Synthesis of a 2-Aminohexahydrobenzoxazole Analogue Related to Trehazolin

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2-Aminohexahydrobenzoxazole analogue **1a**, related to trehazolin (**2**) was synthesized using the Ferrier reaction as a key step. The structural elucidation of this compound by NMR analysis indicated that it is an inseparable mixture of three components (**1a–c**) which in turn stems from the propensity of **1a** to partially undergo both transcyclization (**1a** → **1b**) of the aminooxazoline between the hydroxy group at the C-1 position of aminocyclitol in the aglycon moiety and the hydroxy group at the C-2 position of D-glucose moiety and successive transformation (**1b** → **1c**) of the D-glucose moiety from a pyranose to a furanose structure.

Introduction

While the expansion of glycotechnology and glycobiology continues to progress at an unabated pace, there has been much attention paid to manipulating various glycoconjugates as a means to finding suitable glycosidase inhibitors in therapeutic application. In particular, α-glucosidase inhibitors have been implicated to be useful as drugs for diabetes or HIV. Amid these circumstances, studies on the structure–activity relationship of various glycosidase inhibitors are considered to be rudimentary. It is noteworthy that the analogues **4** of allosamidin (**3a**)[1] and allosamizoline (**3b**), possessing a 6-membered aminocyclitol in the aglycon, were synthesized and have been evaluated in terms of the relationship between their enzyme inhibitory activity and the framework of the aglycon.[2] In light of the aforementioned studies of allosamidins, we also attempted similar studies on trehazolin (**2**)[3] (Figure 1).

In Ando's original paper, trehazolin (**2**) was reported to be a unique pseudodisaccharide exhibiting specific inhibitory activities toward various trehalases.[4] At this juncture, the relationship between the structure and the enzyme-specific and strong inhibitory activities was recognized as being of prime significance from both chemical and biological viewpoints. Therefore, various analogues related to trehazolin have been synthesized in order to consolidate the aforementioned relationship and the inhibitory activities of the various kinds of glycosidases under study.[5]

2-Aminohexahydrobenzoxazole analogue **1a** related to trehazolin was designed and synthesized for the purpose of evaluating the inhibitory activities influenced by the framework of the aglycon moiety. Herein, we describe the synthesis of 2-aminohexahydrobenzoxazole analogue **1a** and its chemical properties.

Synthetic Strategy

The retrosynthetic analysis of the target **1a** is shown in Figure 2. On the basis of our previous syntheses of trehazolin and other related compounds, the thiourea derivative I, as the precursor of **1a**, was to be synthesized from azido compound II and D-glucose isothiocyanate derivative III. Compound II would be derived from the enone IV by performing a 1,2-reduction and a stereoselective epoxidation on compound IV and then regiospecific azido opening of the corresponding epoxide. Preceeding this transformation, we selected the Ferrier reaction,[6] which uses a catalytic amount of $Hg(OCOCF_3)_2$ as a key step to synthesize compound IV from methyl α-D-glucopyranoside.

Synthesis and Discussion

Benzyl ether **7**, obtained by the reported methods,[7] which included a regioselective ditosylation at the C-2,6

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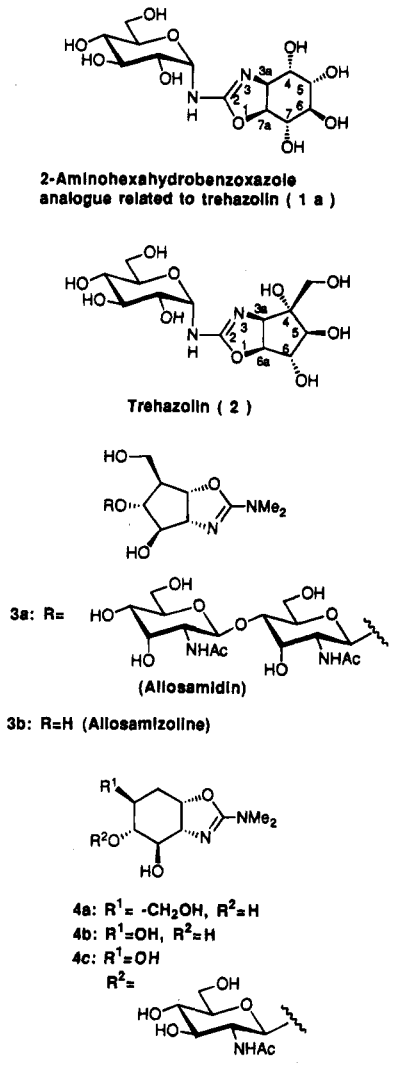

Figure 1. Analogues of allosamidin and trehazolin.

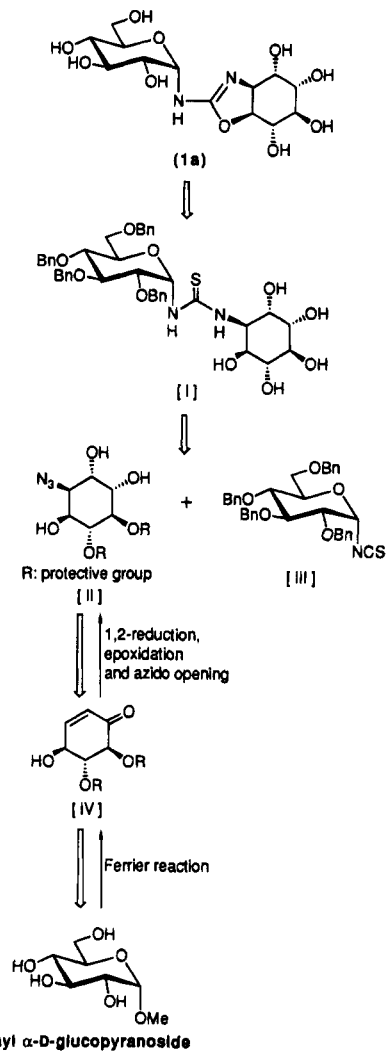

Figure 2. Retrosynthetic analysis of compound **1a**.

positions of methyl α-D-glucopyranoside (**5**) and methoxymethylation with dimethoxymethane and P_2O_5, was converted to the enone **8** via the Ferrier reaction, in which a catalytic amount of $Hg(OCOCF_3)_2$ was used[6] (Scheme 1). A 1,2-reduction of the enone **8** with $NaBH_4$–$CeCl_3 \cdot 7H_2O$ gave allylic alcohol **9**. Epoxidation of compound **9** was conducted stereospecifically, presumably by the free β-hydroxyl group exerting a hydrogen bonding effect in directing the *m*-CPBA β to the ring to yield the epoxy alcohol **10**.[8] After cleavage of the benzyl groups in compound **10** with hydrogenolysis mediated by Pearlman's catalyst ($Pd(OH)_2$ on carbon), azido opening of the corresponding epoxy diol **11** produced an azido alcohol **12** regiospecifically.[9] The regiospecificity of this azido opening can be rationalized on the basis of a preferential diaxial attack of the nucleophile on the most stable conformation of **11**, in accordance with the Fürst–Plattner rule.[10] In addition, the stereochemistry of compound **12** was confirmed by ^{1}H-NMR analysis of the triacetate **13**.[11]

Reduction of the azido group of **12** by hydrogenolysis using $Pd(OH)_2$ on carbon as a catalyst, deprotection of the two methoxymethyl (MOM) groups of the corresponding aminotriol **14**, and the subsequent addition of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl isothiocyanate (**16**)[12] to the six-membered aminocyclitol hydrogen chloride **15** yielded the thiourea derivative **17**, which was treated with 2-chloro-3-ethylbenzoxazolium tetrafluoroborate and triethylamine[13,14] to give **18** (Scheme 2). Finally, hydrogenolysis of **18** using $Pd(OH)_2$ on carbon as a catalyst gave a mixture that was inseparable by chromatography. We expected this mixture to consist of three geometric isomers **1a**, **1d**, and **1e** (Figure 3), predicted from the chemical properties of trehazolin acetylation.[4] Therefore,

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(11) ^{1}H-NMR data for triacetate **13** used to determine the stereochemistry of compound **12** are as follows: ^{1}H-NMR (270 MHz, $CDCl_3$) δ 5.33 (1H, dd, $J_{2,1}$ = 3.3 Hz, $J_{2,3}$ = 4.6 Hz, C2-eqH), 5.22 (1H, dd, $J_{4,5}$ = 9.2 Hz, $J_{4,3}$ = 4.0 Hz, C4-axH), 5.11 (1H, dd, $J_{1,6}$ = 9.2 Hz, $J_{1,2}$ = 3.3 Hz, C1-axH), 4.83–4.70 (4H, m), 4.03 (1H, dd, $J_{3,2}$ = 4.6 Hz, $J_{3,4}$ = 4.0 Hz, C3-eqH), 3.96 (1H, dd, $J_{6,1}$ = 9.2 Hz, $J_{6,5}$ = 8.6 Hz, C6-axH), 3.87 (1H, dd, $J_{5,4}$ = 9.2 Hz, $J_{5,6}$ = 8.6 Hz, C5-axH), 3.40 (3H, s), 3.37 (3H, s), 2.16 (3H, s), 2.14 (3H, s), 2.05 (3H, s).
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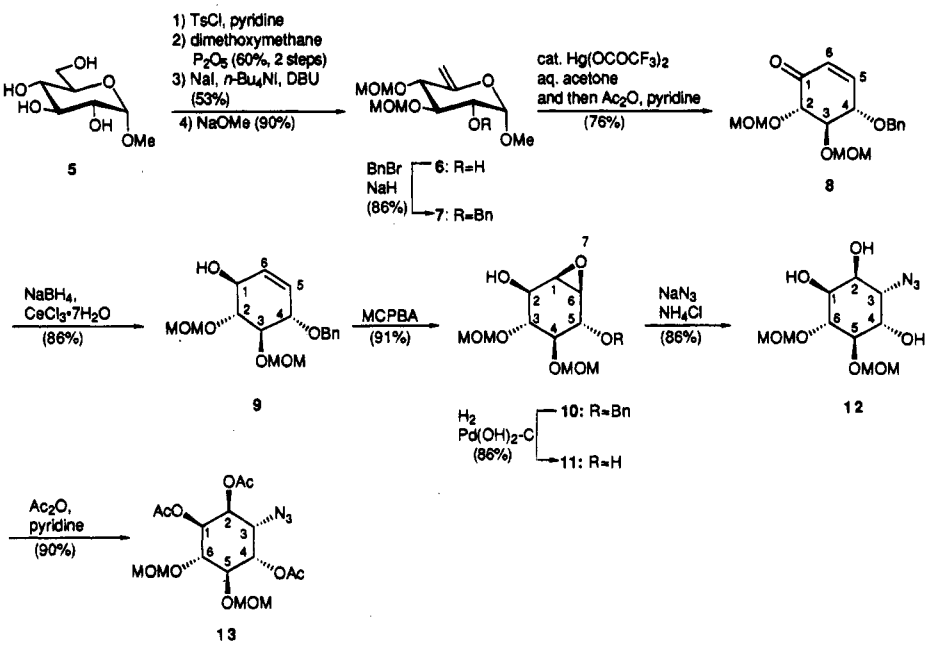
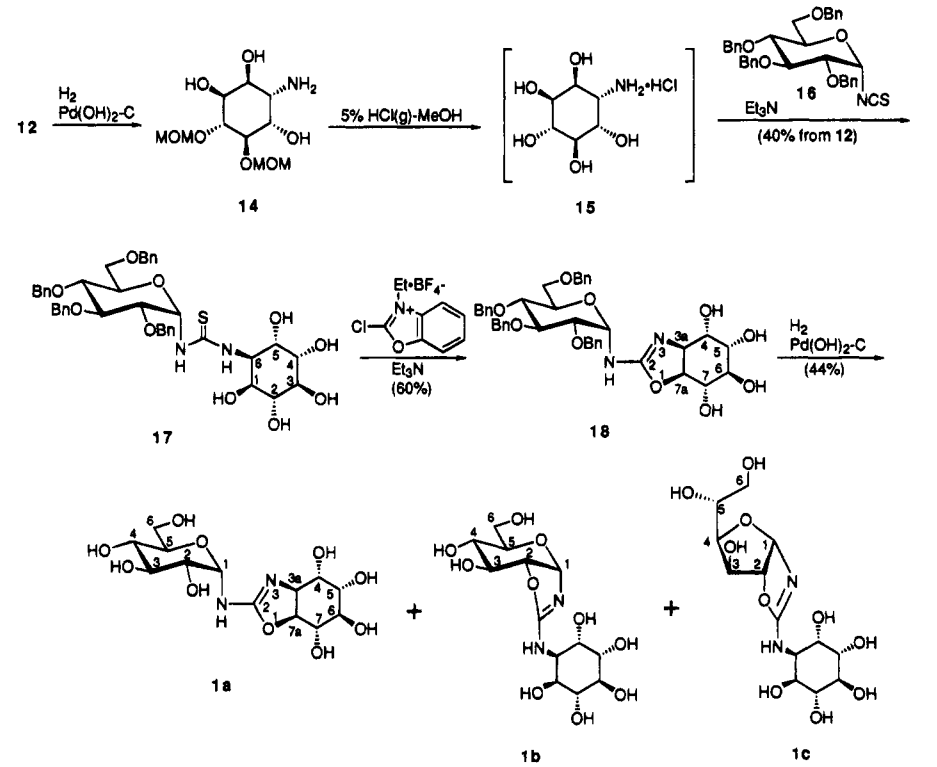
Scheme 1



Scheme 2



structural elucidation of this mixture by NMR was performed to determine the correct structures of the respective components. As a result, surprisingly, the structural elucidation provided evidence that during debenzylation concomitant transcyclization of the aminooxazoline between the hydroxy group at the C-1 position of the aminocyclitol in the aglycon and the C-2 hydroxy group of the D-glucose moiety was occurring, and some ring contraction of the D-glucose moiety to form a furanose ring was also taking place (Scheme 2). The equilibration between **1a**, **1b**, and **1c** (1:1:1−2) was therefore a confluence of the deprotection reaction and the above two reactions involving transcyclization. Structural elucidation of this mixture was carried out by using ^{1}H and ^{13}C-NMR as follows.[15] After identification of the 30 carbons by ^{13}C−^{1}H correlations, their connectivities were analyzed by DQF-COSY,[16] HOHAHA,[17] and HSQC-HOHAHA[18] spectra. The analyses resulted in five partial

(15) The 2D NMR spectra were collected over 1024 points along t2 and 128−256 increments along t1 using spectral windows of 2000 Hz in ^{1}H and 20 000 Hz in ^{13}C. DQF-COSY, HOHAHA, NOESY,[20] HSQC, HMBC, and HSQC-HOHAHA spectra were recorded, the mixing time of HOHAHA and HSQC-HOHAHA spectra was 45 ms, and that of NOESY spectra was 400 ms. All data processing was carried out using NMR1 and NMR2 software on a DEC station 5000/200 computer.

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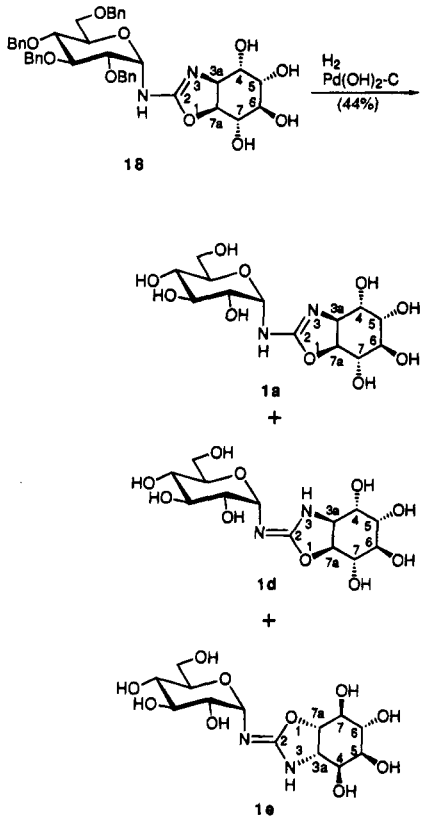

Figure 3. First expected structures of the mixture **1**.

structures consisting of six carbons each, as summarized in Figure 4. In Figure 4, ^1H and ^{13}C chemical shift values of trehazolin are also shown. Considering that these shift values are referenced to external TMS and that all ^1H chemical shifts are of a higher field shifted by about 0.15 ppm than the product mixture, the partial structure **D** was easily assigned to the α-D-glucopyranose moiety by the comparison of ^1H and ^{13}C chemical shift values with that of the α-D-glucopyranose moiety of trehazolin. Observation of the long-range coupling between H-1 and C-4 in the HMBC[19] spectrum of partial structure **A** indicated that it was closed in such a way as to form a five-membered ether ring, the glucofuranose moiety. Since long-range coupling could be observed between H-1 and H-2 of partial structure **A** and the imino carbon at 165.1 ppm, the furanose ring was found to be fused with an oxazoline ring at C-1 and C-2 of the D-glucose moiety. This was consistent with the fact that the chemical shift of H-2 is low-field shifted at 4.88 ppm in partial structure **A** as observed in H-6a of the trehazolin aglycon moiety at 4.77 ppm. Partial structure **B** was assigned to the glucopyranose moiety, as judged by the long-range coupling between H-1 and C-5. The low-field shift of H-1 and H-2, resonating at 5.64 ppm and 4.52 ppm, respectively, strongly suggested that partial structure **B** is a glucopyranose ring fused with an oxazoline ring. In partial structures **C** and **E**, their ^1H NMR signals were observed in the region from 3.50 to 4.70 ppm, and all carbons bearing those protons were shown to be methines by DEPT experiments. Thus, they were considered to form aminocyclitol moieties. To derive the whole structure, long-range correlations with imino carbons, which are expected to be observed around 165 ppm from the known trehazolin-related compounds, were informative, while only four cross signals were observed. In addition to the above-mentioned long-range couplings of H-1 and H-2 of partial structure **A** to an imino carbon at 165.1 ppm, the following long-range couplings were revealed: H-1 of partial structure **B** to an imino carbon at 164.5 ppm and H-3a of partial structure **C** to an imino carbon at 162.3 ppm. Although these values were insufficient to imply connectivity in all of the partial structures, it was concluded that at least three fused ring systems with oxazoline rings were present in the mixture, and partial structures **A**–**C** should form such ring systems differently. Further analysis was carried out as follows. Hydrogenolysis to cleave all of the benzyl groups of **18** was expected to produce compound **1a** at first, which corresponded to the combination of an α-D-glucopyranose moiety derived from partial structure **D** and the fused ring system incorporating partial structure **C**. The low-field chemical shift value of H-7a (4.67 ppm) in partial structure **C** was explained by the fused oxazoline ring. The existence of the fused ring system consisting of a D-glucopyranose moiety attributable to the partial structure **B** and an oxazoline ring indicated that cleavage of the oxazoline ring of **1a** was followed by transcyclization with the hydroxy group of its D-glucose moiety. As a result, an aminocyclitol, which was in good agreement with partial structure **E**, was liberated. This corresponds to the structure of compound **1b**.

Furthermore, if the transformation from glucopyranose to glucofuranose had occurred in **1b**, compound **1c**, which consists of partial structures **A** and **E**, might be produced. It seems probable that the aminocyclitol moieties of **1b** and **1c** have almost identical chemical shift values. In accordance with the above argument, partial structures **A**–**E** could be assigned as the structures **1a**–**c**, and their relative abundance was explained by interconversion between these structures. This observation represents quite an interesting chemical property which we plan to exploit in future synthetic studies of other trehazolin derivatives.

Since the components **1a**–**c** are all structurally related to trehazolin, their activities were expected to be comparable, if not superior, to that of trehazolin. However, disappointingly, the inhibitory activity of the mixture was as follows: IC_{50} (silkworm trehalase), >100 μg/mL; IC_{50} (porcine trehalase), >100 μg/mL. It is obvious that the tetrahydrocyclopentoxazole skeleton of trehazolin plays an important role in trehazolin's strong and specific inhibitory activity toward various trehalases.

Conclusion

We completed the synthesis of the 2-aminohexahydrobenzoxazole analogue **1a** related to trehazolin from D-glucose, utilizing a catalytic Ferrier reaction as a key step. The resulting product was a mixture of three components, and it was proved through structural elucidation with NMR that an equilibrium mixture of compounds **1a**–**c** was present, arising from the intramolecular transcyclization of **1a**. These studies revealed

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(21) The absence of a C–C bond line means that the order of carbon atoms was not determined although they belong to the same spin system as judged from the HSQC-HOHAHA spectrum. In addition, ^1H and ^{13}C chemical shifts of the components from **A** to **E** are referenced to internal TSP and internal dioxane (67.8 ppm), respectively, and the chemical shifts of trehazolin are referenced to external TMS.

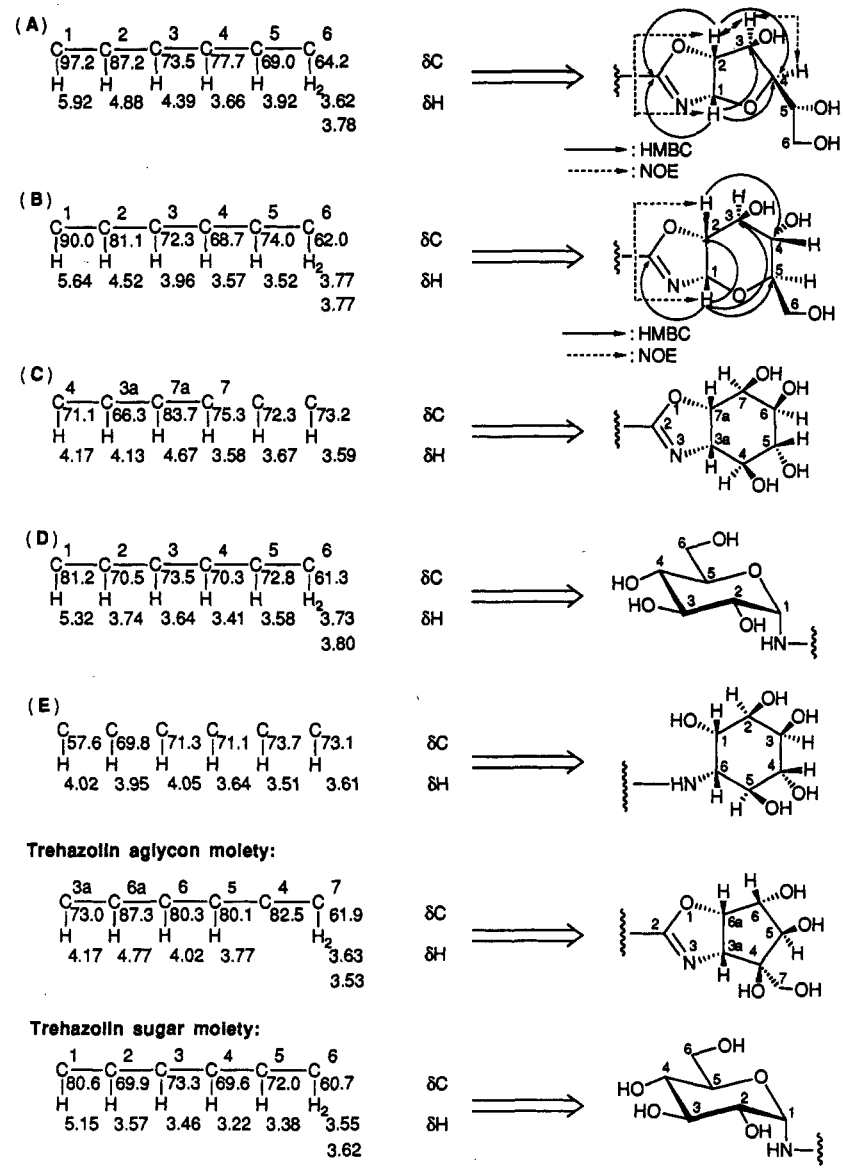

Figure 4. Partial structures of the inseparable mixture 1[21] and trehazolin.

that the 5,5-ring fused constructions of trehazolin and its related compounds were thermodynamically more stable than the 5,6-ring fused ones and indicated that such chemical properties as the potent carbodiimide function in the aminooxazoline framework might influence the generation of strong and enzyme-specific inhibitory activities toward various trehalases.

Experimental Section

General Method. Melting points are uncorrected. 270 MHz ^{1}H-NMR spectra were recorded using tetramethylsilane as an internal reference. Elemental analyses were performed by the Institute of Science and Technology, Inc. Analytical chromatography was performed on Merck Art 5715 silica gel 60-F_{254} plates. Flash chromatography was performed on Merck Art 9385 silica gel 60 (230–400 mesh). THF was distilled from $LiAlH_4$ and used immediately thereafter. Et_2O was dried by passage through ICN Alumina N-Super I. CH_2Cl_2 and $CHCl_3$ were dried by passage through ICN Alumina B-Super I. DMF and pyridine were dried by storage over 4 Å molecular sieves. MeCN was dried by storage over 3 Å molecular sieves. All other commercial reagents were used directly as received.

Methyl 6-Deoxy-3,4-bis-*O*-(methoxymethyl)-α-D-*xylo*-5-hexenopyranoside (6). To a solution of **5** (50 g, 0.26 mol) in pyridine (1000 mL) was added TsCl (108 g, 0.57 mol) at 0 °C, and the mixture was stirred at rt for 21 h. After completion of the reaction, the mixture was poured into H_2O and was extracted three times with EtOAc. The combined organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give 127 g of a crude methyl 2,6-bis-*O*-(*p*-toluenesulfonyl)-α-D-glucopyranoside as a pale yellow syrup: ^{1}H-NMR ($CDCl_3$) δ 7.85–7.60 (4H, m), 7.40–7.20 (4H, m), 4.63 (1H, d, J = 3.3 Hz), 4.61–4.30 (5H, m), 3.91 (1H, t, J = 9.2 Hz), 3.77–3.68 (1H, m), 3.46 (1H, t, J = 9.2 Hz), 3.24 (3H, s), 2.45 (6H, s); R_f = 0.73 (EtOAc). To a solution of the crude methyl 2,6-bis-*O*-(*p*-toluenesulfonyl)-α-D-glucopyranoside (33.3 g, 66.2 mmol) in $CHCl_3$ (410 mL) and dimethoxymethane (410 mL) was added P_2O_5 (330 g, 2.32 mol) at 0 °C, and the mixture was stirred for 1 h at 0 °C. After completion of the reaction, the reaction mixture was poured into cooled saturated aqueous $NaHCO_3$ and extracted three times with EtOAc. The combined organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (3:1) gave 21.9 g (60%) of methyl 3,4-bis-*O*-(methoxymethyl)-2,6-bis-*O*-(*p*-toluenesulfonyl)-α-D-glucopyranoside as a colorless syrup: R_f = 0.44 (hexane:EtOAc = 3:1); ^{1}H-NMR ($CDCl_3$) δ 7.80 (4H, m), 7.35 (4H, m), 4.77 (1H, d, J = 3.6 Hz), 4.59–4.49 (4H, m), 4.38–4.31 (1H, m), 4.24 (1H, dd, J = 9.2, 3.3 Hz), 4.18–4.10 (1H, m), 3.93 (1H, t, J = 9.2

Hz), 3.80–3.72 (1H, m), 3.40–3.34 (1H, m), 3.32 (3H, s), 3.28 (3H, s), 3.19 (3H, s), 2.46 (6H, s); MS (EI) *m/z* 590 (M^+), 545 (M^+ – MOM). To a solution of methyl 3,4-bis-*O*-(methoxymethyl)-2,6-bis-*O*-(*p*-toluenesulfonyl)-α-D-glucopyranoside (24.4 g, 41.3 mmol) in DMSO (500 mL) were added NaI (108.2 g, 0.57 mol), *n*-Bu_4NI (7.6 g, 20.7 mmol), and molecular sieves (4 Å powder, 250 g) at rt, and the mixture was stirred at 90 °C. After 2 h, DBU (31 mL, 0.21 mol) was added, and the reaction mixture was stirred at 90 °C for 20 h. After completion of the reaction, the reaction mixture was filtered through Celite, and the filtrate was poured into H_2O and extracted three times with EtOAc. The combined organic layer was, in turn, washed with each of 10% aqueous $Na_2S_2O_3$, H_2O, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (5:1 → 4:1) gave 9.22 g (53%) of methyl 6-deoxy-3,4-bis-*O*-(methoxymethyl)-2-*O*-(*p*-toluenesulfonyl)-α-D-*xylo*-5-hexenopyranoside as a colorless syrup: R_f = 0.32 (hexane:EtOAc = 3:1); ^{1}H-NMR ($CDCl_3$) δ 7.38 (2H, m), 7.36 (2H, m), 4.87–4.78 (2H, m), 4.76–4.70 (3H, m), 4.64 (2H, s), 4.51–4.44 (1H, m), 4.05–3.93 (2H, m), 3.43 (3H, s), 3.34 (3H, s), 3.29 (3H, s), 2.46 (3H, s). To a solution of methyl 6-deoxy-3,4-bis-*O*-(methoxymethyl)-2-*O*-(*p*-toluenesulfonyl)-α-D-*xylo*-5-hexenopyranoside (9.22 g, 22.0 mmol) in MeOH (180 mL) was added NaOMe (4.9 M MeOH solution) (18 mL, 88.2 mmol) at rt, and the mixture was refluxed for 60 h. After completion of the reaction, glacial AcOH (5.1 mL, 89.6 mmol) was added, and the reaction mixture was poured into H_2O. This mixture was extracted three times with EtOAc. The combined organic layer was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (3:1 → 3:2) gave 5.21 g (90%) of **6** as a colorless syrup: R_f = 0.31 (hexane:EtOAc = 3:2); $[\alpha]^{25}_D$ +119° (*c* 1.5, $CHCl_3$); IR ($CHCl_3$) 3380, 1665, 1145, 1030 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.88–4.73 (7H, m), 4.09–4.02 (1H, m), 3.77–3.68 (2H, m), 3.63 (1H, broad s), 3.48 (3H, s), 3.46 (3H, s), 3.45 (3H, s); MS (EI) *m/z* 219 (M^+ – MOM). Anal. Calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.63. Found: C, 49.82; H, 7.70.

Methyl 2-*O*-Benzyl-6-deoxy-3,4-bis-*O*-(methoxymethyl)-α-D-*xylo*-5-hexenopyranoside (7). To a suspension of NaH (227 mg, 5.2 mmol, 55% oil dispersion) in DMF (19 mL) was added a solution of **6** (1.1 g, 4.3 mmol) in DMF (3 mL) at 0 °C, and the mixture was stirred at rt. After 30 min, BnBr (0.62 mL, 5.2 mmol) was added to the mixture at 0 °C, and it was stirred at rt for 1 h. After completion of the reaction, the mixture was poured into H_2O, and it was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (9:1 → 4:1) gave 1.33 g (86%) of **7** as a colorless syrup: R_f = 0.73 (hexane:EtOAc = 3:2); $[\alpha]^{25}_D$ +30.6° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3010, 1670, 1150, 1085, 1040, 1010 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.40–7.30 (5H, m), 4.88 (1H, d, *J* = 3.3 Hz), 4.93–4.57 (8H, m), 4.03–3.92 (2H, m), 3.54 (1H, dd, *J* = 9.2, 3.3 Hz), 3.46 (3H, s), 3.44 (3H, s), 3.39 (3H, s); MS (EI) *m/z* 309 (M^+ – MOM). Anal. Calcd for $C_{18}H_{26}O_7$: C, 61.00; H, 7.40. Found: C, 60.85; H, 7.43.

[2*S*-(2α,3β,4α)]-4-(Benzyloxy)-2,3-bis(methoxymethoxy)-5-cyclohexen-1-one (8). To a solution of **7** (794 mg, 2.46 mmol) in aqueous acetone (24 mL, acetone:H_2O = 2:1) was added $Hg(OCOCF_3)_2$ (70 mg, 0.16 mmol) at rt, and the mixture was stirred at rt for 15 h. After completion of the reaction, acetone was evaporated under reduced pressure, and saturated aqueous $NaHCO_3$ was added to the residue. The mixture was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a residue. To a solution of this residue in pyridine (16 mL) was added Ac_2O (8 mL) at rt, and the mixture was stirred at rt for 21 h. After completion of the reaction, the reaction mixture was concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (9:1 → 4:1) gave 801 mg (76%) of **8** as a colorless syrup: R_f = 0.23 (hexane:EtOAc = 4:1); $[\alpha]^{25}_D$ +101° (*c* = 1.2, $CHCl_3$); IR ($CHCl_3$) 2990, 1695 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.40–7.30 (5H, m), 6.83 (1H, dd, *J* = 9.9, 2.0 Hz), 6.04 (1H, dd, *J* = 9.9, 2.0 Hz), 4.95–4.85 (4H, m), 4.78 (2H, s), 4.38 (1H, dt, *J* = 7.9, 2.2 Hz), 4.22 (1H, d, *J* = 11.2 Hz), 4.06 (1H, dd, *J* = 11.2, 7.9 Hz), 3.50 (3H, s), 3.43 (3H, s); MS (EI) *m/z* 322 (M^+), 291 (M^+ – OMe). Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.60; H, 6.89.

[1*S*-(1α,2β,3α,4β)]-4-(Benzyloxy)-2,3-bis(methoxymethoxy)-5-cyclohexen-1-ol (9). To a solution of **8** (794 mg, 2.46 mmol) in MeOH (16 mL) was added $CeCl_3 \cdot 7H_2O$ (1.38 g, 3.69 mmol) at 0 °C. After 5 min, $NaBH_4$ (112 mg, 2.95 mmol) was added to the mixture, and it was stirred for 30 min, with the temperature kept at 0 °C. After completion of the reaction, glacial AcOH (0.18 mL) was added to the reaction mixture, and it was poured into saturated aqueous $NaHCO_3$. The mixture was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (4:1) gave 685 mg (86%) of **9** as a colorless syrup: R_f = 0.26 (hexane:EtOAc = 2:1); $[\alpha]^{25}_D$ +189° (*c* 1.2, $CHCl_3$); IR ($CHCl_3$) 3420, 2990, 2880 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.45–7.25 (5H, m), 5.78–5.66 (2H, m), 4.88 (1H, d, *J* = 6.6 Hz), 4.84 (1H, d, *J* = 6.6 Hz), 4.82 (1H, d, *J* = 7.3 Hz), 4.78 (1H, d, *J* = 7.3 Hz), 4.69 (1H, d, *J* = 11.2 Hz), 4.63 (1H, d, *J* = 11.2 Hz), 4.26–4.07 (3H including OH, m), 3.82 (1H, dd, *J* = 10.6, 7.9 Hz), 3.48 (3H, s), 3.42 (1H, dd, *J* = 10.6, 7.3 Hz), 3.41 (3H, s); MS (EI) *m/z* 293 (M^+ – OMe), 279 (M^+ – MOM). Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.75; H, 7.46.

[1*R*-(1α,2β,3α,4β,5α,6α)]-5-(Benzyloxy)-3,4-bis(methoxymethoxy)-7-oxabicyclo[4.1.0]heptan-2-ol (10). To a solution of **9** (110 mg, 0.34 mmol) in CH_2Cl_2 (3 ml) was added *m*-CPBA (88 mg, 0.41 mmol) at rt, and the mixture was stirred at rt for 88 h. After completion of the reaction, the reaction mixture was poured into 20% aqueous Na_2SO_3, and saturated aqueous $NaHCO_3$ was added to the mixture. This mixture was extracted twice with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (4:1 → 2:1) gave 105 mg (91%) of **10** as a colorless syrup: R_f = 0.33 (hexane:EtOAc = 1:1); $[\alpha]^{25}_D$ +131° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 3400, 3000, 2900, 2880, 1150, 1075, 1030 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.40–7.25 (5H, m), 4.83–4.67 (6H, m), 4.08 (1H, broad s), 3.94 (1H, dd, *J* = 7.9, 2.0 Hz), 3.81 (1H, d, *J* = 7.9 Hz), 3.56 (1H, dd, *J* = 9.9, 7.9 Hz), 3.46 (3H, s), 3.47–3.43 (1H, m), 3.37 (3H, s), 3.35 (1H, dd, *J* = 9.9, 7.9 Hz), 3.24 (1H, d, *J* = 3.3 Hz); MS (EI) *m/z* 295 (M^+ – MOM). Anal. Calcd for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 59.72; H, 7.01.

[1*R*-(1α,2β,3α,4β,5α,6α)]-3,4-Bis(methoxymethoxy)-7-oxabicyclo[4.1.0]heptane-2,5-diol (11). To a solution of **10** (1.13 g, 3.31 mmol) in MeOH (23 mL) was added 20% $Pd(OH)_2$ on carbon (3.95 g) at rt, and the mixture was hydrogenolyzed at rt for 1 h. After completion of the reaction, this reaction mixture was filtered and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with hexane–EtOAc (1:1) gave 711 mg (86%) of **11** as a colorless syrup: R_f = 0.14 (hexane:EtOAc = 1:2); $[\alpha]^{25}_D$ +171° (*c* 1.1, $CHCl_3$); IR ($CHCl_3$) 3410, 3000, 2940, 2890, 1145, 1130, 1070, 1025 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.80–4.66 (4H, m), 4.30–4.10 (1H, broad s), 3.95 (1H, dd, *J* = 7.9, 2.0 Hz), 3.92 (1H, d, *J* = 7.9 Hz), 3.95–3.75 (1H, broad s), 3.50–3.44 (1H, m), 3.47 (3H, s), 3.45 (3H, s), 3.41 (1H, dd, *J* = 10.6, 7.9 Hz), 3.26 (1H, d, *J* = 3.3 Hz), 3.21 (1H, dd, *J* = 10.6, 7.9 Hz); MS (EI) *m/z* 219 (M^+ – OMe), 205 (M^+ – MOM). Anal. Calcd for $C_{10}H_{18}O_7$: C, 48.00; H, 7.25. Found: C, 48.07; H, 7.27.

[1*S*-(1α,2α,3β,4β,5α,6β)]-3-Azido-5,6-bis(methoxymethoxy)cyclohexane-1,2,4-triol (12). To a solution of **11** (766 mg, 3.1 mmol) in DMF (20 mL) were added NaN_3 (2.39 g, 36.7 mmol) and NH_4Cl (1.97 g, 36.7 mmol) at rt, and the mixture was stirred at 100 °C for 13 h. After completion of the reaction, the reaction mixture was poured into H_2O, and the mixture was extracted twice with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with CH_2Cl_2–MeOH (15:1) gave 772 mg (86%) of **12** as a colorless syrup: R_f = 0.32 (CH_2Cl_2:MeOH = 19:1); $[\alpha]^{25}_D$

+93.2° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 3360, 2970, 2860, 2820, 2080, 1260, 1020 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 4.81–4.67 (4H, m), 4.14 (1H, dd, J = 4.0, 3.3 Hz), 4.06 (1H, t, J = 3.3 Hz), 4.05 (1H, dd, J = 8.6, 4.0 Hz), 3.67 (1H, dd, J = 8.6, 3.3 Hz), 3.60 (1H, dd, J = 9.2, 8.6 Hz), 3.50 (1H, dd, J = 9.2, 8.6 Hz), 3.47 (6H, s), 3.20–2.40 (3H, broad s); MS (EI) *m/z* 294 (M^+ + 1), 262 (M^+ − OMe), 248 (M^+ − MOM). Anal. Calcd for $C_{10}H_{19}N_3O_7$: C, 40.95; H, 6.53; N, 14.33. Found: C, 40.90; H, 6.48; N, 14.39.

***N*-[[1*S*-(1α,2β,3α,4β,5β,6α)]-1,2,3,4,5-Pentahydroxycyclohex-6-yl]-*N'*-(2,3,4,6-tetra-*O*-α-D-glucopyranosyl)thiourea (17).** To a solution of **12** (302 mg, 1.03 mmol) in MeOH (6 mL) was added 20% $Pd(OH)_2$ on carbon (101 mg) at rt, and the mixture was hydrogenolyzed at rt for 1 h. After completion of the reaction, this reaction mixture was filtered and concentrated *in vacuo* to give a crude product of **14**. Subsequently, 10% methanolic hydrogen chloride (3 mL) was added to a solution of **14** at rt, and the mixture was stirred at 50 °C for 30 min. The mixture was evaporated *in vacuo*, and the residue **15** was dried under reduced pressure. Next, 2,3,4,6-tetra-*O*-benzyl-1-deoxy-α-D-glucopyranosyl isothiocyanate (**16**) (601 mg, 1.03 mmol) and Et_3N (0.18 mL, 1.26 mmol) were added to a solution of the residue **15** in THF (4.8 mL) and H_2O (0.6 mL) at 0 °C, and the mixture was stirred at rt for 16 h. After completion of the reaction, the reaction mixture was evaporated *in vacuo*, and the residue was chromatographed on silica gel. Elution with CH_2Cl_2–MeOH (10:1) gave 314 mg (40%, over three steps) of **17** as a white foamy glass: R_f = 0.39 (CH_2Cl_2:MeOH = 9:1); $[\alpha]^{25}_D$ +75.4° (*c* 1.4, $CHCl_3$); IR ($CHCl_3$) 3600–3200 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 7.50–7.00 (22H, m), 4.95–4.05 (12H, m), 4.00–3.25 (14H, m). Anal. Calcd for $C_{41}H_{48}N_2O_{10}S$: C, 64.72; H, 6.36; N, 3.68; S, 4.21. Found: C, 64.56; H, 6.64; N, 3.55; S, 4.00.

2,3,4,6-Tetra-*O*-benzyl-1-deoxy-1-[[[3a*S*-(3aα,4α,5α,6β,7α,7aα)]-4,5,6,7-tetrahydroxy-3a,4,5,6,7,7a-hexahydrobenzoxazol-2-yl]amino]-α-D-glucopyranose (18). To a solution of 2-chloro-3-ethylbenzoxazolium tetrafluoroborate (140 mg, 0.49 mmol) in MeCN (5.0 mL) was added a solution of **17** (250 mg, 0.33 mmol) in MeCN (4.5 mL) at 0 °C under N_2. After the mixture was stirred for 1 h, Et_3N (0.14 mL, 0.99 mmol) was added, with the temperature kept at 0 °C, and the resulting mixture was stirred for 1 h. After completion of the reaction, the reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude product, which was chromatographed on silica gel. Elution with CH_2Cl_2–MeOH (8:1) gave 144 mg (60%) of **18** as a white foamy glass: R_f = 0.24 (MeCN:H_2O = 99:1); $[\alpha]^{25}_D$ +79.1° (*c* 1.2, $CHCl_3$); IR (KBr) 3600–3100, 1665, 1450, 1070 cm^{-1}; ^1H-NMR ($CDCl_3$:CD_3OD = 1:1) δ 7.38–7.23 (18H, m), 7.20–7.12 (2H, m), 5.40 (1H, d, J = 5.3 Hz), 4.94 (1H, d, J = 11.2 Hz), 4.79 (2H, d, J = 11.2 Hz), 4.76–4.62 (4H, m), 4.56 (1H, d, J = 11.9 Hz), 4.52 (1H, d, J = 9.9 Hz), 4.47 (1H, d, J = 11.9 Hz), 4.15 (1H, dd, J = 8.6, 5.3 Hz), 4.07 (1H, dd, J = 5.3, 2.5 Hz), 3.89 (1H, dd, J = 9.9, 8.6 Hz), 3.77 (1H, dd, J = 9.2, 5.3 Hz), 3.73–3.50 (6H, m). Anal. Calcd for $C_{41}H_{46}N_2O_{10}$: C, 67.75; H, 6.38; N, 3.85. Found: C, 67.77; H, 6.23; N, 3.57.

1-Deoxy-1-[[[3a*S*-(3aα,4α,5α,6β,7α,7aα)]-4,5,6,7-tetrahydroxy-3a,4,5,6,7,7a-hexahydrobenzoxazol-2-yl]amino]-α-D-glucopyranose (1a) and Isomers 1b and 1c. To a solution of **18** (33 mg, 0.04 mmol) in MeOH (6.7 mL) was added 20% $Pd(OH)_2$ on carbon (1.0 g) at 24 °C, and the mixture was hydrogenolyzed at 60 °C for 30 min. After completion of the reaction, this reaction mixture was filtered and concentrated *in vacuo* to give a crude product, which was chromatographed on Amberlite CG-50 (NH_4^+ type/H^+ type = 3/2, 5 mL). Elution with 0.5 M aqueous NH_3 gave 5.9 mg (44%) of **1** as a white powder: R_f = 0.29 (MeCN:H_2O:AcOH = 13:5:2); $[\alpha]^{25}_D$ +70.3° (*c* 0.37, H_2O); IR (KBr) 3380, 1656, 1591 cm^{-1}; ^1H-NMR (since large-scale overlapping of peaks occurred, herein the only observable H–H coupling constants of sugar moieties of respective components of mixture **1** are given) **1a** δ 5.32 (d, J = 5.0 Hz, H-1, 3.74 (dd, J = 9.0, 5.0 Hz, H-2), 3.64 (dd, J = 9.0, 10.0 Hz, H-3), 3.41 (t, J = 10.0 Hz, H-4); **1b** δ 5.64 (d, J = 6.4 Hz, H-1), 4.52 (dd, J = 5.7, 6.4 Hz, H-2), 3.96 (dd, J = 6.5, 5.7 Hz, H-3), 3.57 (dd, J = 9.0, 6.5 Hz, H-4); **1c** δ 5.92 (d, J = 5.0 Hz, H-1); FAB-MS positive *m/z* 367 ($M + H)^+$, negative *m/z* 365 ($M - H)^-$; high resolution mass calcd for $C_{13}H_{23}O_{10}N_2$ 367.1357, found *m/z* 367.1355 ($M + H)^+$.

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