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Strategy for designing selective α-L-rhamnosidase inhibitors: Synthesis and biological evaluation of L-DMDP cyclic isothioureas

Shota Miyawaki [a], Yuki Hirokami [a], Kyoko Kinami [a], Masako Hoshino [a], Daisuke Minehira [a], Daiki Miyamoto [b], Robert J. Nash [c], George W. J. Fleet [d], Isao Adachi [a], Naoki Toyooka [b], Atsushi Kato [a,*]

[a] Department of Hospital Pharmacy, University of Toyama, Toyama 930-0194, Japan
[b] Graduate School of Science and Engineering, University of Toyama, Toyama 930-8555, Japan
[c] Institute of Biological, Environmental and Rural Sciences/Phytoquest Limited, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, United Kingdom
[d] Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Oxford OX1 3TA, United Kingdom

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ABSTRACT

This study shows that the cyclization of L-DMDP thioureas to bicyclic L-DMDP isothioureas improved α-L-rhamnosidase inhibition which was further enhanced by increasing the length of the alkyl chain. The addition of a long alkyl chain, such as decyl or dodecyl, to the nitrogen led to the production of highly potent inhibitors of α-L-rhamnosidase; it also caused broad inhibition spectrum against β-glucosidase and β-galactosidase. In contrast, the corresponding *N*-benzyl-L-DMDP cyclic isothioureas display selective inhibition of α-L-rhamnosidase; 3′,4′-dichlorobenzyl-L-DMDP cyclic isothiourea (**3r**) was found to display the most potent and selective inhibition of α-L-rhamnosidase, with IC_{50} value of 0.22 μM, about 46-fold better than the positive control 5-*epi*-deoxyrhamnojirimycin (5-*epi*-DRJ; IC_{50} = 10 μM) and occupied the active-site of this enzyme (K_i = 0.11 μM). Bicyclic isothioureas of *ido*-L-DMDP did not inhibit α-L-rhamnosidase. These new mimics of L-rhamnose may affect other enzymes associated with the biochemistry of rhamnose including enzymes involved in progression of tuberculosis.

1. Introduction

Human tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. Despite the introduction of the effective four-drug [isoniazid (INH), rifampicin (REF), pyrazinamide (PZA) and ethambutol (EMB)] combination therapy regimen, TB infection remains a major global health problem complicated by escalating rates of antibiotic resistance. The cell wall of *Mycobacterium tuberculosis* is composed of arabinogalactan and peptidoglycan regions. In the linker part, an L-rhamnosyl residue has a crucial structural role in the attachment of arabinogalactan to peptidoglycan. Therefore, mimics of L-rhamnose that are able to act as inhibitors either of thymidine diphosphate-(dTDP)-rhamnose biosynthesis from dTDP-glucose or reconstruction of the mycobacterial cell wall by α-L-rhamnosidase may have potential as novel carbohydrate-based chemotherapeutic agents for the treatment of TB infection.[1–4] Rhamnose has no role in mammalian metabolism so that selective α-L-rhamnosidase inhibitors should not have any deleterious effect on humans.

Iminosugars are sugar mimics with a nitrogen atom in place of the ring oxygen of monosaccharides and inhibit various glycosidases in a reversible and competitive manner.[5–7] In general, iminosugars inhibit glycosidases by mimicking the pyranosyl and furanosyl moiety of the corresponding substrate. Based on this general strategy, mimics of rhamnopyranose and/or of rhamnofuranose are suitable structures for modifying rhamnose metabolism. However, as shown in Figure 1, although L-rhamnopyranose and 1-deoxyrhamnojirimycin (DRJ) have common topographical features, DRJ did not inhibit α-L-rhamnosidase (less than 50% inhibition at 750 μM); in contrast, 5-*epi*-DRJ was found to be a potent inhibitor against α-L-rhamnosidase, with an IC_{50} value 5.0 μM.[8,9] The presence of the additional hydroxymethyl group such as in α-homonojirimycin often leads to an increase in either their inhibitory potency and/or specificity but α-homo-5-*epi*-DRJ has only a marginal inhibitory effect against this enzyme.[8,10] Therefore, the structural requirements of α-L-rhamnosidase inhibitors are not yet as well resolved compared to α-glucosidase and α-galactosidase inhibitors.[11–14] Based on the analogy that the structural features of D-mannosidase which is responsible for inhibition by 1,4-dideoxy-1,4-imino-D-mannitol (DIM) are mirrored by the properties that cause inhibition of α-L-rhamnosidase, a wide range of mimics of L-rhamnofuranose have been synthesized. L-DIM is a moderate inhibitor with IC_{50} of 52.8 μM and L-6-deoxy-DIM, which is a faithful mimic of L-rhamnofuranose, is a more potent and

* Corresponding author.

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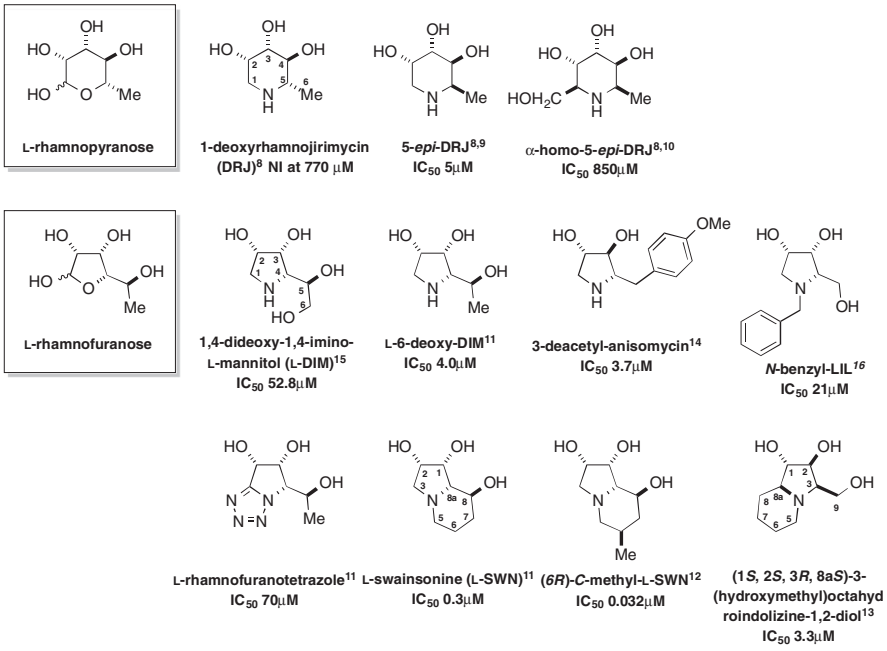


Figure 1. Structures of iminosugar α-L-rhamnosidase inhibitors.

highly specific inhibitor of α-L-rhamnosidase, with an IC_{50} value of 4.0 μM.[11,15] We have previously reported that *N*-benzyl-1,4-dideoxy-1,4-imino-L-lyxitol (*N*-benzyl-LIL) is a moderate inhibitor of α-L-rhamnosidase, with an IC_{50} value of 21 μM, whereas the parent compound, LIL, exhibited less than 50% inhibition of α-L-rhamnosidase even at concentrations as high as 1000 μM.[16] Similarly, its enantiomer, *N*-benzyl-1,4-dideoxy-1,4-imino-D-lyxitol (*N*-benzyl-DIL) was a moderate competitive inhibitor of α-L-rhamnosidase. These results suggested that an *N*-benzyl group will lead to the enhancement of α-L-rhamnosidase inhibition.

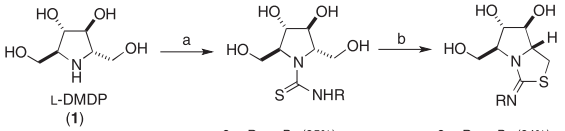
Bicyclic mimics, L-rhamnofuranotetrazole, also showed moderate inhibition, with IC_{50} value of 70 μM.[11] It is interesting to note that inhibition of rhamnosidase by L-swainsonine (IC_{50} = 0.3 μM) is better than that of L-6-deoxy-DIM or L-DIM.[11] Furthermore, (6*R*)-*C*-methyl-L-swainsonine (IC_{50} = 0.034 μM) is a significantly more potent α-L-rhamnosidase inhibitor than L-swainsonine.[12] L-Swainsonine may be regarded as a bicyclic derivative of L-6-deoxy-DIM or L-DIM. Thus, 'cyclization of pyrrolidine-based iminosugars' also causes a significant improvement in inhibition. Similarly, the bicyclic indolizidine iminosugar, (1*S*,2*S*,3*R*,8a*S*)-3-(hydroxymethyl)octahydroindolizine-1,2-diol is the only iminosugar with a *trans* diol configuration in a pyrrolidine ring to inhibit α-L-rhamnosidase with IC_{50} = 3.3 μM.[13] It is noteworthy that 3-deacetyl-anisomycin bearing a (2*S*,3*S*)-*trans* configuration in a pyrrolidine ring has the same potent inhibition as those with a (2*S*,3*R*)-*cis* motif, with IC_{50} values of 3.7 and 3.2 μM, respectively.[17,18] We have previously reported that the enantiomeric (2*R*,5*R*)-bis(dihydroxymethyl)-(3*R*,4*R*)-dihydroxypyrrolidine (DMDP) derivatives showed no inhibition of rhamnosidase. None of the parent 10 stereoisomers of DMDP were potent rhamnosidase inhibitors.[19] However, we considered that a further improvement in activity might be gained where the iminosugars have a longer side chain or bulky aromatic group. To test the hypotheses that a *trans*-diol in the pyrrolidines, and that bicyclic analogues with longer alkyl chain may inhibit rhamnosidase, a series of enantiomeric DMDP and L-DMDP cyclic isothioureas with long alkyl side chains or aromatic groups were prepared. We herein describe the synthesis and biological evaluation and structure–activity relationship of novel L-DMDP cyclic isothiourea derivatives as powerful and selective inhibitors of α-L-rhamnosidase.

2. Results and discussion

2.1. Chemistry

The synthesis began with the known imino sugar L-DMDP (**1**), prepared from D-xylose.[19] L-DMDP (**1**) was treated with corresponding isothiocyanates in the presence of NEt_3 to give the thiourea derivatives **2a**–**t**. Acid treatment of **2a**–**t** by using conc. HCl in MeOH gave the cyclic isothiourea derivatives **3a**–**t** (Scheme 1); because of the C_2 symmetry in L-DMDP, only one bicyclic analogue can be formed. To confirm the importance of the absolute stereochemistry of pyrrolothiazole nucleus for the inhibitory effects against the glycosidases, we synthesized the corresponding enantiomers of **2a**–**e** and **3a**–**e** from DMDP (Scheme 2).

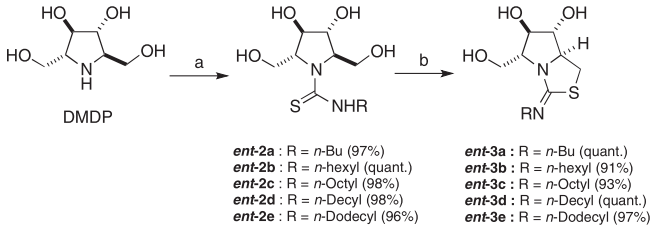
Furthermore, we also synthesized the diastereomers *ido*-**2r** and *ido*-**3r** of most potent inhibitors of **2r** and **3r** from the *ido*-L-DMDP



2a : R = *n*-Bu (95%)
2b : R = *n*-Hexyl (96%)
2c : R = *n*-Octyl (85%)
2d : R = *n*-Decyl (98%)
2e : R = *n*-Dodecyl (63%)
2f : R = Benzyl (86%)
2g : R = 3-Phenylpropyl (83%)
2h : R = 5-Phenylpentyl (93%)
2i : R = 7-Phenylheptyl (92%)
2j : R = 9-Phenylnonyl (92%)
2k : R = 4-Methylbenzyl (89%)
2l : R = 4-Methoxybenzyl (77%)
2m : R = 4-Fluorobenzyl (70%)
2n : R = 4-Chlorobenzyl (85%)
2o : R = 2-Chlorobenzyl (68%)
2p : R = 3-Chlorobenzyl (67%)
2q : R = 2,4-Dichlorobenzyl (62%)
2r : R = 3,4-Dichlorobenzyl (85%)
2s : R = 3,5-Dichlorobenzyl (84%)
2t : R = 3,4,5-Trichlorobenzyl (77%)

3a : R = *n*-Bu (94%)
3b : R = *n*-Hexyl (98%)
3c : R = *n*-Octyl (quant.)
3d : R = *n*-Decyl (95%)
3e : R = *n*-Dodecyl (quant.)
3f : R = Benzyl (99%)
3g : R = 3-Phenylpropyl (quant.)
3h : R = 5-Phenylpentyl (quant.)
3i : R = 7-Phenylheptyl (quant.)
3j : R = 9-Phenylnonyl (quant.)
3k : R = 4-Methylbenzyl (quant.)
3l : R = 4-Methoxybenzyl (quant.)
3m : R = 4-Fluorobenzyl (quant.)
3n : R = 4-Chlorobenzyl (quant.)
3o : R = 2-Chlorobenzyl (quant.)
3p : R = 3-Chlorobenzyl (quant.)
3q : R = 2,4-Dichlorobenzyl (quant.)
3r : R = 3,4-Dichlorobenzyl (quant.)
3s : R = 3,5-Dichlorobenzyl (quant.)
3t : R = 3,4,5-Trichlorobenzyl (quant.)

Scheme 1. Reagents and conditions: (a) RNCS, NEt_3, pyridine, rt; (b) concd HCl, MeOH, 50 °C.



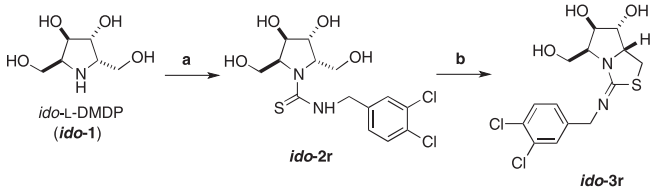
Scheme 2. Reagents and conditions: (a) RNCS, NEt_3, pyridine, rt; (b) concd HCl, MeOH, 50 °C.

series, prepared from tri-*O*-acetyl-D-glucal[20] (Scheme 3). *ido*-L-DMDP is the only other diastereomer of DMDP with C_2 symmetry and the same absolute configuration at C3 and C4 of the pyrrolidine ring.

2.2. Biological results and discussion

2.2.1. Comparison of 4 pyrrolidine iminosugar cyclic isothiourea derivatives as glycosidase inhibitors

Recently, Fernandez and Mellet et al.[21] designed a new class of piperidine- or pyrrolidine- iminosugars as glycosidase inhibitors—sp^2-iminosugars. They were characterized by the intramolecular nitrogen being part of a pseudoamide functionality. Furthermore, they reported that *N*-octyl-DAB and LAB isothiourea derivatives showed good inhibition against β-glucocerebrosidase, with IC_{50} values 7.3 and 8.6 μM, respectively.[22] In this study, we selected DMDP (***ent*-1**) and its enantiomer L-DMDP (**1**) as the parent iminosugars (Schemes 1 and 2). DMDP (***ent*-1**), a widespread naturally occurring pyrrolidine iminosugar, is a β-D-fructofuranose mimic.[5,23] As shown in Table 1, DMDP (***ent*-1**) itself was a potent inhibitor of yeast α-glucosidase and bovine liver β-galactosidase (IC_{50} = 7.8 and 9.7 μM, respectively), whereas it showed no inhibition of human lysosome β-glucocerebrosidase. In contrast, its mirror image L-DMDP (**1**) did not show any significant inhibition toward any of these enzymes though it is an inhibitor of intestinal maltase.[19] A side-by-side comparison of 4 cyclic isothioureas, derived from DAB, DMDP (***ent*-1**), LAB, and L-DMDP (**1**) revealed that *N*-octyl-DMDP cyclic isothiourea (***ent*-3c**) gave significant inhibition against human lysosome β-glucocerebrosidase (IC_{50} = 2.4 μM), which was about 3-times better inhibition than *N*-octyl DAB and LAB cyclic isothioureas[35] (Table 1). Cyclization of these three thioureas to the corresponding bicyclic isothioureas clearly increased their inhibition of β-glucocerebrosidase. However, *N*-octyl-L-DMDP cyclic isothiourea (**3c**) has the same cyclic framework but did not inhibit this enzyme. Moreover, we were intrigued by the fact that *N*-octyl-L-DMDP cyclic isothiourea (**3c**) showed potent and selective inhibition toward *Penicillium decumbens* α-L-rhamnosidase, with IC_{50} value of 1.6 μM (Table 1). This behavior was clearly different from previously reported cyclic isothioureas. On the basis of these findings, it appeared that L-DMDP cyclic isothioureas might be a new class of highly potent and selective inhibitors of α-L-rhamnosidase.



Scheme 3. Reagents and conditions: (a) 3,4-Dichlorobenzylisothiocyanate, NEt_3, pyridine, rt (94%) (b) concd HCl, MeOH, 50 °C (quant.).

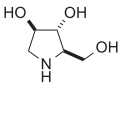
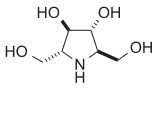
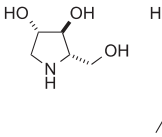
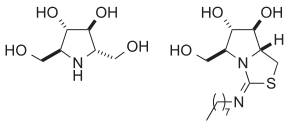
2.2.2. Comparison of human lysosome β-glucocerebrosidase inhibition by *N*-alkyl-DMDP thioureas (*ent*-2a–*ent*-2e) and cyclic isothiourea (*ent*-3a–*ent*-3e)

In this study, we showed that *N*-octyl-DMDP cyclic isothiourea (***ent*-3c**) was 3-times better as an inhibitor of human lysosome β-glucocerebrosidase than *N*-octyl-DAB and -LAB cyclic isothiourea (Table 1). Thus, we next investigated the effect of *N*-alkyl chain length of DMDP thiourea and cyclization to isothiourea derivatives (Scheme 2) on the activity of this enzyme (Table 2). Comparison of DMDP thiourea (***ent*-2a–*ent*-2e**) and DMDP cyclic isothiourea (***ent*-3a–*ent*-3e**) revealed that inhibition potency against β-glucocerebrosidase gradually increased with increasing length of the alkyl chain. The inhibition potency is likely to change drastically beyond octyl or decyl chain length. It is noteworthy that open ring structure DMDP thioureas were much better inhibitors than DMDP cyclic isothioureas. Especially, *N*-decyl-DMDP thiourea (***ent*-2d**) and *N*-dodecyl-DMDP thiourea (***ent*-2e**) showed very strong inhibition against this enzyme, with IC_{50} values of 0.74 and 0.16 μM, respectively. These results were markedly different from previously reported thiourea and cyclic isothiourea derivatives.[23] All 1-deoxynojirimycin (DNJ), DAB or LAB-based *N*-octyl thiourea gave very weak (10^{-4} M order) inhibition of β-glucocerebrosidase,[22] whereas their closed ring *N*-octyl cyclic isothiourea derivatives were reported with IC_{50} of 7.3–12.4 μM. We focused on parent compound DMDP (***ent*-1**) which itself showed 58-fold better inhibition than DAB against bovine liver β-glucosidase, with IC_{50} value of 9.7 μM. In contrast, DAB was a much stronger inhibitor of yeast α-glucosidase than DMDP (***ent*-1**). These results clearly suggested that DMDP (***ent*-1**) itself might lead to β-glucosidase directed inhibitors. The target enzyme β-glucocerebrosidase cleaves the β-glycosidic bond of glucosylceramide to release ceramide and glucose. Therefore, its active site comprises both sugar and hydrophobic moieties.[24] It can be considered that DMDP (***ent*-1**) has suitable interactions with the sugar part but itself does not have a hydrophobic moiety. The introduction of long alkyl chains such as *N*-decyl- and *N*-dodecyl brought favorable recognition by the hydrophobic pocket and thereby stabilized the DMDP-ring orientation, leading to an increase in inhibition.

2.2.3. Comparison of α-L-rhamnosidase inhibition by *N*-alkyl-L-DMDP thiourea (2a–2e) and cyclic isothiourea (3a–3e)

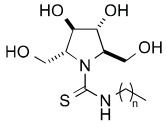
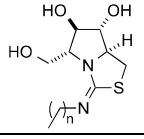
In the first experiment, we discovered for the first time that L-DMDP cyclic isothiourea is a potent and selective inhibitor of α-L-rhamnosidase. We next focused on the nitrogen and whether extending the alkyl chain at this position (Scheme 1) would influence the inhibition by L-DMDP cyclic isothioureas (Table 3). In order to check if the change from open ring thioureas to cyclic isothioureas had an influence on inhibition of α-L-rhamnosidase, we tested the L-DMDP thiourea derivatives (**2a**–**2e**). Of these compounds, open ring structure L-DMDP thiourea derivatives (**2a**–**2e**) showed no effect against *Penicillium decumbens* α-L-rhamnosidase but showed a broad inhibition spectrum against β-glucosidases and β-galactosidase. In sharp contrast, ring closed L-DMDP cyclic isothiourea derivatives (**3a**–**3e**) showed potent inhibition of *Penicillium decumbens* α-L-rhamnosidase. These results showed that the bicyclic isothiourea structure is necessary for this inhibition. It is the *opposite* trend to that of β-glucocerebrosidase inhibition by *N*-decyl-DMDP thiourea (***ent*-2d**) and *N*-dodecyl-DMDP thiourea (***ent*-2e**). We found that the inhibitory potency of L-DMDP cyclic isothiourea derivatives against α-L-rhamnosidase was enhanced with increasing length of the alkyl chain. *N*-Dodecyl-L-DMDP cyclic isothiourea (**3e**) showed very potent inhibition against α-L-rhamnosidase (IC_{50} = 0.46 μM) but it also showed potent inhibition against bovine liver β-glucosidase and β-galactosidase, with IC_{50} values of 9.5 and 11 μM. On the basis of these findings, it is clear that the addition of a long alkyl chain to the

Table 1
Concentration of DAB, DMDP, LAB, L-DMDP and their cyclic isothiourea derivatives giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)							
	D-form				L-form			
	Previous report (Ref. 22)		This study		Previous report (Ref. 22)		This study	
								
	DAB	*N*-Octyl-DAB cyclic isothiourea	DMDP (*ent*-**1**)	*N*-Octyl-DMDP cyclic isothiourea (*ent*-**3c**)	LAB	*N*-Octyl-LAB cyclic isothiourea	L-DMDP (**1**)	*N*-Octyl-L-DMDP cyclic isothiourea (**3c**)
α-L-Rhamnosidase								
Penicillium decumbens	>2000	>2000	[a]NI	NI	>2000	>2000	[a]NI	1.6
α-Glucosidase								
Yeast	0.17	125	7.8	NI	10.3	>2000	NI	NI
β-Glucosidase								
Almond	35	3.1	10	NI	32	3.6	NI	NI
Bovine liver	572	59	9.7	63	>2000	1.4	NI	NI
Human lysosome	>2000	7.3	NI	2.4	>2000	8.6	NI	NI
α-Galactosidase								
Coffee beans	>2000	>2000	NI	NI	>2000	>2000	NI	NI
β-Galactosidase								
E. coli	>2000	>2000	NI	NI	>2000	>2000	NI	NI
α-Mannosidase								
Jack bean	>2000	>2000	NI	NI	>2000	>2000	NI	NI
β-Mannosidase								
Helix pomatia	>2000	>2000	721	NI	>2000	>2000	NI	NI
Amyloglucosidase								
A.niger	4.4	244	116	NI	40	120	NI	NI

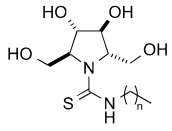
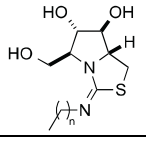
[a] NI: No inhibition (less than 50% inhibition at 1000 μM).

Table 2
Concentration of DMDP thiourea and DMDP cyclic isothiourea derivatives giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)									
	DMDP thiourea derivatives					DMDP cyclic isothiourea derivatives				
	n = 					n = 				
	3 *ent*-**2a**[a]	**5** *ent*-**2b**[a]	**7** *ent*-**2c**[a]	**9** *ent*-**2d**[b]	**11** *ent*-**2e**[b]	**3** *ent*-**3a**[a]	**5** *ent*-**3b**[a]	**7** *ent*-**3c**[a]	**9** *ent*-**3d**[b]	**11** *ent*-**3e**[b]
α-L-Rhamnosidase										
Penicillium decumbens	NI	NI	NI	NI	NI	NI	494	NI	NI	67
α-Glucosidase										
Rice	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Rat intestinal maltase	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-Glucosidase										
Almond	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Bovine liver	NI	NI	689	NI	23	NI	NI	63	63	63
Human lysosome	NI	NI	12	0.74	0.16	532	16	2.4	1.0	1.0
β-Galactosidase										
Bovine liver	NI	1000	414	100	24	NI	NI	NI	93	32
Amyloglucosidase										
A.niger	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
α-L-Fucosidase										
Bovine kidney	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Trehalase										
Porcine kidney	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-Glucuronidase										
E. coli	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

[a] NI: No inhibition (less than 50% inhibition at 1000 μM).
[b] NI: No inhibition (less than 50% inhibition at 100 μM).

Table 3
Concentration of L-DMDP thiourea and L-DMDP cyclic isothiourea derivatives giving 50% inhibition of various glycosidases

	IC_{50} (μM)									
	L-DMDP thiourea derivatives					L-DMDP cyclic isothiourea derivatives				
	 n =					 n =				
Enzyme	**3** **2a**[a]	**5** **2b**[a]	**7** **2c**[a]	**9** **2d**[b]	**11** **2e**[b]	**3** **3a**[a]	**5** **3b**[a]	**7** **3c**[a]	**9** **3d**[b]	**11** **3e**[b]
α-L-Rhamnosidase										
Penicillium decumbens	NI	NI	NI	NI	NI	37	5.3	1.6	0.77	0.46
α-Glucosidase										
Rice	741	NI	1000	NI	NI	339	418	NI	NI	NI
Rat intestinal maltase	1000	NI	1000	NI	NI	NI	799	NI	NI	NI
β-Glucosidase										
Almond	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Bovine liver	939	NI	178	16	36	NI	NI	NI	NI	9.5
Human lysosome	NI	NI	NI	65	73	NI	NI	NI	NI	NI
β-Galactosidase										
Bovine liver	473	572	266	61	30	719	599	NI	36	11
Amyloglucosidase										
A.niger	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
α-L-Fucosidase										
Bovine kidney	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Trehalase										
Porcine kidney	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-Glucuronidase										
E. coli	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

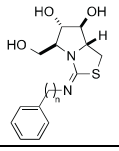
[a] NI: No inhibition (less than 50% inhibition at 1000 μM).
[b] NI: No inhibition (less than 50% inhibition at 100 μM).

nitrogen of L-DMDP cyclic isothiourea led to the production of highly potent inhibitors of α-L-rhamnosidase. However, these long alkyl chains not only broadened the inhibition spectrum but also decreased water-solubility and caused unexpected cell membrane damage. Thus, we turned our strategy from introducing a simple *n*-alkyl chain at the nitrogen to introducing a benzyl substituent at this position. Consequently, we selected *N*-benzyl-L-DMDP cyclic isothioureas because they were found to display more selective inhibition of α-L-rhamnosidase than introduction of simple *n*-alkyl chains (Tables 3 and 4). The terminal aromatic ring increased the specificity but reduced the potency of rhamnosidase inhibition; the *N*-benzyl derivative was a weaker inhibitor than *N*-dodecyl-L-DMDP cyclic isothiourea (**3e**). Thus, we next investigated the effect of halogen, methyl, and methoxy substituents on the aromatic ring (Table 5). Comparison of **3f** and **3k**–**3t** revealed that the α-L-rhamnosidase inhibition of 4′-methyl (**3k**), 4′-methoxy (**3l**), 4′-fluoro derivatives slightly increased over un-substituted **3f**, whereas it was clear that chloro substituents (**3n**) on the benzene ring led to highly potent inhibitors. Thus, we next focused on the number and positions of chloro groups on the terminal benzene ring and whether increasing the number could influence the inhibition. As shown in Table 4, we finally discovered that 3′,4′-dichlorobenzyl-L-DMDP cyclic isothiourea (**3r**) had an IC_{50} value of 0.22 μM against *Penicillium decumbens* α-L-rhamnosidase. It was a 46-fold better inhibitor than the positive control 5-*epi*-deoxyrhamnojirimycin (5-*epi*-DRJ; IC_{50} = 10 μM). For confirmation, we also synthesized 3′,4′-dichlorobenzyl 2,5-dideoxy-2,5-imino-L-iditol cyclic isothiourea (***ido*-3r**) but found it did not show such the inhibition (9.3% inhibition at 1000 μM) (Scheme 3). These results support our suggestion that the configuration of the parent iminosugar is a vital factor for α-L-rhamnosidase inhibition. To understand the structural basis of the interaction of 3′,4′-dichlorobenzyl-L-DMDP cyclic isothiourea (**3r**) and 5-*epi*-DRJ with *Penicillium decumbens* α-L-rhamnosidase, we determined the mode of inhibition and the inhibition constant (K_i) of both compounds from Lineweaver–Burk plots. 3′,4′-Dichlorobenzyl-L-DMDP cyclic isothiourea (**3r**) and 5-*epi*-DRJ were found to inhibit this enzyme in a competitive manner, with K_i values of 0.11 and 4.2 μM, respectively.

3. Conclusion

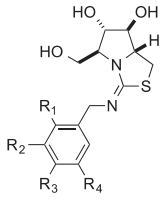
In this study, we designed and synthesized a series of DMDP and L-DMDP cyclic isothiourea. Previous reports with limited structural diversity have shown cyclization from thiourea to cyclic isothiourea generally increased β-glucocerebrosidase inhibition. We performed this experiment to test our hypothesis that 'cyclisation of pyrrolidine-based iminosugars' would bring improvement in the inhibition activity toward α-L-rhamnosidase inhibition, which was based on our previous discovery that bicyclic L-swainsonine was better than that of L-6-deoxy-DIM or L-DIM.[11] For the purpose of clarifying the difference in the inhibition spectrum between pyrrolidine thioureas and pyrrolidine cyclic isothioureas, we performed side-by-side comparisons. The main features are: (a) cyclization from L-DMDP thiourea to L-DMDP cyclic isothiourea resulted in α-L-rhamnosidase inhibition. (b) introduction of a long alkyl chain to the nitrogen of L-DMDP cyclic isothioureas can lead to the production of highly potent inhibitors of α-L-rhamnosidase. However, it caused not only a broad spectrum of inhibition but also decreased water-solubility, which induced unexpected cell membrane damage. (c) *N*-benzyl L-DMDP cyclic isothioureas displayed selective inhibition of α-L-rhamnosidase and more than in the case of introducing the simple *n*-alkyl chain units. (d) 3′,4′-dichlorobenzyl L-DMDP cyclic isothiourea (**3r**) showed potent inhibition of *Penicillium decumbens* α-L-rhamnosidase, with IC_{50} value of 0.22 μM. It was a 46-fold better inhibitor than the positive control 5-*epi*-deoxyrhamnojirimycin (5-*epi*-DRJ; IC_{50} = 10 μM). (e) **3r** inhibited this enzyme in a competitive manner, with K_i value of 0.11 μM. These new mimics of L-rhamnose may affect other

Table 4
Concentration of *N*-alkylbenzyl-L-DMDP cyclic isothiourea derivatives giving 50% inhibition of various glycosidases

	IC_{50} (μM)				
					
	n =				
	1	**3**	**5**	**7**	**9**
Enzyme	**3f**	**3g**	**3h**	**3i**	**3j**
α-L-Rhamnosidase					
Penicillium decumbens	2.2	6.8	1.8	1.0	0.87
α-Glucosidase					
Rice	[a]NI	NI	NI	NI	NI
Rat intestinal maltase	NI	NI	NI	NI	NI
β-Glucosidase					
Almond	NI	NI	NI	NI	NI
Bovine liver	NI	NI	NI	NI	33
Human lysosome	NI	NI	NI	NI	NI
β-Galactosidase					
Bovine liver	NI	NI	NI	NI	13
Amyloglucosidase					
A.niger	NI	NI	NI	NI	NI
α-L-Fucosidase					
Bovine kidney	NI	NI	NI	NI	NI
Trehalase					
Porcine kidney	NI	NI	NI	NI	NI
β-Glucuronidase					
E. coli	NI	NI	NI	NI	NI

[a] NI: No inhibition (less than 50% inhibition at 100 μM).

Table 5
Concentration of *N*-alkylbenzyl-L-DMDP cyclic isothiourea derivatives giving 50% inhibition of α-L-rhamnosidase



Compounds	R_1	R_2	R_3	R_4	IC_{50} (μM)
3f	H	H	H	H	2.2
3k	H	H	Me	H	1.2
3l	H	H	OMe	H	1.6
3m	H	H	F	H	1.8
3n	H	H	Cl	H	0.89
3o	Cl	H	H	H	2.5
3p	H	Cl	H	H	0.70
3q	Cl	H	Cl	H	0.80
3r	H	Cl	Cl	H	0.23
3s	H	Cl	H	Cl	0.70
3t	H	Cl	Cl	Cl	0.45
5-*epi*-DRJ					10

enzymes associated with the biochemistry of rhamnose including enzymes involved in progression of tuberculosis.

4. Experimental

4.1. Chemistry

4.1.1. General information

Flash chromatography was performed with Kanto Kagaku silicagel 60N (63–210 nm). NMR spectra were recorded on a JEOL JNM-A400 or JEOL JNM-ECX500 spectrometer in the solvent indicated. Chemical shifts (δ) are given in ppm downfield from referenced with CH_3OH (3.30 ppm) and $CHCl_3$ (7.26 ppm) as an internal standard. Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; t, triplet; quint, quintuplet; m, multiplet; br, broad and coupling constants are given in (J) Hz. High resolution mass spectral data was obtained on a JEOL MStation JMS-700. All commercial reagents were used as received unless otherwise noted.

4.1.1.1. General procedure for the synthesis of thiourea derivatives (2a–t). To a stirred solution of L-DMDP (0.3 mmol) in pyridine (4.4 ml), NEt_3 (0.36 mmol) was added corresponding isothiocyanate (0.33 mmol) at room temperature, and the reaction mixture was stirred for 21 h. The solvent was evaporated and the residue was co-evaporated several times with toluene. The crude product was chromatographed on silica gel (2 g, CH_2Cl_2/MeOH = 20:1) to give **2a–t**.

4.1.1.2. (2*S*,3*S*,4*S*,5*S*)-3,4-Dihydroxy-2,5-bis(hydroxymethyl) pyrrolidine-1-carbothioic acid butylamide (2a). Colorless oil; 95%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.13 (2H, s), 4.02 (2H, br), 3.86 (2H, br), 3.69–3.62 (1H, m), 3.52–3.45 (1H, m), 3.34–3.30 (2H, m), 1.63–1.56 (2H, m), 1.42–1.33 (2H, m), 0.95 (3H, t, J = 7.3 Hz); ^{13}C NMR (100 MHz, MeOH-d_4): δ 181.46, 79.78, 72.87, 61.70, 46.32, 32.33, 21.24, 14.24; IR (neat): 3268, 2956, 2931, 1559, 1069 cm^{-1}; MS (FAB): m/z 279 (M^++1); HRMS (FAB) Calcd for $C_{11}H_{23}N_2O_4S$ 279.1379; Found: 279.1376 (M^++1); $[\alpha]_D^{21}$ −25.4 (c 0.4, MeOH).

4.1.1.3. (2*S*,3*S*,4*S*,5*S*)-3,4-Dihydroxy-2,5-bis(hydroxymethyl) pyrrolidine-1-carbothioic acid hexylamide (2b). Colorless oil; 96%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.13 (2H, s), 4.03 (2H, br), 3.87 (2H, br), 3.68–3.61 (1H, m), 3.51–3.44 (1H, m), 3.34–3.30 (2H, m), 1.63–1.58 (2H, m), 1.37–1.33 (6H, m), 0.91 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, MeOH-d_4): δ 181.41, 79.95, 72.70, 61.68, 46.59, 32.76, 30.12, 27.80, 23.66, 14.39; IR (neat): 3310, 2928, 2858, 1558, 1068 cm^{-1}; MS (FAB): m/z 307 (M^++1); HRMS (FAB) Calcd for $C_{13}H_{27}N_2O_4S$ 307.1692; Found 307.1694 (M^++1); $[\alpha]_D^{18}$ −26.5 (c 0.9, MeOH).

4.1.1.4. (2*S*,3*S*,4*S*,5*S*)-3,4-Dihydroxy-2,5-bis(hydroxymethyl) pyrrolidine-1-carbothioic acid octylamide (2c). Colorless oil; 85%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.12 (2H, s), 4.02 (2H, br), 3.86 (2H, br), 3.68–3.61 (1H, m), 3.51–3.44 (1H, m), 3.34–3.29 (2H, m), 1.63–1.59 (2H, m), 1.34–1.30 (10H, m), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, MeOH-d_4): δ 181.37, 79.95, 72.44, 61.66, 46.58, 33.00, 30.48, 30.37, 30.14, 28.11, 23.71, 14.45; IR (neat): 3290, 2924, 2854, 1558, 1068 cm^{-1}; MS (FAB): m/z 335 (M^++1); HRMS (FAB) Calcd for $C_{15}H_{31}N_2O_4S$ 335.2005; Found 335.2003 (M^++1); $[\alpha]_D^{25}$ −21.7 (c 0.3, MeOH).

4.1.1.5. (2*S*,3*S*,4*S*,5*S*)-3,4-Dihydroxy-2,5-bis(hydroxymethyl) pyrrolidine-1-carbothioic acid decylamide (2d). White solid; mp; 98–99 °C; 98%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.13 (2H, s), 4.03 (2H, br), 3.86 (2H, br), 3.70–3.61 (1H, m), 3.51–3.44 (1H, m), 3.34–3.29 (2H, m), 1.61 (2H, t, J = 6.7 Hz), 1.34–1.29 (14H, m), 0.89 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, MeOH-d_4): δ 181.36, 79.78, 72.84, 61.68, 46.60, 33.07, 30.72, 30.53, 30.47, 30.16, 28.12, 23.74, 14.50; IR (KBr): 3287, 2942, 2858, 1559, 1052 cm^{-1}; MS (FAB): m/z 363 (M^++1); HRMS (FAB) Calcd for $C_{17}H_{35}N_2O_4S$ 363.2318; Found: 363.2315 (M^++1); $[\alpha]_D^{18}$ −25.7 (c 0.75, MeOH).

4.1.1.6. (2*S*,3*S*,4*S*,5*S*)-3,4-Dihydroxy-2,5-bis(hydroxymethyl) pyrrolidine-1-carbothioic acid dodecylamide (2e). White solid; mp; 99–100 °C; 63%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.13 (2H, s), 4.03 (2H, br), 3.87 (2H, br), 3.68–3.61 (1H, m), 3.52–3.45 (1H, m), 3.34–3.29 (2H, m), 1.61–1.60 (2H, m), 1.34–1.29 (18H, m), 0.89

(3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, MeOH-d_4): δ 181.38, 79.84, 72.59, 61.68, 46.58, 33.07, 30.79, 30.75, 30.70, 30.52, 30.48, 30.15, 28.11, 23.73, 14.46; IR (KBr): 3284, 2921, 2851, 1558, 1064 cm^{-1}; MS (FAB): m/z 391 (M$^+$+1); HRMS (FAB) Calcd for $C_{19}H_{39}N_2O_4S$ 391.2631; Found: 391.2635 (M$^+$+1); $[\alpha]_D^{18}$ −23.2 (c 0.7, MeOH).

4.1.1.7. General procedure for the synthesis of cyclic isothiourea derivatives (3a–t). To a stirred solution of **2a–t** (0.18 mmol) in MeOH (3.7 ml) was added conc.HCl (5 drops) at room temperature, and the reaction mixture was stirred for 24 h at 50 °C. After cooling, the solvent was evaporated and the residue was co-evaporated several times with MeOH. The crude product was chromatographed on silica gel (2 g, CH_2Cl_2/MeOH = 15:1) to give **3a–t**.

4.1.1.8. (5*S*,6*S*,7*S*,7a*R*)-3-Butylimino-5-hydroxymethyl-tetrahydro-pyrrolo[1,2-*c*]thiazole-6,7-diol (3a). Colorless oil; 94%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.47–4.40 (1H, m), 3.97–3.91 (3H, m), 3.79–3.60 (4H, m), 3.46–3.36 (2H, m), 1.67–1.61 (2H, m), 1.45–1.36 (2H, m), 0.96 (3H, t, J = 7.4 Hz); ^{13}C NMR (125 MHz, MeOH-d_4): δ 171.93, 80.47, 79.45, 71.73, 68.58, 63.72, 49.09, 35.75, 32.09, 20.68, 13.82; IR (neat): 3226, 2957, 2859, 1652 cm^{-1}; MS (FAB): m/z 261 (M$^+$+1); HRMS (FAB) Calcd for $C_{11}H_{21}N_2O_3S$ 261.1273; Found: 261.1274 (M$^+$+1); $[\alpha]_D^{30}$ +120.8 (c 0.4, MeOH).

4.1.1.9. (5*S*,6*S*,7*S*,7a*R*)-3-Hexylimino-5-hydroxymethyl-tetrahydro-pyrrolo[1,2-*c*]thiazole-6,7-diol (3b). Colorless oil; 98%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.25 (1H, br), 3.93–3.83 (3H, m), 3.69–3.64 (3H, m), 3.52–3.47 (1H, m), 3.37–3.34 (2H, m), 1.62–1.58 (2H, m), 1.38–1.28 (6H, m), 0.91 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.87, 80.46, 79.42, 71.77, 68.60, 63.70, 49.21, 35.82, 32.33, 30.02, 27.18, 23.47, 14.31; IR (neat): 3260, 2940, 2852, 1653 cm^{-1}; MS (FAB): m/z 289 (M$^+$+1); HRMS (FAB) Calcd for $C_{13}H_{25}N_2O_3S$ 289.1586; Found: 289.1588 (M$^+$+1); $[\alpha]_D^{19}$ +109.3 (c 0.4, MeOH).

4.1.1.10. (5*S*,6*S*,7*S*,7a*R*)-5-Hydroxymethyl-3-octylimino-tetrahydro-pyrrolo[1,2-*c*]thiazole-6,7-diol (3c). Colorless oil; quant.; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.41–4.37 (1H, m), 3.96–3.91 (3H, m), 3.77–3.56 (4H, m), 3.44–3.34 (2H, m), 1.66–1.60 (2H, m), 1.34–1.31 (10H, m), 0.91–0.88 (3H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.91, 80.47, 79.45, 71.75, 68.62, 63.74, 49.21, 35.80, 32.89, 30.20, 30.13, 30.08, 27.54, 23.66, 14.40; IR (neat): 3290, 2926, 2856, 1653 cm^{-1}; MS (FAB): m/z 317 (M$^+$+1); HRMS (FAB) Calcd for $C_{15}H_{29}N_2O_3S$ 317.1889; Found:317.1899 (M$^+$+1); $[\alpha]_D^{18}$ +97.1 (c 0.65, MeOH).

4.1.1.11. (5*S*,6*S*,7*S*,7a*R*)-3-Decylimino-5-hydroxymethyl-tetrahydro-pyrrolo[1,2-*c*]thiazole-6,7-diol (3d). Colorless oil; 95%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.40 (1H, br), 3.96–3.93 (3H, m), 3.77–3.57 (4H, m), 3.42–3.34 (2H, m), 1.64 (2H, br), 1.30 (14H, br), 0.91–0.87 (3H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.78, 80.42, 79.31, 71.73, 68.54, 63.65, 49.36, 35.84, 32.92, 30.54, 30.46, 30.32, 30.11, 30.02, 27.47, 23.63, 14.43; IR (neat): 3286, 2923, 2854, 1653 cm^{-1}; MS (FAB): m/z 345 (M$^+$+1); HRMS (FAB) Calcd for $C_{17}H_{33}N_2O_3S$ 345.2212; Found: 345.2211 (M$^+$+1); $[\alpha]_D^{19}$ +99.5 (c 0.7, MeOH).

4.1.1.12. (5*S*,6*S*,7*S*,7a*R*)-3-Dodecylimino-5-hydroxymethyl-tetrahydro-pyrrolo[1,2-*c*]thiazole-6,7-diol (3e). Colorless oil; quant.; ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.42–4.39 (1H, m), 3.96–3.91 (3H, m), 3.77–3.57 (4H, m), 3.45–3.35 (2H, m), 1.66–1.62 (2H, m), 1.29 (18H, br), 0.91–0.87 (3H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.89, 80.48, 79.44, 71.79, 68.63, 63.72, 49.43, 35.82, 33.04, 30.72, 30.66, 30.53, 30.45, 30.18, 30.08, 27.54, 23.70, 14.45; IR (neat): 3271, 2923, 2853, 1653 cm^{-1}; MS (FAB): m/z 373 (M$^+$+1); HRMS (FAB) Calcd for $C_{19}H_{37}N_2O_3S$ 373.2525; Found: 373.2528 (M$^+$+1); $[\alpha]_D^{29}$ +98.5 (c 0.55, MeOH).

4.1.1.13. (5*S*,6*S*,7*S*,7a*R*)-3-Benzylimino-5-hydroxymethyl-tetrahydro-pyrrolo[1,2-*c*]thiazole-6,7-diol (3f). Colorless oil; 99%; ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.44–7.33 (5H, m), 4.66 (1H, d, J = 15.0 Hz), 4.53 (1H, d, J = 15.0 Hz), 4.49–4.42 (1H, m), 3.96–3.93 (3H, m), 3.81–3.61 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 172.33, 136.39, 130.11, 129.55, 128.75, 80.55, 79.48, 72.01, 68.73, 63.60, 52.40, 36.01; IR (neat): 3293, 2913, 1636, 1453, 1420 cm^{-1}; MS (EI): m/z 294 (M$^+$+); HRMS (EI) Calcd for $C_{14}H_{18}N_2O_3S$ 294.1038; Found: 294.1037 (M$^+$+); $[\alpha]_D^{19}$ +90.4 (c 0.4, MeOH).

4.1.1.14. (5*S*,6*S*,7*S*,7a*R*)-5-Hydroxymethyl-3-[(3-phenylpropyl)imino]tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3g). Pale yellow oil; quant; ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.30–7.17 (5H, m), 4.46–4.39 (1H, m), 3.99–3.92 (3H, m), 3.81–3.57 (4H, m), 3.41–3.32 (2H, m), 2.76–2.70 (2H, m), 2.01–1.94 (2H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.88, 141.81, 129.60, 129.44, 127.33, 80.45, 79.39, 71.69, 68.51, 63.72, 48.70, 35.82, 33.50, 31.66; IR (neat): 3275, 2926, 1647, 1636 cm^{-1}; MS (EI): m/z 322 (M$^+$+); HRMS (EI) Calcd for $C_{16}H_{22}N_2O_3S$ 322.1350; Found: 322.1350 (M$^+$+); $[\alpha]_D^{24}$ +93.0 (c 0.95, MeOH).

4.1.1.15. (5*S*,6*S*,7*S*,7a*R*)-5-Hydroxymethyl-3-[(5-phenylpentyl)imino]tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3h). Pale yellow oil; quant; ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.26–7.14 (5H, m), 4.44–4.41 (1H, m), 3.97–3.92 (3H, m), 3.78–3.58 (4H, m), 3.42–3.31 (2H, m), 2.64–2.60 (2H, m), 1.69–1.62 (4H, m), 1.44–1.38 (2H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.88, 143.45, 129.42, 129.33, 126.78, 80.46, 79.43, 71.74, 68.58, 63.71, 49.29, 36.57, 35.80, 32.04, 29.90, 26.99; IR (neat): 3278, 2931, 1647, 1637 cm^{-1}; MS (EI): m/z 350 (M$^+$+); HRMS (EI) Calcd for $C_{18}H_{26}N_2O_3S$ 350.1664; Found: 350.1660 (M$^+$+); $[\alpha]_D^{25}$ +89.2 (c 1.1, MeOH).

4.1.1.16. (5*S*,6*S*,7*S*,7a*R*)-5-Hydroxymethyl-3-[(7-phenylheptyl)imino]tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3i). Pale yellow oil; quant; ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.25–7.11 (5H, m), 4.44–4.39 (1H, m), 3.96–3.90 (3H, m), 3.78–3.58 (4H, m), 3.49–3.31 (2H, m), 2.59 (2H, t, J = 7.6 Hz), 1.63–1.62 (4H, m), 1.36 (6H, br); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.91, 143.83, 129.40, 129.26, 126.66, 80.46, 79.45, 71.72, 68.59, 63.73, 49.34, 36.82, 35.77, 32.57, 30.02, 29.98, 27.43; IR (neat): 3275, 2926, 1646, 1636 cm^{-1}; MS (EI): m/z 378 (M$^+$+); HRMS (EI) Calcd for $C_{20}H_{30}N_2O_3S$ 378.1977; Found: 378.1975 (M$^+$+); $[\alpha]_D^{25}$ +88.1 (c 0.75, MeOH).

4.1.1.17. (5*S*,6*S*,7*S*,7a*R*)-5-Hydroxymethyl-3-[(9-phenylnonyl)imino]tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3j). Pale yellow oil; quant; ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.25–7.10 (5H, m), 4.41–4.37 (1H, m), 3.96–3.90 (3H, m), 3.76–3.56 (4H, m), 3.43–3.30 (2H, m), 2.58 (2H, t, J = 7.7 Hz), 1.64–1.61 (4H, m), 1.32 (10H, br); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.38, 143.93, 129.38, 129.24, 126.62, 80.46, 79.51, 71.44, 68.54, 63.86, 49.71, 36.89, 35.68, 32.71, 30.44, 30.23, 30.18, 30.11, 27.53; IR (neat): 3254, 2925, 2853, 1640 cm^{-1}; MS (EI): m/z 406 (M$^+$+); HRMS (EI) Calcd for $C_{22}H_{34}N_2O_3S$ 406.2290; Found: 406.2285 (M$^+$+); $[\alpha]_D^{26}$ +84.4 (c 0.95, MeOH).

4.1.1.18. (5*S*,6*S*,7*S*,7a*R*)-5-(Hydroxymethyl)-3-[(4-methylbenzyl)imino]tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3k). Pale yellow oil; quant;^{1}H NMR (400 MHz, MeOH-d_4): δ 7.24–7.19 (4H, m), 4.61 (1H, d, J = 15.0 Hz), 4.50–4.46 (2H, m), 3.99–3.94 (3H, m), 3.82–3.62 (4H, m), 2.32 (3H, s); ^{13}C NMR (100 MHz, MeOH-

d_4): δ 172.22, 139.62, 133.30, 130.67, 128.79, 80.54, 79.50, 71.94, 68.73, 63.63, 52.23, 36.02, 21.14; IR (neat): 3284, 2926, 1636 cm^{-1}; MS (EI): *m/z* 308 (M^++); HRMS (EI) Calcd for $C_{15}H_{20}N_2O_3S$ 308.1195; Found: 308.1193 (M^++); $[\alpha]_D^{26}$ +92.8 (*c* 1.0, MeOH).

4.1.1.19. (5*S*,6*S*,7*S*,7a*R*)-5-(Hydroxymethyl)-3-[(4-methoxybenzyl)imino]tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3l). Colorless oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.28–7.25 (2H, m), 6.95–6.91 (2H, m), 4.58 (1H, d, *J* = 14.7 Hz), 4.47–4.41 (2H, m), 3.95–3.92 (3H, m), 3.78–3.60 (7H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 172.03, 161.37, 130.34, 128.19, 115.41, 80.53, 79.50, 71.85, 68.69, 63.63, 55.79, 51.99, 36.00; IR (neat): 3274, 2922, 1636, 1513, 1252 cm^{-1}; MS (EI): *m/z* 324 (M^++); HRMS (EI) Calcd for $C_{15}H_{20}N_2O_4S$ 324.1144; Found: 324.1138 (M^++); $[\alpha]_D^{26}$ +93.5 (*c* 0.95, MeOH).

4.1.1.20. (5*S*,6*S*,7*S*,7a*R*)-3-[(4-Fluorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3m). Pale yellow oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.40–7.36 (2H, m), 7.15–7.11 (2H, m), 4.64 (1H, d, *J* = 15.3 Hz), 4.53 (1H, d, *J* = 15.3 Hz), 4.51–4.46 (1H, m), 3.96–3.93 (3H, m), 3.79–3.62 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 172.28, 164.09 (d, *J* = 245.1 Hz), 132.46, 130.96 (d, *J* = 7.6 Hz), 116.81 (d, *J* = 21.9 Hz), 80.56, 79.46, 72.05, 68.74, 63.54, 51.61, 36.05; IR (neat): 3326, 2917, 1634, 1511, 1222 cm^{-1}; MS (EI): *m/z* 312 (M^++); HRMS (EI) Calcd for $C_{14}H_{17}FN_2O_3S$ 312.0944; Found: 312.0947 (M^++); $[\alpha]_D^{27}$ +107.6 (*c* 1.0, MeOH).

4.1.1.21. (5*S*,6*S*,7*S*,7a*R*)-3-[(4-Chlorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-pyrrolo[1,2-*c*]-thiazole-6,7-diol (3n). Pale yellow oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.39 (2H, d, *J* = 8.6 Hz), 7.33 (2H, d, *J* = 8.6 Hz), 4.61 (1H, d, *J* = 15.3 Hz), 4.51 (1H, d, *J* = 15.3 Hz), 4.44–4.38 (1H, m), 3.97–3.91 (3H, m), 3.80–3.57 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.52, 135.83, 135.14, 130.39, 130.07, 80.56, 79.57, 71.58, 68.62, 63.75, 52.31, 35.89; IR (neat): 3353, 2913, 1639, 1093 cm^{-1}; MS (EI): *m/z* 328 (M^++); HRMS (EI) Calcd for $C_{14}H_{17}ClN_2O_3S$ 328.0648; Found: 328.0651 (M^++); $[\alpha]_D^{26}$ +87.2 (*c* 1.0, MeOH).

4.1.1.22. (5*S*,6*S*,7*S*,7a*R*)-3-[(2-Chlorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-1*H*-pyrrolo-[1,2-*c*]thiazole-6,7-diol (3o). Colorless oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.48–7.36 (4H, m), 4.73 (1H, d, *J* = 15.3 Hz), 4.65 (1H, d, *J* = 15.3 Hz), 4.50–4.44 (1H, m), 4.00–3.93 (3H, m), 3.83–3.64 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 172.48, 134.71, 133.80, 131.52, 131.46, 131.02, 128.71, 80.48, 79.38, 71.88, 68.71, 63.68, 50.47, 36.16 MS (EI): *m/z* 328 (M^++); HRMS (EI) Calcd for $C_{14}H_{17}ClN_2O_3S$ 328.0648; Found: 328.0651 (M^++); IR (neat): 3356, 2905, 1636 cm^{-1}; $[\alpha]_D^{27}$ +80.5 (*c* 1.05, MeOH).

4.1.1.23. (5*S*,6*S*,7*S*,7a*R*)-3-[(3-Chlorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-1*H*-pyrrolo-[1,2-*c*]thiazole-6,7-diol (3p). Colorless oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.40–7.27 (4H, m), 4.65 (1H, d, *J* = 15.3 Hz), 4.53 (1H, d, *J* = 15.3 Hz), 4.48–4.42 (1H, m), 3.98–3.94 (3H, m), 3.82–3.60 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.94, 139.15, 135.81, 131.62, 129.47, 128.72, 127.13, 80.58, 79.55, 71.83, 68.71, 63.66, 52.13, 35.94; IR (neat): 3286, 2917, 1635 cm^{-1}; MS (EI): *m/z* 328 (M^++); HRMS (EI) Calcd for $C_{14}H_{17}ClN_2O_3S$ 328.0648; Found: 328.0651 (M^++); $[\alpha]_D^{27}$ +92.8 (*c* 1.0, MeOH).

4.1.1.24. (5*S*,6*S*,7*S*,7a*R*)-3-[(2,4-Dichlorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-1*H*-pyrrolo-[1,2-*c*]thiazole-6,7-diol (3q). Pale yellow oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.49 (1H, s), 7.42 (1H, d, *J* = 8.3 Hz), 7.34 (1H, d, *J* = 8.3 Hz), 4.60–4.50 (2H, m), 4.30–4.24 (1H, m), 3.94–3.86 (3H, m), 3.76–3.49 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 169.23, 135.49, 135.24, 134.96, 131.98, 130.34, 128.62, 80.46, 79.78, 70.00, 68.20, 64.42, 52.38, 35.48; IR (neat): 3368, 2900, 1636 cm^{-1}; MS (EI): *m/z* 362 (M^++); HRMS (EI) Calcd for $C_{14}H_{16}Cl_2N_2O_3S$ 362.0259; Found: 362.0255 (M^++); $[\alpha]_D^{26}$ +63.3 (*c* 0.9, MeOH).

4.1.1.25. (5*S*,6*S*,7*S*,7a*R*)-3-[(3,4-Dichlorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-1*H*-pyrrolo-[1,2-*c*]thiazole-6,7-diol (3r). Colorless oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.57–7.55 (2H, m), 7.30 (1H, d, *J* = 8.6 Hz), 4.66 (1H, d, *J* = 15.9 Hz), 4.55 (1H, d, *J* = 15.9 Hz), 4.52–4.46 (1H, m), 3.98–3.95 (3H, m), 3.83–3.63 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 172.61, 137.32, 133.88, 133.41, 132.18, 130.86, 128.65, 80.61, 79.49, 72.26, 68.84, 63.50, 51.04, 36.12; IR (neat): 3353, 2919, 1632 cm^{-1}; MS (EI): *m/z* 362 (M^++); HRMS (EI) Calcd for $C_{14}H_{16}Cl_2N_2O_3S$ 362.0259; Found: 362.0255 (M^++); $[\alpha]_D^{25}$ +80.4 (*c* 0.95, MeOH).

4.1.1.26. (5*S*,6*S*,7*S*,7a*R*)-3-[(3,5-Dichlorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-1*H*-pyrrolo-[1,2-*c*]thiazole-6,7-diol (3s). Pale yellow oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.43 (1H, d, *J* = 1.8 Hz), 7.34 (2H, s), 4.64 (1H, d, *J* = 15.6 Hz), 4.53 (1H, d, *J* = 15.6 Hz), 4.48–4.42 (1H, m), 3.98–3.94 (3H, m), 3.83–3.60 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.83, 141.10, 136.62, 129.22, 127.34, 80.59, 79.58, 71.78, 68.71, 63.68, 51.81, 35.92; IR (neat): 3346, 2905, 1634 cm^{-1}; MS (EI): *m/z* 362 (M^++); HRMS (EI) Calcd for $C_{14}H_{16}Cl_2N_2O_3S$ 362.0259; Found: 362.0255 (M^++); $[\alpha]_D^{26}$ +71.7 (*c* 0.9, MeOH).

4.1.1.27. (5*S*,6*S*,7*S*,7a*R*)-3-[(3,4,5-trichlorobenzyl)imino]-5-(Hydroxymethyl)tetrahydro-1*H*-pyrrolo-[1,2-*c*]thiazole-6,7-diol (3t). Pale yellow oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.51 (2H, s), 4.60 (1H, d, *J* = 15.8 Hz), 4.51 (1H, d, *J* = 15.8 Hz), 4.45–4.40 (1H, m), 3.97–3.91 (3H, m), 3.81–3.57 (4H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.42, 138.68, 135.50, 131.70, 129.23, 80.59, 79.63, 71.57, 68.67, 63.75, 51.72, 35.85; IR (neat): 3367, 2901, 1636 cm^{-1}; MS (EI): *m/z* 396 (M^++); HRMS (EI) Calcd for $C_{14}H_{15}Cl_3N_2O_3S$ 395.9869; Found: 395.9858 (M^++); $[\alpha]_D^{24}$ +56.9 (*c* 0.95, MeOH).

4.1.1.28. Synthesis of *ido*-2r. The compound ***ido*-2r** was synthesized by the same procedure for the synthesis of **2r** from *ido*-L-DMDP instead of L-DMDP as described above.

4.1.1.29. (2*S*,3*R*,4*R*,5*S*)-*N*-(3,4-Dichlorobenzyl)-3,4-dihydroxy-2,5-bis(hydroxymethyl)pyrrolidine-1-carbothioamide (*ido*-2r). Pale yellow solid; mp; 188–190 °C; 94%; ^1H NMR (400 MHz, MeOH-d_4): δ 7.52(1H, s), 7.43 (1H, d, *J* = 8.0 Hz), 7.28 (1H, dd, *J* = 8.0, 2.2 Hz), 4.94 (1H, d, *J* = 15.3 Hz), 4.74 (1H, d, *J* = 15.3 Hz), 4.43 (2H, br), 4.23 (2H, br), 3.75 (2H, br), 3.34–3.30 (2H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 182.27, 141.53, 133.02, 131.53, 131.33, 130.81, 128.65, 75.48, 75.34, 63.43, 59.41; IR (neat): 3357, 2934, 1522, 1348, 1028 cm^{-1}; MS (FAB): *m/z* 381 (M^++1); HRMS (FAB) Calcd for $C_{14}H_{19}Cl_2N_2O_4S$ 381.0442; Found: 381.0443 (M^++1); $[\alpha]_D^{17}$ −43.7 (*c* 1.0, MeOH).

4.1.1.30. Synthesis of *ido*-3r. The compound ***ido*-3r** was synthesized by the same procedure for the synthesis of **3r** from ***ido*-2r** instead of **2r** as described above.

4.1.1.31. (5*S*,6*R*,7*R*,7a*R*)-3-[(3,4-Dichlorobenzyl)imino]-5-(hydroxymethyl)tetrahydro-1*H*-pyrrolo-[1,2-*c*]thiazole-6,7-diol (*ido*-3r). Colorless oil; quant; ^1H NMR (400 MHz, MeOH-d_4): δ 7.56 (1H, s), 7.53 (1H, d, *J* = 2.5 Hz), 7.28 (1H, d, *J* = 8.5 Hz), 4.62–4.50 (4H, m), 4.08–4.05 (1H, m), 3.92–3.74 (4H, m), 3.54–3.49 (1H, m); ^{13}C NMR (100 MHz, MeOH-d_4): δ 172.75, 137.87, 133.84, 133.21, 132.13, 130.75, 128.52, 81.27, 74.37, 72.97, 67.23, 62.70, 51.48, 29.00; IR (neat): 3294, 2930, 1637 cm^{-1}; MS (EI): *m/z* 362 (M^++); HRMS (EI) Calcd for $C_{14}H_{16}Cl_2N_2O_3S$ 362.0259; Found: 362.0264 (M^++); $[\alpha]_D^{17}$ +122.2 (*c* 1.0, MeOH).

4.2. Biological assays

The enzymes α-L-rhamnosidase (from *Penicillium decumbens*), α-glucosidase (from yeast), β-glucosidase (from almond and bovine liver), α-galactosidase (from coffee beans), β-galactosidase (from *Escherichia coli* and bovine liver), α-mannosidase (from Jack bean), β-mannosidase (from *Helix pomatia*), α-L-fucosidase (from bovine kidney), trehalase (from porcine kidney), β-glucuronidases (from *E. coli*), amyloglucosidase (from *Aspergillus niger*), *p*-nitrophenyl glycosides, and various disaccharides were purchased from Sigma–Aldrich Co. Brush border membranes were prepared from the rat small intestine according to the method of Kessler et al.,[25] and were assayed at pH 6.8 for rat intestinal maltase using maltose. The inhibitory activity toward β-glucocerebrosidase was measured with Cerezyme (Genzyme; Boston, MA) as the enzyme source and 4-methylumbelliferyl-β-D-glucopyranoside (Sigma–Aldrich Co; St. Louis, Mo, USA) as substrate. The reaction mixture consisted 100 mM McIlvaine buffer (pH 5.2), 0.25% sodium taurocholate and 0.1% Triton X-100 (Nacalai Tesque Inc; Kyoto, Japan), and the appropriate amount of enzyme. The reaction mixture was pre-incubated at 0 °C for 45 min, and the reaction was started by the using 3 mM substrate solution, followed by incubation at 37 °C for 30 min. The reaction was stopped by the addition of 1.6 mL of the solution of 400 mM Glycine-NaOH solution (pH 10.6). The released 4-methylumbelliferone was measured (excitation 362 nm, emission 450 nm) with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). For rat intestinal maltase, porcine kidney trehalase, and *A. niger* amyloglucosidase activities, the reaction mixture (0.2 mL) contained 25 mM maltose and the appropriate amount of enzyme, and the incubations were performed for 10–30 min at 37 °C. The reaction was stopped by heating at 100 °C for 3 min. After centrifugation (600 g; 10 min), 0.05 mL of the resulting reaction mixture were added to 3 mL of the Glucose CII-test Wako (Wako Pure Chemical Ind., Osaka, Japan). The absorbance at 505 nm was measured to determine the amount of the released D-glucose. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction mixture (1 mL) contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by addition of 2 mL of 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.10.015.

References and notes

1. Ma, Y.; Stern, R. J.; Scherman, M. S.; Vissa, V. D.; Yan, W.; Jones, V. C.; Zhang, F.; Franzblau, S. G.; Lewis, W. H.; McNeil, M. R. *Antimicrob. Agents Chemother.* **2001**, *45*, 1407.
2. Babaoglu, K.; Page, M. A.; Jones, V. C.; McNeil, M. R.; Dong, C.; Naismith, J. H.; Lee, R. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3227.
3. Ma, Y.; Pan, F.; NcNeil, M. *J. Bacteriol.* **2002**, *184*, 3392.
4. Lucas, R.; Balbuena, P.; Errey, J. C.; Squire, M. A.; Gurcha, S. S.; McNeil, M.; Besra, G. S.; Davis, B. G. *ChemBioChem* **2008**, *9*, 2197.
5. Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645.
6. Asano, N. *Glycobiology* **2003**, *13*, 93R.
7. Nash, R. J.; Kato, A.; Yu, C.-Y.; Fleet, G. W. J. *Future Med. Chem.* **2011**, *3*, 1513.
8. Shilvock, J. P.; Wheatley, J. R.; Davis, B.; Nash, R. J.; Griffiths, R. C.; Jones, M. G.; Müller, M.; Crook, S.; Watkin, D. J.; Smith, C.; Besra, G. S.; Brennan, P. J.; Fleet, G. W. J. *Tetrahedron Lett.* **1996**, *37*, 8569.
9. Davis, B. G.; Hull, A.; Smith, C.; Nash, R. J.; Watson, A. A.; Winkler, D. A.; Griffiths, R. C.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **1998**, *9*, 2947.
10. Shilvock, J. P.; Wheatley, J. R.; Nash, R. J.; Watson, A. A.; Griffiths, R. C.; Butters, T. D.; Muller, M.; Watkin, D. J.; Winkler, D. A.; Fleet, G. W. J. *J. Chem. Soc., Perkin Trans. 1* **1999**, *19*, 2735.
11. Davis, B.; Bell, A. A.; Nash, R. J.; Watson, A. A.; Griffiths, R. C.; Jones, M. G.; Smith, C.; Fleet, G. W. J. *Tetrahedron Lett.* **1996**, *37*, 8565.
12. Håkansson, A. E.; van Ameijde, J.; Horne, G.; Nash, R. J.; Wormald, M. R.; Kato, A.; Besra, G. S.; Gurcha, S.; Fleet, G. W. J. *Tetrahedron Lett.* **2008**, *49*, 179.
13. Gomez, L.; Garrabou, X.; Joglar, J.; Bujons, J.; Parella, T.; Vilaplana, C.; Cardona, P. J.; Clapes, P. *Org. Biomol. Chem.* **2012**, *10*, 6309.
14. Concia, A. L.; Gomez, L.; Bujons, J.; Parella, T.; Vilaplana, C.; Cardona, P. J.; Joglar, J.; Clapes, P. *Org. Biomol. Chem.* **2013**, *11*, 2005.
15. Håkansson, A. E.; van Ameijde, J.; Guglielmini, L.; Horne, G.; Nash, R. J.; Evinson, E. L.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007**, *18*, 282.
16. Mercer, T. B.; Jenkinson, S. F.; Bartholomew, B.; Nash, R. J.; Miyauchi, S.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2009**, *20*, 2368.
17. Chapman, T. M.; Courtney, S.; Hay, P.; Davis, B. G. *Chem. Eur. J.* **2003**, *9*, 3397.
18. Kim, J. H.; Curtis-Long, M. J.; Seo, W. D.; Lee, J. H.; Lee, B. W.; Yoon, Y. J.; Kang, K. Y.; Park, K. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4282.
19. Ayers, B. J.; Ngo, N.; Jenkinson, S. F.; Martinez, R. F.; Shimada, Y.; Adachi, I.; Weymouth-Wilson, A. C.; Kato, A.; Fleet, G. W. *J. Org. Chem.* **2012**, *77*, 7777.
20. Ansari, A. A.; Vankar, Y. D. *J. Org. Chem.* **2013**, *78*, 9383.
21. (a) Garcia-Moreno, M. I.; Diaz-Perez, P.; Ortiz Mellet, C.; Garcia Fernandez, J. *Chem. Commun.* **2002**, 848; (b) Diaz-Perez, P.; Garcia-Moreno, M. I.; Mellet, C. O.; Fernandez, J. M. G. *Synlett* **2003**, 341; (c) Aguilar, M.; Diaz-Perez, P.; Garcia-Moreno, M. I.; Mellet, C. O.; Fernandez, J. M. G. *J. Org. Chem.* **2008**, *73*, 1995; (d) Aguilar-Moncayo, M.; Mellet, C. O.; Fernandez, J. M. G.; Garcia-Moreno, M. I. *J. Org. Chem.* **2009**, *74*, 3595; (e) Aguilar-Moncayo, M.; Gloster, T. M.; Turkenburg, J. P.; Garcia-Moreno, M. I.; Mellet, C. O.; Davies, G. J.; Fernandez, J. M. G. *Org. Biomol. Chem.* **2009**, *7*, 2738; (f) Sanchez-Fernandez, E. M.; Garcia Fernandez, J. M.; Mellet, C. *Chem. Commun.* **2016**, 5497.
22. Mena-Barragan, T.; Garcia-Moreno, M. I.; Nanba, E.; Higaki, K.; Concia, A. L.; Clapes, P.; Garcia Fernandez, J. M.; Ortiz Mellet, C. *Eur. J. Med. Chem.* **2016**, *121*, 880.
23. Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265.
24. Kato, A.; Nakagome, I.; Sato, K.; Yamamoto, A.; Adachi, I.; Nash, R. J.; Fleet, G. W.; Natori, Y.; Watanabe, Y.; Imahori, T.; Yoshimura, Y.; Takahata, H.; Hirono, S. *Org. Biomol. Chem.* **2016**, *14*, 1039.
25. Kessler, M.; Acuto, O.; Strelli, C.; Murer, H.; Semenza, G. A. *Biochem. Biophys. Acta* **1978**, *506*, 136.