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Synthesis and Inhibitory Activity of Glycosidase Inhibitors, Glycosylamino-Oxazolines

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Abstract—In connection with structural modification of the trehalase inhibitor trehazolin (**1**), as a new-type of glycohydrolase inhibitor, some glycosylamino-oxazolines were designed and synthesized. Among three oxazolines β-*galacto* (**3**), β-*gluco* (**5**) and α-*manno*-types (**6**) obtained in stable form, the latter **6** has been shown to possess a moderate inhibitory activity against α-mannosidase.

Introduction

A potent and specific trehalase inhibitor trehazolin[1] (**1**) possesses a pseudo-disaccharide structure composed of α-D-glucopyranosylamine and trehazolamine, 1D-(1,3/2,4,5)-5-amino-1-*C*-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol,[2,3] linked by way of a cyclic isourea group. On the basis of the structural feature of **1**, the α-D-glucopyranose and aminocyclitol moieties may be assumed to correspond with two α-D-glucopyranose residues as the mimic of symmetric α,α-trehalose (Fig. 1). This assumption has been partly supported by a series of studies[3–6] on the structural modification of **1**, leading to a discovery of some potent α-glucosidase inhibitors, modified 3-amino-2-oxa-4-azabicyclo[3.3.0]-oct-3-enes.[7] On the other hand, the cyclic isourea moiety would constitute the charge distribution part for its binding to the active site of the enzymes. Therefore, in addition, it is likely to design new sugar-hydrolase inhibitors of hexopyranoses having cyclic isourea functions.

Several *N*-alkyl β-glycosylamines have been known[8] as strong inhibitors against β-glucosidase and β-galactosidase. However, these compounds are rather unstable and readily hydrolyzed to give rise to sugars and amines. Therefore, five glycosylamino oxazolines: α-(**2**) and β-galacto-(**3**), and α-(**4**) and β-gluco-(**5**), and α-mannopyranosyl oxazolines (**6**) have been designed as the respective glycohydrolase inhibitors and synthetic attempts carried out (Fig. 1).

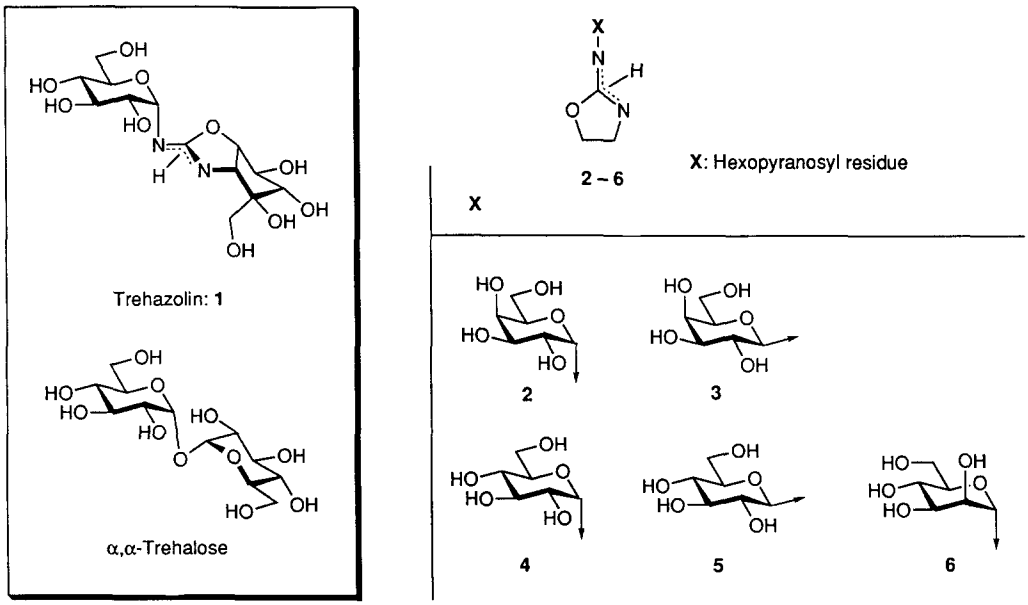


Figure 1.

Results and Discussion

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosylisothiocyanate[4] (**7**) was allowed to couple with 2-aminoethanol to give the corresponding thiourea derivative **8** in good yield. Cyclization of **8** with yellow mercury(II) oxide[3] in diethyl ether afforded the isourea **9** in 94%. A synthesis of the glucosyl isomer **12** has previously been described[4] by us. Deprotection of **9** and **12** by removal of the benzyl ether groups however did not provide the desired α-glycopyranosyloxazolines **2** and **4**. Thus, generation of free **2** and **4** resulted in rearrangement of the isourea rings to the bicyclic sugar oxolane rings through intramolecular attack of the 2-hydroxyl groups, first giving rise to more stable bicyclic pyranoid-oxazolines **10** and **13** and then easily equilibrating with the tautomeric furanoid-oxazolines **11** and **14**, respectively (Fig. 2). Their ^{1}H NMR spectra indicated roughly a 1:1 mixture of each tautomer. The similar rearrangement has so far been observed in the case of trehazolin analogues,[3] in which the interconversion between the bicyclo[4.3.0]nonane and [3.3.0]octane rings containing the cyclic isoureas is possible.

On the other hand, the β-galacto-(**3**), β-gluco-(**5**), and α-mannopyranosyloxazolines (**6**), where the 2-hydroxyl functions were situated *trans* to the oxazoline rings were obtained purely and characterized as the per-*N,O*-acetyl derivatives (Fig. 3). Thus, 2,3,4,6-tetra-*O*-benzyl-β-D-galacto-(**15**) and glucopyranosylisothiocyanates (**16**) were allowed to couple with 2-aminoethanol to give the respective thioureas **17** (~100%) and **18** (~100%). Cyclization of compounds **17** and **18** was carried out similarly with yellow mercury(II) oxide to give the protected oxazolines **19** (96%) and **20** (~100%), respectively. Deprotection of **19** and **20** was carried out under Birch conditions to afford the free oxazolines **3** (58%) and **5** (88%), which were characterized as the *N,O*-pentaacetyl derivatives **3a** and **5a**, respectively. Their ^{1}H NMR spectra supported the assigned structures.

The α-mannosyl isomer **6** was prepared from the isothiocyanate[9] **21** in the following sequence (Fig. 4): coupling with 2-aminoethanol [→the thiourea **22** (~100%)], cyclization with HgO [→the oxazoline **23** (~100%)] and deprotection of benzyl ether groups [→the free base **6** (94%)]. The structure of **6** was also confirmed by converting it into the *N,O*-pentaacetyl derivative **6a**.

Three glycosylamino-oxazolines (**3**, **5** and **6**), along with structurally related trehazolin (**1**) and its analogues **24** (α-*galacto* type) and **25** (α-*manno* type), were tested for enzyme-inhibitory activities against six sugar hydrolases: α-(*E. coli*) and β-galactosidases (*E. coli* and bovin liver), α-(baker's yeast) and β-glucosidases (almonds), and α-mannosidase (jack beans).

The α-mannopyranosylamino-oxazoline (**6**) is only a weak α-mannosidase inhibitor. In contrast to β-glucopyranosylamine derivatives,[8] the β-anomers **3** and **5** accessible in this study did not show any notable potency against all enzymes (Table 1). Trehazolin (**1**) and the analogues **24** and **25** are very weak α-glucosidase inhibitors, while **25** possesses similar moderate inhibition against α-mannosidase as **6**. Therefore, the branched chain cyclopentanepolyol

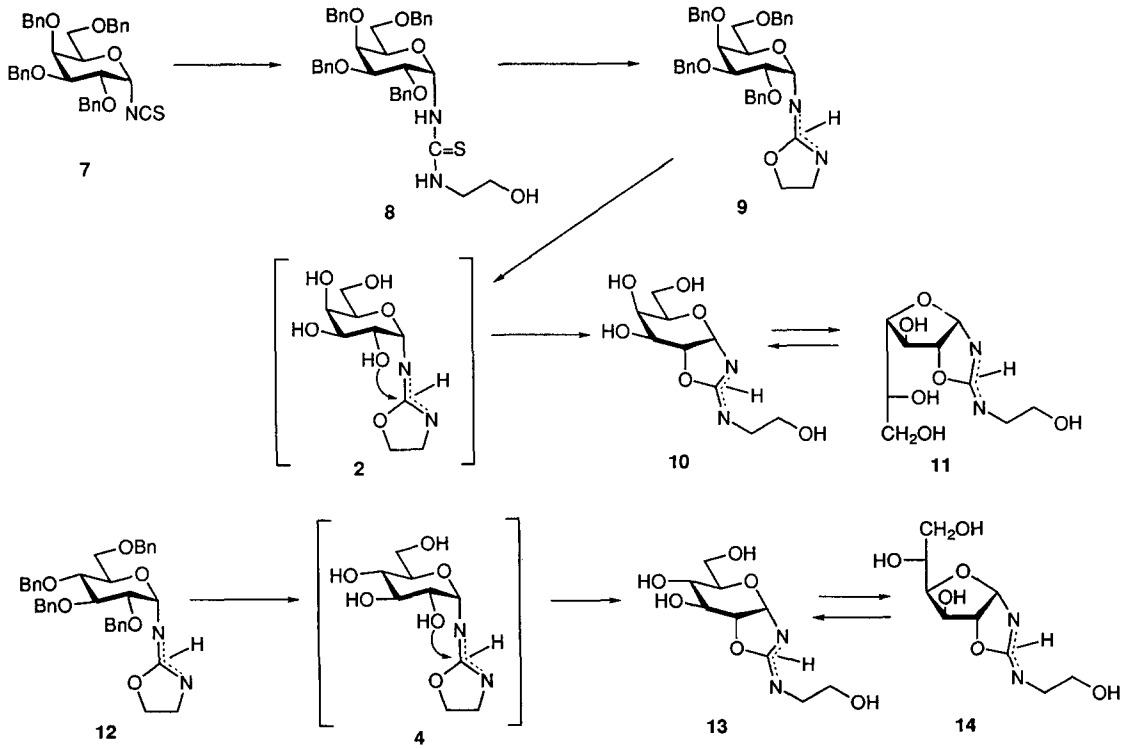


Figure 2.

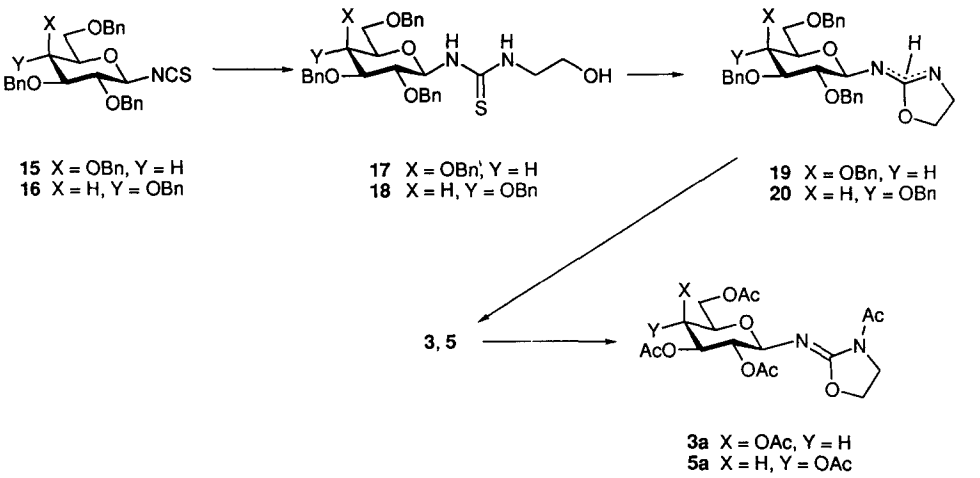


Figure 3.

moiety of **25** does not play an essential role in exhibiting inhibitory potency against α-mannosidase. On the other hand, concerning inhibition of α-glucosidase observed for **1**, **24** and **25**, the cyclitol moieties containing the cyclic isoureas seem to be important, rather than the hexopyranosyl residues.

Considering the present results, although enough reference compounds have not yet been surveyed, the aminocyclopentanepolyol part including the isourea structure has convincingly been shown to play a more important role, i.e. acting as a glycone part in the transition state of hydrolysis to exert inhibitory activity. This postulation may accord with the consideration derived from the structural modification[5,6] of trehazolin (**1**). Furthermore, it was demonstrated that this part itself would be a lead structure for development of new potent glycosidase inhibitors.[7]

Experimental

General methods

Melting points were determined on a MEL-TEMP capillary melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded for solutions in deuteriochloroform with internal tetramethylsilane (TMS) as a reference, hexadeuteriodimethylsulfoxide with internal TMS as a reference, or dideuterium oxide with internal acetone (δ 2.08) as a reference with a JEOL JNM-GX 270 FT (270 MHz) instrument. IR spectra were measured with a JASCO IR-810 (neat) or Hitachi Bio-Rad Digital Lab FTS-65 (KBr disk) spectrometer. TLC was performed on silica gel 60 F-254 (E. Merck, Darmstadt). The silica gel used for column chromatography was Wakogel C-300 (Wako Junyaku Kogyo Co., Osaka, Japan; 200–300 mesh) or silica gel 60 KO 70 (Katayama Kagaku Kogyo Co., Osaka, Japan; 70–230 mesh) Organic solutions were dried over anhydrous Na_2SO_4 and concentrated at <45 °C under diminished pressure.

Bioassay

Glycosidases and corresponding nitrophenyl glycosides were purchased from Sigma. 96-Well microplates were used for bioassay and the absorbance of nitrophenol produced by the enzyme reaction was measured with 405 nm at several concentrations of the samples to calculate IC_{50} values.

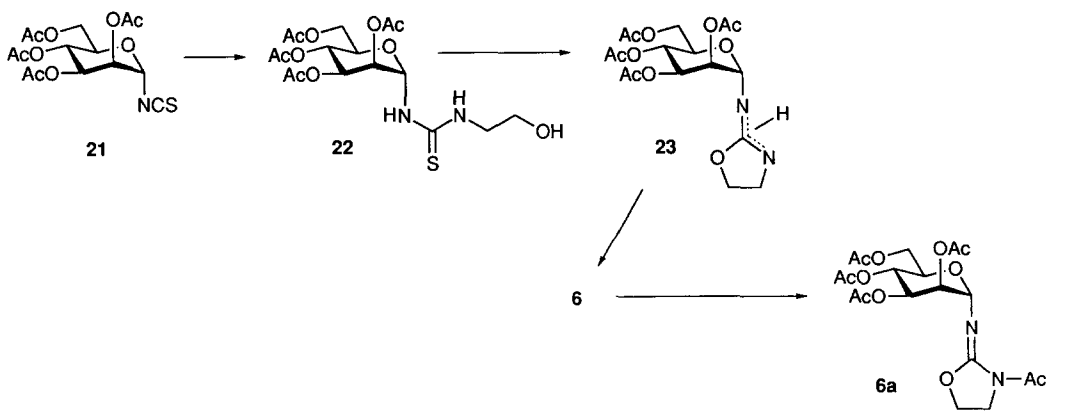
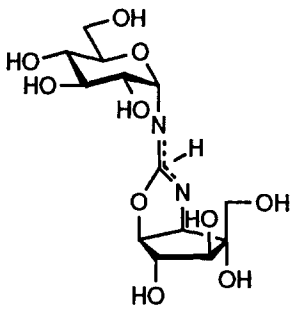


Figure 4.

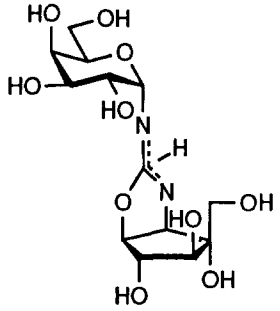
Table 1. Inhibitory activities of glycosylisoureas against several glycohydrolases

Compound	IC_{50} (M)					
	α-glucosidase[a] (Baker's yeast)	β-glucosidase[b] (Almonds)	α-mannosidase[c] (Jack beans)	α-galactosidase[d] (*E. coli*)	β-galactosidase[e] (*E. coli*)	β-galactosidase[e] (Bovin liver)
3	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$
5	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$
6	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	9.8×10^{-5}	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$	$>4.0\times10^{-4}$
1	6.0×10^{-5}	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$
24	2.1×10^{-5}	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$
25	3.0×10^{-5}	$>2.7\times10^{-4}$	1.0×10^{-4}	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$	$>2.7\times10^{-4}$

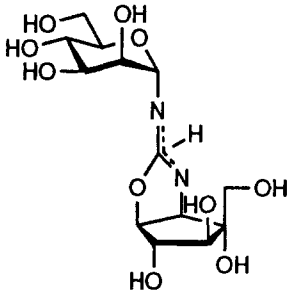
[a]0.66 mM *p*-Nitrophenyl α-D-glucopyranoside, 0.1 M potassium phosphate buffer, pH 6.8.
[b]0.33 mM *p*-Nitrophenyl β-D-glucopyranoside, 0.1 M acetate buffer, pH 5.0.
[c]20 mM *p*-Nitrophenyl α-D-mannopyranoside, 0.1 M acetate buffer, pH 4.5.
[d]9.9 mM *p*-Nitrophenyl α-D-galactopyranoside, 0.1 M potassium phosphate buffer, pH 6.5.
[e]20 mM *o*-Nitrophenyl β-D-galactopyranoside, 0.1 M 2-mercaptoethanol, 50 mM potassium phosphate buffer including 1.3 mM $MgCl_2$, pH 7.3.



Trehazolin: **1**



4'-Epitrehazolin: **24**



2'-Epitrehazolin: **25**

***N*-(2-Hydroxyethyl)-*N'*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)thiourea (8).** To a solution of the isothiocyanate[4] **7** (107 mg, 0.185 mmol) in CH_2Cl_2:MeOH (2 mL, 2:1, v/v) was added 2-aminoethanol (14.7 μL, 1.3 molar equiv) at room temperature. The mixture was stirred for 3 h at room temperature and then evaporated. The residue was chromatographed on a silica gel (3 g) with EtOAc:toluene (1:2, v/v) as eluent to afford **18** (133 mg, ~100%) as a syrup, $[\alpha]_D^{26}+80.6°$ (*c* 2.37; $CHCl_3$), ν_{max} (neat) 3330 (OH and NH) and 1550 (NH) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 7.62 (1H, br, s, NH), 7.36–7.19 (20 H, m, 4 Ph), 6.56 (1H, s, N'H), 5.19 (1H, d, $J_{1',2'}=5.1$ Hz, 1'-H), 4.89 and 4.51 (each 1H, ABq, $J_{gem}=11.7$ Hz, $PhC\underline{H}_2$), 4.80 and 4.69 (each 1H, ABq, $J_{gem}=11.9$ Hz, $PhC\underline{H}_2$), 4.73 and 4.65 (each 1H, ABq, $J_{gem}=11.6$ Hz, $PhC\underline{H}_2$), 4.41 and 4.36 (each 1H, ABq, $J_{gem}=11.7$ Hz, $PhC\underline{H}_2$), 4.09 (1H, dd, $J_{1',2'}=5.1$, $J_{2',3'}=9.5$ Hz, 2'-H), 3.92 (1H, dd, $J_{5',6'}=3.3$ and 8.1 Hz, 5'-H), 3.84–3.41 (4H, m, 2×1- and 2×2-H), 3.74 (1H, d, $J_{3',4'}=2.6$ Hz, 4'-H), 3.69 (1H, dd, $J_{2',3'}=9.5$, $J_{3',4'}=2.6$ Hz, 3'-H), 3.57 (1H, dd, $J_{5',6'}=8.1$, $J_{gem}=10.1$ Hz, 6'-H), 3.19 (1H, dd, $J_{5',6'}=3.3$, J_{gem}10.1 Hz, 6'-H), 2.54 (1H, br, s, OH). Anal. calcd for $C_{37}H_{42}N_2O_6S$: C, 69.13; H, 6.59; N, 4.36%. Found C, 69.25; H, 6.30; N, 4.40%.

2-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)amino-1-oxa-3-azacyclopent-2-ene (9). To a mixture of the thiourea **8** (76.0 mg, 0.118 mmol) in diethyl ether (2 mL) were added three portions of yellow mercury oxide (each 70 mg, 0.35 mmol, 3 equiv, total 210 mg, 1.05 mmol, 9 equiv) at room temperature. The mixture was stirred for 17 h at room temperature and then filtered through a bed of Celite. The bed was thoroughly washed with ethanol, the filtrate and washings were combined and evaporated to give **9** (67.7 mg, 94.2%) as a syrup, $[\alpha]_D^{27}+48.7°$ (*c* 0.87; $CHCl_3$), ν_{max} (neat) 3200 (NH), 1680 (C=N) and 1540 (NH) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 7.38–7.22 (20H, m, 4 Ph), 5.47 (1H, d, $J_{1',2'}=4.8$ Hz, 1'-H), 4.86 and 4.58 (each 1H, ABq, $J_{gem}=11.5$ Hz, $PhC\underline{H}_2$), 4.71 (2H, s, $PhC\underline{H}_2$), 4.68 and 4.62 (each 1H, ABq, $J_{gem}=11.5$ Hz, $PhC\underline{H}_2$), 4.49 and 4.40 (each 1H, ABq, $J_{gem}=11.5$ Hz, $PhC\underline{H}_2$), 4.23 (2H, t, $J=8.4$ Hz, 4- or 5-H), 4.12 (1H, dd, $J_{1',2'}=4.8$, $J_{2',3'}=9.5$ Hz, 2'-H), 4.02–3.62 (7H, m, 4- or 5-, 3'-, 4'-, 5' and 2×6'-H). Anal. calcd for $C_{37}H_{40}N_2O_6$: C, 73.01; H, 6.62; N, 4.60%. Found C, 73.45; H, 6.60; N, 4.69%.

Mixture of (1*S*,3*R*,4*S*,5*S*,6*R*)-8-(2-hydroxyethyl)-amino-3-hydroxymethyl-2,7-dioxa-9-azabicyclo[4.3.0]-non-8-ene-4,5-diol (10) and (1*R*,5*R*,7*S*,8*S*)-7-[(1*R*)-1,2-dihydroxyethyl]-3-(2-hydroxyethyl)amino-2,6-dioxa-4-azabicyclo[3.3.0]oct-3-ene-8-ol (11). To a stirred mixture of sodium (123 mg, 5.36 mmol, 100 equiv) and liquid ammonia (5 mL) was added a solution of the isourea **9** (32.6 mg, 0.0536 mmol) in THF (1 mL) at −78 °C. After 20 min, NH_4Cl (430 mg, 8.04 mmol, 150 equiv) was added to the mixture and ammonia was allowed to evaporate spontaneously at

room temperature. The residue was diluted with water (5 mL) and washed with $CHCl_3$ (5 mL × 2). The water layer was chromatographed on a column of Dowex 50W-X2 (H^+) resin (20 mL) with 0.5 M aq NH_4OH as eluent to afford a mixture of **10** and **11** (10.5 mg, 78.9%) as a white solid: ^1H NMR (270 MHz, D_2O) (inter alia) for **10**, δ_H 5.59 (0.4 H, d, $J_{1,6}=6.2$ Hz, 1-H), 4.41 (0.4 H, dd, $J_{1,6}=6.2$, $J_{5,6}=6.6$ Hz, 6-H), 3.80–3.74 (0.4, H, m, 3-H); for **11**, δ_H 5.79 (0.6 H, $J_{1,5}=5.9$ Hz, 5-H), 4.82 (0.6 H, $J_{1,5}=5.9$, $J_{1,8}=2.2$ Hz, 1-H), 4.27 (0.6 H, dd, $J_{1,8}=2.2$, $J_{7,8}=4.4$ Hz, 8-H).

Mixture of (1*S*,3*R*,4*R*,5*S*,6*R*)-8-(2-hydroxyethyl)-amino-3-hydroxymethyl-2,7-dioxa-9-azabicyclo[4.3.0]-non-8-ene-4,5-diol (13) and (1*R*,5*S*,7*R*,8*S*)-7-[(1*R*)-1,2-dihydroxyethyl]-3-(2-hydroxyethylamino-2,6-dioxa-4-azabicyclo[3.3.0]oct-3-ene-8-ol (14). The isourea[4] **12** (33.3 mg, 0.0546 mmol) was O-debenzylated, as in the preparation of **10** and **11**, to give a mixture of **13** and **14** (11.5 mg, 84.6%) as a white solid: ^1H NMR (270 MHz, D_2O) (inter alia) for **13**, δ_H 5.50 (0.6 H, d, $J_{1,6}=6.8$ Hz, 1-H), 4.36 (0.6 H, dd, $J_{1,6}=6.8$, $J_{5,6}=5.5$ Hz, 6-H), 3.80 (0.6 H, dd, $J_{4,5}=5.9$, $J_{5,6}=5.5$ Hz, 5-H); for **14** δ_H 5.79 (0.4 H, $J_{1,5}=5.1$ Hz, 5-H), 4.74 (0.4 H, d, $J_{1,5}$ Hz, 1-H), 4.25 (0.4 H, d, $J_{7,8}=2.6$ Hz, 8-H).

***N*-(2-Hydroxyethyl-*N'*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)thiourea (17).** The isothiocyanate[4] **15** (78.0 mg, 0.134 mmol) was allowed to couple with 1.3 molar equiv of 2-aminoethanol, as in the preparation of **8**, to afford **17** (86.2 mg, ~100%) as a syrup, $[\alpha]_D^{26}+25°$ (*c* 1.13; $CHCl_3$), ν_{max} (neat) 3320 (OH and NH) and 1560 (NH) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 7.33–7.14 (20H, m, 4 Ph), 5.43 (1H, br, s, 1'-H), 4.98 and 4.61 (each 1H, ABq, $J_{gem}=11.7$ Hz, $PhCH_2$), 4.71 (2H, s, $PhCH_2$), 4.64 and 4.60 (each 1H, ABq, $J_{gem}=11.7$ Hz, $PhCH_2$), 4.38 and 4.27 (each 1H, ABq, $J_{gem}=11.9$ Hz, $PhCH_2$), 4.02 (1H, dd, $J_{1',2'}=9.2$, $J_{2',3'}=9.7$ Hz, 2'-H), 3.86 (1H, d, $J_{3',4'}=2.0$ Hz, 4'-H), 3.77–3.15 (7H, m, 2 × 1-, 2 × 2-, 5'-, and 2 × 6'-H), 3.66 (1H, dd, $J_{2',3'}=9.7$, $J_{3',4'}=2.0$ Hz, 3'-H), 2.85 (1H, br, s, OH). Anal. calcd for $C_{37}H_{42}N_2O_6S$: C, 69.13; H, 6.59; N, 4.36%. Found C, 69.00; H, 6.68; N, 4.50%.

2-(2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)amino-1-oxa-3-azacyclopent-2-ene (19). The thiourea **17** (86.2 mg, 0.134 mmol) was treated with yellow HgO, as in the preparation of **9**, to give **19** (80.6 mg, 96.1%) as a syrup, $[\alpha]_D^{27}+3.3°$ (*c* 1.28; $CHCl_3$), ν_{max} (neat) 3300 and 3200 (NH) and 1680 (C=N) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 7.36–7.21 (20H, m, 4 Ph), 4.92 and 4.60 (each 1H, ABq, $J_{gem}=11.7$ Hz, $PhCH_2$), 4.84 and 4.76 (each 1H, ABq, $J_{gem}=11.4$ Hz, $PhCH_2$), 4.83 (1H, br, d, $J_{1',2'}=7.2$ Hz, 1'-H), 4.74 and 4.69 (each 1H, ABq, $J_{gem}=12.1$ Hz, $PhCH_2$), 4.44 and 4.39 (each 1H, ABq, $J_{gem}=11.9$ Hz, $PhCH_2$), 4.29–4.15 (2H, m, 4- or 5-H), 3.98 (1H, d, $J_{3',4'}=2.4$ Hz, 4'-H), 3.76–3.54 (6H, m, 4- or 5-, 2'-, 5'-, 2 × 6'-H), 3.62 (1H, dd, $J_{2',3'}=9.3$, $J_{3',4'}=2.4$ Hz, 3'-H). Anal. calcd for $C_{37}H_{40}N_2O_6 \cdot 0.5H_2O$: C, 71.94; H, 6.69; N, 4.53%. Found C, 71.95; H, 6.55; N, 4.59%.

2-(β-D-Galactopyranosyl)amino-1-oxa-3-azacyclopent-2-ene (3). The isourea **19** (25.5 mg, 0.419 mmol) was debenzylated, as in the preparation of **10** and **11**, to give **3** (6.0 mg, 57.7%) as a white solid, $[\alpha]_D^{29}+7.8°$ (*c* 0.29; water), ν_{max} (KBr disk) 3400 (OH and NH), 1670 (C=N) and 1550 (NH) cm^{-1}, ^1H NMR (270 MHz, D_2O, ref. acetone) δ_H 4.50 (1H, $J_{1',2'}=8.8$ Hz, 1'-H), 4.35 (2H, t, $J=8.4$ Hz, 4- or 5-H), 3.82 (1H, d, $J_{3',4'}=3.1$ Hz, 4'-H), 3.63–3.57 (5H, m, 4- or 5-, 5'-, 2 × 6'-H), 3.54 (1H, dd, $J_{2',3'}=9.9$, $J_{3',4'}=3.1$ Hz, 3'-H). 3.45 (1H, dd, $J_{1',2'}=8.8$, $J_{2',3'}=9.9$ Hz, 2'-H.

3-*N*-Acetyl-2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)imino-1-oxa-3-azacyclopentane (3a). The oxazoline **3** (3.3 mg, 0.0133 mmol) was acetylated conventionally to afford **3a** (2.1 mg, 34.4%) as a syrup, $[\alpha]_D^{29}+35.4°$ (*c* 0.11; $CHCl_3$), ν_{max} (neat) 1750 (OAc) and 1680 (NAc and C=N) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 5.44 (1H, dd, $J_{3',4'}=3.7$, $J_{4',5'}=1.1$ Hz, 4'-H), 5.30 (1H, dd, $J_{1',2'}=8.4$, $J_{2',3'}=10.6$ Hz, 2'-H), 5.08 (1H, dd, $J_{2',3'}=10.6$, $J_{3',4'}=3.7$ Hz, 3'-H), 4.90 (1H, d, $J_{1',2'}=8.4$ Hz, 1'-H), 4.46–4.27 (2H, m, 4- or 5-H), 4.18–4.10 (2H, m, 2 × 6'-H), 4.10–3.89 (3H, m, 4- or 5- and 5'-H), 2.54, 2.18, 2.05, 2.01 and 1.99 (each 3H, 5 s, 5 Ac). Anal. calcd for $C_{19}H_{26}N_2O_{11}$: C, 49.78; H, 5.72; N, 6.11%. Found C, 49.68; H, 5.91; N, 5.83%.

***N*-(2-Hydroxyethyl)-*N'*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)thiourea (18).** To a solution of the isothiocyanate[4] **16** (200 mg, 0.344 mmol) in CH_2Cl_2:MeOH (4 mL, 2:1, v/v) was added 2-aminoethanol (29 μL, 0.447 mmol, 1.3 molar equiv) at room temperature. The mixture was stirred for 3 h at room temperature and then evaporated to give a crystalline residue, which was chromatographed on a column of a silica gel (5 g) with EtOAc:toluene (1:2, v/v) as eluent to afford **18** (221 mg, ~100%) as crystals, mp 137–138 °C (from toluene), $[\alpha]_D^{23}-4.5°$ (*c* 0.57; $CHCl_3$), ν_{max} (neat) 3320 (OH and NH) and 1550 (NH) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 7.29–7.05 (20 H, m, 4 Ph), 6.97 (1H, br, s, NH), 5.61 (1H, br, s, NH), 4.90–4.33 (9H, m, 1'-H and 4 $PhCH_2$), 3.85–3.35 (9H, m, 2 × 1-, 2 × 2-, 2'-, 3'-, 4'-, and 2 × 6'-H), 3.27 (1H, br, s, 5'-H), 2.66 (1H, br, s, OH). Anal. calcd for $C_{37}H_{42}N_2O_6S$: C, 69.13; H, 6.59; N, 4.36%. Found C, 69.26; H, 6.64; N, 4.24%.

2-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)amino-1-oxa-3-azacyclopent-2-ene (20). To a mixture of the thiourea **18** (72.2 mg, 0.112 mmol) in diethyl ether (2 mL) were added three portions of yellow HgO (each 73.0 mg, total 219 mg, 1.01 mmol, 9 molar equiv) at room temperature. The mixture was stirred for 18 h at room temperature and filtered through a bed of Celite. The bed was washed with EtOH thoroughly and the filtrate and washings were combined and evaporated to give **20** (68.4 mg, ~100%) as a syrup, $[\alpha]_D^{21}+2.2°$ (*c* 1.79; $CHCl_3$), ν_{max} (neat) 1700, 1680 and 1640 (C=N) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 7.35–7.10 (20 H, m, 4 Ph), 4.97 (1H, br, d, $J_{1',2'}=8.4$ Hz, 1'-H), 4.93–4.79 (5H, m), 4.57 and 4.46 (each 1H, ABq, $J_{gem}=12.1$ Hz, $PhCH_2$), 4.51 (1H, d, $J_{gem}=12.8$ Hz), 4.27–4.16 (2H, m), 3.75–3.51 (7H, m), 3.33 (1H, dd,

$J_{1',2'}=8.4$, $J_{2',3'}=8.4$ Hz, 2'-H). Anal. calcd for $C_{37}H_{40}N_2O_6$: C, 73.01; H, 6.62; N, 4.60%. Found C, 72.73; H, 6.70; N, 4.50%.

2-(β-D-Glucopyranosyl)amino-1-oxa-3-azacyclopent-2-ene (5). The isourea **20** (68.4 mg, 0.112 mmol) was debenzylated, as in the preparation of the mixture of two isoureas **10** and **11**, to give **5** (24.4 mg, 87.5%) as a white solid, $[\alpha]_D^{29}-31.0°$ (*c* 1.22; water), ν_{max} (KBr disk) 3390 (OH and NH), 1670 (C=N) and 1560 (NH) cm^{-1}, ^1H NMR (270 MHz, D_2O, ref. acetone) δ_H 4.54 (1H, d $J_{1',2'}=8.8$ Hz, 1'-H), 4.36–4.24 (2H, m, 4- or 5-H), 3.75 (1H, dd, $J_{5',6'}=2.2$, $J_{gem}=12.5$ Hz, 6'-H), 3.60–3.53 (2H, m, 4- or 5-H), 3.58 (1H, dd, $J_{5',6'}=5.1$, $J_{gem}=12.5$ Hz, 6'-H), 3.39 (1H, dd, $J_{2',3'}=8.8$, $J_{3',4'}=8.8$ Hz, 3'-H), 3.36 (1H, ddd, $J_{4',5'}=9.9$, $J_{5',6'}=2.2$ and 5.1 Hz, 5'-H), 3.26 (1H dd, $J_{3',4'}=8.8$, $J_{4',5'}=9.9$ Hz, 4'-H), 3.20 (1H, dd, $J_{1',2'}=8.8$, $J_{2',3'}=8.8$ Hz, 2'-H).

3-*N*-Acetyl-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-imino-1-oxa-3-azacyclopentane (5a). The oxazoline **5** (22.3 mg, 0.0898 mmol) was acetylated with acetic anhydride (0.5 mL) in pyridine (1 mL) for 6 h at room temperature. After evaporation of excess reagents, column chromatography of silica gel (3 g) with acetone:toluene (1:4, v/v) gave **5a** (23.3 mg, 56.6%) as a syrup, $[\alpha]_D^{29}-3.9°$ (*c* 1.17; $CHCl_3$), ν_{max} (neat) 1760 and 1750 (OAc), 1700 and 1680 (NAc and C=N) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 5.26 (1H, dd, $J_{2',3'}=9.5$, $J_{3',4'}=9.2$ Hz, 3'-H), 5.15 (1H, dd, $J_{3',4'}=9.2$, $J_{4',5'}=9.9$ Hz, 4'-H), 6.10 (1H, dd, $J_{1',2'}=8.4$, $J_{2',3'}=9.5$ Hz, 2'-H), 4.93 (1H, d, $J_{1',2'}=8.4$ Hz, 1'-H), 4.46–4.28 (2H, m, 4- or 5-H), 4.28 (1H, dd, $J_{5',6'}=4.4$, $J_{gem}=12.5$ Hz, 6'-H, 4.12 (1H, dd, $J_{5',6'}=2.6$, $J_{gem}=12.5$ Hz, 6'-H), 4.09–3.88 (2H, m, 4- or 5-H), 3.80 (1H, ddd, $J_{4',5'}=9.9$, $J_{5',6'}=2.6$ and 4.4 Hz, 5'-H), 2.52, 2.09, 2.03, 2.02 and 2.00 (each 3H, 5 s, 5 Ac). Anal. calcd for $C_{19}H_{26}N_2O_{11}$: C, 49.78; H, 5.72; N, 6.11%. Found C, 50.05; H, 6.00; N, 5.88%.

***N*-(2-Hydroxyethyl)-*N'*-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)thiourea (22).** The isothiocyanate[9] **21** (62.3 mg, 0.138 mmol) was allowed to couple with 1.3 molar equiv of 2-aminoethanol, as in the preparation of the thiourea **8**, to give **22** (59.6 mg, ~100%) as a syrup, $[\alpha]_D^{26}+60.4°$ (*c* 1.27; $CHCl_3$), ν_{max} (neat) 3350 (OH and NH), 1750 (OAc) and 1550 (NH) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 7.55 and 7.47 (each 1H, 2 br s, 2 NH), 5.56 (1H, br s, 1'-H), 5.37 (1H, br s, 2'-H), 5.34–5.25 (2H, m, 3'- and 4'-H), 4.33 (1H, dd, $J_{5',6'}=5.1$, $J_{gem}=12.5$ Hz, 6'-H), 4.18 (1H, dd, $J_{5',6'}=2.9$, $J_{gem}=12.5$ Hz, 6'-H), 4.07 (1H, br s, 5'-H), 3.90–3.70 (4H, m, 2×1- and 2×2-H), 3.06 (1H, br s, OH), 2.18, 2.11, 2.07 and 2.04 (each 3H, 4 s, 4 Ac). Anal. calcd for $C_{17}H_{26}N_2O_{10}S$: C, 45.33; H, 5.82; N, 6.22%. Found C, 45.08; H, 6.17; N, 6.06%.

2-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)amino-1-oxa-3-azacyclopent-2-ene (23). The thiourea **22** (62.3 mg, 0.138 mmol) was treated with yellow HgO, as in the preparation of **9**, to give **23** (57.6 mg, ~100%) as a syrup, $[\alpha]_D^{27}+42.4°$ (*c* 0.83; $CHCl_3$), ν_{max} (neat) 3370 (NH), 1750 (OAc), 1680 (C=N) and 1550 (NH) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 5.60–5.05 (3H, m, 1'-, 2'- and 3'-H), 5.30 (1H, dd, $J=9.5$ and 9.9 Hz, 4'-H), 4.43 (2H, t, $J=7.7$ Hz, 4- or 5-H), 4.29 (1H, dd, $J_{5',6'}=4.4$, $J_{gem}=14.4$ Hz, 6'-H), 4.29–4.22 (1H, m, 5'-H), 4.08 (1H, dd, $J_{5',6'}=5.0$, $J_{gem}=14.4$ Hz, 6'-H), 3.65 (2H, t, $J_{gem}=7.7$ Hz, 4- or 5-H), 2.17, 2.10, 2.03 and 1.99 (each 3H, 4 s, 4 Ac). Anal. calcd for $C_{17}H_{24}N_2O_{10}$: C, 49.04; H, 5.81; N, 6.73%. Found C, 48.50; H, 6.01; N, 6.54%.

2-(α-D-Mannopyranosyl)amino-1-oxa-3-azacyclopent-2-ene (6). The isourea **23** (57.6 mg, 0.138 mmol) was treated with 1 M methanolic NaOMe (0.2 mL) in MeOH (1 mL) for 2 h at −15 °C. The reaction mixture was charged on a column of Dowex 50W-X2 (H^+) resin (2 mL) and eluted with 0.5 M aq NH_4OH to afford **6** (9.2 mg, 93.9%) as a white solid, $[\alpha]_D^{27}+122°$ (*c* 0.43; water), ν_{max} (KBr disk) 3410 (OH and NH), 1660 (C=N) and 1550 (NH) cm^{-1}, ^1H NMR (270 MHz, D_2O, ref acetone) δ_H 4.99 (1H, d, $J_{1',2'}=2.0$ Hz, 1'-H), 4.28 (2H, t, $J=8.5$ Hz, 4- or 5-H), 3.80 (1H, dd, $J_{1',2'}=2.0$, $J_{2',3'}=3.3$ Hz, 2'-H), 3.70 (1H, dd, $J_{5',6'}=2.2$, $J_{gem}=12.1$ Hz, 6'-H), 3.69 (1H, dd, $J_{2',3'}=3.3$, $J_{3',4'}=9.9$ Hz, 3'-H), 3.59 (1H, dd, $J_{5',6'}=5.5$, $J_{gem}=12.1$ Hz, 6'-H), 3.53 (2H, dt, $J=2.2$, 8.5 and 8.5 Hz, 4- or 5-H), 3.52 (1H, dd, $J_{3',4'}=9.9$, $J_{4',5'}=8.4$ Hz, 4'-H), 3.44 (1H, ddd, $J_{4',5'}=8.4$, $J_{5',6'}=2.2$ and 5.5 Hz, 5'-H).

3-*N*-Acetyl-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)imino-1-oxa-3-azacyclopentane (6a). The oxazoline **6** (6.0 mg, 0.0242 mmol) was acetylated conventionally to give **6a** (11.1 mg, ~100%) as a syrup, $[\alpha]_D^{26}+62.5°$ (*c* 0.57; $CHCl_3$), ν_{max} (neat) 1750 (OAc) and 1680 (NAc and C=) cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ_H 5.39–5.34 (3H, m, 1'-, 3'- and 4'-H), 5.17 (1H, dd, $J=2.0$ and 2.7 Hz, 2'-H), 4.47–4.32 (2H, m, 4- or 5-H), 4.30–4.25 (1H, m, 5'-H), 4.29 (1H, dd, $J_{5',6'}=4.2$, $J_{gem}=13.7$ Hz, 6'-H), 4.11 (1H, dd, $J_{5',6'}=4.2$, $J_{gem}=13.7$ Hz, 6'-H), 4.03 (2H, t, $J=8.1$ Hz, 4- or 5-H), 2.61, 2.18, 2.11, 2.06 and 1.99 (each 3H, 5 s, 5 Ac). Anal. calcd for $C_{19}H_{26}N_2O_{11}$: C, 49.78; H, 5.72; N, 6.11%. Found C, 49.73; H, 5.98; N, 5.97%.

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