Synthesis, Inhibition Properties, and Theoretical Study of the New Nanomolar Trehalase Inhibitor 1-Thiatrehazolin: Towards a Structural Understanding of Trehazolin Inhibition

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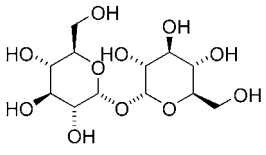
A new trehazolin analogue, 1-thiatrehazolin, has been synthesized from carbohydrate precursors by a highly efficient route based on our previously developed ketone/oxime ether reductive carbocyclization reaction for the construction of the cyclitol ring and an intramolecular nucleophilic displacement reaction for the construction of the thiazoline ring. 1-Thiatrehazolin is a very potent, slow, tight-binding trehalase inhibitor. A structural model for trehalase inhibition by trehazolin and its analogues, based on the experimental results and supported by theoretical calculations, is proposed.

Introduction

α,α-Trehalose (**1**) is a non-reducing disaccharide formed by two molecules of α,α-1,1-linked D-glucose and widespread throughout a large variety of organisms including bacteria, yeast, fungi, insects, nematodes, and plants. In bacteria, fungi, insects, and nematodes it serves as source of energy and carbon, while in yeast and plants it might also have a signaling function.[1] In addition, trehalose has been shown to play a protecting role against different stress conditions. Trehalase (EC 3.2.1.28) is a very specific enzyme that hydrolyzes trehalose to two glucose units, an essential process in the life functions of various organisms, in particular in fungi, insects, and nematodes. Accordingly, trehalase inhibitors are of potential interest for crop protection. Several trehalase inhibitors have been isolated from natural sources, including deoxynojirimycin (**2**),[2] validamycins (**3**),[3] validoxylamines (**4**),[4] trehazolin (**5**),[5] salbostatin (**6**),[6] and calystegin B4 (**7**).[7] Among these natural inhibitors, trehazolin is the most potent and specific. It has a unique pseudodisaccharide structure consisting of an α-D-glucopyranose moiety bonded to an aminocyclopentitol (trehazolamine, **8**) through a fused 2-aminooxazoline ring. The chemistry and biochemistry of **5** have been thoroughly investigated.[8,9] Inhibition of trehalases by trehazolin is of the reversible, competitive type with respect to trehalose.[10,11] Trehalases are inverting glycosidases; this suggests the presence of a catalytic acid group, together with a nucleophilic water molecule, in the active site of the enzyme.[12] Kinetic studies performed with porcine kidney trehalase in the presence of two types of competitive inhibitors[13] support the earlier hypothesis[14] that the active center of the enzyme may comprise two subsites, one for catalysis and one for recognition, acting separately on each glucose unit of trehalose. This conclusion could probably be extended to other trehalases. Although there is no structural information on any enzyme–inhibitor complex for **5** yet available, the cyclitol moiety seems to mimic the transition state leading to the high-energy glucopyranosyl intermediate involved in the enzymatic hydrolysis reaction. It has been proposed[8] that the anomeric nitrogen of **5** interacts with the catalytic acid group, while the nitrogen or the oxygen atom of



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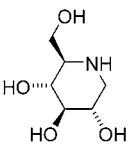

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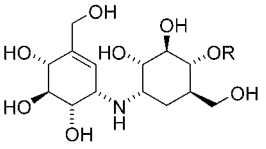
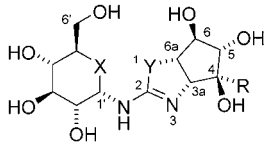
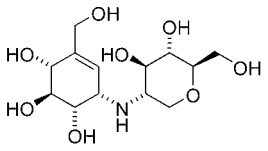


3, Validamycin A (R = β-D-glucose)
4, Validoxylamine A (R = OH)



5, X = Y = O, R = CH_2OH
9, X = CH_2, Y = O, R = CH_2OH
10, X = CH_2, Y = NH, R = H
11, X = O, Y = S, R = CH_2OH



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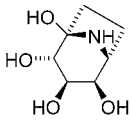

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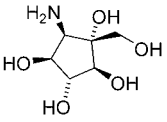

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the oxazoline ring acts as a surrogate for the nucleophilic water molecule. Extensive structure–activity relationship studies have shown that the inhibitory potency is highly sensitive to subtle structural modifications at the pyranose or cyclopentitol moieties.[8] Only the 5a′-carba-analogue **9**[15] conserves the nanomolar inhibitory activity of the parent compound against trehalases, while also possessing higher chemical stability. Very little is known, however, about the effect of structural modifications at the oxazoline ring. The only analogue of this type described is compound **10**,[16] which contains an imidazoline ring, but also has two other concomitant structural modifications: a 5a-carbaglucose—to confer stability towards hydrolytic cleavage without compromising activity—and a 4-de(hydroxymethyl)cyclitol moiety, a modification that has been shown[17] to lower the inhibitory activity by 100 times with respect to parent **5**. Since compound **10** is 1000 times less potent than trehazolin against silkworm trehalase, it can be concluded from the above that the oxazoline to imidazoline modification is clearly deleterious to inhibitory activity, by roughly an order of magnitude. With this in mind, we decided to prepare the corresponding thiazoline analogue **11** and to assay its inhibitory activity against trehalase.

Results

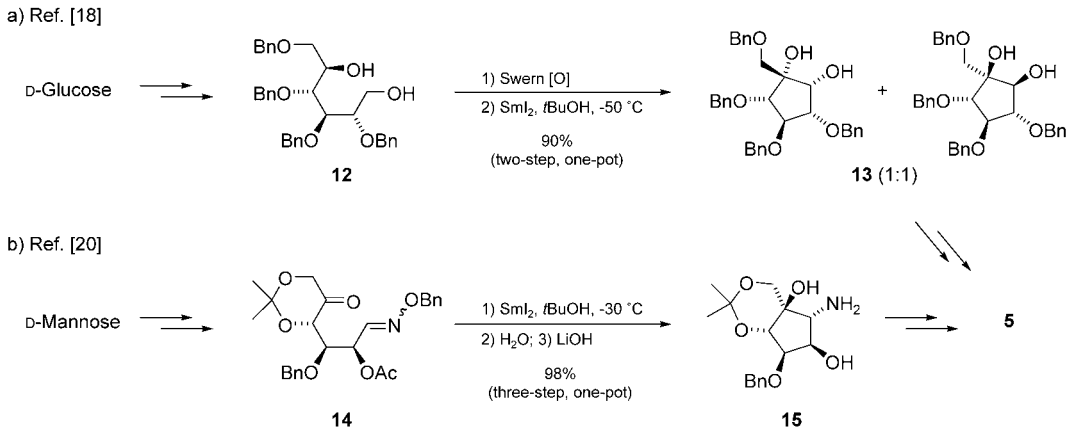
Synthesis of 11

For the preparation of **11**, we relied on earlier synthetic work developed by our group en route to **5**. We have previously described two different synthetic approaches to **5** from readily available carbohydrate precursors, based on a reductive carbocyclization reaction promoted by samarium diiodide as a key step. In the first approach,[18] a highly efficient two-step, one-pot oxidation-reductive coupling sequence[19] served to transform the 1,5-diol **12**, derived from D-glucose, into a 1:1 mixture of carbocyclic *cis*-diols **13**, from which trehazolamine (**8**) and then the final target **5** were readily prepared by simple synthetic manipulations (Scheme 1 a). A second and more efficient route[20] (Scheme 1 b) was developed later, and utilized a carbonyl-oxime ether reductive carbocyclization with subsequent N–O reductive cleavage, a highly efficient one-pot sequence first described by our group in 1995.[21] Thus, treatment of keto-oxime **14**, readily available from D-mannose,[20] with an excess of samarium diiodide (>4 equiv) promoted a very high yielding tandem process that consisted of a completely stereoselective reductive carbocyclization followed by the in situ N–O reductive cleavage of the resultant carbocyclic hydroxylamine, triggered upon addition of water to the reaction mixture containing excess SmI_2. Subsequent addition of LiOH produced the in situ hydrolysis of the ester group to afford aminocyclopentitol **15** as a single diastereoisomer in an almost quantitative overall yield.[20] The high efficiency and complete diastereoselectivity of this tandem process is quite remarkable and underscores the utility and mildness of samarium diiodide in the promotion of selective transformations on highly functionalized substrates. The synthesis of **5** was completed via an intermediate urea derivative of **15**, from which the oxazoline ring was constructed through an intramolecular S_N2 reaction that also served to adjust the final stereochemistry of the carbocycle.

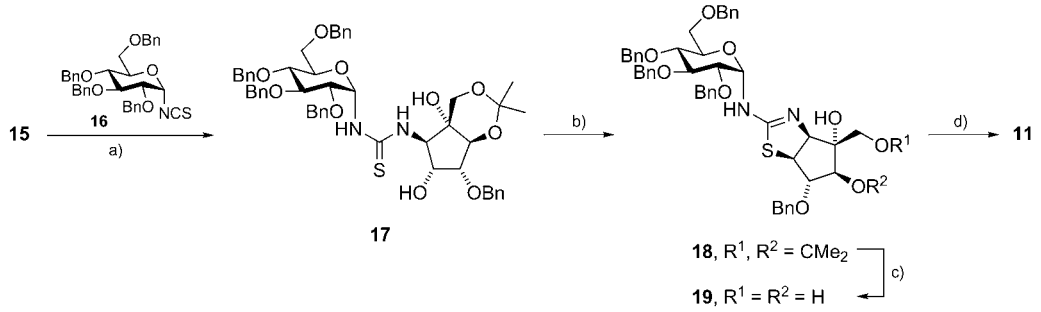
Thiazoline analogue **11** was readily prepared from aminocyclopentitol **15**[20] as follows (Scheme 2). Treatment of **15** with α-D-glucosyl isothiocyanate **16**[22] afforded thiourea **17**[20] in an almost quantitative yield. Treatment of **17** with triflic anhydride and pyridine under our previously optimized conditions[20] produced a smooth cyclization to afford the 2-aminothiazoline **18** in very good yield with concomitant inversion of stereochemistry at the center bearing the secondary hydroxy group through the intramolecular S_N2 displacement of a transient triflate by the vicinal thiocarbonyl group. Complete deprotection of **18** finally afforded our target 1-thiatrehazolin (**11**).

Enzymatic studies

Analogue **11** was tested as inhibitor against commercially available porcine kidney trehalase and its activity was compared to that of synthetic **5**[20] measured under identical experimental conditions (Table 1). Compound **11** is a nanomolar in-



Scheme 1. *Our two former synthetic approaches to trehazoline (**5**) from carbohydrate precursors with a ketyl radical reductive carbocyclization reaction as a key step.*



Scheme 2. *Synthesis of 1-thiatrehazolin (**11**) from aminocyclopentitol **15**. a) THF, RT, 96%; b) Tf_2O, Py, CH_2Cl_2, −40°C to RT, 93%; c) cat. pTsOH, CH_2Cl_2/MeOH, RT, 89%; d) Na, NH_3/THF, −35°C, quant.*

Table 1. *Inhibitory activity against porcine trehalase (measured at 37°C at pH 6.2) and pK_a values measured for synthetic **5** and **11**.*

Compound	IC_{50} [nM][a]	K_I [nM]	pK_a[b]
5	15.5[c] (5.1)	2.1[d]	6.3[e]
11	83.0 (20.0)	30.4	5.8

[a] In brackets, the value measured after 30 min of preincubation of the inhibitor with the enzyme at 37°C. [b] Measured by ^1H NMR titration in D_2O at 25°C. [c] Reported for natural **5** against porcine trehalase: IC_{50} = 16 nM (ref. [10]), 19 nM (ref. [11]). [d] Reported for natural **5** against silkworm trehalase: K_i = 10 nM (ref. [10]). [e] Ref. [5b].

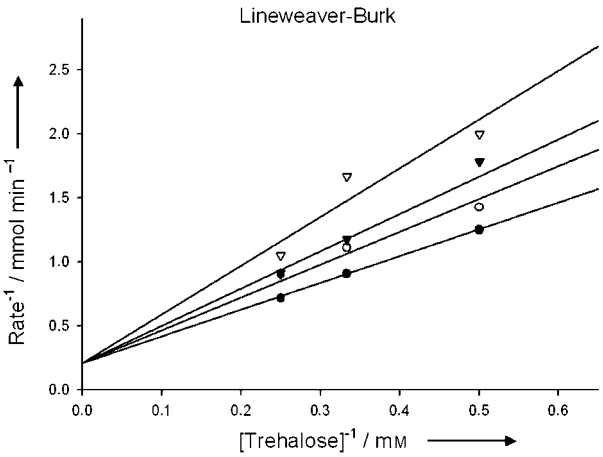


Figure 1. *Lineweaver–Burk plots of commercial pig kidney trehalase activities in the presence of **11**. Concentrations of **11** were 0 μM (●), 7 μM (○), 12 μM (▼), and 25 μM (▽).*

hibitor of this enzyme, although with an IC_{50} value approximately five times higher than that of parent **5**. Like **5**,[10] **11** also presents a slow inhibition onset, its activity increasing upon preincubation with the enzyme (see Table 1). Slow binding is a widespread phenomenon among potent enzyme inhibitors, the inhibition process occurring over a period of minutes and not at diffusion-controlled rates.[23] In the case of **11**, inhibition reaches a maximum within 30 min, while potentiation proceeds over up to 6 h in the case of **5**.[10] In order to test the reversibility of the inhibition, trehalase preincubated with **11** at 37°C for 30 min was dialyzed at 4°C against sodium citrate/Na_2HPO_4 buffer (pH 6.2). Dialysis gradually restored trehalase activity, the percentages of regained activity with respect to a control experiment in the absence of inhibitor being 65 and 100% after 3 and 18 h, respectively. Recovery has been reported to proceed much more slowly in the case of **5**,[10] taking 48 h of dialysis at 4°C to restore only 24% of the initial activity observed without inhibitor. Lineweaver–Burk plots (Figure 1) show that, as reported for natural **5**,[11] **11** inhibits porcine trehalase competitively with respect to trehalose. From Dixon plots, K_i values were obtained for **11** and for synthetic **5** (Table 1).

Discussion

According to our kinetic studies, 1-thiatrehazolin (**11**) can be classified as a slow, tight-binding, competitive trehalase inhibitor, similar to its parent compound **5**. This kind of inhibition is usually indicative of (reversible) covalent attachment of the inhibitor to the enzyme or of a conformational transition of the enzyme between two states that bind the inhibitor with different affinities,[23,24] although recent studies indicate that it could also be a consequence of relatively slow on- and off-binding rates between enzyme and inhibitor.[25] The aminooxazo(thiazo)line moiety is a potential electrophilic locus (at C-2) for covalent attachment of an active site nucleophile. However, kinetic studies with silkworm trehalase seem to rule out such a possibility for **5**.[10] Our observation that analogue **11** is also a slow, tight-binding inhibitor supports this conclusion, since the introduction of the isosteric –S– group would be expected to result in a substantial reduction of the net positive charge at C-2 (see below), lessening its electrophilic character significantly.[26] The 2-aminooxazo(thiazo)line moiety, however, is perfectly suited to form a bidentate complex with the active site carboxylic acid, as shown in Figure 2 (or a salt bridge after net proton transfer from the catalytic acid to the heterocycle). Such a complex would be expected to be stronger for **5** than for its thiazoline analogue **11**, as shown by DFT B3LYP/6-311+G(d,p) quantum mechanical calculations performed on a simple model for this interaction in the gas phase. In this theoretical study, we have used acetic acid to represent the enzyme cata-

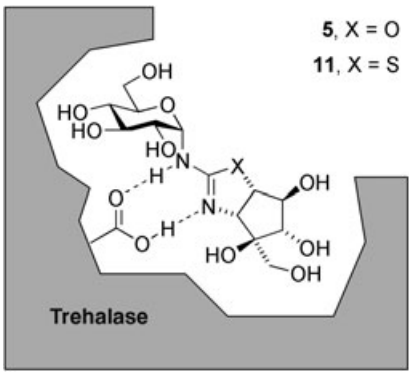
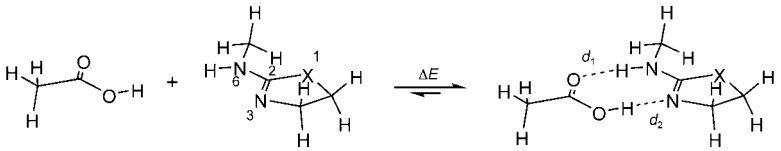


Figure 2. *Proposed model for the complex of inhibitors* ***5*** *(X = O) and* ***11*** *(X = S) with trehalase.*

lytic acid group and 2-(methylamino)oxazoline or -thiazoline as models for **5** or **11**, respectively. For the theoretical study we considered only the 2-(methylamino)-tautomer of the heterocycles, since previous calculations[26,27] at various levels of theory have shown that 2-aminooxazo(thiazo)line is more stable than the alternative 2-iminooxazo(thiazo)lidine tautomer by about 2 kcal mol^{-1}. To simplify the analysis, we neglected the *Z*/*E* isomerization of the methylamino group, and only the *Z* isomer was included in the calculations. The optimized geometries of the complexes at this level of theory show that 2-(methylamino)oxazoline forms a tighter complex than 2-(methylamino)thiazoline with acetic acid, as revealed by the smaller $d_1 + d_2$ sum of H-bond distances calculated for the former (Table 2). Accordingly, the enthalpies, free energies, and equilibrium constants calculated for the complexation reaction in the gas phase reveal that the oxazoline derivative forms the more stable complex (Table 2). No net proton transfer from acetic acid to the heterocycle with formation of a salt bridge is predicted at this level of theory in the gas phase. Our theoretical results for this model interaction parallel the experimentally measured pK_a values for **5** and **11** (see Table 1) and those reported[28] for 2-aminooxazoline (pK_a = 9.37) and 2-aminothiazoline (pK_a = 8.70). The calculated equilibrium constant (K_d) for the dissociation of the oxazoline complex in the gas phase is about an order of magnitude lower than that of the thiazoline complex (see Table 2), in close parallel to the corresponding K_i values measured for **5** and its thio analogue **11**. This is what could be expected if this single interaction were the main energy component responsible for the difference between the complexation reactions of each inhibitor with trehalase. Recent studies[29] on the trehalase inhibitory activity of a series of simple 2-(arylamino)oxazoline and -thiazoline derivatives have shown that the oxazoline compounds have IC_{50} values that are approximately an order of magnitude lower than those of their corresponding thiazoline analogues, as we have observed for **5** and **11**, a result that can also be explained on the basis of our complexation model. Also in favor of this model is the observation that the oxazoline to imidazoline modification has a deleterious effect on inhibition, as seen for analogue **10**.[16] A 2-aminoimidazoline analogue would be expected to have a higher pK_a value than **5** and hence to be fully protonated at the pH of the enzyme assay and therefore unable to interact efficiently with the catalytic acid group. Ando's observation[10] that trehazolin is a slightly worse inhibitor of silkworm trehalase at lower pH (IC_{50} = 27 nM at pH 6.2; IC_{50} = 52 nM at pH 5.4) also supports our proposal that the neutral molecule is the active form of the inhibitor.

For a more in-depth understanding of the possible differential binding interactions of inhibitors **5** and **11** with trehalase, we have also compared the calculated atomic charge distributions of the two model heterocycles. NBO population analysis[30] shows two major differences in the electronic charge distributions in the iso(thio)urea regions of 2-(methylamino)oxazoline and 2-(methylamino)thiazoline (Table 3). Firstly, as advanced above, the C-2 atom carries a lower positive charge in the thio derivative. Secondly, while the oxygen atom is highly negatively charged, the electropositive sulfur atom carries a positive charge. The fact that inhibition of trehalase is only slightly affected by changing X from oxygen to sulfur, in spite of their very different atomic charges and van der Waals radii, supports the conclusion that X is probably not directly involved in significant interactions with residues in the catalytic site of the enzyme.

Table 2. *Computed hydrogen bond distances, energies, enthalpies, free energies, and dissociation equilibrium constants for the model complexation reaction of acetic acid with 2-(methylamino)oxazoline and 2-(methylamino)-thiazoline at the B3LYP/6-311+G(d,p) level in the gas phase (at 298.15 K).*



X	d_1 [Å]	d_2 [Å]	ΔE[a] [kcal mol^{-1}]	$\Delta H°$ [kcal mol^{-1}]	$\Delta G°$ [kcal mol^{-1}]	K_d[b]
O	1.882	1.628	−17.45	−16.38	−6.00	3.97×10^{-5}
S	1.876	1.651	−16.59	−15.52	−4.92	2.46×10^{-4}

[a] At 0 K. [b] The equilibrium constant for the dissociation process was computed from the $-\Delta G°$ values (reverse reaction) by use of the equation: $K_d = \exp[-(-\Delta G°)/RT]$.

Table 3. *B3LYP/6-311+G(d,p) natural atomic charges calculated for 2-(methylamino)oxazoline (X = O) and 2-(methylamino)thiazoline (X = S) (see Table 2 for atom numbering).*

X	X-1	C-2	N-3	N-6
O	−0.574	0.727	−0.596	−0.641
S	0.163	0.328	−0.564	−0.643

Conclusion

In summary, we have synthesized a new trehazolin analogue, 1-thiatrehazolin, by a very efficient route that features a highly stereoselective reductive tandem process promoted by samarium diiodide for the construction of the cyclitol moiety and a mild and high-yielding intramolecular nucleophilic displacement reaction for the construction of the thiazoline ring. 1-Thiatrehazolin is a nanomolar, slow, tight-binding inhibitor of porcine trehalase. From our experimental results and those reported in the literature for related compounds, a structural model for the inhibition of trehalase by trehazolin and its analogues has been proposed, supported by theoretical calculations.

Experimental Section

Thiourea 17: A solution of **16**[22] (227 mg, 0.39 mmol) in THF (4 mL) was added dropwise to a solution of **15** (121 mg, 0.39 mmol) in THF (6 mL). After the system had been stirred for 4 h at 30 °C, the solvent was removed at reduced pressure and the crude product was purified by flash chromatography (EtOAc/hexane 1:2) to afford **17** (334 mg, 96%) as a white solid. $R_f = 0.42$ (EtOAc/hexane 1:1); m.p. 81–82 °C; $[\alpha]_D^{22} = +149.1$ ($c = 0.9$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.48$ (d, $^3J(H,H) = 6.0$ Hz, 1H), 7.40–7.23 (m, 22H), 7.14–7.11 (m, 2H), 6.65 (d, $^3J(H,H) = 1.6$ Hz, 1H), 5.17 (dd, $^3J(H,H) = 4.8$, 1.7 Hz, 1H), 5.08 (s, 1H), 4.90 (d, $^3J(H,H) = 11.0$ Hz, 1H), 4.85–4.77 (m, 3H), 4.64 (s, 2H), 4.58 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.58 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.53 (dd, $^3J(H,H) = 9.9$, 6.0 Hz, 1H), 4.45 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.45 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.16 (td, $^3J(H,H) = 10.4$, 4.4 Hz, 1H), 4.03 (s, 1H), 3.85–3.67 (m, 4H), 3.66 (s, 2H), 3.58 (dd, $^3J(H,H) = 10.4$, 1.9 Hz, 1H), 3.53–3.44 (m, 2H), 2.67 (d, $^3J(H,H) = 10.7$ Hz, 1H), 1.70 (s, 1H), 1.41 (s, 3H), 1.22 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 186.0$, 138.1, 137.7, 137.3, 137.2, 136.7, 128.6–127.7 (25C), 99.3, 81.7, 81.3, 79.8, 77.5, 77.3, 77.0, 76.4, 76.1, 75.9, 75.0, 73.4, 72.3, 72.1, 71.0, 70.8, 68.2, 64.5, 26.3, 22.3 ppm; IR (KBr): $\tilde{\nu} = 3400$, 3000, 1545, 1370, 1080, 700 cm^{-1}; elemental analysis calcd (%) for $C_{51}H_{58}N_2O_{10}S$: C 68.74, H 6.56, N 3.14, S 3.60; found: C 68.71, H 6.80, N 3.41, S 3.52.

Aminothiazoline 18: Pyridine (37 μL, 0.46 mmol) and triflic anhydride (25 μL, 0.15 mmol) were added at −40 °C to a solution of **17** (100 mg, 0.11 mmol) in CH_2Cl_2 (3 mL). After stirring at this temperature for 1 h, the mixture was diluted with CH_2Cl_2 (5 mL), and aqueous saturated $NaHCO_3$ was added. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3×5 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated at reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane 1:1) to afford **18** (91 mg, 93%) as a colorless oil. $R_f = 0.18$ (EtOAc/hexane 1:1); $[\alpha]_D^{22} = +95.3$ ($c = 2.2$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.35$–7.23 (m, 23H), 7.15–7.10 (m, 2H), 5.35 (m, 1H), 4.90 (d, $^3J(H,H) = 10.8$ Hz, 1H), 4.80 (d, $^3J(H,H) = 11.0$ Hz, 1H), 4.77 (d, $^3J(H,H) = 11.0$ Hz, 1H), 4.73 (d, $^3J(H,H) = 8.7$ Hz, 1H), 4.63 (d, $^3J(H,H) = 12.1$ Hz, 1H), 4.59–4.41 (m, 7H), 4.25 (dd, $^3J(H,H) = 8.4$, 3.7 Hz, 1H), 4.19 (d, $^3J(H,H) = 12.1$ Hz, 1H), 4.04–3.99 (m, 2H), 3.82–3.62 (m, 6H), 1.42 (s, 3H), 1.27 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 159.1$, 138.6, 138.4, 138.1, 137.4 (2C), 128.5–127.6 (25C), 99.0, 92.3, 85.3, 82.1 (2C), 80.4, 78.4 (2C), 77.3, 75.6, 74.9, 73.4, 72.7, 72.4, 70.3, 68.4, 65.1, 56.7, 26.8, 21.6 ppm; elemental analysis calcd (%) for $C_{51}H_{56}N_2O_9S$: C 70.16, H 6.47, N 3.21, S 3.67; found: C 69.98, H 6.61, N 3.14, S 3.51.

Aminothiazoline 19: *p*TsOH (24 mg, 0.16 mmol) was added to a solution of **18** (96 mg, 0.12 mmol) in MeOH/CH_2Cl_2 (1:1, 8 mL). After being stirred at RT for 12 h, the mixture was diluted with CH_2Cl_2 (15 mL), and aqueous saturated $NaHCO_3$ was added. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3×15 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated at reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane 5:1) to afford **19** (81 mg, 89%) as a colorless oil. $R_f = 0.37$ (EtOAc); $[\alpha]_D^{22} = +83.6$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.36$–7.12 (m, 25H), 5.24 (d, $^3J(H,H) = 4.1$ Hz, 1H), 4.90 (d, $^3J(H,H) = 10.9$ Hz, 1H), 4.79 (d, $^3J(H,H) = 11.0$ Hz, 1H), 4.75 (d, $^3J(H,H) = 10.2$ Hz, 1H), 4.66–4.57 (m, 5H), 4.50 (d, $^3J(H,H) = 9.4$ Hz, 1H), 4.46 (d, $^3J(H,H) = 11.7$ Hz, 1H), 4.14 (d, $^3J(H,H) = 7.2$ Hz, 1H), 4.02 (dd, $^3J(H,H) = 10.4$, 5.7 Hz, 1H), 3.85–3.62 ppm (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 162.8, 138.6, 138.2, 138.0, 137.7, 137.3, 128.4- 127.5 (25C), 90.4, 82.2, 81.8, 81.0, 80.5, 80.3, 78.3, 77.3, 75.5, 74.9, 73.4, 72.7, 72.4, 70.4, 68.5, 64.0, 52.9 ppm.

1-Thiatrehazolin (11): A solution of **19** (98 mg, 0.12 mmol) in THF (8 mL) was added at −78 °C to a solution of Na (228 mg, 9.91 mmol) in NH_3 (20 mL). The reaction mixture was stirred at −78 °C to −35 °C for 12 h, NH_4Cl (263 mg) was added, and the mixture was allowed to warm to RT. The mixture was partitioned between water (10 mL) and CH_2Cl_2 (25 mL), the aqueous phase was washed with CH_2Cl_2 (2×5 mL), and the water was removed at reduced pressure. The residue was purified by ion-exchange chromatography on Dowex 50W-H$^+$ with elution with NH_4OH (1 M) to afford **11** (45 mg, 100%) as a white solid after lyophilization. $R_f =$ 0.40 (CH_3CN/AcOH/H_2O 6:1:3); $[\alpha]_D^{22} = +$ 107.2 ($c = 0.7$ in CH_3OH); ^{1}H NMR (400 MHz, D_2O, 25 °C, TMS): $\delta = 5.44$ (d, $^3J(H,H) = 5.2$ Hz, 1H), 4.66 (d, $^3J(H,H) = 8.4$ Hz, 1H), 4.12–4.05 (m, 2H), 3.92–3.89 (m, 1H), 3.85–3.61 (m, 6H), 3.57–3.52 (m, 1H), 3.40 ppm (t, $^3J(H,H) =$ 9.3 Hz, 1H); ^{13}C NMR (100 MHz, D_2O, 25 °C, TMS): $\delta = 161.9$, 83.5, 82.5, 81.5, 81.3, 81.2, 73.2, 72.3, 70.0, 69.6, 62.8, 60.7, 54.4 ppm; IR (KBr): $\tilde{\nu} = 3434$, 1629, 1033 cm^{-1}; MS (FAB): *m/z* (%): 383 (100) $[M+H]^+$.

Enzyme assays: α,α-Trehalase (EC 3.2.1.28) from porcine kidney was purchased from Sigma (0.7 U mg^{-1}). The reaction (45 μL total volume) was started by addition of enzyme (0.7 mU, 5 μL) to sodium citrate (20 mM)/Na_2HPO_4 (40 mM) buffer (1:1, pH 6.2) containing bovine serum albumin (0.2 mg mL^{-1}), α,α-trehalose (4, 3, 2, and 1 mM), and various concentrations of the inhibitor (1-thiatrehazolin: 0, 7, 12, and 25 nM; trehazolin: 0, 0.5, 2.5, and 5 nM). After incubation of the mixture at 37 °C for 40 min, the reaction was stopped by placing the mixture over boiling water for 3 min. The reaction mixture was then cooled in ice/water, and denatured protein was removed by centrifugation at 12000 rpm for 5 min. The concentration of D-glucose in the supernatant was determined by the glucose oxidase–peroxidase method (Glucose Trinder 100, from Sigma).[31] Lineweaver–Burk analysis of the kinetic data gave $K_m =$ 3.7 nM, $V_{max} = 2.8$ mmol min^{-1} for the enzymatic hydrolysis of trehalose without inhibitor and $K_m = 10.1$ nM, $V_{max} = 4.8$ mmol min^{-1} in the presence of **11**.

For the dialysis experiments, the enzyme and 1-thiatrehazolin (at two concentrations: 3.5 and 7 nM) were preincubated at 37 °C for 30 min. The reaction mixture was then dialyzed against sodium citrate/Na_2HPO_4 buffer (pH 6.2) at 4 °C. Trehalase activity was determined as above after 3 h and 18 h of dialysis.

Theoretical calculations: Ab initio calculations were carried out with the aid of the Gaussian 98[32] program package at the density

functional (B3LYP) level of theory with use of the 6-311+G(d,p) standard basis set. After geometry optimization, analytical frequency calculations were carried out to determine the nature of the stationary points found and to obtain thermochemical properties by standard procedures.

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[1] A. D. Elbein, Y. T. Pan, I. Pastuszak, D. Carroll, *Glycobiology* **2003**, *13*, 17R–27R.
[2] S. Murao, S. Miyata, *Agric. Biol. Chem.* **1980**, *44*, 219–221.
[3] N. Asano, T. Yamaguchi, Y. Kameda, K. Matsui, *J. Antibiot.* **1987**, *40*, 526–532.
[4] Y. Kameda, N. Asano, T. Yamaguchi, K. Matsui, *J. Antibiot.* **1987**, *40*, 563–565.
[5] a) T. Nakayama, T. Amachi, S. Murao, T. Sakai, T. Shin, P. T. M. Kenny, T. Iwashita, M. Zagorski, H. Komura, K. Nomoto, *J. Chem. Soc. Chem. Commun.* **1991**, 919–921; b) O. Ando, H. Satake, K. Itoi, A. Sato, M. Nakajima, S. Takahashi, H. Haruyama, Y. Ohkuma, T. Kinoshita, R. Enokita, *J. Antibiot.* **1991**, *44*, 1165–1168.
[6] L. Vertesy, H.-W. Fehlhaber, A. Schulz, *Angew. Chem.* **1994**, *106*, 1936–1937; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1844–1846.
[7] N. Asano, A. Kato, H. Kizu, K. Matsui, A. A. Watson, R. J. Nash, *Carbohydr. Res.* **1996**, *293*, 195–204.
[8] A. Berecibar, C. Grandjean, A. Siriwardena, *Chem. Rev.* **1999**, *99*, 779–844.
[9] Y. Kobayashi, *Carbohydr. Res.* **1999**, *315*, 3–15.
[10] O. Ando, M. Nakajima, M. Kifune, H. Fang, K. Tanzawa, *Biochim. Biophys. Acta* **1995**, *1244*, 295–302.
[11] S. V. Kyosseva, Z. N. Kyossev, A. D. Elbein, *Arch. Biochem. Biophys.* **1995**, *316*, 821–826.
[12] a) J. Defaye, H. Driguez, B. Henrissat, E. Bar-Guilloux, *Carbohydr. Res.* **1983**, *124*, 265–273; b) K. H. Clifford, *Eur. J. Biochem.* **1980**, *106*, 337–340.
[13] N. Asano, A. Kato, K. Matsui, *Eur. J. Biochem.* **1996**, *240*, 692–698.
[14] a) J. Defaye, H. Driguez, B. Henrissat, E. Bar-Guilloux in *Mechanism of Saccharide Polymerization and Depolymerization* (Ed.: J. J. Marshall), Academic Press, New York, **1980**, pp. 331–353; b) J. Defaye, H. Driguez, B. Henrissat, E. Bar-Guilloux, *Nouv. J. Chim.* **1980**, *4*, 59–68.
[15] C. Uchida, H. Kitahashi, S. Watanabe, S. Ogawa, *J. Chem. Soc. Perkin Trans. 1* **1995**, 1707; C. Uchida, S. Ogawa, *Carbohydr. Lett.* **1994**, *1*, 77–81.
[16] C. Uchida, T. Yamagishi, H. Kitahashi, Y. Iwaisaki, S. Ogawa, *Bioorg. Med. Chem.* **1995**, *3*, 1605–1624.
[17] C. Uchida, H. Kitahashi, T. Yamagishi, Y. Iwaisaki, S. Ogawa, *J. Chem. Soc. Perkin Trans. 1* **1994**, 2775–2785.
[18] I. Storch de Gracia, H. Dietrich, S. Bobo, J. L. Chiara, *J. Org. Chem.* **1998**, *63*, 5883–5889.
[19] a) J. L. Chiara, I. Storch de Gracia, A. Bastida, *Chem. Commun.* **2003**, 1874–1875; b) J. L. Chiara, N. Valle, *Tetrahedron: Asymmetry* **1995**, *6*, 1895–1898; c) J. L. Chiara, M. Martin-Lomas, *Tetrahedron Lett.* **1994**, *35*, 2969–2973.
[20] I. Storch de Gracia, S. Bobo, M. D. Martin-Ortega, J. L. Chiara, *Org. Lett.* **1999**, *1*, 1705–1708.
[21] a) J. L. Chiara, C. Destabel, P. Gallego, J. Marco-Contelles, *J. Org. Chem.* **1996**, *61*, 359–360; J. Marco-Contelles, P. Gallego, M. Rodriguez-Fernandez, N. Khiar, C. Destabel, M. Bernabe, A. Martinez-Grau, J. L. Chiara, *J. Org. Chem.* **1997**, *62*, 7397–7412; b) J. L. Chiara in *Carbohydrate Mimics: Concepts and Methods* (Ed.: Y. Chapleur), Wiley-VCH, New York, **1998**, pp. 123–156; c) S. Bobo, I. Storch de Gracia, J. L. Chiara, *Synlett* **1999**, 1551–1554; d) J. L. Chiara, J. Marco-Contelles, N. Khiar, P. Gallego, C. Destabel, M. Bernabe, *J. Org. Chem.* **1995**, *60*, 6010–6011.
[22] M. J. Camarasa, P. Fernández-Resa, M. T. García-López, F. Gómez de las Heras, P. P. Méndez-Castrillón, A. San Félix, *Synthesis* **1984**, 509–510.
[23] J. F. Morrison, C. T. Walsh, *Adv. Enzymol. Relat. Areas Mol. Biol.* **1988**, *61*, 201.
[24] C. Dash, V. Vathipadiekal, S. P. George, M. Rao, *J. Biol. Chem.* **2002**, *277*, 17978–17986.
[25] a) A. Bulow, I. W. Plesner, M. Bols, *Biochim. Biophys. Acta* **2001**, *1545*, 207–215; b) A. Lohse, T. Hardlei, A. Jensen, I. W. Plesner, M. Bols, *Biochem. J.* **2000**, *349*, 211–215.
[26] M. Remko, O. A. Walsh, W. G. Richards, *Chem. Phys. Lett.* **2001**, *336*, 156–162.
[27] a) N. Marchand-Geneste, A. Carpy, *THEOCHEM* **1999**, *465*, 209–217; b) J. Ouhabi, C. Jarry, Z. Abdelhadi, A. Carpy, *Arch. Pharm. (Weinheim, Ger.)* **1997**, *330*, 367–371.
[28] O. Gawron, J. Keil, *Arch. Biochem. Biophys.* **1960**, *89*, 293–295.
[29] X. Qian, Z. Liu, Z. Li, Z. Li, G. Song, *J. Agric. Food Chem.* **2001**, *49*, 5279–5284.
[30] NBO Version 3.1, E. D. Glendening, A. E. Reed, J. E. Carpenter, F. Weinhold.
[31] B. B. Bauminger, *J. Clin. Pathol.* **1974**, *27*, 1015–1017.
[32] Gaussian 98 (Revision A.3), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh PA, 1998.

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