMR spectra were acquired on Varian Mercury NMR-spectrometer (300 MHz). Exact mass measurements were performed on JEOL LC-Mate double-focusing mass spectrometer (Peabody, MA, USA) equipped with electrospray ionization source at a resolving power of 5000 with polyethyleneglycol as an internal reference. The MS and MS/MS spectra were obtained on Varian 1200LC triple quadrupole mass spectrometer (Walnut Creek, CA, USA) with electrospray ionization source in positive mode. Spectrometer Beckman Du-65 was used for the enzymatic hydrolysis studies. Elemental analyses were done by Desert Analytics, Tucson, AZ, and Robertson Microlit Laboratories, Madison, NJ. All solvents were purified by conventional techniques prior to use. Starting materials and acids **14, 15, 17–19** were purchased from Aldrich, Sigma, Lancaster, TCI, and Across Organics.

4.1. Dimethyl 4-cyclohexene-*cis*-1,2-dicarboxylate (8)

A mixture of *cis*-1,2,3,6-tetrahydrophthalic anhydride (10 g, 66 mmol), 50 mL of MeOH and 2 mL of concd H_2SO_4 was heated at reflux for 5 h. The solution was neutralized with solid $NaHCO_3$ and was filtered. MeOH was removed on rotary evaporator to yield 12.7 g (97%) of light-yellow oil that was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (m, 2H, H3, H6), 2.55 (m, 2H, H3, H6), 3.05 (m, 2H, H1, H2), 3.69 (s, 6H, CH_3), 5.68 (t, 2H, H4, H5); ^{13}C NMR ($CDCl_3$): δ 25.93 (C3, C6), 39.90 (C1, C2), 52.01 (OCH_3), 125.31 (C4, C5), 173.94 (C=O); MS/MS, m/z (%): 79.2 (100), 107.1 (19), 139.2 (21), 167.2 (85), 199.2 (21) $[M + H]^+$.

4.2. Epoxidation: diethyl 7-oxabicyclo[4.1.0]heptane-*trans*-3,4-dicarboxylate (12)

Diethyl 4-cyclohexene-*trans*-1,2-dicarboxylate (5 g, 22.1 mmol; prepared as described before[5]) was dissolved in 150 mL of dry

CH_2Cl_2, and *m*-CPBA (8.2 g, 33 mmol; 70% tech. grade) was added in small portions at 0 °C while stirring. The stirring continued at 0 °C for 10 h. After consumption of the starting material (TLC, $CHCl_3$), 100 mL of $CHCl_3$ and 200 mL of saturated Na_2CO_3 were added. The mixture was stirred for 30 min, the organic phase was separated, washed with 4 × 100 mL of saturated Na_2CO_3, dried for 12 h over anhyd Na_2SO_4, and evaporated to yield 3.8 g of yellowish oil, which was purified by column chromatography (EtOAc–hexane 1:3) to give 3.2 g (60%) of pure epoxide **12**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, 3H, CH_3), 1.245 (t, 3H, CH_3), 1.88 (ddd, H5a), 2.05 (dd, H2a), 2.31 (ddd, H2e), 2.46 (ddd, H5e), 2.59 (dt, H3), 2.82 (dt, H4), 3.19 (t, H1), 3.25 (m, H6), 4.13 (m, 4H, OCH_2); ^{13}C NMR ($CDCl_3$): δ 14.13 (CH_3), 26.41, 27.25 (C2, C5), 37.80, 40.12 (C3, C4), 50.33, 51.86 (C1, C6), 60.74 (OCH_2), 173.71, 174.65 (C=O); MS/MS, *m/z* (%): 79.2 (18), 151.5 (10), 169.2 (33), 197.4 (39), 243.2 (100) $[M+H]^+$.

4.3. Dimethyl 7-oxabicyclo[4.1.0]heptane-cis-3,4-dicarboxylates (9-*anti*) and (9-*syn*)

Epoxides **9-*anti*** and **9-*syn*** were prepared from 10 g (50.4 mmol) of the dimethyl ester **8** as described above for epoxide **12**, and were separated by column chromatography (EtOAc–hexane, 1:1).

4.3.1. Dimethyl (1*R**,3*R**,4*S**,6*S**)-7-oxabicyclo[4.1.0]heptane-3,4-dicarboxylate (9-*anti*):

Yield 5.6 g (52%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.17–2.33 (m, 4H; H2, H5), 2.92 (m, 2H; H3, H4), 3.24 (m, 2H; H1, H6), 3.68 (s, 6H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.83 (C2, C5), 37.69 (C3, C4), 51.54 (C1, C6), 51.97 (OCH_3), 173.48 (C=O); MS/MS, *m/z* (%): 67.1 (18), 85.1 (25), 95.0 (100), 123.1 (33), 155.0 (54), 183.0 (26), 215.0 (44) $[M+H]^+$.

4.3.2. Dimethyl (1*R**,3*S**,4*R**,6*S**)-7-oxabicyclo[4.1.0]heptane-3,4-dicarboxylate (9-*syn*):

Yield 2.8 g (26%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (m, 2H; H2, H5), 2.64–2.79 (m, 4H; H2–H5), 3.17 (m, 2H; H1, H6), 3.69 (s, 6H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.83 (C2, C5), 37.59 (C3, C4), 50.93 (C1, C6), 51.85 (OCH_3), 172.97 (C=O); MS/MS, *m/z* (%): 67.2 (23), 85.1 (20), 95.2 (100), 123.1 (43), 155.1 (44), 183.0 (28), 215.2 (61) $[M+H]^+$.

4.4. General procedure for the reaction of epoxides with *n*-alkanol

Epoxide (9.3 mmol) was dissolved in 15 mL of dry alcohol with addition of a catalytic amount of conc. H_2SO_4, and stirred under nitrogen till complete consumption of the starting material (TLC; EtOAc–hexane, 1:1). The reaction mixture was diluted with 30 mL of $CHCl_3$ and neutralized with saturated $NaHCO_3$. The aqueous layer was separated and washed with 3 × 20 mL of $CHCl_3$. Combined organic layers were dried over Na_2SO_4, the solvent was removed on rotary evaporator, and the product was purified by column chromatography.

4.4.1. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4,5-dihydroxycyclohexane-1,2-dicarboxylate (10a)

Eluent EtOAc–hexane (3:1); yield: 83%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (ddd, *J* = 13.5, 10.5, 4.9 Hz, 1H; H3a), 1.89 (q, *J* = 12.0 Hz, 1H; H6a), 2.30 (dt, *J* = 13.2, 4.1 Hz, 1H; H6e), 2.40 (dt, *J* = 13.5, 3.7 Hz, 1H; H3e),), 2.61 (dt, *J* = 12.1, 4.3 Hz, 1H; H1), 3.1 (br s, 2H; OH), 3.27 (q, *J* = 4.0 Hz, 1H; H2), 3.43 (dt, *J* = 4.7, 8.8 Hz, 1H; H5), 3.50 (dt, *J* = 4.1, 8.6 Hz, 1H; H4), 3.67 (s, 3H; CH_3), 3.68 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.91 (C6), 33.04 (C3), 40.17 (C2), 41.94 (C1), 52.23 (OCH_3), 71.75 (C5), 74.18 (C4), 173.39, 173.52 (C=O); MS/MS, *m/z* (%): 78.9 (51), 96.6 (18), 123.2 (20), 150.7 (22), 168.8 (100), 215.0 (16), 232.8 (41) $[M+H]^+$.

4.4.2. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-methoxycyclohexane-1,2-dicarboxylate (10b)

Eluent EtOAc–hexane (1:1); yield: 64%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.63 (ddd, *J* = 13.6, 11.0, 5.1 Hz, 1H; H3a), 1.80 (q, *J* = 12.0 Hz, 1H; H6a), 2.46 (m, 2H; H3e + H6e), 2.57 (dt, *J* = 11.8, 4.1 Hz, 1H; H1), 2.6 (br s, 1H, OH), 3.02 (ddd, *J* = 10.5, 8.3, 4.4 Hz, 1H; H5), 3.27 (q, *J* = 4.2 Hz, 1H; H2), 3.41 (s, 3H, OCH_3), 3.54 (ddd, *J* = 11.0, 8.4, 4.4 Hz, 1H; H4), 3.68 (s, 3H; CO_2CH_3), 3.70 (s, 3H; CO_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.54 (C6), 32.13 (C3), 40.50 (C2), 41.54 (C1), 51.91 (OCH_3), 51.96 (OCH_3), 56.49 (CH_3, ether), 69.91 (C4), 83.05 (C5), 172.92, 173.09 (C=O); MS/MS, *m/z* (%): 84.8 (10), 95.1 (54), 123.2 (57), 137.2 (19), 155.2 (67), 183.2 (44), 215.0 (100), 247.2 (57) $[M + H]^+$. Anal. Calcd for $C_{11}H_{18}O_6$ (246.26): C, 53.65; H, 7.37. Found: C, 53.78; H, 7.22.

4.4.3. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-ethoxycyclohexane-1,2-dicarboxylate (10c)

Eluent EtOAc–hexane (1:1); yield: 49%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (t, *J* = 7.0 Hz, 3H; CH_3), 1.61 (ddd, *J* = 13.5, 11.0, 5.2 Hz, 1H; H3a), 1.81 (q, *J* = 12.0 Hz, 1H; H6a), 2.42 (dt, *J* = 14.2, 4.1 Hz, 1H; H6e), 2.45 (dt, *J* = 13.8, 4.1 Hz, 1H; H3e), 2.55 (dt, *J* = 11.8, 4.2 Hz, 1H; H1), 2.65 (br s, 1H; OH), 3.10 (ddd, *J* = 10.6, 8.4, 4.4 Hz, 1H; H5), 3.27 (q, *J* = 4.1 Hz, 1H; H2), 3.41 (ddd, *J* = 14.0, 9.1, 6.9 Hz, 1H; OCH_2), 3.51 (ddd, *J* = 11.3, 8.5, 4.2 Hz, 1H; H4), 3.66 (s, 3H; OCH_3), 3.68 (s, 3H; OCH_3), 3.72 (ddd, *J* = 14.0, 9.1, 7.1 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.55 (CH_3), 27.35 (C6), 32.13 (C3), 40.54 (C2), 41.63 (C1), 51.95 (OCH_3), 64.23 (OCH_2), 69.89 (C4), 81.48 (C5), 172.96, 173.09 (C=O). MS/MS, *m/z* (%): 79.1 (23), 95.1 (38), 123.2 (37), 137.2 (19), 155.2 (18), 169.2 (85), 183.2 (38), 201.1 (59), 229.1 (100), 261.2 (33) $[M+H]^+$.

4.4.4. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-propyloxycyclohexane-1,2-dicarboxylate (10d)

Eluent EtOAc–hexane (1:1); yield: 87%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (t, *J* = 7.4 Hz, 3H; CH_3), 1.57 (sextet, *J* = 7.1 Hz, 2H; CH_2), 1.61 (ddd, *J* = 13.5, 11.0, 5.2 Hz, 1H; H3a), 1.79 (q, *J* = 12.0 Hz, 1H; H6a), 2.41 (dt, *J* = 13.9, 4.2 Hz, 1H; H6e), 2.46 (dt, *J* = 13.9, 4.1 Hz, 1H; H3e), 2.55 (dt, *J* = 12.1, 4.3 Hz, 1H; H1), 2.65 (br s, 1H; OH), 3.08 (ddd, *J* = 10.5, 8.3, 4.1 Hz, 1H; H5), 3.26 (q, *J* = 4.2, 1H; H2), 3.31 (dt, *J* = 9.1, 6.6 Hz, 1H; OCH_2), 3.52 (ddd, *J* = 11, 8.2, 4.0 Hz, 1H; H4), 3.61 (dt, *J* = 9.1, 6.6 Hz, 1H; OCH_2), 3.66 (s, 3H; OCH_3), 3.68 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.59 (CH_3), 23.28 (CH_2, propyl), 27.35 (C6), 32.10 (C3), 40.53 (C2), 41.63 (C1), 51.93 (OCH_3), 69.93 (OCH_2), 70.66 (C4), 81.65 (C5), 172.98, 173.13 (C=O); MS/MS, *m/z* (%): 79.2 (22), 155.1 (17), 169.2 (47), 201.1 (100), 243.0 (44), 275.2 (14) $[M+H]^+$.

4.4.5. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-butyloxycyclohexane-1,2-dicarboxylate (10e)

Eluent EtOAc–hexane (1:1); yield: 52%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, *J* = 7.3 Hz, 3H; CH_3, Bu), 1.36 (br sextet, 2H; CH_2, Bu), 1.56 (m, 2H; CH_2, Bu), 1.62 (ddd, *J* = 13.5, 11.0, 5.2 Hz, 1H; H3a), 1.80 (q, *J* = 12.0 Hz, 1H; H6a), 2.43 (dt, *J* = 13.8, 4.0 Hz, 1H; H6e), 2.48 (dt, *J* = 13.5, 4.0 Hz, 1H; H3e), 2.56 (dt, *J* = 11.8, 4.2 Hz, 1H; H1), 3.09 (ddd, *J* = 10.6, 8.4, 4.4 Hz, 1H; H5), 3.28 (q, *J* = 4.2 Hz, 1H; H2), 3.36 (dt, *J* = 9.1, 6.6 Hz, 1H; OCH_2, Bu), 3.53 (ddd, *J* = 11.1, 8.3, 4.3 Hz, 1H; H4), 3.67 (dt, *J* = 9.1, 6.6 Hz, 1H; OCH_2, Bu), 3.68 (s, 3H; OCH_3), 3.70 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.88 (CH_3, Bu), 19.35 (CH_2, Bu), 27.34 (C6), 32.15 (C3 + CH_2), 40.56 (C2), 41.66 (C1), 51.94 (OCH_3), 68.73 (OCH_2; Bu), 69.95 (C4), 81.72 (C5), 172.97, 173.12 (C=O); MS/MS, *m/z* (%): 79.2 (10), 155.1 (19), 169.2 (35), 201.1 (100), 257.1 (48), 289.1 (23) $[M+H]^+$.

4.4.6. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-pentyloxycyclohexane-1,2-dicarboxylate (10f)

Eluent EtOAc–hexane (1:2); yield: 27%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 7.0 Hz, 3H; CH_3), 1.32 (br m, 4H; CH_2, pentyl), 1.56 (m, 2H; CH_2, pentyl), 1.61 (m, 1H; H3a), 1.79 (q, J = 12.0 Hz, 1H; H6a), 2.42 (dt, J = 14.3, 4.1 Hz, 1H; H6e), 2.47 (dt, J = 13.6, 4.0 Hz, 1H; H3e), 2.54 (dt, J = 12.1, 4.2 Hz, 1H; H1), 2.61 (br s, 1H; OH), 3.07 (ddd, J = 10.7, 8.5, 4.3 Hz, 1H; H5), 3.27 (q, 4.1 Hz, 1H; H2), 3.34 (dt, J = 9.0, 6.6 Hz, 1H; OCH_2), 3.51 (ddd, J = 10.4, 8.8, 4.4 Hz, 1H; H4), 3.65 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2), 3.67 (s, 3H; OCH_3), 3.69 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.02 (CH_3), 22.52 (CH_2, pentyl), 27.31 (C6), 28.34, 29.75, (CH_2, pentyl), 32.13 (C3), 40.56 (C2), 41.64 (C1), 51.95 (OCH_3), 69.05 (OCH_2), 69.97 (C4), 81.72 (C5), 172.96, 173.10 (C=O); MS/MS, *m/z* (%): 79.2 (12), 155.1 (15), 169.2 (35), 201.1 (100), 271.2 (28), 303.0 (26) $[M+H]^+$.

4.4.7. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-hexyloxycyclohexane-1,2-dicarboxylate (10g)

Eluent EtOAc–hexane (1:2); yield: 44%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J = 6.7 Hz, 3H; CH_3), 1.3 (br m, 6H; CH_2, hexyl), 1.57 (m, 2H; CH_2, hexyl), 1.62 (ddd, J = 13.8, 11.0, 5.1 Hz, 1H; H3a), 1.81 (q, J = 12.0 Hz, 1H; H6a), 2.42 (dt, J = 13.8, 4.1 Hz, 1H; H6e), 2.47 (dt, J = 13.8, 4.1 Hz, 1H; H3e), 2.56 (dt, J = 11.8, 4.1 Hz, 1H; H1), 2.68 (br s, 1H; OH), 3.09 (ddd, J = 10.7, 8.4, 4.4 Hz, 1H; H5), 3.27 (q, J = 4.3 Hz, 1H; H2), 3.35 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.53 (ddd, J = 10.7, 8.5, 4.4 Hz, 1H; H4), 3.66 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.68 (s, 3H; OCH_3), 3.70 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.19 (CH_3), 22.77, 26.02 (CH_2, hexyl), 27.50 (C6), 30.20, 31.85 (CH_2, hexyl), 32.26 (C3), 40.69 (C2), 41.78 (C1), 51.19 (OCH_3), 69.24 (OCH_2), 70.08 (C4), 81.83 (C5), 173.15, 173.30 (C=O); MS/MS, *m/z* (%): 79.0 (8), 169.2 (29), 201.2 (100), 285.3 (27), 317.1 (18) $[M+H]^+$.

4.4.8. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-heptyloxycyclohexane-1,2-dicarboxylate (10h)

Eluent EtOAc–hexane (1:2); yield: 52%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.9 Hz, 3H; CH_3), 1.28 (br m, 8H; CH_2, heptyl), 1.57 (m, 2H; CH_2, heptyl), 1.62 (ddd, J = 13.5, 11.0, 5.0 Hz, 1H; H3a), 1.80 (q, J = 12.0 Hz, 1H; H6a), 2.42 (dt, J = 14.3, 4.1 Hz, 1H; H6e), 2.47 (dt, J = 13.8, 4.0 Hz, 1H; H3e), 2.55 (dt, J = 12.1, 4.2 Hz, 1H; H1), 2.63 (br s, 1H; OH), 3.09 (ddd, J = 10.7, 8.5, 4.2 Hz, 1H; H5), 3.27 (q, J = 4.2 Hz, 1H; H2), 3.35 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.52 (ddd, J = 10.9, 8.7, 4.4 Hz, 1H; H4), 3.66 (s, 3H; OCH_3), 3.66 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.07 (CH_3), 22.62, 26.14 (CH_2, heptyl), 27.23 (C6), 29.14, 30.08, 31.81, (CH_2, heptyl), 32.17 (C3), 40.55 (C2), 41.65 (C1), 51.95 (OCH_3), 69.07 (OCH_2), 69.95 (C4), 81.71 (C5), 172.97, 173.13 (C=O); MS/MS, *m/z* (%): 79.0 (13), 123.2 (11), 155.2 (11), 169.2 (89), 183.2 (17), 201.2 (100), 331.2 (10) $[M+H]^+$.

4.4.9. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-octyloxycyclohexane-1,2-dicarboxylate (10i)

Eluent EtOAc–hexane (1:2); yield: 42%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 6.8 Hz, 3H; CH_3), 1.25 (br m, 10H; CH_2, octyl), 1.55 (m, 2H; CH_2, octyl), 1.62 (ddd, J = 13.8, 11.3, 5.2 Hz, 1H; H3a), 1.79 (q, J = 12.0 Hz, 1H; H6a), 2.42 (dt, J = 14.0, 4.0 Hz, 1H; H6e), 2.47 (dt, J = 13.8, 4.1 Hz, 1H; H3e), 2.55 (dt, J = 12.1, 4.1 Hz, 1H; H1), 2.61 (br s, 1H; OH), 3.07 (ddd, J = 10.6, 8.5, 4.3 Hz, 1H; H5), 3.25 (q, J = 4.2 Hz, 1H; H2), 3.33 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2), 3.51 (ddd, J = 11.8, 8.6, 4.4 Hz, 1H; H4), 3.64 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.66 (s, 3H; OCH_3), 3.68 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.07 (CH_3), 22.66, 26.19 (CH_2, octyl), 27.34 (C6), 29.26, 29.45, 30.09, 31.84 (CH_2, octyl), 32.15 (C3), 40.57 (C2), 41.68 (C1), 51.94 (OCH_3), 69.09 (OCH_2), 69.96 (C4), 81.74 (C5), 172.96, 173.11 (C=O); MS/MS, *m/z* (%): 169.2 (38), 201.2 (100), 313.1 (17), 345.0 (9) $[M+H]^+$.

4.4.10. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-nonyloxycyclohexane-1,2-dicarboxylate (10j)

Eluent EtOAc–hexane (1:2); yield: 31%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 6.7 Hz, 3H; CH_3), 1.26 (br m, 12H; CH_2, nonyl), 1.56 (m, 2H; CH_2, nonyl), 1.61 (ddd, J = 13.5, 11.0, 5.0 Hz, 1H; H3a), 1.79 (q, J = 12.1 Hz, 1H; H6a), 2.22 (br s, 1H; OH), 2.42 (dt, J = 14.0, 4.0 Hz, 1H; H6e), 2.47 (dt, J = 13.8, 4.2 Hz, 1H; H3e), 2.54 (dt, J = 11.8, 4.2 Hz, 1H; H1), 3.07 (ddd, J = 10.7, 8.5, 4.4 Hz, 1H; H5), 3.26 (q, J = 4.5 Hz, 1H; H2), 3.34 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.52 (ddd, J = 11.1, 8.4, 4.1 Hz, 1H; H4), 3.62 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.67 (s, 3H; OCH_3), 3.69 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.26 (CH_3), 22.84, 26.35 (CH_2, nonyl), 27.51 (C6), 29.44, 29.66, 29.73, 30.26 (CH_2, nonyl), 32.21 (C3), 40.74 (C2), 41.82 (C1), 52.10 (OCH_3), 69.26 (OCH_2), 70.13 (C4), 81.90 (C5), 17.3.14, 173.28 (C=O); MS/MS, *m/z* (%): 79.0 (15), 169.2 (42), 183.2 (9), 201.2 (100), 327.1 (21), 359.3 (18) $[M+H]^+$.

4.4.11. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-decyloxycyclohexane-1,2-dicarboxylate (10k)

Eluent EtOAc–hexane (1:3); yield: 62%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, J = 6.7 Hz, 3H; CH_3), 1.25 (m, 14H; CH_2, decyl), 1.52 (m, 2H; CH_2, decyl), 1.59 (ddd, J = 13.5, 11.0, 5.0 Hz, 1H; H3a), 1.78 (q, J = 11.9 Hz, 1H; H6a), 2.39 (dt, J = 14.3, 4.0 Hz, 1H; H6e), 2.43 (dt, J = 13.8, 4.1 Hz, 1H; H3e), 2.53 (dt, J = 11.8, 4.1 Hz, 1H; H1), 2.67 (br s, 1H; OH), 3.06 (ddd, J = 10.6, 8.5, 4.4 Hz, 1H; H5), 3.23 (q, J = 4.2 Hz, 1H; H2), 3.32 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.50 (ddd, J = 10.4, 8.7, 4.3 Hz, 1H; H4), 3.62 (dt, J = 9.0, 6.6 Hz, 1H; OCH_2), 3.64 (s, 3H; OCH_3), 3.66 (s, 3H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.07 (CH_3), 22.69, 26.19 (CH_2, decyl), 27.35 (C6), 29.33, 29.50, 29.61, 29.85, 30.10, 31.91 (CH_2, decyl), 32.08 (C3), 40.50 (C2), 41.61 (C1), 51.91 (OCH_3), 69.10 (OCH_2), 69.89 (C4), 81.63 (C5), 172.98, 173.14 (C=O); MS/MS, *m/z* (%): 169.2 (26), 183.2 (9), 201.2 (100), 341.1 (9), 373.2 (20) $[M+H]^+$.

4.4.12. Dimethyl (1*S**,2*R**,4*R**,5*R**)-4-hydroxy-5-dodecyloxycyclohexane-1,2-dicarboxylate (10l)

Eluent EtOAc–hexane (1:3); yield: 22%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 6.6 Hz, 3H; CH_3), 1.28 (m, 18H; CH_2, dodecyl), 1.55 (m, 2H; CH_2, dodecyl), 1.60 (ddd, J = 13.8, 11.3, 5.2 Hz, 1H; H3a), 1.79 (q, J = 12.0 Hz, 1H; H6a), 2.41 (dt, J = 14.3, 3.7 Hz, 1H; H6e), 2.46 (dt, J = 13.8, 4.1 Hz, 1H; H3e), 2.53 (dt, J = 12.1, 4.1 Hz, 1H; H1), 2.67 (br s, 1H; OH), 3.07 (ddd, J = 10.5, 8.3, 4.1 Hz, 1H; H5), 3.26 (q, J = 4.1 Hz, 1H; H2), 3.33 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.51 (ddd, J = 11.1, 8.7, 4.5 Hz, 1H; H4), 3.64 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2), 3.68 (br s, 6H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.07 (CH_3), 24.79, 29.32, 29.56, 29.60, 29.62, 29.64, 29.70, 30.05, 32.11, 37.65 (CH_2), 40.53 (C2), 41.63 (C1), 51.53 (OCH_3), 69.09 (OCH_2), 69.91 (C4), 81.69 (C5), 173.10, 173.46 (C=O); MS/MS, *m/z* (%): 183.2 (17), 201.2 (51), 215.0 (100), 369.1 (15), 401.1 (18) $[M+H]^+$.

4.4.13. Dimethyl (1*R**,2*S**,4*R**,5*R**)-4-hydroxy-5-butyloxycyclohexane-1,2-dicarboxylate (11)

Eluent EtOAc–hexane (1:1); yield: 32%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, J = 7.3 Hz, 3H; CH_3, Bu), 1.35 (br sextet, J = 7.4 Hz, 2H; CH_2, Bu), 1.55 (m, 2H; CH_2, Bu), 1.56 (m, H6a), 1.93 (dt, J = 13.5, 10.2 Hz, 1H; H3a), 2.30 (dt, J = 13.8, 4.4 Hz, 1H; H3e), 2.46 (dt, J = 13.7, 4.5 Hz, 1H; H6e), 2.70 (dt, J = 10.6, 4.5 Hz, 1H; H2), 3.21 (dt, J = 7.7, 3.6 Hz, 1H; H5), 3.24 (q, J = 4.3 Hz, 1H; H1), 3.39 (dt, J = 9.2, 6.6 Hz, 1H; OCH_2, Bu), 3.55 (ddd, J = 9.6, 7.7, 4.4 Hz, 1H; H4), 3.60 (dt, J = 9.3, 6.6 Hz, 1H; OCH_2, Bu), 3.70 (s, 6H; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.87 (CH_3, Bu), 19.35 (CH_2, Bu), 28.61 (C6), 29.89 (C3), 32.18 (CH_2, Bu), 40.54 (C1), 41.18 (C2), 51.95, 52.01 (OCH_3), 69.11 (OCH_2; Bu), 71.39 (C4), 79.01 (C5), 173.31, 173.64 (C=O); MS/MS, *m/z* (%): 79.2 (12), 155.1 (22), 169.2 (45), 201.1 (100), 257.1 (42), 289.1 (33) $[M+H]^+$.

4.4.14. Diethyl (1*S**,2*S**,4*S**,5*S**)-4-hydroxy-5-butyloxycyclohexane-1,2-dicarboxylate (13)

Eluent EtOAc–hexane (1:2); yield: 54%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, J = 7.3 Hz, 3H; CH_3, Bu), 1.24 (t, J = 7.2 Hz, 3H; CH_3, Et), 1.25 (t, J = 7.2 Hz, 3H; CH_3, Et), 1.37 (br sextet, J = 7.4 Hz, 2H; CH_2, Bu), 1.54 (br quintet, J = 7.8 Hz, 2H; CH_2, Bu), 1.84 (m, 2H; H3a, H6a), 2.07 (m, 2H; H3e, H6e), 3.03 (dt, J = 4.1, 8.3 Hz, 1H; H1), 3.09 (dt, J = 4.2, 8.3 Hz, 1H; H2), 3.34 (dt, J = 3.3, 5.8 Hz, 1H; H5), 3.38 (dt, J = 9.1, 6.5 Hz, 1H; OCH_2, Bu), 3.57 (dt, J = 9.1, 6.5 Hz, 1H; OCH_2, Bu), 3.82 (br q, J = 4.5 Hz, 1H; H4), 4.14 (m, 4H, OCH_2; Et); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.88 (CH_3; Bu), 14.18, 14.20 (CH_3; Et), 19.37 (CH_2; Bu), 27.28 (C6), 30.66 (C3), 32.17 (CH_2; Bu), 39.36 (C2), 39.77 (C1), 60.67, 60.71 (OCH_2; Et), 68.05(C4), 68.83 (OCH_2; Bu), 77.35 (C5), 174.36, 174.41 (C=O); MS/MS, *m/z* (%): 79.2 (10), 123.5 (14), 151.2 (18), 169.2 (15), 179.5 (48), 197.3 (28), 215.3 (75), 271.3 (100), 317.2 (21) $[M+H]^+$.

4.5. General procedure for the hydrolysis of esters

Diester **10**, **11,** or **13** (3 mmol) was dissolved in 3 mL of MeOH, and 1 M KOH (10 mL) was added. The mixture was stirred at rt till consumption of the starting material (20 h), then concentrated to 1/3 of the volume on a rotary evaporator, and acidified with 6 M HCl to pH ~ 1. The aqueous layer was separated and extracted with 4 × 8 mL EtOAc. Combined organic extracts were dried over Na_2SO_4 for 6 h, the solvent was removed on rotary evaporator, and the product was purified by column chromatography (EtOAc–hexane–AcOH, 7:3:0.5). To completely remove the eluent from the product, dry toluene (2–3 mL) was added and evaporated three times.

4.5.1. (1*S**,2*R**,4*R**,5*R**)-4,5-dihydroxycyclohexane-1,2-dicarboxylic acid (5a). [14]

Yield: 28%; mp 149–150 °C (lit.:[14] 151 °C). ^{1}H NMR (300 MHz, D_2O): δ 1.74 (ddd, J = 13.7, 11.0, 5.5 Hz, 1H; H3a), 1.82 (q, J = 12.1 Hz, 1H; H6a), 2.30 (dt, J = 13.6, 3.7 Hz, 1H; H6e), 2.40 (dt, J = 13.5, 3.7 Hz, 1H; H3e), 2.89 (dt, J = 12.1, 4.4 Hz, 1H; H1), 3.38 (q, J = 4.1 Hz, H1; H2), 3.54 (dt, J = 4.3, 8.7 Hz, 1H; H4), 3.57 (dt, J = 4.5, 8.8 Hz, 1H; H5); ^{13}C NMR (75 MHz, D_2O): δ 30.84 (C6), 32.81 (C3), 41.09 (C1/C2), 41.12 (C1/C2), 71.18 (C4), 73.14 (C5), 177.30, 177.68 (C=O); MS/MS, *m/z* (%): 68.6 (42), 79.2 (100), 96.7 (46), 122.5 (23), 141.2 (18), 149.0 (49), 169.2 (83), 187.1 (45), 205.0 (33) $[M+H]^+$; HRMS (APCI): $C_8H_{12}O_6$ requires $[M+H]^+$ 205.0712; found 205.0722.

4.5.2. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-methoxycyclohexane-1,2-dicarboxylic acid (5b)

Yield: 56%. ^{1}H NMR (300 MHz, CD_3OD): δ 1.63 (ddd, J = 13.5, 9.9, 5.0 Hz, 1H; H3a), 1.86 (dt, J = 13.2, 10.3 Hz, 1H; H6a), 2.31 (dt, J = 8.6, 4.4 Hz, 1H; H6e), 2.35 (dt, J = 8.9, 4.5 Hz, 1H; H3e), 2.65 (dt, J = 10.7, 4.3 Hz, 1H; H1), 3.08 (ddd, J = 9.4, 7.7, 4.2 Hz, 1H; H5), 3.11 (m, 1H; H2), 3.40 (s, 3H; OCH_3), 3.57 (ddd, J = 10.1, 7.8, 4.3 Hz, 1H; H4); ^{13}C NMR (75 MHz, CD_3OD): δ 28.6 (C6), 33.84 (C3), 41.62 (C2), 42.54 (C1), 57.32 (OCH_3), 70.93 (C4), 83.97 (C5), 177.28, 177.44 (C=O); MS/MS, *m/z* (%): 79.1 (41), 95.1 (73), 109.2 (18), 123.1 (71), 141.3 (37), 155.3 (36), 162.9 (17), 169.1 (42), 177.1 (39), 183.1 (37), 201.2 (82), 219.1 (100) $[M+H]^+$; HRMS: $C_9H_{14}O_6$ requires $[M+Na]^+$ 241.0688; found 241.0714.

4.5.3. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-ethoxycyclohexane-1,2-dicarboxylic acid (5c)

Yield: 67%; mp 169–171 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.85 (t, J = 7.05 Hz, 3H; CH_3), 1.67 (ddd, J = 14.0, 9.6, 4.9 Hz, 1H; H3a), 1.92 (br q, J = 11 Hz, 1H; H6a), 2.27 (dt, J = 13.5, 4.2 Hz, 1H; H6e), 2.35 (dt, J = 13.6, 4.8 Hz, 1H; H3e), 2.68 (dt, J = 10.3, 4.75 Hz, 1H; H1), 3.09 (q, J = 4.8 Hz, 1H; H2), 3.17 (ddd, J = 9.1, 7.7, 4.1 Hz, 1H; H5), 3.51 (dt, J = 9.1, 7.1 Hz, 1H; OCH_2), 3.55 (m, 1H; H4), 3.69 (dt, J = 9.1, 7.1 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 15.84 (CH_3), 29.10 (C6), 33.46 (C3), 41.23 (C2), 42.22 (C1), 65.81 (OCH_2), 70.86 (C4), 81.84 (C5), 177.04, 177.14 (C=O); MS/MS, *m/z* (%): 100.4 (37), 112.4 (27), 125.9 (29), 141.6 (41), 169.1 (58), 186.5 (28), 215.3 (71), 232.7 (100) $[M+H]^+$; HRMS: $C_{10}H_{16}O_6$ requires $[M+Na]^+$ 255.0845; found 255.0813.

4.5.4. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-propyloxycyclohexane-1,2-dicarboxylic acid (5d)

Yield: 84%; mp 137–140 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 0.92 (t, J = 7.4 Hz, 3H; CH_3), 1.57 (sextet, J = 7.0 Hz, 2H; CH_2, propyl), 1.64 (ddd, J = 13.8, 9.6, 5.0 Hz, 1H; H3a), 1.88 (q, J = 11.0 Hz, 1H; H6a), 2.27 (dt, J = 1.8, 4.1 Hz, 1H; H6e), 2.36 (ddd, J = 14.0, 5.2, 4.4 Hz, 1H; H3e), 2.68 (dt, J = 10.5, 5.0, 1H; H1), 3.08 (q, J = 4.6 Hz, 1H; H2), 3.16 (ddd, J = 9.1, 7.7, 4.1 Hz, 1H; H5), 3.43 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2), 3.56 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2), 3.58 (m, 1H; H4); ^{13}C NMR (75 MHz, CD_3OD): δ 10.94 (CH_3), 24.28 (CH_2, propyl), 29.43 (C6), 33.59 (C3), 41.41 (C2), 42.73 (C1), 70.90 (C4), 72.33 (OCH_2), 82.16 (C5), 177.39, 177.67 (C=O); MS/MS, *m/z* (%): 79.1 (7), 169.1 (52), 187.1 (81), 229.2 (40), 247.1 (100) $[M+H]^+$; HRMS: $C_{11}H_{18}O_6$ requires $[M+Na]^+$ 269.1001; found 269.1008.

4.5.5. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-butyloxycyclohexane-1,2-dicarboxylic acid (5e)

Yield: 82%; mp 133–136 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 0.92 (t, J = 7.3 Hz, 3H; CH_3, Bu), 1.38 (sextet, J = 7.3 Hz, 2H; CH_2, Bu), 1.54 (quintet, J = 7.0 Hz, 2H; CH_2, Bu), 1.68 (ddd, J = 14.0, 9.5, 5.0 Hz, 1H; H3a), 1.94 (dt, J = 13.2, 9.6 Hz, 1H; H6a), 2.25 (dt, J = 13.8, 4.1 Hz, 1H; H6e), 2.34 (ddd, J = 13.7, 5.9, 4.1 Hz, 1H; H3e), 2.70 (dt, J = 9.8, 4.4 Hz, 1H; H1), 3.08 (q, J = 4.8 Hz, 1H; H2), 3.16 (ddd, J = 8.8, 7.4, 4.1 Hz, 1H; H5), 3.46 (dt, J = 9.4, 6.5 Hz, 1H; OCH_2, Bu), 3.57 (m, 1H; H4), 3.61 (dt, J = 9.4, 6.5 Hz, 1H; OCH_2, Bu); ^{13}C NMR (75 MHz, CD_3OD): δ 14.30 (CH_3, Bu), 20.36 (CH_2, Bu), 28.99 (C6), 33.14 (C3), 33.33 (CH_2, Bu), 41.03 (C2), 41.93 (C1), 70.39 (OCH_2; Bu), 70.74 (C4), 81.84 (C5), 176.92, 177.03 (C=O); MS/MS, *m/z* (%): 79.1 (16), 97.1 (6), 169.1 (92), 187.1 (100), 243.2 (10), 261.1 (8) $[M+H]^+$; HRMS: $C_{12}H_{20}O_6$ requires $[M+Na]^+$ 283.1158; found 283.1155.

4.5.6. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-pentyloxycyclohexane-1,2-dicarboxylic acid (5f)

Yield: 57%; mp 151–153 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 0.91 (t, J = 7.2 Hz, 3H; CH_3), 1.32 (m, 4H; CH_2, pentyl), 1.57 (m, 2H; CH_2, pentyl), 1.69 (ddd, J = 14.0, 9.5, 5.0 Hz, 1H; H3a), 1.94 (q, J = 9.6 Hz, 1H; H6a), 2.26 (dt, J = 13.5, 4.2 Hz, 1H; H6e), 2.34 (ddd, J = 13.5, 5.6, 4.1 Hz, 1H; H3e), 2.70 (dt, J = 9.9, 4.7 Hz, 1H; H1), 3.09 (q, J = 5.0 Hz, 1H; H2), 3.16 (ddd, J = 9.1, 7.7, 4.1 Hz, 1H; H5), 3.45 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.56 (m, 1H; H4), 3.60 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 14.40 (CH_3), 23.62, 29.48, 33.87 (CH_2, pentyl), 28.97 (C6), 33.15 (C3), 41.04 (C2), 41.89 (C1), 70.71 (OCH_2), 70.74 (C4), 81.86 (C5), 176.84, 176.97 (C=O); MS/MS *m/z* (%): 123.4 (24), 151.2 (15), 169.2 (100), 187.1 (68), 257.2 (10), 275.2 (7) $[M+H]^+$. Anal. Calcd for $C_{13}H_{22}O_6$ (274.31): C, 56.92; H, 8.08. Found: C, 56.75; H, 8.18.

4.5.7. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-hexyloxycyclohexane-1,2-dicarboxylic acid (5g)

Yield: 97%; mp 160–164 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 0.90 (t, J = 6.8 Hz, 3H; CH_3), 1.32 (m, 6H; CH_2, hexyl), 1.5 (m, 2H; CH_2, hexyl), 1.68 (ddd, J = 14.0, 9.5, 5.0 Hz, 1H; H3a), 1.93 (q, J = 9.6 Hz, 1H; H6a), 2.26 (dt, J = 13.5, 4.1 Hz, 1H; H6e), 2.35 (ddd, J = 13.8, 5.5, 4.1 Hz, 1H; H3e), 2.69 (dt, J = 8.9, 4.3 Hz, 1H; H1), 3.09 (q, J = 4.7 Hz, 1H; H2), 3.16 (ddd, J = 9.0, 7.6, 4.1 Hz, 1H; H5), 3.46 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.56 (m, 1H; H4), 3.61 (dt,

J = 9.1, 6.7 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 14.41 (CH_3), 23.72, 26.94 (CH_2, hexyl), 29.05 (C6), 31.16, 32.94 (CH_2, hexyl), 33.25 (C3), 41.11 (C2), 42.02 (C1), 70.74 (OCH_2), 70.80 (C4), 81.94 (C5), 176.95, 177.04 (C=O); MS/MS, *m/z* (%): 79.4 (11), 85.8 (9), 97.1 (11), 123.4 (10), 169.0 (60), 187.1 (100), 270.9 (14), 288.8 (63) $[M+H]^+$. Anal. Calcd for $C_{14}H_{24}O_6$ (288.34): C, 58.32; H, 8.39. Found: C, 58.22; H, 8.50.

4.5.8. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-heptyloxycyclohexane-1,2-dicarboxylic acid (5h)

Yield: 66%; mp 153–157 °C. ^1H NMR (300 MHz, CD_3OD): δ 0.90 (t, J = 6.9 Hz, 3H; CH_3), 1.32 (m, 8H; CH_2, heptyl), 1.55 (m, 2H; CH_2, heptyl), 1.66 (ddd, J = 14.0, 9.6, 5.0 Hz, 1H; H3a), 1.91 (dt, J = 10.9 Hz, 1H; H6a), 2.27 (dt, J = 13.5, 4.1 Hz, 1H; H6e), 2.35 (ddd, J = 13.5, 5.2, 4.1 Hz, 1H; H3e), 2.68 (dt, J = 10.2, 4.6 Hz, 1H; H1), 3.08 (q, J = 4.6 Hz, 1H; H2), 3.16 (ddd, J = 9.0, 7.6, 4.2 Hz, 1H; H5), 3.46 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.57 (m, 1H; H4), 3.61 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 14.42 (CH_3), 23.73, 27.27 (CH_2, heptyl), 29.05 (C6), 30.35, 31.19, 33.05 (CH_2, heptyl), 33.27 (C3), 41.11 (C2), 42.02 (C1), 70.73 (OCH_2), 70.78 (C4), 81.92 (C5), 177.05, 177.09 (C=O); MS/MS, *m/z* (%): 79.2 (8), 169.1 (47), 187.1 (100), 285.0 (23), 302.2 (75) $[M+H]^+$. Anal. Calcd for $C_{15}H_{26}O_6$ (302.36): C, 59.58; H, 8.67. Found: C, 59.49; H, 8.69.

4.5.9. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-octyloxycyclohexane-1,2-dicarboxylic acid (5i)

Yield: 66%; mp 143–145 °C. ^1H NMR (300 MHz, CD_3OD): δ 0.90 (t, J = 6.9 Hz, 3H; CH_3), 1.31 (m, 10H; CH_2, octyl), 1.56 (br quin, J = 6.8 Hz, 2H; CH_2, octyl), 1.68 (ddd, J = 13.7, 9.3, 4.9 Hz, 1H; H3a), 1.93 (dt, J = 13.5, 9.6 Hz, 1H; H6a), 1.93 (dt, J = 13.5, 9.6 Hz, 1H; H6a), 2.26 (dt, J = 13.5, 4.2, 1H; H6e), 2.35 (ddd, J = 13.5, 5.2, 4.1 Hz, 1H; H3e), 2.69 (dt, J = 10.1, 4.7 Hz, 1H; H1), 3.09 (q, J = 5.0 Hz, 1H; H2), 3.16 (ddd, J = 9.1, 7.6, 4.1 Hz, 1H; H5), 3.46 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.56 (m, 1H; H4), 3.61 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 14.44 (CH_3), 23.74, 27.27 (CH_2, octyl), 29.05 (C6), 30.45, 30.64, 31.19, 33.06, (CH_2, octyl), 33.27 (C3), 41.11 (C2), 42.00 (C1), 70.74 (OCH_2), 70.80 (C4), 81.95 (C5), 176.92, 177.02 (C=O); MS/MS, *m/z* (%): 123.1 (15), 151.0 (18), 169.0 (100), 187.1 (73), 299.3 (12), 317.0 (13) $[M+H]^+$. Anal. Calcd for $C_{16}H_{28}O_6$ (316.39): C, 60.74; H, 8.92. Found: C, 60.50; H, 8.92.

4.5.10. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-nonyloxycyclohexane-1,2-dicarboxylic acid (5j)

Yield: 21%; mp 132–135 °C. ^1H NMR (300 MHz, CD_3OD): δ 0.89 (t, J = 6.8 Hz, 3H; CH_3), 1.30 (m, 12H; CH_2, nonyl), 1.56 (br quin, J = 6.7 Hz, 2H; CH_2, nonyl), 1.68 (ddd, J = 14.0, 9.5, 4.9 Hz, 1H; H3a), 1.93 (q, J = 10.8 Hz, 1H; H6a), 2.26 (dt, J = 13.7, 4.3 Hz, 1H; H6e), 2.35 (ddd, J = 13.5, 5.5, 4.1 Hz, 1H; H3e), 2.69 (dt, J = 10.9, 4.7 Hz, 1H; H1), 3.09 (q, J = 4.8 Hz, 1H; H2), 3.16 (ddd, J = 8.9, 7.7, 4.2 Hz, 1H; H5), 3.45 (dt, J = 9.2, 6.6 Hz, 1H; OCH_2), 3.56 (m, 1H; H4), 3.61 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 14.43 (CH_3), 23.73, 27.25 (CH_2, nonyl), 29.09 (C6), 30.44, 30.66, 30.73, 31.18, 33.07 (CH_2, nonyl), 33.29 (C3), 41.16 (C2), 42.09 (C1), 70.74 (OCH_2), 70.81 (C4), 81.96 (C5), 177.01, 177.07 (C=O). MS/MS, *m/z* (%): 123.1 (10), 151.2 (18), 169.0 (86), 187.1 (48), 313.2 (38), 331.2 (100) $[M+H]^+$; HRMS: $C_{17}H_{30}O_6$ requires $[M+Na]^+$ 353.1940; found 353.1954.

4.5.11. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-decyloxycyclohexane-1,2-dicarboxylic acid (5k)

Yield: 89%; mp 149–152 °C. ^1H NMR (300 MHz, CD_3OD): δ 0.89 (t, J = 6.74 Hz, 3H; CH_3), 1.31 (m, 14H; CH_2, decyl), 1.56 (br quin, J = 6.7 Hz, 2H; CH_2, decyl), 1.68 (ddd, J = 14.0, 9.6, 5.0 Hz, 1H; H3a), 1.93 (q, J = 13.0 Hz, 1H; H6a), 2.26 (dt, J = 13.5, 4.2 Hz, 1H; H6e), 2.35 (ddd, J = 13.7, 5.5, 4.1 Hz, 1H; H3e), 2.69 (dt, J = 9.9, 4.6 Hz, 1H; H1), 3.09 (q, J = 4.7 Hz, 1H; H2), 3.16 (ddd, J = 9.1, 7.6, 4.1 Hz, 1H; H5), 3.45 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2), 3.57 (m, 1H; H4), 3.61 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 14.44 (CH_3), 23.75, 27.25 (CH_2, decyl), 29.02 (C6), 30.48, 30.67, 30.75, 30.78, 31.17, 33.09 (CH_2, decyl), 33.22 (C3), 41.09 (C2), 41.99 (C1), 70.73 (OCH_2), 70.78 (C4), 81.91 (C5), 176.96, 177.03 (C=O); MS/MS, *m/z* (%): 85.1(10), 94.9 (9), 122.9 (37), 140.9 (10), 151.2 (22), 169.1 (75), 187.1 (91), 281.6 (10), 326.9 (67), 345.2 (100) $[M+H]^+$. Anal. Calcd for $C_{18}H_{32}O_6$ (344.44): C, 62.77; H, 9.36. Found: C, 63.00; H, 9.51.

4.5.12. (1*S**,2*R**,4*R**,5*R**)-4-Hydroxy-5-dodecyloxycyclohexane-1,2-dicarboxylic acid (5l)

Yield: 25%; mp 146–149 °C. ^1H NMR (300 MHz, CD_3OD): δ 0.89 (t, J = 6.7 Hz, 3H; CH_3), 1.31 (m, 18H; CH_2, dodecyl), 1.55 (m, 2H; CH_2, dodecyl), 1.69 (ddd, J = 14.0, 9.6, 4.9 Hz, 1H; H3a), 1.93 (q, J = 12.1 Hz, 1H; H6a), 2.22 (dt, J = 13.8, 4.1 Hz, 1H; H6e), 2.36 (dt, J = 13.2, 3.6 Hz, 1H; H3e), 2.69 (dt, J = 10.2, 4.7 Hz, 1H; H1), 3.09 (q, J = 4.7 Hz, 1H; H2), 3.17 (ddd, J = 9.1, 7.7, 4.1 Hz, 1H; H5), 3.45 (dt, J = 9.1, 6.6 Hz, 1H; OCH_2), 3.56 (m, 1H; H4), 3.61 (dt, J = 9.1, 6.7 Hz, 1H; OCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 14.03 (CH_3), 23.74, 27.25 (CH_2, decyl), 29.01 (C6), 30.48, 30.56, 30.67, 30.77, 30.78, 31.18, 32.39, 33.09 (CH_2, decyl), 34.55 (C3), 41.12 (C2), 41.97 (C1), 70.74 (OCH_2), 70.79 (C4), 81.93 (C5), 176.97, 177.2 (C=O); MS/MS, *m/z* (%): 123.2 (8), 151.2 (11), 169.2 (53), 187.2 (100), 355.2 (11), 373.2 (22) $[M+H]^+$; HRMS: $C_{20}H_{36}O_6$ requires $[M+Na]^+$ 395.2410; found 395.2180.

4.5.13. (1*R**,2*S**,4*R**,5*R**)-4-Hydroxy-5-butyloxycyclohexane-1,2-dicarboxylic acid (6)

Yield: 80%; mp 131–133 °C. ^1H NMR (300 MHz, CD_3OD): δ 0.92 (t, J = 7.3 Hz, 3H; CH_3, Bu), 1.38 (br sextet, J = 7.3 Hz, 2H, CH_2; Bu), 1.54 (m, 2H, CH_2; Bu), 1.56 (m, H6a), 1.94 (dt, J = 13.5, 10.6 Hz, 1H; H3a), 2.19 (dt, J = 13.5, 4.4 Hz, 1H; H3e), 2.45 (dt, J = 13.8, 4.2 Hz, 1H; H6e), 2.66 (dt, J = 11.0, 4.3 Hz, 1H; H2), 3.14 (q, J = 4.4 Hz, 1H; H1), 3.16 (m, H5), 3.49 (m, H4), 3.50 (dt, J = 9.4, 6.3 Hz, 1H; OCH_2, Bu), 3.57 (dt, J = 9.1, 6.3 Hz, 1H; OCH_2, Bu); ^{13}C NMR (75 MHz, CD_3OD): δ 14.27 (CH_3, Bu), 20.35 (CH_2, Bu), 30.98 (C6), 32.45 (C3), 33.34 (CH_2, Bu), 41.78 (C1), 42.22 (C2), 70.62 (OCH_2; Bu), 73.01 (C4), 80.65 (C5), 176.76, 176.99 (C=O); MS/MS, *m/z* (%): 79.0 (7), 81.1 (22), 97.2 (7), 123.1 (7), 141.2 (23), 169.2 (42), 187.2 (27), 225.2 (55), 243.2 (64), 261.1 (100) $[M+H]^+$; HRMS: $C_{12}H_{20}O_6$ requires $[M+Na]^+$ 283.1158; found 283.1165.

4.5.14. (1*S**,2*S**,4*S**,5*S**)-4-Hydroxy-5-butyloxycyclohexane-1,2-dicarboxylic acid (7)

Yield: 90%. ^1H NMR (300 MHz, CD_3OD): δ 0.94 (t, J = 7.4 Hz, 3H; CH_3, Bu), 1.41 (sextet, J = 7.3 Hz, 2H; CH_2, Bu), 1.55 (br quintet, J = 7.0 Hz, 2H; CH_2, Bu), 1.83 (br t, J = 13 Hz, 2H; H3a, H6a), 1.91 (dt, J = 13.5, 4.1 Hz, 1H; H3e), 2.20 (dt, J = 13.8, 3.7 Hz, 1H; H6e), 2.83 (dt, J = 11.3, 3.6 Hz, 1H; H1), 2.93 (dt, J = 4.5, 11.1 Hz, 1H; H2), 3.42 (br q, J = 3.3 Hz, 1H; H5), 3.45 (dt, J = 9.2, 6.3 Hz, 1H; OCH_2, Bu), 3.56 (dt, J = 9.3, 6.3 Hz, 1H; OCH_2, Bu), 3.87 (br q, J = 3.3 Hz, 1H; H4); ^{13}C NMR (75 MHz, CD_3OD): δ 14.26 (CH_3; Bu), 20.44 (CH_2; Bu), 28.49 (C6), 31.84 (C3), 33.31 (CH_2; Bu), 40.04 (C2), 40.52 (C1), 67.52 (C4), 69.84 (OCH_2; Bu), 77.39 (C5), 178.96, 179.08 (C=O); MS/MS, *m/z* (%): 80.9 (8), 123.1 (7), 141.2 (11), 151.0 (13), 169.2 (33), 187.2 (39), 225.2 (56), 243.2 (100), 261.1 (14) $[M+H]^+$; HRMS: $C_{12}H_{20}O_6$ requires $[M+Na]^+$ 283.1158; found 283.1193.

4.5.15. 4-Cyclohexene-*trans*-1,2-dicarboxylic acid (16)

Yield: 86%. ^1H NMR (300 MHz, $CDCl_3$): δ 2.21 (m, 2H, H3, H6), 2.50 (m, 2H, H3, H6), 2.85 (m, 2H, H1, H2), 5.73 (br d, 2H, H4,

H5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.00 (C3, C6), 41.80 (C1, C2), 125.20 (C4, C5), 181.80 (C=O).

4.6. General procedure for the glycosidase activity and inhibition assay

The enzyme activities were assayed using several glycosidases in multi-enzyme complexes isolated from fungi *P. canescens* (α-D- and β-D-galactosidase, β-D-glucosidase, and α-L- and β-D-fucosidase) and *A. oryzae* (α-D- and β-D-galactosidase, α-D- and β-D-glucosidase, and α-L- and β-D-fucosidase).[8]

All assays were performed in a standard way[9] by spectrophotometric monitoring (at 400 nm, with Beckman Du-65 spectrometer) the release of *p*-nitrophenol from the corresponding *p*-nitrophenyl glycosides at 30 °C. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per minute. Enzyme and substrate concentrations were chosen so that the degree of hydrolysis was never more than 20%, and in most cases was less than 10%, over the course of the assay. The method used to measure the rate of the reaction assumes that the amount of the substrate is high enough, such that the disappearance over a given period is insignificant, that is the rate of the reaction is close to linear for the first stage of the reaction.

An enzyme (5 mg in 1 mL) was added to 3.5 mL of 0.2 M acetic buffer (pH 4.2), and the mixture was incubated for 30 min at 30 °C. The reaction was initiated with addition of a proper substrate (0.5 mL of 20 mM glycopyranoside), and aliquots were taken after 2, 5, 10, and 15 min. The reaction was terminated by addition of 1 mL of 1 M Na_2CO_3 to 0.5 mL aliquot solution. The concentration of the released *p*-nitrophenol was determined with the spectrophotometer at a 400 nm wavelength using molar extinction coefficient 18.3 mM^{-1} cm^{-1}.

The same protocol was used for determination of inhibitory activity with the following changes: 1 mL of enzyme solution, 0.5 mL inhibitor solution, and 3.0 mL of 0.2 M acetic buffer (pH 4.2) were incubated for 1 h at 30 °C before addition of 0.5 mL of substrate (20 mM). The inhibition was estimated as a loss of enzymatic activity (in %; Table 1).

4.7. Kinetic parameters (K_M and K_M^{app})

Six substrate solutions were prepared with various concentrations up to saturation point for each enzyme: the final substrate concentration after all additions varied from 0.01 mM to 2.00 mM. Reaction rates were measured as described above. Control experiments with no enzyme were performed to exclude the errors due to the substrate spontaneous hydrolysis. Enzyme behavior (at pH 4.2 and 30 °C) abided the Michaelis–Menten equation. Parameters K_M and K_M^{app} were calculated from Lineweaver–Burk (double-reciprocal) plot[15,16] and Cornish-Bowden–Eisenthal (direct linear) plot.[17,18]

The parameter IC_{50} was determined from a graph, log[I] versus *V* (enzyme activity), using a constant concentration of substrate and varying concentration of inhibitor.[16,19] The K_M and IC_{50} values were used to calculate K_i by Eq. (1). The same values for K_i were determined from Eq. (2)

$$K_i = K_M IC_{50}/(K_M + [S]_{fin}) \quad (1)$$

$$K_i = K_M[I]/(K_M^{app} - K_M) \quad (2)$$

Three-five independent trials using freshly prepared substrate and enzyme solutions were performed to obtain each parameter. Values for V_{max} and K_M were reproducible within ±5% and ±10%, respectively, and the standard error was ±10% for the IC_{50} values and ±15% for the K_i values.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.11.009.

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