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Trihydroxy-2-thiaquinolizidine derivatives as a new class of bicyclic glycosidase inhibitors

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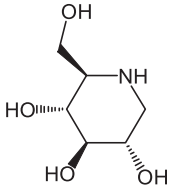
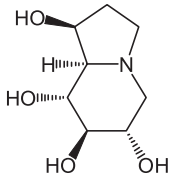
Abstract—Trihydroxy-2-thiaquinolizidines, a new class of bicyclic dideoxy-iminohexitol glycosidase inhibitor derivatives with nominally the D-*gluco*, L-*ido*, D-*manno* and L-*gulo* configurations were synthesized. X-ray analyses indicated that the preferred conformation for D-*gluco* and D-*manno* derivatives was a flat *trans*-fused system. Unlike deoxynojirimycin, the compound with D-*gluco* configuration was selective for α-glucosidases (yeast and rice) and showed no inhibitory activity towards β-glucosidase (almond), α-galactosidase (green coffee beans), α-galactosidase (*E. coli*) and α-mannosidase (jack bean), while the L-*ido* derivative was specific for β-glucosidase (almond).

1. Introduction

Despite their promise, glycosidase inhibitors such as deoxynojirimycin **1** and castanospermine **2**, (generally known as iminoalditols or aza-sugars) have not realized their full clinical potential. This is largely because of a lack of commercially viable syntheses and difficulty in preparing a comprehensive palette of variant structures. In some cases such as deoxynojirimycin there is also the problem of too low specificity.[1,2] Iminoalditols are typically plant alkaloids and many of the possible drug candidates are available in only small exploratory amounts. The potential medical applications for these compounds and their derivatives are numerous and range from diabetes[3–6] and other metabolic disorders through antimicrobials,[7–11] cancer,[12] autoimmune diseases,[13–18] neurological[19] and metabolic[20,21] disorders. Because of their rigidity and the added interaction of the second ring, bicyclic systems such as castanospermine **2** and swainsonine **3** are especially interesting.



1



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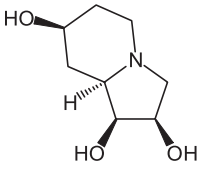

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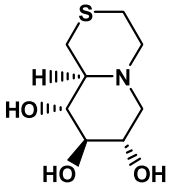
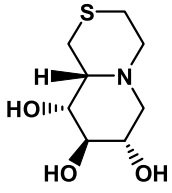
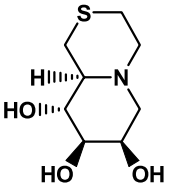


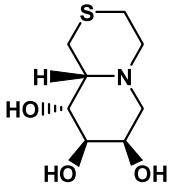
4a



4b



5a



5b

As part of our ongoing work on the development of strategies for the preparation of glycosidase inhibitors, we explored the possibility of synthesizing analogs of deoxynojirimycin **1** and related compounds in which O-6 were replaced by a sulfur atom. We also envisaged bridging the 6-position to the ring nitrogen with a 2-carbon fragment to form a trihydroxy-2-thiaquinolizidine ring system thus increasing rigidity and lipophilicity. Such systems have never been reported before but hold great promise because the formation of a carbon–carbon bond is circumvented as in **2** and **3**. The presence of sulfur (closely related to oxygen) at a position that is normally oxygenated is also a decided advantage. If such systems could be reached using a general strategy, analogs with differing configurations at the various

Keywords: Glycosidase inhibitors; Aza-sugars; Iminoalditols; Glucosidases; Mannosidases; Enzymology; Drugs.
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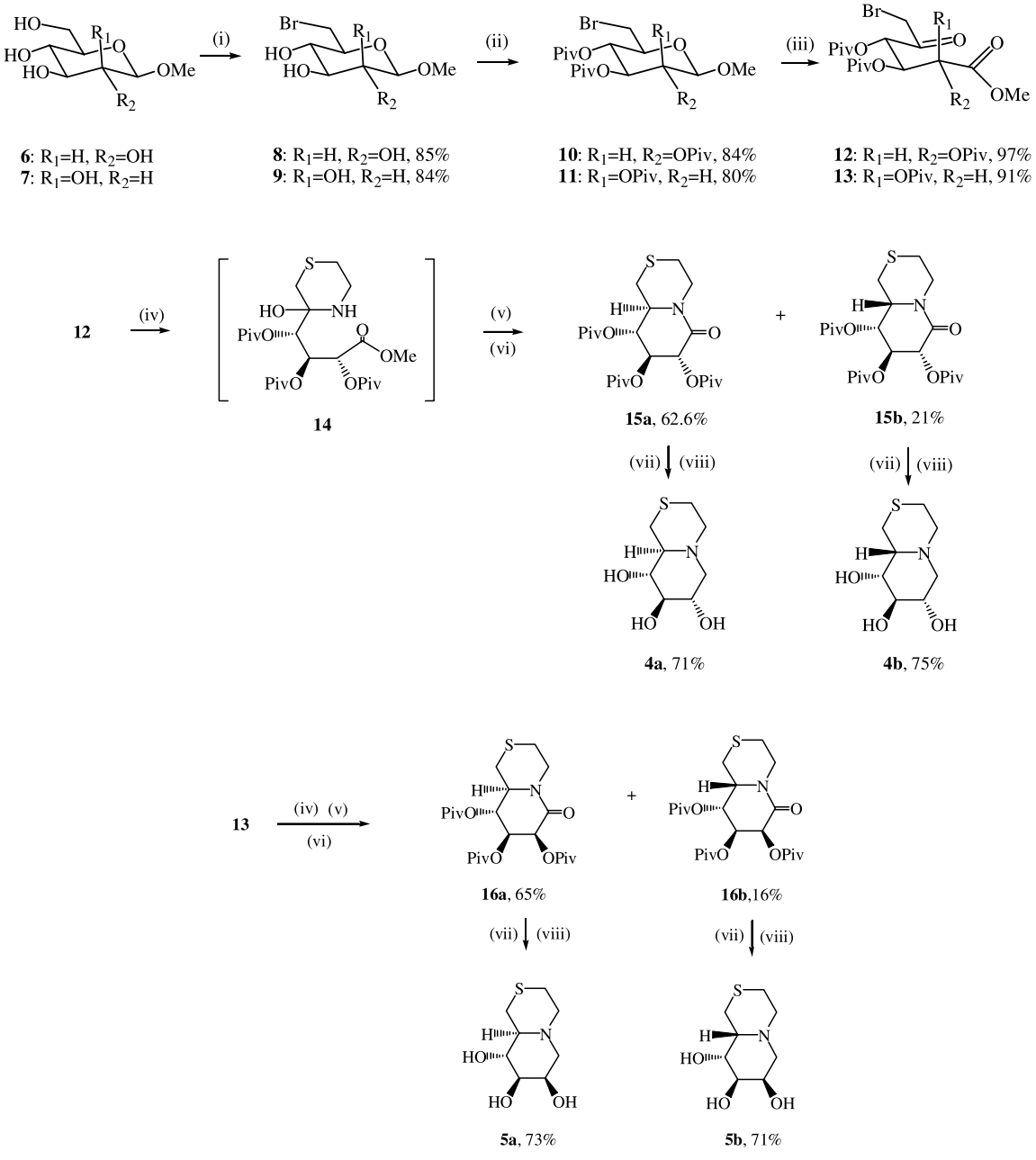
carbon centers and differing substitution patterns could be made, increasing the chance of obtaining compounds with useful therapeutic potential. The strategy could, in principle, be extended to iminopentitol analogs. A total of four such systems (**4a**, **4b**, **5a**, and **5b**) were prepared corresponding to the D-*gluco*, L-*ido*, D-*manno*, L-*gulo* configurations. The synthesis is illustrated below in Scheme 1.

The key feature of the reaction scheme is the oxidation of a 6-bromo β-glycoside to give a 6-bromo-5-ulosonic acid alkyl ester (Scheme 1). This oxidation has been reported to give the keto–ester functionality in high yield.[22] Reaction of this α-halo ketone with an α-aminothiol should lead to rapid thioether formation and immediate cyclization to an aminal which could quickly form an imine. Reduction of the aminal or imine by hydride (e.g., borane or cyanoborohydride) should yield an amine which could then be cyclized to form a lactam. Reduction of the lactam should yield the thiaquinolizidine system.

2. Results

The reaction sequence involved the preparation of a peracylated 6-bromo-6-deoxy-glycoside **10** which was obtained by selective conversion of the primary hydroxyl group of methyl β-D-glucoside to a bromo-group followed by treatment with pivaloyl chloride (trimethylacetyl chloride) in pyridine to protect the remaining hydroxyl groups. The pivaloyl group was selected over the acetyl



Scheme 1. Synthesis of trihydroxy-2-thiaquinolizidine derivatives. (i) Ph_3P, CBr_4, pyridine; (ii) PivCl, pyridine; (iii) CrO_3, Ac_2O, HOAc; (iv) $HS(CH_2)_2NH_2$, CH_3OH; (v) $NaCNBH_3$, CH_3OH; (vi) Na_2CO_3, $CHCl_3$; (vii) BH_3–THF; (viii) $NaOCH_3$, CH_3OH.

group because of the partial deacylation of acetates by aminoethanethiol, resulting in difficulty in purification and low yield. Oxidation of **10** with chromium trioxide in acetic acid afforded a 5-ulosonic acid ester **12**. The oxidation of acetylated β-glycopyranosides by chromium trioxide has been reported by Angyal and James[22] to afford 5-keto esters, independent of the configurations on C2, C3, or C4. The oxidation is specific to β-glycopyranosides, while α-glycopyranosides are not attacked. Treatment of **12** with 2-aminoethanethiol yielded the aminal **14** directly which underwent reduction by $NaBCNH_3$ and cyclization to the lactam **15a** and the L-*ido* isomer **15b**. There was some variability in the actual amount of lactam **15b** ranging from traces to 2.5:1 in favor of the D-*gluco* analog. Reduction of the lactams with borane and deacylation yielded the desired compounds **4a** and **4b**.The final and intermediate products were characterized by a very high degree of crystallinity.

The same reaction sequence was applied to methyl β-D-mannopyranoside (**7**), and the D-*manno* lactam **16a** and L-*gulo* lactam **16b** was obtained in 4:1 ratio in favor of the D-*manno* lactam. Reduction of lactams and deprotection yielded products **5a** and **5b** in good yields. X-ray analysis of **4a** and **5a** (Fig. 1) indicated two six-membered rings with relaxed chair conformation for both D-*gluco* and D-*manno* products. A *trans*-diequatorial type fusion between the rings gives the molecules an overall flat geometry. The expected intermediate oxocarbenium species is very flat because of the double bond character between the ring oxygen and C-1.

The inhibitory activity of four trihydroxy-2-thiaquinolizidines against a series of enzymes was tested. Enzymes were assayed according to standard procedures[23] by following the hydrolysis of nitrophenyl glycosides spectrophotometrically or by evaluating the reducing sugar formed in some glucosidase assays. The enzymes used were α-glucosidase (yeast and rice), β-glucosidase (almond), α-galactosidase (green coffee beans), β-galactosidase (*E. coli*) and α-mannosidase (jack beans).

Table 1 shows the inhibition constants (mM) for compounds **4a**, **4b**, **5a**, and **5b**. Compound **4a** displayed competitive inhibition against both yeast and rice α-glucosidase with K_i of 330 and 900 μM, respectively. No inhibitory activity towards β-glucosidase (almond), α-galactosidase (green coffee beans), β-galactosidase (*E. coli*) and α-mannosidase (jack bean) was observed. Compound **4b** was tested against α-glucosidase (yeast), β-glucosidase and α-mannosidase. It showed an opposite inhibition pattern to compound **4a**. It only inhibits β-glucosidase with K_i value 1 mM without inhibiting α-glucosidase and α-mannosidase. Compound **5a** and **5b** were also tested for α-mannosidase (jack beans), but no inhibition was observed.

3. Discussion

The synthetic strategy for the preparation of trihydroxy-2-thiaquinolizidines proved to be quite efficient and direct. The relative ease of preparation of these analogs and the generality of the method open the possibility for the preparation of a clinically relevant series of selective inhibitory compounds. The inhibition results indicated that compound **4a** was active only against α-glucosidases. No inhibition of β-glucosidases was observed. This is consistent with the observation that deoxynojirimycin type inhibitors with nitrogen atom at the ring oxygen position are more selective for α-glucosidase.[24–26] According to the stereoelectronic requirements, in α-glycosidases, the positively charged leaving group and the lone pair of the ring oxygen are positioned antiperiplanar and cooperatively facilitate the glycosidic bond cleavage. Thus, oxocarbenium ion can be formed directly, and this oxocarbenium ion with positive charge at ring oxygen is an important transition state for α-glucosidases. For β-glycosidases, the glycosidic bond cleavage cannot receive aid form the lone pair of the ring oxygen, so the ring could flip to a boat (**17**) or other conformations (**18**) to facilitate the bond cleavage. Substrate distortion is generally the case for β-glycosidases.[27–31]

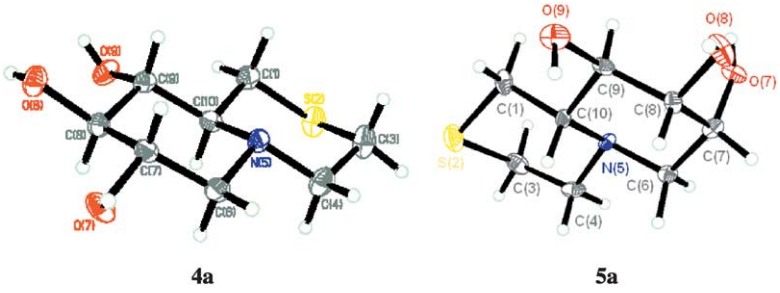


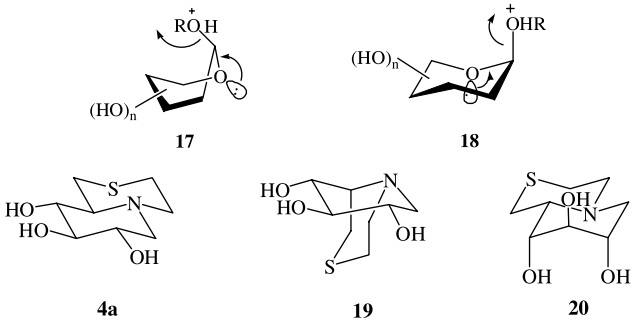
Figure 1. X-ray structures of 7(*S*),8(*R*),9(*R*),10(*S*)-trihydroxy-2-thiaquinolizidine **4a** and 7(*R*),8(*R*),9(*R*),10(*S*)-trihydroxy-2-thiaquinolizidine **5a** showing the *trans*-type ring junction and overall flat geometry.

Table 1. Inhibition constants (mM) for compounds **4a**, **4b**, **5a**, and **5b**

Enzymes	4a	4b	5a	5b
α-Glucosidase (yeast)	0.33	ni	—	—
α-Glucosidase (rice)	0.9	—	—	—
β-Glucosidase (almond)	ni	1.0	—	—
α-Galactosidase (green coffee beans)	ni	—	—	—
β-Galactosidase (*E. coli*)	ni	—	—	—
α-Mannosidase (jack beans)	ni	ni	ni	ni

ni, No inhibition observed in this concentration range; —, not determined.

Compound **4a**, with its rigid bicyclic structure, is locked in its *trans*-fused chair conformation, so it cannot flip or change to other conformations. Therefore, it showed no inhibition against β-glucosidase. In contrast, compound **4b** showed specific inhibition against β-glucosidase while no inhibition for α-glucosidase. This is almost certainly due to a difference in conformation to the D-*gluco* compound brought about by flattening or ring inversion to place the thiomethyl group in an equatorial position. Compound **4b** should be more stable in conformation **20** instead of conformation **19**. Conformation **20** corresponds to the one that matches the transition state for a β-glycoside undergoing hydrolysis under stereoelectronic control.



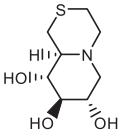
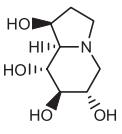
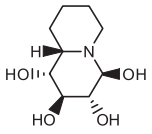
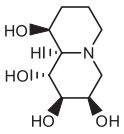
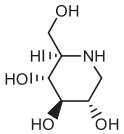
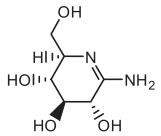
The activities and specificities of the known aza-bicyclic systems and key monocyclic systems are shown in Table 2. Compared to deoxynojirimycin **1**, compound **4a** is a relatively weak inhibitor, but it showed specificity for α-glucosidase. No inhibitory activity towards β-glucosidase (almond), α-galactosidase (green coffee beans), α-galactosidase (*E. coli*) and α-mannosidase (jack bean) was observed. One of the major problems with the use of iminosugars and their derivatives as inhibitors is the lack of specificity. Hence the last two entries in Table 2 have low K_i values but show poor specificity. Compound **4a** showed very specific activity against α-glucosidase, and **4b** showed specificity against β-glucosidase. The specificity comes from the structural rigidity, which prevents **4a** from distortion to boat or other conformations that are important for mimicking the β-glucosidase transition state. Compound **4b** is expected to exist in a conformation that favors β-glucosidase inhibition. Compound **5a** and **5b** showed no activity against α-mannosidase (jack beans). This is probably because of the rigid structure. It is also possible that the second 6-member ring might interfere with any change in ring geometry that might be necessary to facilitate binding of the inhibitor to the active site of the enzyme.

Castanospermine is one of the most active bicyclic systems. It showed poor activity against yeast α-glucosidase but strongly inhibited the rice enzyme. However, it showed non-selectivity by inhibiting almond β-glucosidase. It has relatively flexible structure compared to **4a** and **4b**, and it presented a twisted boat conformation of the 6-member ring when bounded to Exo-β-(1,3)-gluconase.[36] This distortion cannot be made for compound **4a** which has a *trans*-diequatorial type fusion between the rings. Compound **22**, which is the slightly ring-expanded version of the potent α-mannosidase inhibitor swainsonine (**3**) showed complete loss of inhibition of α-mannosidase (Table 2). As a general rule, decalin-type bicyclic systems show much reduced or no inhibitory activity compared to their acyclic or octahydroindene-type analogs. Therefore, one important conclusion that can be made is that structural flexibility leads to nonspecific inhibitory activity. Monocyclic systems generally showed poor specificity by inhibiting both α- and β-glycosidases because of their flexible structures. Castanospermine (**2**) and the thiaquinolizidine described here (**4a**) are the most impressive of the reported bicyclic aza-type iminosugar derivatives with a nitrogen atom at the ring junction. Compound **4a** was superior against and selective for α-glucosidases compared to castanospermine. Thiaquinolizidines, therefore represent a significant advancement in this area.

4. Conclusion

Trihydroxy-2-thiaquinolizidines are bicyclic systems where reasonable inhibitory activity and absolute specificity for one anomer were obtained. Although only modest inhibitory activity was observed, the specificity is extremely high. This is a very important point. For iminopentitol glycosidase inhibitors, the general rule is that high inhibitory activity always comes along with low specificity tremendously limiting their utility as drugs. Modest inhibitors combined

Table 2. Comparison of inhibition activity K_i, μM (IC_{50}, μM) for iminoalditol transition state analogs

Enzymes	 **4a**	 **2**[1]	 **21**[32]	 **22**[33]	 **1**[1]	 **23**[34,35]
α-Glucosidase (yeast)	330	>1500	—	IC_{50}>2000	12.6	~10
α-Glucosidase (rice)	900	0.015	—	—	0.01	—
β-Glucosidase (almond)	ni	1.5	ni	IC_{50}>2000	47	8
α-Galactosidase (green coffee beans)	ni	—	ni	—	—	~10
β-Galactosidase (*E. coli*)	ni	—	—	—	—	~10
α-Mannosidase (jack beans)	ni	—	—	IC_{50}>2000	—	9

—, Not determined; ni, no inhibition observed.

with efficient drug delivery strategies to keep local concentrations high enough would constitute an excellent therapeutic approach. This study also identifies a strategy for changing the specificity of an inhibitor by inverting the stereochemistry at a critical position to increase the stability of inverted ring structures. The thiaquinolizidines described here are relatively easily accessible. Their ease of preparation and specificity therefore represent significant potential for further therapeutic advancement.

5. Experimental

5.1. General procedures

Melting points were measured on a Ficher–Johns melting point apparatus. Optical rotations were measured ($\lambda=$ 589 nm) at room temperature using a Jasco P-1010 polarimeter. IR spectra were recorded on a FT-IR instrument. The ^{1}H (and ^{13}C) NMR spectra were recorded at 500 (125.5) MHz on a Varian VXR spectrometer. The HRFABMS mass spectra were obtained using a Jeol HX-110 double-focusing mass spectrometer operating in positive ion mode.

5.2. Inhibition assays

Inhibitory potency was determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the corresponding nitrophenyl α- or β-D-glycopyranoside. The glycosidases used were α-glucosidase (yeast), α-glucosidase (rice), β-glucosidase (almond), α-galactosidase (green coffee beans), β-galactosidase (*E. coli*) and α-mannosidase (jack beans). All enzymes were purchased from Sigma. Each assay was performed in a phosphate or an acetate buffer at the optimal pH for each enzyme. Inhibition studies (except rice α-glucosidase) were performed by adding the inhibitor to a final concentration of 0.05–11 mM to the respective buffer solutions along with enzyme. The solutions were incubated at 37 °C before adding substrates to the reactions. The absorbance of the resulting mixture was determined at 400 nm (for *p*-nitrophenol).

For rice α-glucosidase inhibition, maltose was used as the substrate, and the assay was based on the glucose oxidase/peroxidase enzyme procedure. In this assay, the glucose released from maltose can be oxidized by glucose oxidase to generate D-gluconic acid and hydrogen peroxide. Under the catalysis of peroxidase, hydrogen peroxide reacts with dianisidine to give the oxidized form which forms a brown color. The absorbance of the solution was determined at 500 nm for oxidized *o*-dianisidine. The assay was performed in sodium acetate buffer at pH 4.0 at 37 °C. The inhibitor was added to a final concentration of 0.4 and 8.9 mM to the substrate solution. The enzyme was added to the solution at 37 °C, and the reaction was stopped after 10 and 30 min by adding dilute perchloric acid solution. The glucose oxidase/peroxidase solution was pipetted into the reaction mixture, and incubate at 37 °C for 30 min. The absorbance of the solution was determined at 500 nm.

5.3. Synthetic methods

5.3.1. Methyl 6-bromo-6-deoxy-β-D-glucopyranoside.[37] (8) To a stirred solution of methyl β-D-glucopyranoside **6** (10.15 g, 50 mmol) in anhydrous pyridine (300 mL) at 0 °C were added triphenylphosphine (26.2 g, 100 mmol) and carbon tetrabromide (24.87 g, 75 mmol). The resulting mixture was protected from moisture and stirred at 0 °C for 10 min. It was then allowed to warm to 65 °C and was stirred for an additional 4 h. Methanol (10 mL) was added to decompose any excess reagent. The solvent was removed by evaporation and the residue was purified by column chromatography (CH_2Cl_2, followed by 20:1 CH_2Cl_2/MeOH). Crystallization from methanol-hexanes afforded white crystalline solid (10.96 g, 85%), mp 149–150 °C, lit. mp[38] 154 °C, $[\alpha]_D^{20}$ −27.6° (*c* 0.22, H_2O).

5.3.2. Methyl 6-bromo-6-deoxy-2,3,4-tri-*O*-pivaloyl-β-D-glucopyranoside (10). Pivaloylation of **8** (6.73 g, 26 mmol) by trimethylacetyl chloride (28.8 mL, 32.4 mmol) in pyridine (300 mL) at room temperature for 2 days afforded an white solid **6** (11.2 g, 84%), mp 109–110 °C, $[\alpha]_D^{20}$ −2.4° (*c* 0.31, $CHCl_3$). IR (CH_3Cl) ν_{max} 2971.7, 1745.6, 1140.9 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.29 (1H, t, $J=9.5$ Hz), 4.99 (2H, t, $J=9.7$ Hz), 4.42 (1H, d, $J=$ 8.0 Hz), 3.70 (1H, m), 3.50 (3H, s), 3.39–3.12 (2H, m), 1.14 (9H, s), 1.13 (9H, s), 1.08 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 177.14, 176.63, 176.51, 101.36, 73.7, 71.9, 71.2, 70.8, 57.1, 38.8, 38.7, 30.6 ppm; HRFABMS ($M+H^+$) Calcd 509.1750, found 509.1736.

5.3.3. Methyl 6-bromo-2,3,4-tri-*O*-pivaloyl-5-keto-ester (12). To a solution of **10** (1 g, 1.96 mmol) in acetic acid (100 mL) and acetic anhydride (10 mL), chromium trioxide (1.18 g, 11.8 mmol) was added and the suspension was stirred at room temperature for 3 h. The mixture was then poured slowly into cold water (500 mL). The water was extracted 5 times with CH_2Cl_2 and the combined organic phase was washed with brine, saturated sodium bicarbonate and dried (Na_2SO_4), concentrated. The resulting residue was passed through a small pad of silica gel to give **12** as a colorless oil (1 g, 97%), $[\alpha]_D^{20}$ +36.5° (*c* 0.12 $CHCl_3$), IR (CH_2Cl_2) ν_{max} 2975.85, 1743.63, 1132.00 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.72, (1H, t, $J=4.8$ Hz), 5.57 (1H, d, $J=4.5$ Hz), 5.23 (1H, d, $J=5.0$ Hz), 4.12 (1H, d, $J=$ 14.0 Hz), 4.01 (1H, d, $J=13.5$ Hz), 3.72 (3H, s), 1.25 (9H, s), 1.21 (9H, s), 1.18 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 194.5, 177.1, 176.9, 176.8, 167.1, 72.9, 70.2, 69.4, 52.7, 38.9, 38.8, 38.7, 31.6, 27.0, 26.9 ppm; HRFABMS ($M+H^+$) Calcd 523.1543, found 523.1530.

5.3.4. Lactam (15a) and (15b). A solution of **7** (7 g, 13.4 mmol) and 2-aminoethanethiol (1.24 g, 16.1 mmol) in methanol (250 mL) was stirred at room temperature for 1 h, followed by addition of sodium cyanoboron hydride (1.26 g, 20 mmol). The reaction mixture was stirred overnight and sodium carbonate was added to facilitate the lactam cyclization. After stirred for several hours, the suspension was filtered and acetic acid (2 mL) was added and concentrated. The residue was purified by column chromatography (10:1 hexanes/acetone) to yield two lactam diastereomers **15a** and **15b** (4.64 g, 73.6%), the ratio is 2.5:1.

Lactam **15a** (3.31 g, 52.6%) was given as a white solid, mp 188–190 °C, $[\alpha]_D^{20}$ +12.6° (*c* 0.1 $CHCl_3$), IR ($CHCl_3$) ν_{max} 1744.54, 1685.34 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 5.53 (1H, t, J=10.5 Hz), 5.30 (1H, d, J=11.0 Hz), 5.19 (1H, dd, J=10.5, 8 Hz), 4.94 (1H, dt, J=13.5, 3 Hz), 3.53 (1H, ddd, J=9.8, 8.4, 3.4 Hz), 2.87 (1H, td, J=14.3, 2.5 Hz Hz), 2.66 (1H, td, J=13.0, 3.0 Hz), 2.61–2.49 (3H, m); 1.21 (9H, s), 1.17 (9H, s), 1.12 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 177.4, 177.1, 176.6, 164.3, 70.4, 69.4, 67.8, 60.2, 44.7, 38.9, 38.7, 31.8, 27.1, 26.6 ppm. HRFABMS (M+H^+) calcd 472.2369, found 472.2379.

Lactam **15b** (1.33 g, 21.0%) was given as a white solid, mp 179–181 °C. IR (CH_2Cl_2) ν_{max} 1741.07, 1679.15, 1137.70 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.76 (1H, t, J=10.3 Hz), 5.27 (1H, dd, J=11.5, 6.3 Hz), 4.78 (1H, m), 4.02 (1H, ddd, J=11.8, 6.3, 2.0 Hz), 3.07 (1H, t, J=12.3 Hz), 2.98–2.88 (3H, m), 2.50 (1H, d, J=13.0 Hz), 2.35 (1H, m), 1.20 (9H, s), 1.17 (9H, s), 1.14 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 177.4, 176.8, 163.7, 67.7, 67.0, 59.2, 47.1, 38.9, 38.7, 27.7, 27.1, 27.1, 27.0, 26.3 ppm. HRFABMS (M+H^+) calcd 472.2369, found 472.2371.

5.3.5. 7(*S*),8(*R*),9(*R*),10(*S*)-Trihydroxy-2-thiaquinolizidine (4a). A solution of lactam **15a** (2 g, 4.24 mmol) and BH_3–THF (20 mL, 1.5 M) in anhydrous THF (30 mL) was refluxed for 4 h and the TLC and NMR showed the completion of the reduction. The solvent was removed and methanol was added and the mixture concentrated 3 times. The residue was dissolved in methanol (30 mL), followed by addition of NaOMe (0.15 g, 2.8 mmol). The reaction was stirred for 8 h to remove remaining ester groups and concentrated. The residue was applied to an ion exchange column (Dowex 50WX8-400, 30 g), which was washed with water (50 mL) and eluted with NH_4OH (50 mL). The elution was concentrated and purified by column chromatography (15:1 CH_2Cl_2/MeOH) to afford a white solid (0.62 g, 71%), mp 235–237 °C; $[\alpha]_D^{20}$ +20.2° (*c* 0.06 H_2O); IR (KBr) ν_{max} 3355.78, 3275.61 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.50 (1H, ddd, 11.0, 9.1, 4.9 Hz), 3.25 (1H, t, J=9.3 Hz), 3.12 (1H, dt, J=12.5, 3.0 Hz), 3.06 (1H, t, J=9.5 Hz), 2.93 (1H, dt, J=14.0, 2.5 Hz), 2.84 (1H, dd, J=11.5, 5.0 Hz), 2.75 (1H, td, J=13.0, 3.0 Hz), 2.52 (1H, m), 2.45 (1H, t, J=12.3 Hz), 2.43 (1H, m), 2.19 (1H, t, J=11.3 Hz), 2.13 (1H, td, J=10.0, 2.5 Hz); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 77.9, 74.1, 68.5, 65.6, 59.4, 55.7, 29.3, 26.3 ppm. HRFABMS (M+H^+) calcd 206.0851, found 206.0849.

5.3.6. 7(*S*),8(*R*),9(*R*),10(*R*)-Trihydroxy-2-thiaquinolizidine (4b). The title compound was obtained by the same method as **4a** from lactam **15b** (75% from **15b**). ^1H NMR (500 MHz, D_2O) δ 3.51–2.42 (2H, m), 3.06 (2H, d, J=10.0 Hz), 2.90 (4H, dd, J=26.0, 12.5 Hz), 2.82 (2H, t, J=10.5 Hz), 2.60 (2H, dd, J=12.0, 4 Hz), 2.20 (2H, d, J=14.0 Hz). HRFABMS (M+H^+) calcd 206.0851, found 206.0849.

Manno-derivatives were obtained in the same fashion as *gluco*-derivatives.

5.3.7. Methyl 6-bromo-6-deoxy-β-D-mannopyranoside (9). The title compound was obtained as a white solid (84%). ^1H NMR (500 MHz, D_2O) δ 3.91 (1H, d, J=2 Hz), 3.75 (1H, d, J=2.5 Hz), 3.73 (1H, d, J=2.5 Hz), 3.571 (2H, dd, J=11.8, 5.8 Hz), 3.569 (1H, d, J=1.5 Hz), 3.46 (3H, s), 3.43 (1H, m); ^{13}C NMR (125.5 MHz, D_2O) δ 101.41, 74.89, 72.83, 70.47, 68.96, 57.24, 32.95 ppm.

5.3.8. Methyl 6-bromo-6-deoxy-2,3,4-tri-*O*-pivaloyl-β-D-mannopyranoside (11). The title compound was obtained as white solid (80%), mp 130–132. ^1H NMR (500 MHz, $CDCl_3$) δ 5.41 (1H, dd, J=3, 0.75 Hz), 5.24 (1H, t, J=10 Hz), 5.08 (1H, dd, J=10, 3 Hz), 4.57 (1H, d, J=1 Hz), 3.68 (1H, m), 3.49 (3H, s), 3.47–3.39 (2H, m), 1.24 (9H, s), 1.15 (9H, s), 1.09 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 177.28, 177.22, 176.80, 99.74, 73.82, 70.85, 68.31, 68.21, 57.18, 39.06, 38.84, 38.75, 31.08, 27.16, 27.04, 27.02 ppm. HRFABMS (M+H^+) Calcd 509.1750, found 509.1748.

5.3.9. Methyl 6-bromo-2,3,4-tri-*O*-pivaloyl-5-keto-ester (13). The title compound was obtained as a colorless oil (91%). ^1H NMR (500 MHz, $CDCl_3$) δ 5.73 (1H, dd, J=8.5, 3 Hz), 5.71 (1H, d, J=2.5 Hz), 5.01 (1H, d, J=9 Hz), 4.13 (1H, d, J=14 Hz), 4.00 (1H, d, J=13.5 Hz) 3.69 (3H, s), 1.24 (9H, s), 1.21 (9H, s), 1.15 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 194.78, 176.90, 176.85, 176.59, 167.76, 73.16, 68.99, 68.85, 52.75, 38.91, 38.81, 38.69, 38.91, 38.81, 38.69, 31.38, 26.96, 26.86, 26.84 ppm. HRFABMS (M+H^+) Calcd 523.1543, found 523.1545.

5.3.10. Lactam 16a. The title compound was obtained as white solid (65%), mp 136–138. ^1H NMR (500 MHz, $CDCl_3$) δ 5.67 (1H, d, J=3.5 Hz), 5.39 (1H, m), 5.01 (1H, m), 4.99 (1H, t, J=3 Hz), 3.66 (1H, dt, J=11.5, 2 Hz), 2.99 (1H, m), 2.85 (1H, td, J=13, 2 Hz), 2.74 (1H, td, J=13, 2.5 Hz), 2.59 (1H, dt, J=13.5, 2 Hz), 2.46 (1H, m), 1.22 (9H, s), 1.21 (9H, s), 1.20 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 176.94, 176.86, 176.36, 163.27, 69.43, 68.18, 66.51, 62.75, 46.54, 38.91, 38.85, 38.81, 31.06, 27.18, 27.08, 26.98, 26.66 ppm. HRFABMS (M+H^+) calcd 472.2369, found 472.2366.

5.3.11. Lactam 16b. The title compound was obtained as white solid (16%), mp 182–184. ^1H NMR (500 MHz, $CDCl_3$) δ 5.65 (1H, d, J=2.5 Hz), 5.34 (1H, t, J=5 Hz), 5.31 (1H, m), 5.04 (1H, dt, J=8.5, 3 Hz), 3.77 (1H, m), 2.79 (1H, td, J=13.5, 2 Hz), 2.68 (2H, m), 2.53 (1H, m), 2.39 (1H, dt, J=13.5, 2 Hz), 1.25 (9H, s), 1.20 (9H, s), 1.18 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 177.12, 176.66, 176.23, 165.11, 67.13, 66.90, 66.34, 58.60, 44.57, 39.14, 38.94, 38.85, 28.78, 27.19, 27.11, 27.04 ppm. HRFABMS (M+H^+) calcd 472.2369, found 472.2367.

5.3.12. 7(*R*),8(*R*),9(*R*),10(*S*)-Trihydroxy-2-thiaquinolizidine (5a). The title compound was obtained as white solid (73% from **16a**), mp 144–145. ^1H NMR (500 MHz, D_2O) δ 3.93 (1H, m), 3.43 (1H, dd, J=10, 3.5 Hz), 3.35 (1H, t, J=9.5 Hz), 3.06 (1H, dt, J=12.5, 3 Hz), 2.90 (1H, dt, J=8.5, 2.5 Hz), 2.81–2.74 (2H, m), 2.51–2.44 (2H, m), 2.39–2.32 (2H, m), 2.05 (1H, t, J=9 Hz); ^{13}C NMR (125.5 MHz, D_2O) δ 73.90, 71.15, 67.48, 66.24, 59.37, 55.87, 28.77, 26.02 ppm. HRFABMS (M+H^+) calcd 206.0851, found 206.0851.

5.3.13. 7(*R*),8(*R*),9(*R*),10(*R*)-Trihydroxy-2-thiaquinolizidine (5b). The title compound was obtained by the same

way as **51a** (71% from **16b**). ^{1}H NMR (500 MHz, D_2O) δ 3.97 (1H, m), 3.87 (1H, s), 3.69 (1H, d, $J=2.0$ Hz), 3.10 (1H, d, $J=12.5$ Hz), 2.84 (2H, dd, $J=13.5$, 11.0 Hz), 2.76 (1H, m), 2.61 (2H, m), 2.44 (2H, t, $J=11.0$ Hz), 2.36 (1H, d, $J=14.0$ Hz). ^{13}C NMR (125.5 MHz, D_2O) δ 72.1, 70.5, 64.3, 60.4, 56.8, 54.4, 28.4, 26.0. HRFABMS ($M+H^+$) calcd 206.0851, found 206.0850.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 231490. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 36033 or e-mail: deposit@ccdc.cam.ac.uk].

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