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Note

Synthesis of 6,7-dideoxy-7-isothiocyanatoheptoses: stable fully unprotected monosaccharide isothiocyanates

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Abstract

Methyl 6,7-dideoxy-7-isothiocyanato-α-D-*gluco*(*manno*)(*galacto*)-heptopyranosides have been synthesized in four steps by homologation of the respective methyl hexopyranosides via the corresponding heptopyranosyduruononitriles. Neither intra- nor intermolecular thiocarbamate formation was observed, even under rather strenuous acidic or basic conditions. The reducing derivative 6,7-dideoxy-7-isothiocyanato-α-D-*gluco*-heptopyranose was also a stable compound in aqueous solution in the absence of base. Formation of a six-membered intramolecular cyclic thiocarbamate was achieved in DMF solution in the presence of triethylamine. The title compounds are the first examples of stable fully unprotected monosaccharide isothiocyanates.

Keywords: Sugar isothiocyanates; Sugar thiocarbamates; Sugar nitriles; Heptoses, homologation

The ability of isothiocyanate groups to react with the amino group of lysine residues makes sugar isothiocyanates attractive candidates to specifically label the proteins involved in the transport of carbohydrate substrates across cell membranes [1]. Fully unprotected glycosyl isothiocyanates were first used for this purpose [2–4]. Although they were found to behave as potent irreversible inhibitors of monosaccharide translocation in the human erythrocyte, the results were strongly perturbed by fast decomposition of the reagents under physiological conditions [5]. Since the primary hydroxyl group is not essential for membrane binding [6], 6-deoxy-6-isothiocyanato hexoses were further proposed as suitable affinity reagents [7]. Yet, we showed later that these compounds are actually unstable and react intramolecularly through the furanose form to give the corresponding 6,5-(cyclic thiocarbamates) [8].

A systematic investigation of the stability of monosaccharide derivatives bearing both isothiocyanate and hydroxyl groups [9–11] revealed that, as a general rule, β- and γ-hydroxyisothiocyanate segments undergo spontaneous or base-induced ring closure to the corresponding oxazolidine or tetrahydrooxazine heterocycle, respectively. Interestingly, 6-deoxy-6-isothiocyanato hexopyranosides were found to be stable provided OH-4 was blocked, such as in cyclomaltooligosaccharides [12–14]. No seven- or higher-mem-

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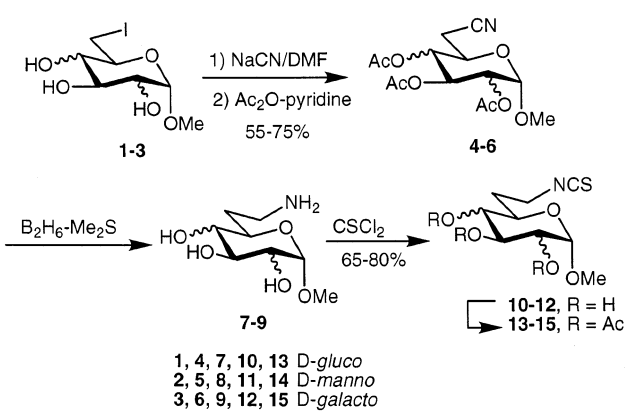
bered cyclic thiocarbamates nor intermolecular thiocarbamates were formed even under rather strenuous conditions, suggesting that isothiocyanate and hydroxyl groups distant by three carbon atoms or more can coexist in a sugar molecule. 6,7-Dideoxy-7-isothiocyanato heptose homologues fulfill these structural requirements while keeping intact the hydroxylation profile at the pyranoid ring. Thus, it was of interest to check their stability for a variety of configurational patterns at different temperature and pH conditions.

Chain elongation of methyl α-D-glucopyranoside, α-D-mannopyranoside and α-D-galactopyranoside was achieved in a straightforward manner following the methodology reported by Defaye and Gadelle [15] in the cyclomaltooligosaccharide (cyclodextrin) series. Nucleophilic displacement of iodine in the 6-deoxy-6-iodoglycopyranosides **1**–**3** by cyanide anion and acetylation of the reaction mixtures afforded the corresponding tri-*O*-acetylheptopyranosyduruononitriles **4**–**6** in 55–75% yield (Scheme 1). Formation of methyl 3,6-anhydro-α-D-galactopyranoside [16] as a side-product was observed in the D-*galacto* series. Nevertheless, since direct replacement of the primary hydroxyl group by iodine in glycopyranosides can be effected in high yield with no need for protection/deprotection steps [17–19], this procedure looks more convenient for our purposes than other reported methodologies using tosylate [20], triflate [21] or cyclic sulfate [22] as leaving groups. Reduction of the cyano group was accomplished using borane–dimethyl sulfide complex to give the methyl 7-amino-6,7-dideoxy-α-D-heptopyranosides **7**–**9** as hygroscopic solids that were transformed into the target isothiocyanates **10**–**12** by reaction with thiophosgene in water–acetone (Scheme 1).

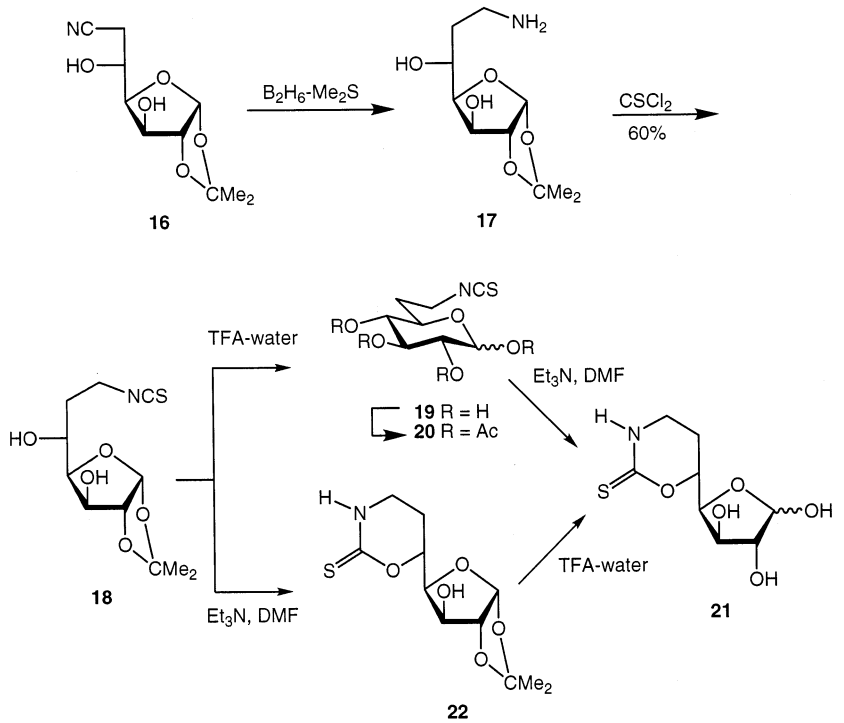


Scheme 1.

As expected, compounds **10**–**12** were stable in water solution and tolerated both acidic (trifluoroacetic acid) and basic pH conditions (triethylamine). They remained unchanged even after heating at 80 °C in *N*,*N*-dimethylformamide (DMF) solution in the presence of triethylamine[1], conditions that caused instantaneous cyclic thiocarbamate formation in the case of the related 6-deoxy-6-isothiocyanato hexopyranosides [9]. Conventional acetylation led to the triacetates **13**–**15** as the sole reaction products.

To implement this strategy in the access of fully unprotected reducing sugar isothiocyanates, 6,7-dideoxy-1,2-*O*-isopropylidene-7-isothiocyanato-α-D-*gluco*-heptofuranose (**18**) was synthesized from the known D-*gluco*-heptofuranurononitrile **16** [23] via the amine **17**, following an analogous reaction sequence (Scheme 2). Acid hydrolysis of the acetal protecting group afforded the corresponding D-*gluco*-heptopyranose isothiocyanate **19** as an equilibrium mixture of the α and β anomers. In contrast to what was reported for the 6-deoxy-6-isothiocyanto-α-D-glucose homologue [8,9], compound **19** was found to be stable in neutral or acidic aqueous solution. Conventional acetylation afforded the corresponding mixture of anomeric tetraacetates **20**. In the presence of base, slow formation of the six-membered cyclic thiocarbamate **21**, resulting from intramolecular nucleophilic addition of OH-5 to the heterocumulene functionality in the furanose form, was observed. Fast closure of the oxazine ring was achieved in *N*,*N*-dimethylformamide–triethylamine at 80 °C. Compound **21** was alternatively prepared by triethylamine-catalyzed cyclization of **18** (→ **22**) and subsequent deacetylation.

[1] At higher temperatures, partial decomposition of DMF took place with formation of dimethylamine that eventually reacted with the isothiocyanate group to give the corresponding *N*′,*N*′-dimethylthioureido sugars. In any case, no products resulting from reaction with hydroxyl groups were detected even at 110 °C.



Scheme 2.

The presence of the isothiocyanate group in compounds **10**–**15** and **18**–**20** was confirmed by the very characteristic strong IR absorption at 2180–2120 cm^{-1} and the δ_{NCS} ^{13}C resonance at 131.0–130.0 ppm [24]. Structural proofs for the six-membered cyclic thiocarbamate structure of compound **20** were provided by both the ^{1}H and ^{13}C NMR spectra. The ^{13}C chemical shifts of C-5 and C-7 showed strong deshielding and shielding effects indicative of the O-5–C(=S)NH–C-7 bridge [24]. The signal at 187.7 ppm confirmed the presence of the thiocarbonyl group. The coupling constants around the oxazine ring were in agreement with a conformation close to a chair, with the furanoid substituent in equatorial disposition.

In summary, 6,7-dideoxy-7-isothiocyanato-heptose derivatives are the first examples of stable fully unprotected monosaccharide isothiocyanates. They represent an alternative to the currently used sugar isothiocyanate conjugates, i.e., glycosides bearing the NCS group in the aglycon moiety, for biological studies [25–27] and in neoglycoconjugate synthetic strategies without protecting groups [28].

1. Experimental

General methods.—A Perkin–Elmer model 141 MC polarimeter and 1 dm tubes were used for measurement of specific rotations. Infrared spectra were recorded on a Bomem Michelson MB-120 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 (125.7) and 300 (75.5) MHz with Bruker 500 AMX and 300 AMX spectrometers, respectively. Chemical shifts are given in ppm with reference to tetramethylsilane as internal standard. Assignments of ^{1}H and ^{13}C signals were assisted by 1D TOCSY, 2D COSY and HETCOR experiments. FABMS were taken in a Kratos MS-80 RFA instrument. The operating conditions were the following: the primary beam consisted of Xe atoms with a maximum energy of 8 keV; the samples were dissolved in *m*-nitrobenzyl alcohol (O-protected derivatives) or thioglycerol (fully unprotected compounds), and the positive ions were separated and accelerated over a potential of 7 kV; NaI was added as cationizing agent. Thin-layer chromatography (TLC) was performed with E. Merck precoated TLC plates, Silica Gel 30F-245, with visualization

by UV light and by charring with 10% H_2SO_4. Column chromatography was carried out with Silica Gel 60 (E. Merck, 230–400 mesh). Microanalyses were performed by the Instituto de Investigaciones Químicas (CSIC, Seville).

General procedure for the preparation of methyl heptopyranosidurononitriles.—To a stirred soln of methyl 6-deoxy-6-iodo-α-D-gluco(*manno*, *galacto*)pyranoside [17–19] (**1**–**3**; 1.8 g, 4.18 mmol) in DMF (30 mL) was added NaCN (0.41 g, 8.36 mmol). The soln was stirred overnight at 70 °C, then concd and acetylated by treatment with 1:1 Ac_2O–pyridine (30 mL) for 5 h. Conventional work-up and purification by column chromatography (1:1 EtOAc–toluene) gave **4**–**6** as amorphous solids.

Methyl 2,3,4-tri-O-*acetyl*-α-D-gluco-*heptopyranosidurononitrile* (**4**).—Yield: (1.00 g, 73%); $[\alpha]_D^{20} + 42.6°$ (*c* 1.8, CH_2Cl_2); IR (KBr): 2947, 2257, 1759, 1371, 1240, and 1045 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.45 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 4.95 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.88 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 4.86 (dd, 1 H, H-2), 4.04 (ddd, 1 H, $J_{5,6a}$ 2.0, $J_{5,6b}$ 4.0 Hz, H-5), 3.43 (s, 3 H, OMe), 2.59–2.57 (m, 2 H, H-6a, H-6b), 2.06, 2.05, and 2.00 (3 s, 12 H, 3 MeCO); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.9, 169.7, 169.6 (3 CO), 115.9 (C-7), 96.6 (C-1), 71.6 (C-2), 70.4 (C-4), 69.3 (C-3), 65.0 (C-5), 55.6 (OMe), 20.8 (C-6), and 20.5 (3 *Me*CO); FABMS: *m*/*z* 352 (100%, $[M + Na]^+$). Anal. Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 51.00; H, 5.72; N, 4.20.

Methyl 2,3,4-tri-O-*acetyl*-α-D-manno-*heptopyranosidurononitrile* (**5**).—Yield: 0.81 g (60%); $[\alpha]_D^{20} + 4.6°$ (*c* 1.1, CH_2Cl_2); IR (KBr): 2942, 2843, 2257, 1755, 1371, 1223, and 1086 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.11 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.7 Hz, H-3), 5.06 (dd, 1 H, $J_{1,2}$ 1.6 Hz, H-2), 4.96 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 4.57 (d, 1 H, H-1), 3.86 (ddd, 1 H, $J_{5,6a}$ 4.8, $J_{5,6b}$ 7.2 Hz, H-5), 3.28 (s, 3 H, OMe), 2.55 (dd, 1 H, $J_{6a,6b}$ 16.7 Hz, H-6a), 2.47 (dd, 1 H, H-6b), 2.09, 2.08, and 2.00 (3 s, 12 H, 3 MeCO); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.7, 169.6, 169.4 (3 CO), 116.2 (C-7), 98.3 (C-1), 68.9 (C-4), 68.6 (C-3), 68.3 (C-2), 66.1 (C-5), 54.9 (OMe), 20.8 (C-6), and 20.5 (3 *Me*CO); FABMS: *m*/*z* 352 (100%, $[M + Na]^+$). Anal. Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 50.92; H, 5.92; N, 4.26.

Methyl 2,3,4-tri-O-*acetyl*-α-D-galacto-*heptopyranosidurononitrile* (**6**).—Yield: 0.75 g (55%); $[\alpha]_D^{20} + 143.5°$ (*c* 0.9, CH_2Cl_2); IR (KBr): 2947, 2255, 1758, 1379, 1236, and 1060 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.39 (dd, 1 H, $J_{3,4}$ 10.6, $J_{4,5}$ 1.0 Hz, H-4), 5.35 (t, 1 H, $J_{2,3}$ 10.6 Hz, H-3), 5.15 (dd, 1 H, $J_{1,2}$ 3.6 Hz, H-2), 5.03 (d, 1 H, H-1), 4.29 (ddd, 1 H, $J_{5,6a}$ 8.1, $J_{5,6b}$ 4.8 Hz, H-5), 3.46 (s, 3 H, OMe), 2.61 (dd, 1 H, $J_{6a,6b}$ 16.8 Hz, H-6a), 2.52 (dd, 1 H, H-6b), 2.19, 2.09, and 1.99 (3 s, 12 H, 3 MeCO); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.2, 169.7 (3 CO), 116.2 (C-7), 97.1 (C-1), 69.2 (C-4), 67.4 (C-2), 67.1 (C-3), 64.2 (C-5), 55.7 (OMe), 21.2, 20.6, 20.4 (3 *Me*CO) and 19.8 (C-6); FABMS: *m*/*z* 352 (100%, $[M + Na]^+$). Anal. Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 51.11; H, 5.99; N, 4.60.

General procedure for the preparation of methyl 7 - amino - 6,7 - dideoxy heptopyranosides.—To a soln of the corresponding nitrile **4**–**6** (1.88 g, 5.7 mmol) in dry 1,2-dimethoxyethane (60 mL), commercial borane–methyl sulfide complex (9.4 mL, 94 mmol) was added and the mixture was stirred at 40 °C under Ar for 1 day. After cooling at 0 °C, MeOH (30 mL) was added and the soln was stirred for 1 day before the addition of water (20 mL). The solvents were evaporated and MeOH (3 × 20 mL) was added and evaporated to give a hygroscopic solid residue which was checked by ^{13}C NMR and FABMS and used without further purification in the next step.

Methyl 7-amino-6,7-dideoxy-α-D-gluco-*heptopyranoside* (**7**).—^{13}C NMR (75.5 MHz, D_2O): δ 99.3 (C-1), 73.0 (C-2), 72.9 (C-3), 71.3 (C-4), 69.3 (C-5), 55.2 (OMe), 36.9 (C-7), and 28.4 (C-6); FABMS: *m*/*z* 230 (100%, $[M + Na]^+$).

Methyl 7-amino-6,7-dideoxy-α-D-manno-*heptopyranoside* (**8**).—^{13}C NMR (75.5 MHz, D_2O): δ 101.0 (C-1), 70.6 (C-2), 70.3 (C-3), 69.9 (C-4, C-5), 54.9 (OMe), 37.0 (C-7), and 28.4 (C-6); FABMS: *m*/*z* 230 (20%, $[M + Na]^+$).

Methyl 7-amino-6,7-dideoxy-α-D-galacto-heptopyranoside (**9**).—^{13}C NMR (75.5 MHz, D_2O): δ 99.6 (C-1), 70.9 (C-2), 69.5 (C-3), 68.7 (C-4), 68.1 (C-5), 55.2 (OMe), 37.3 (C-7), and 29.6 (C-6); FABMS: *m/z* 230 (100%, $[M+Na]^+$).

General procedure for the preparation of methyl 6,7-dideoxy-7-isothiocyanato heptopyranosides.—To a heterogeneous mixture of the respective crude amine **7**–**9** (5.75 mmol) in 1:1 water–acetone (30 mL) and $CaCO_3$ (2.3 g, 23 mmol) was added $CSCl_2$ (1.32 g, 0.88 mL, 11.5 mmol). The mixture was vigorously stirred for 2 h at room temperature (rt), then concd and the residue purified by column chromatography (20:1 → 9:1 CH_2Cl_2–MeOH) to afford the isothiocyanates **10**–**12** as amorphous solids. Conventional acetylation of **10**–**12** (0.5 g, 2.0 mmol) with 1:1 Ac_2O–pyridine (8 mL) at rt led to the corresponding triacetates **13**–**15** in virtually quantitative yield.

Methyl 6,7-dideoxy-7-isothiocyanato-α-D-gluco-heptopyranoside (**10**).—Yield: 1.14 g (80%), R_f 0.41 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ + 208.0° (*c* 0.7, CH_2Cl_2); IR (KBr): 3362, 3206, 2924, 2182, 2120, 1352, 1138, and 1045 cm^{-1}; ^{1}H NMR (300 MHz, CD_3O): δ 4.65 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 3.76–3.68 (m, 2 H, H-7a, H-7b), 3.65 (td, 1 H, $J_{5,6a}$ 2.2, $J_{4,5} = J_{5,6b}$ 9.5 Hz, H-5), 3.59 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 3.42 (s, 3 H, OMe), 3.40 (dd, 1 H, H-2), 3.08 (t, 1 H, H-4), 2.26 (dddd, 1 H, $J_{6a,6b}$ 14.6, $J_{6a,7a}$ 9.2, $J_{6a,7b}$ 6.7 Hz, H-6a), 1.70 (ddt, 1 H, $J_{6b,7a} = J_{6b,7b}$ 5.3 Hz, H-6b); ^{13}C NMR (75.5 MHz, CD_3OD): δ 131.4 (NCS), 101.1 (C-1), 75.4 (C-4), 74.8 (C-3), 73.5 (C-2), 69.0 (C-5), 55.8 (OMe), 42.5 (C-7), and 33.1 (C-6); FABMS: *m/z* 272 (100%, $[M+Na]^+$). Anal. Calcd for $C_9H_{15}NO_5S$: C, 43.36; H, 6.07; N, 5.62; S, 12.86. Found: C, 43.45; H, 6.18; N, 5.50; S, 12.63.

Methyl 6,7-dideoxy-7-isothiocyanato-α-D-manno-heptopyranoside (**11**).—Yield: 0.92 g (65%); R_f 0.39 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ + 111.3° (*c* 0.95, MeOH); IR (KBr): 3343, 2930, 2842, 2184, 2106, 1441, and 1055 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 4.61 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.80–3.72 (m, 2 H, H-7a, H-7b), 3.79 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 3.64 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 3.53 (td, 1 H, $J_{5,6a}$ 2.4, $J_{4,5} = J_{5,6b}$ 9.4 Hz, H-5), 3.43 (t, 1 H, H-4), 3.39 (s, 3 H, OMe), 2.28 (dddd, 1 H, $J_{6a,6b}$ 14.4, $J_{6a,7a}$ 8.9, $J_{6a,7b}$ 6.4 Hz, H-6a), 1.76 (ddt, 1 H, $J_{6b,7a} = J_{6b,7b}$ 5.3 Hz, H-6b); ^{13}C NMR (75.5 MHz, CD_3OD): δ 130.8 (NCS), 102.7 (C-1), 72.3 (C-2), 72.0 (C-3, C-4), 69.9 (C-5), 55.3 (OMe), 43.2 (C-7), and 33.0 (C-6); FABMS: *m/z* 272 (100%, $[M+Na]^+$). Anal. Calcd for $C_9H_{15}NO_5S$: C, 43.36; H, 6.07; N, 5.62. Found: C, 43.10; H, 5.97; N, 5.50.

Methyl 6,7-dideoxy-7-isothiocyanato-α-D-galacto-heptopyranoside (**12**).—Yield: 1.0 g (60%); R_f 0.37 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ + 175.7° (*c* 1, MeOH); IR (KBr): 3415, 2935, 2842, 2183, 2113, 1402, and 1047 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ 4.68 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-1), 3.89 (dd, 1 H, $J_{4,5}$ 0, $J_{5,6a}$ 10.2, $J_{5,6b}$ 3.4 Hz, H-5), 3.66–3.37 (m, 5 H, H-2, H-3, H-4, H-7a, H-7b), 3.41 (s, 3 H, OMe), 2.09 (ddt, 1 H, $J_{6a,6b}$ 17.7, $J_{6a,7a} = J_{6a,7b}$ 5.0 Hz, H-6a), 1.85 (dddd, 1 H, $J_{6b,7a}$ 6.4, $J_{6b,7b}$ 9.5 Hz, H-6b); ^{13}C NMR (125.5 MHz, CD_3OD): δ 132.3 (NCS), 101.6 (C-1), 72.4 (C-2), 71.5 (C-3), 70.0 (C-4), 68.1 (C-5), 55.8 (OMe), 42.9 (C-7), and 32.3 (C-6); FABMS: *m/z* 272 (100%, $[M+Na]^+$). Anal. Calcd for $C_9H_{15}NO_5S$: C, 43.36; H, 6.07; N, 5.62. Found: C, 43.36; H, 5.99; N, 5.59.

Methyl 2,3,4-tri-O-acetyl-6,7-dideoxy-7-isothiocyanato-α-D-gluco-heptopyranoside (**13**).—Yield: 0.73 g (98%), syrup; R_f 0.61 (1:2 EtOAc–petroleum ether); $[\alpha]_D^{20}$ + 13.7° (*c* 1.0, CH_2Cl_2); IR (KBr): 2938, 2845, 2182, 2110, 1735, 1370, 1223, and 1044 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.47 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 4.92 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.84 (m, 2 H, H-2, H-4), 3.93 (ddd, 1 H, $J_{4,5}$ 9.8 Hz, $J_{5,6a}$ 2.5, $J_{5,6b}$ 10.5 Hz, H-5), 3.74 (ddd, 1 H, $J_{7a,7b}$ 14.3, $J_{6a,7a}$ 9.2, $J_{6b,7a}$ 6.5 Hz, H-7a), 3.68 (ddd, $J_{6a,7b}$ 5.4, $J_{6b,7b}$ 4.5 Hz, 1 H, H-7b), 3.43 (s, 3 H, OMe), 1.96 (dddd, 1 H, $J_{6a,6b}$ 14.2 Hz, H-6a), 1.77 (dddd, 1 H, H-6b), 2.08, 2.07, and 2.01 (3 s, each 3 H, 3 MeCO); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 170.0, 169.8, 169.7 (3 CO), 130.9 (NCS), 96.4 (C-1), 71.8 (C-2), 70.8 (C-4), 69.7 (C-3), 65.1 (C-5), 55.4 (OMe), 40.8 (C-7), 31.3 (C-6), and 20.5 (3 *Me*CO); FABMS: *m/z* 398 (100%, $[M+Na]^+$). Anal. Calcd for $C_{15}H_{21}NO_8S$: C, 47.99; H, 5.64; N, 3.73; S, 8.54. Found: C, 48.10; H, 5.72; N, 3.73; S, 8.29.

Methyl 2,3,4-tri-O-acetyl-6,7-dideoxy-7-isothiocyanato-α-D-manno-heptopyranoside (**14**).—Yield: 0.71 g (96%), syrup; R_f 0.28 (3:2 EtOAc–petroleum ether); $[\alpha]_D^{20} + 56.0°$ (*c* 1.0, CH_2Cl_2); IR (KBr): 2943, 2196, 2117, 1751, 1379, 1226, and 1053 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.25 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.4 Hz, H-2), 5.10 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 4.67 (d, 1 H, H-1), 3.90 (td, 1 H, $J_{5,6a}$ 2.8, $J_{4,5} = J_{5,6b}$ 9.3 Hz, H-5), 3.76 (ddd, 1 H, $J_{7a,7b}$ 14.2, $J_{6a,7a}$ 9.2, $J_{6b,7a}$ 6.8 Hz, H-7a), 3.69 (ddd, 1 H, $J_{6a,7b}$ 5.6, $J_{6b,7b}$ 4.2 Hz, H-7b), 3.43 (s, 3 H, OMe), 3.32 (dd, 1 H, H-4), 1.93 (dddd, 1 H, $J_{6a,6b}$ 14.3 Hz, H-6a), 1.86 (dddd, 1 H, H-6b), 2.15, 2.08, and 1.99 (3 s, each 3 H, 3 MeCO); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.9, 169.8, 169.7 (3 CO), 130.8 (NCS), 98.3 (C-1), 69.4 (C-4), 69.1 (C-2), 68.4 (C-3), 66.3 (C-5), 55.3 (OMe), 40.9 (C-7), 31.4 (C-6), 20.8, 20.7, and 20.5 (3 *Me*CO); FABMS: *m/z* 398 (100%, $[M + Na]^+$). Anal. Calcd for $C_{15}H_{21}NO_8S$: C, 47.99; H, 5.64; N, 3.73. Found: C, 47.91; H, 5.64; N, 3.66.

Methyl 2,3,4-tri-O-acetyl-6,7-dideoxy-7-isothiocyanato-α-D-galacto-heptopyranoside (**15**).—Yield: 0.70 g (95%), syrup; R_f 0.53 (1:2 EtOAc–petroleum ether); $[\alpha]_D^{20} + 144°$ (*c* 1.0, CH_2Cl_2); IR (KBr): 2947, 2121, 1756, 1374, 1231, and 1056 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.38 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.4 Hz, H-3), 5.34 (dd, 1 H, $J_{4,5}$ 1.3, H-4), 5.13 (dd, 1 H, $J_{1,2}$ 3.7 Hz, H-2), 4.98 (d, 1 H, H-1), 4.14 (dd, 1 H, $J_{5,6a}$ 2.9, $J_{5,6b}$ 10.4 Hz, H-5), 3.73 (ddd, 1 H, $J_{7a,7b}$ 14.4, $J_{6a,7a}$ 9.0, $J_{6b,7a}$ 6.0 Hz, H-7a), 3.66 (ddd, 1 H, $J_{6a,7b}$ 5.5, $J_{6b,7b}$ 4.5 Hz, H-7b), 3.44 (s, 3 H, OMe), 1.89 (dddd, 1 H, $J_{6a,6b}$ 14.2 Hz, H-6a), 1.76 (dddd, 1 H, H-6b), 2.16, 2.10, and 1.99 (3 s, each 3 H, 3 MeCO); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.9, 169.8, 169.7 (3 CO), 130.8 (NCS), 97.0 (C-1), 70.9 (C-4), 70.8 (C-5), 67.9 (C-2), 67.5 (C-3), 55.5 (OMe), 41.2 (C-7), 31.5 (C-6), 20.7, and 20.5 (3 *Me*CO); FABMS: *m/z* 376 (10%, $[M+H]^+$). Anal. Calcd for $C_{15}H_{21}NO_8S$: C, 47.99; H, 5.64; N, 3.73. Found: C, 47.53; H, 5.61; N, 3.68.

6-Deoxy-1,2-O-isopropylidene-α-D-gluco-heptofuranurononitrile (**16**).—To a soln of 1,2-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-α-D-glucofuranose [23] (1.73 g, 4.62 mmol) in dimethylacetamide (10 mL) was added sodium cyanide (0.34 g, 7 mmol) and the mixture was stirred at 80 °C for 1.5 h. The solvent was evaporated and the residue purified by column chromatography (2:1 EtOAc–petroleum ether) to afford 16 (0.95 g, 90%) as an amorphous solid having $[\alpha]_D^{20} - 8.6°$ (*c* 1.0, MeOH); lit. [23] $[\alpha]_D^{20} - 15.2°$ (*c* 1.5, pyridine); ^{13}C NMR (75.5 MHz, CD_3OD): δ 121.6 (C-7), 115.4 (CMe_2), 108.7 (C-1), 89.1 (C-2), 85.8 (C-4), 77.1 (C-3), 67.7 (C-5), 29.5 (C-6), 28.9, and 26.8 (CMe_2); FABMS: *m/z* 230 (55%, $[M + H]^+$).

7-Amino-6,7-dideoxy-1,2-O-isopropylidene-α-D-gluco-heptofuranose (**17**).—Compound **17** was prepared from nitrile **16** (1.58 g, 5.7 mmol) by reduction with borane–dimethylsulfide complex (9.4 mL, 94 mmol), following the procedure described above for the preparation of **7**–**9**, and isolated as a hygroscopic solid which was directly used in the next step without further purification. ^{13}C NMR (125.7 MHz, D_2O): 112.6 (CMe_2), 104.6 (C-1), 84.4 (C-2), 82.6 (C-4), 73.1 (C-3), 65.9 (C-5), 36.8 (C-7), 31.4 (C-6), 25.4, and 25.0 (CMe_2); FABMS: *m/z* 234 (100%, $[M + Na]^+$).

6,7-Dideoxy-1,2-O-isopropylidene-7-isothiocyanato-α-D-gluco-heptofuranose (**18**).—Isothiocyanation of the crude amine **17** (5.75 mmol) with thiophosgene (1.32 g, 0.88 mL, 11.5 mmol), as described above for the preparation of **10**–**12**, and column chromatography of the reaction mixture (1:1 EtOAc–petroleum ether) afforded **18** (0.95 g, 60%) as an amorphous solid; $[\alpha]_D^{20} - 2.9°$ (*c* 1.0, MeOH); R_f 0.34 (1:1 EtOAc–petroleum ether); IR (KBr): 2991, 2928, 2189, 2109, and 1379 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 5.96 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.50 (d, 1 H, $J_{2,3}$ 0 Hz, H-2), 4.30 (t, 1 H, $J_{OH,3} = J_{3,4}$ 2.5 Hz, H-3), 4.26 (dddd, 1 H, $J_{OH,5}$ 3.5, $J_{4,5}$ 4.5, $J_{5,6a}$ 2.9, $J_{5,6b}$ 10.3 Hz, H-5), 3.97 (dd, 1 H, H-4), 3.81 (d, 1 H, OH-5), 3.79 (ddd, 1 H, $J_{7a,7b}$ 14.0, $J_{6a,7a}$ 9.0, $J_{6b,7a}$ 6.0 Hz, H-7a), 3.71 (ddd, 1 H, $J_{6a,7b}$ 5.5, $J_{6b,7b}$ 4.5 Hz, H-7b), 2.91 (d, 1 H, OH-3), 2.03 (dddd, 1 H, $J_{6a,6b}$ 14.2 Hz, H-6a), 1.94 (dddd, 1 H, H-6b), 1.47, and 1.30 (2 s, each 3 H, CMe_2); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 131.0 (NCS), 111.9 (CMe_2), 104.8 (C-1), 85.4 (C-2), 80.8 (C-4), 75.7 (C-3), 67.8 (C-5), 41.7 (C-7), 32.7 (C-6), 26.8, and 26.1 (CMe_2); FABMS: *m/z* 276 (100%, [M +

Na]$^+$). Anal. Calcd for $C_{11}H_{17}NO_5S$: C, 47.99; H, 6.22; N, 5.09. Found: C, 47.96; H, 6.53; N, 5.02.

6,7-Dideoxy-7-isothiocyanato-D-gluco-*heptopyranose* (**19**).—Compound **18** (300 mg, 1.08 mmol) was treated with 90% TFA–water (3 mL) at rt in a rotatory evaporator until evolution of acetone ceased (10–15 min). Concentration, elimination of traces of TFA by coevaporation with water, and final lyophilization yielded **19** (224 mg, 87%) as a white foam. Compound **19** existed as a 1:1.2 α:β anomeric equilibrium mixture in D_2O soln (H-1 integration); $[\alpha]_D^{20} + 60.6°$ (*c* 0.7, MeOH); R_f 0.40 (45:5:3 EtOAc–EtOH–water); IR (KBr): 3190, 2888, 2832, 2197, 2117, 1649, and 1545 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): α anomer, δ 5.05 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 3.83 (td, 1 H, $J_{5,6a}$ 2.6, $J_{4,5} = J_{5,6b}$ 9.7 Hz, H-5), 3.64 (m, 2 H, H-7a, H-7b), 3.60 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.7 Hz, H-3), 3.31 (dd, 1 H, H-2), 3.02 (t, 1 H, H-4), 2.21 (dtd, 1 H, $J_{6a,6b}$ 13.9, $J_{6a,7a} = J_{6a,7b}$ 8.8 Hz, H-6a), 1.68 (ddt, 1 H, $J_{6b,7a}$ 9.7, $J_{6b,7b}$ 5.8 Hz, H-6b); β anomer, δ 4.43 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.68 (m, 2 H, H-7a, H-7b), 3.29 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 3.28 (m, 1 H, H-5), 3.09 (dd, 1 H, H-2), 3.06 (t, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 2.24 (m, 1 H, H-6a), 1.69 (m, 1 H, H-6b); ^{13}C NMR (125.7 MHz, CD_3OD): α anomer, δ 133.4 (NCS), 96.3 (C-1), 78.1 (C-4), 77.3 (C-3), 76.4 (C-2), 71.4 (C-5), 45.0 (C-7), 35.9 (C-6); β anomer, δ 133.4 (NCS), 100.7 (C-1), 80.4 (C-3), 78.9 (C-2), 77.7 (C-4), 76.2 (C-5), 44.9 (C-7), 35.9 (C-6); FABMS: *m*/*z* 236 (100%, [M + H]$^+$). Anal. Calcd for $C_8H_{13}NO_5S$: C, 40.84; H, 5.57; N, 5.95. Found: C, 40.55; H, 5.48; N, 5.70.

1,2,3,4-Tetra-O-*acetyl-6,7-dideoxy-7-isothiocyanato-α- and β*-D-gluco-*heptopyranose* (**20**).—Conventional acetylation of **19** (150 mg, 0.64 mmol) with 1:1 Ac_2O–pyridine (2 mL) gave syrupy **20** (233 mg, 85%) as an inseparable mixture of the corresponding α and β anomers; α:β ratio 1:1.4; R_f 0.50 (1:1 EtOAc–petroleum ether); IR (KBr): 2951, 2197, 2125, 1760, 1379, and 1228 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): α anomer, δ 6.28 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.46 (t, 1 H, $J_{3,4} = J_{2,3}$ 9.4 Hz, H-3), 5.05 (dd, 1 H, H-2), 4.89 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 4.05 (td, 1 H, $J_{5,6a}$ 2.1, $J_{5,6b}$ 9.4 Hz, H-5), 3.69 (ddd, 1 H, $J_{7a,7b}$ 14.3, $J_{6a,7a}$ 10.3, $J_{6b,7a}$ 3.8 Hz, H-7a), 3.57 (dt, 1 H, $J_{6a,7b} = J_{6b,7b}$ 5.2 Hz, H-7b), 2.21–1.98 (MeCO), 1.90 (dddd, 1 H, $J_{6a,6b}$ 14.3 Hz, H-6a), 1.72 (dddd, 1 H, H-6b); β anomer, δ 5.66 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.23 (t, 1 H, $J_{3,4} = J_{2,3}$ 9.8 Hz, H-3), 5.09 (dd, 1 H, H-2), 4.88 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.69 (td, 1 H, $J_{5,6a}$ 9.8, $J_{5,6b}$ 2.9 Hz, H-5), 3.63 (dd, 2 H, $J_{6a,7a} = J_{6b,7a}$ 7.6, $J_{6a,7b} = J_{6b,7b}$ 5.3 Hz, H-7a, H-7b), 2.21–1.98 (MeCO), 1.94 (dddd, 1 H, $J_{6a,6b}$ 14.6 Hz, H-6a), 1.82 (dddd, 1 H, H-6b); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 169.9–168.7 (CO), 132.0, 131.7 (NCS), 91.8 (C-1 α), 88.7 (C-1 β), 72.7 (C-3 β), 71.5 (C-5 β), 71.4 (C-4 α), 71.1 (C-4 β), 70.4 (C-2 β), 69.4 (C-2 α), 69.8 (C-3 α), 67.5 (C-5 α), 40.9, (C-7 β), 40.6 (C-7 α), 31.6 (C-6 α), 31.3 (C-6 β), and 20.8-20.2 (*Me*CO); FABMS: *m*/*z* 426 (100%, [M + Na]$^+$). Anal. Calcd for $C_{16}H_{21}NO_9S$: C, 47.64; H, 5.25; N, 3.47. Found: C, 47.56; H, 5.28; N, 3.42.

7-Amino-6,7-dideoxy-D-gluco-*heptofuranose 7,5-(cyclic thiocarbamate)* (**21**).—To a stirred soln of **20** (94 mg, 0.4 mmol) in DMF (10 mL) freshly distilled Et_3N (18 μL, 0.12 mmol) was added and the reaction mixture was heated at 80 °C for 1 h. Evaporation of the solvent and freeze-drying of the residue gave **21** (94 mg, 100%) as a white foam; α:β ratio 1.2:1 (H-1 integration, CD_3OD); $[\alpha]_D^{20} - 35.5°$ (*c* 0.9, MeOH); R_f 0.50 (45:5:3 EtOAc–EtOH–water); ^{1}H NMR (500 MHz, CD_3OD): α anomer, δ 5.40 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.51 (ddd, 1 H, $J_{4,5}$ 8.5, $J_{5,6a}$ 2.9, $J_{5,6b}$ 10.7 Hz, H-5), 4.29 (dd, 1 H, $J_{2,3}$ 2.4, $J_{3,4}$ 3.9 Hz, H-3), 4.10 (dd, 1 H, H-4), 3.96 (dd, 1 H, H-2), 3.34 (m, 2 H, H-7a, H-7b), 2.27 (dddd, 1 H, $J_{6a,6b}$ 14.2, $J_{6a,7a}$ 5.0, $J_{6a,7b}$ 8.1 Hz, H-6a), 1.96 (m, 1 H, H-6b); β anomer, δ 5.10 (bs, 1 H, H-1), 4.67 (ddd, 1 H, $J_{4,5}$ 7.8, $J_{5,6a}$ 2.9, $J_{5,6b}$ 10.9 Hz, H-5), 4.20 (dd, 1 H, $J_{2,3}$ 0.8, $J_{3,4}$ 4.1 Hz, H-3), 4.17 (dd, 1 H, H-4), 4.00 (dd, 1 H, $J_{1,2}$ 0.7 Hz, H-2), 3.34 (m, 2 H, H-7a, H-7b), 2.33 (dddd, 1 H, $J_{6a,6b}$ 14.1, $J_{6a,7a}$ 5.1, $J_{6a,7b}$ 6.4 Hz, H-6a), 1.96 (m, 1 H, H-6b); ^{13}C NMR (125.7 MHz, D_2O): α anomer, δ 185.1 (C=S), 102.3 (C-1), 81.5 (C-4), 77.1 (C-5), 75.7 (C-2), 74.7 (C-3), 39.3 (C-7), 21.2 (C-6); β anomer, δ 185.1 (C=S), 96.7 (C-1), 80.1 (C-2), 78.3 (C-4), 77.2 (C-5), 74.2 (C-3), 39.3 (C-7), 21.7 (C-6). Anal. Calcd

for $C_8H_{13}NO_5S$: C, 40.84; H, 5.57; N, 5.95. Found: C, 40.69; H, 5.51; N, 5.72.

Compound **21** was also obtained in quantitative yield from the acetonide **22** by acid hydrolysis of the isopropylidene group using 90% TFA–water.

*7-Amino-6,7-dideoxy-1,2-*O*-isopropylidene-α-*D*-gluco-heptofuranose 7,5-(cyclic thiocarbamate)* (**22**).—To a stirred soln of **18** (155 mg, 0.55 mmol) in DMF (15 mL) was added Et_3N (25 μL, 0.165 mmol) and the reaction mixture was heated at 80 °C for 2 h, then concd and the residue purified by column chromatography (2:1 EtOAc–petroleum ether) to give **22** (135 mg, 87%) as an amorphous solid; $[\alpha]_D^{20}$ −47.8° (*c* 1.0, MeOH); R_f 0.18 (1:1 EtOAc–petroleum ether); ^{1}H NMR (500 MHz, CD_3OD): δ 5.91 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.56 (ddd, 1 H, $J_{4,5}$ 7.9, $J_{5,6a}$ 2.9, $J_{5,6b}$ 10.1 Hz, H-5), 4.51 (d, 1 H, $J_{2,3}$ 0 Hz, H-2), 4.30 (d, 1 H, $J_{3,4}$ 2.4 Hz, H-3), 4.07 (dd, 1 H, H-4), 3.34 (m, 2 H, H-7a, H-7b), 2.29 (dq, 1 H, $J_{6a,6b}$ 16.3, $J_{6a,7a} = J_{6a,7b}$ 2.9 Hz, H-6a), 1.95 (dddd, 1 H, $J_{6b,7a}$ 6.2, $J_{6b,7b}$ 4.0 Hz, H-6b), 1.47 and 1.29 (2 s, each 3 H, CMe_2); ^{13}C NMR (125.7 MHz, CD_3OD): δ 183.0 (C=S), 111.3 (CMe_2), 104.7 (C-1), 84.9 (C-2), 80.4 (C-4), 74.9 (C-3), 72.9 (C-5), 38.8 (C-7), 25.4, 24.6 (CMe_2), and 22.0 (C-6); FABMS: *m/z* 276 (100%, $[M+H]^+$). Anal. Calcd for $C_{11}H_{17}NO_5S$: C, 47.99; H, 6.22; N, 5.09. Found: C, 48.22; H, 6.22; N, 4.96.

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