Synthesis, Characterization, and Reaction of Crystalline Fischer's Glucopyranosyl Isocyanate

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We have established a simple method for the preparation of crystalline Fischer's glucopyranosyl isocyanate **1**. NMR spectroscopic analysis of **1** allowed the unambiguous determination of the stereochemistry at the anomeric position. Synthetic applications of glucopyranosyl isocyanate **1** were examined by its reactions with amines and alcohols to furnish a wide variety of neoglycoconjugates.

Introduction

The first syntheses of glucopyranosyl isocyanate **1** and glucopyranosyl isothiocyanate **2** were reported by Fischer in 1914.[1] Although Fischer's glucopyranosyl isocyanate **1** appeared to promise a fruitful starting point for the development of new and important syntheses of compounds with biochemical interest,[2] little attention was paid to **1** during the next several decades. The long-standing lack of interest in **1** is in contrast to the attention that glucopyranosyl isothiocyanate **2** receives, which has secured its widespread use in glycoconjugate synthesis.[3] Such an unsatisfactory position for **1** may be partially due to the inconvenience associated with its preparation and handling. In fact, classical Fischer synthesis involves treatment of tetraacetylglucopyranosyl bromide with silver cyanate (AgOCN) in xylene, which is difficult to reproduce.[4] An improved synthesis using thoroughly dried AgOCN has been reported by Piskala, who also proposed the anomeric stereochemistry of **1** to have the β-configuration by conversion into the known *N*-β-D-glucopyranosyl urea.[5]

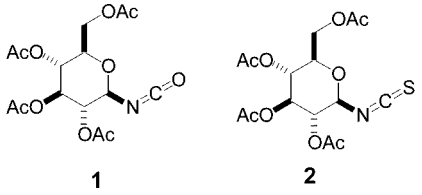
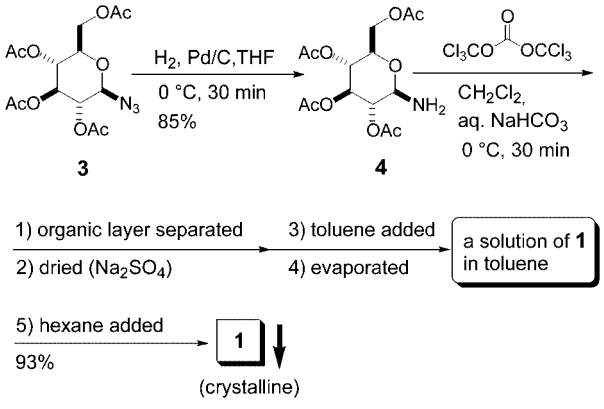


Figure 1. Glucopyranosyl isocyanate **1** and glucopyranosyl isothiocyanate **2**

In our ongoing project to study urea-tethered glycopeptide mimetics, we have developed two methods for the stereospecific synthesis of α- and β-D-glucopyranosyl isocyanates.[6] In these studies, glucopyranosyl isocyanates have been prepared either by oxidation of glucopyranosyl isocyanides or by elimination reaction of glucopyranosyl carbamates. The resulting glucopyranosyl isocyanates have been treated in situ with amines, and only stable urea glucosides were isolated as products. During these research efforts, our attention has focused on the isolation and characterization of glucopyranosyl isocyanates. We are now pleased to report a convenient method for the preparation and isolation of Fischer's glucopyranosyl isocyanate **1**, its characterization by NMR spectroscopy, and its application to the synthesis of neoglycoconjugates.

Results and Discussion

A readily accesible and high-yielding route to **1** is illustrated in Scheme 1, where we have employed the well-known reaction of amines with phosgene equivalents



Scheme 1. Synthesis of Fischer's glucopyranosyl isocyanate **1**

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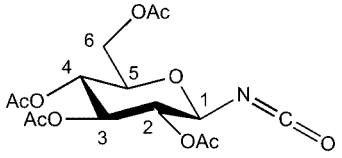
(COX_2) and aqueous sodium hydrogencarbonate ($NaHCO_3$) under Schotten–Baumann conditions. Catalytic hydrogenation of β-D-glucopyranosyl azide **3** (H_2, Pd/C, THF) gave β-D-glucopyranosylamine **4**, which was purified by recrystallization.[7] A solution of **4** and triphosgene ($Cl_3COCO_2CCl_3$) in dichloromethane (CH_2Cl_2) was stirred vigorously with aqueous $NaHCO_3$ at 0 °C for 30 min. The resulting organic layer was separated, dried (Na_2SO_4) and diluted with toluene.[8] Careful evaporation of the dichloromethane furnished a solution of **1** in toluene, which was treated successively with hexane to furnish crystalline **1** in excellent yield (93%).[9] It should be noted that the ready access to the starting material **4** and the commercial availability of triphosgene render this preparative method particularly attractive.

Crystalline **1** has physical data consistent with that of Fischer's isocyanate.[10] Table 1 shows the fully assigned NMR spectroscopic data obtained by using 2D techniques, such as H,H and C,H COSY spectra. The β-anomeric configuration is evident from the coupling constant ($J_{1,2}$ = 9.5 Hz), and the presence of the isocyanate group was confirmed by its characteristic signal in the ^{13}C NMR spectrum that appears at δ = 127.1 ppm. Because of the anomeric NCO-functionality, the chemical shifts of the anomeric proton and carbon atom resonate at δ = 4.81 and 82.7 ppm, respectively. These signals are similar to those of the glucopyranosyl isothiocyanate **2** ($\delta_{H\text{-}1}$ = 5.64 ppm, $J_{1,2}$ = 8.6 Hz; $\delta_{C\text{-}1}$ = 83.47 ppm, δ_{NCS} = 144.22 ppm).[11]

Having established a convenient gram-scale synthesis of **1**,[12] we now proceed to demonstrate that **1** is a useful synthon for the neoglycoconjugate synthesis. As revealed in Table 2, the reaction of **1** with amines, as well as alcohols, proceeded smoothly to afford the corresponding urea and carbamate glucosides in excellent yields (> 89%). The synthetic operation for urea glucosides (Entries 1–5)[13] is quite straightforward: simply treating amines (1.0 equiv.) with **1** (ca. 1.1 equiv.) in toluene at room temperature for 60 min completes the urea glucosidation. After quenching the excess of **1** by the addition of *N*,*N*-dimethylethylenediamine, the resulting reaction mixture was concentrated and then purified by chromatography. Even in the case of sterically hindered amines, such as diisopropylamine (Entry 5), the corresponding urea glucoside **9** was isolated in good yield (89%). When a low-nucleophilic amine, such as aniline, was employed (Entry 6), it was necessary to heat the reaction mixture at 70 °C for 50 min to furnish the urea glucoside **10** in 90% yield. Carbamate-tethered glucosides of terpene alcohols were also prepared, as represented in Entries 7–9. In each case, a slight excess amount of **1** (1.5–2.0 equiv.)

Table 1. Selected NMR spectroscopic data for **1**[a]

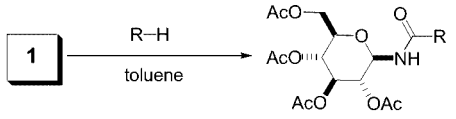


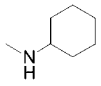
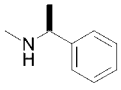
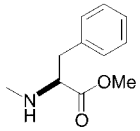
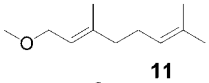
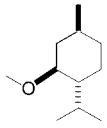
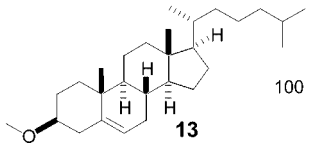
Glucopyranosyl isocyanate **1**

^1H NMR chemical shifts δ, ppm[b]						
H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
4.81 (d)	5.01(t)	5.20 (t)	5.12 (t)	3.77 (ddd)	4.15 (dd)	4.26 (dd)
^1H NMR coupling constants in Hz						
$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
9.5	9.5	9.5	9.5	2.0	5.0	12.5
^{13}C NMR chemical shifts δ, ppm						
C-1	C-2	C-3	C-4	C-5	C-6	NCO
82.7	72.4	72.4	67.8	74.0	61.5	127.1

[a] Measured in $CDCl_3$. [b] Multiplicities in parentheses.

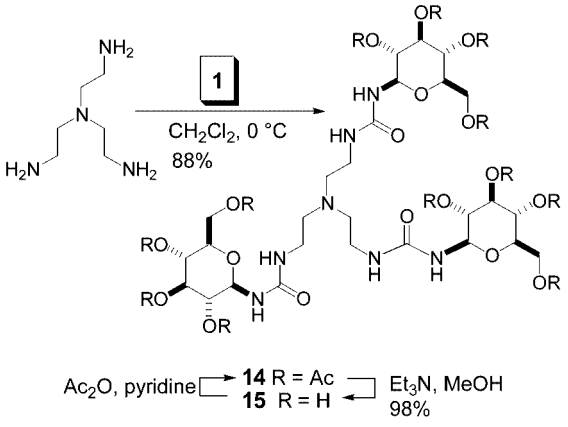
Table 2. Synthesis of urea and carbamate glucosides



Entry	Product (R =)	Temperature (°C)	Equiv. of **1**	% Yield
1	 **5**	r. t.	1.1	92
2	 **6**	r. t.	1.1	94
3	 **7**	r. t.	1.1	90
4	 **8**	r. t.	1.1	90
5	 **9**	r. t.	1.1	89
6	 **10**	70	1.1	90
7	 **11**	100	2.0	98
8	 **12**	100	1.5	91
9	 **13**	100	2.0	98

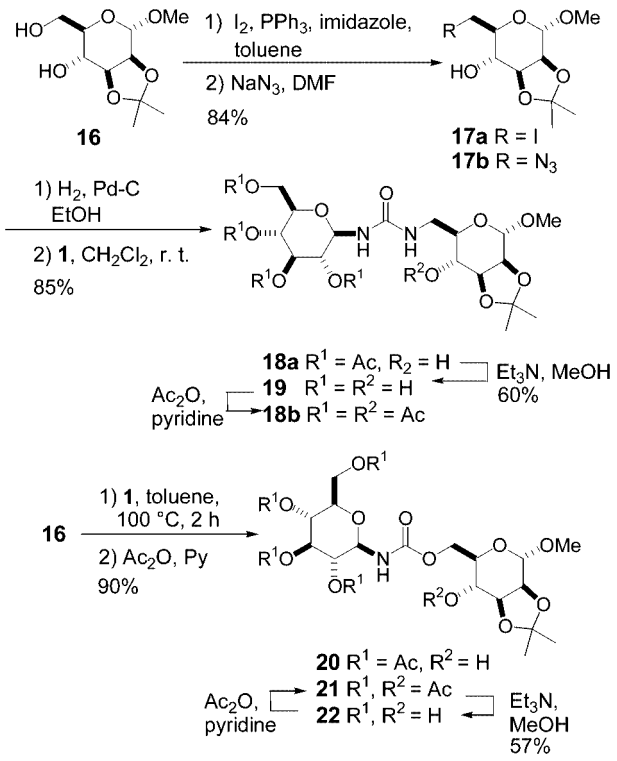
and heating the reaction mixture at 100 °C for 3 h resulted in satisfactory yields (> 91%).

Lindhorst reported the synthesis of thio-bridged cluster glycosides by the reaction of tris(2-aminoethyl)amine with glucopyranosyl isothiocyanate **2**.[14] In his synthesis, it was found that the base-catalyzed O→N migration of acyl groups from the acetyl-protected glucosyl isothiocyanates onto the amino termini of the trivalent amine decreased the yields. To avoid this problem, a large excess of **2** was employed under highly dilute conditions at refluxing temperature. We were interested in this report, because the reaction of **1** with a trivalent amine would offer an opportunity to compare the reactivity of glucopyranosyl isocyanate **1** with its isothiocyanate counterpart. In fact, reaction of **1** with tris(2-aminoethyl)amine in dichloromethane occurred at 0 °C to furnish an 88% yield of **14** without any problematic acyl migration, which reflects the higher reactive nature of **1** relative to **2**. Further deprotection of **14** gave water-soluble C_3-symmetric multivalent glucoside **15** (Scheme 2). It should be noted that no anomerization of the urea−glucoside linkage has ever been observed during the hydrolysis of acetates, which was confirmed by acetylation of **15** to afford **14**.



Scheme 2. Synthesis of a water-soluble C_3-symmetric multivalent glucoside

A further demonstration of the potency of **1** for the synthesis of neoglycoconjugates is shown in Scheme 3. The synthesis of two disaccharides, **18a** and **20**, has been achieved by the coupling reaction of **1** with two sugars prepared from the mannose derivative **16**[15] (Scheme 3). Selective iodination of the primary hydroxy group in **16** (I_2, PPh_3, imidazole),[16] followed by the displacement reaction of the resulting iodide **17a** with sodium azide in DMF, furnished **17b** in 84% yield for the two steps. Catalytic hydrogenation of azide **17b** gave the corresponding amine, which was subsequently treated with **1** in CH_2Cl_2 at room temperature to furnish the urea-tethered disaccharide **18a** in 85% yield. The carbamate-tethered disaccharide **21** was also prepared by heating a reaction mixture of **1** and **16** in toluene at 100 °C for 2 h to afford **20**, the acetylation of which gave rise to **21** in 90% yield.[17] The acetate groups in **18a** and **21**



Scheme 3. Synthesis of urea- and carbamate-tethered disaccharides

were removed by treatment with triethylamine in methanol. Acetylation of the resulting free sugars **19** and **22** yielded the acetates **18b** and **21**, which confirmed that deprotection could be carried out without affecting the urea- and carbamate-glucoside linkages.

Conclusion

In summary, we have established a simple and practical method for the preparation of Fischer's glucopyranosyl isocyanate **1** in a crystalline form. The structure of **1** was fully characterized using NMR spectroscopy techniques. The highly reactive nature of **1** appears to promise its great applicability in the synthesis of urea- and carbamate-tethered neoglycoconjugates, which may find widespread use in the combinatorial library synthesis as well as in the pharmaceutical industries.

Experimental Section

General Procedures: Melting points were recorded with a micro melting point apparatus and are not corrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Infrared spectra were recorded with a JASCO FT/IR-8300 spectrophotometer and are reported in wavenumbers (cm^{-1}). Proton nuclear magnetic resonance (^1H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded with Varian Gemini-2000 spectrometers. ^1H NMR chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS, δ = 0.00 ppm in $CDCl_3$), CHD_2OH (δ = 3.31 ppm in CD_3OH), and

*t*BuOH (δ = 1.24 ppm in D_2O) as internal standards. Data are reported as follows; chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, sext = sextet, br. = broadened, m = multiplet), coupling constants (*J*, given in Hz). ^{13}C NMR chemical shifts (δ) are recorded in parts per million (ppm) relative to $CDCl_3$ (δ = 77.0), CD_3OD (δ = 49.0), and *t*BuOH (δ = 30.29, in D_2O) as internal standards. High-resolution mass spectra (HRMS) are reported in *m*/*z*. Elemental analyses were performed by the Analytical Laboratory at the Graduate School of Bioagricultural Sciences, Nagoya University. For thin-layer chromatography (TLC) analysis, we used Merck precoated TLC plates (silica gel 60 F_{254}, 0.25 mm). Column chromatography was performed on silica gel (Silica gel 60) supplied by E. Merck. Preparative TLC separation was made on plates prepared with a 2-mm layer of silica gel (Silica gel PF_{254}) obtained from E. Merck. Reactions were run under nitrogen when the reactions were sensitive to moisture or oxygen. Dichloromethane was dried with 3-Å molecular sieves, and toluene was stored over 4-Å molecular sieves. Pyridine and triethylamine were stored over anhydrous KOH. All other commercially available reagents were used as received.

Preparation of 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Isocyanate (1): Triphosgene (4.30 g, 14.5 mmol) was added in a single portion to a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (**4,** 5.03 g, 14.5 mmol) in CH_2Cl_2 (160 mL) and saturated aqueous $NaHCO_3$ (100 mL). The biphasic mixture was stirred vigorously in an ice bath for 30 min. The organic layer was separated, washed with brine and dried (Na_2SO_4). Toluene (20 mL) was added to the extracts, and the CH_2Cl_2 was removed carefully by evaporation (water bath temperature: 30 °C; 50 Torr). Hexane was added slowly to the resulting toluene solution, and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isocyanate (**1**) crystallized as a white solid (5.05 g, 93%). M.p. 118−120 °C. $[\alpha]_D^{29} = -6.7$ (*c* = 1.00, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3392, 2944, 2260, 1751, 1539, 1436, 1370, 1235, 1038, 908, 602 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.02 (s, 3 H, Ac), 2.04 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 3.77 (ddd, *J* = 9.5, 5.0, 2.0 Hz, 1 H, H-5), 4.15 (dd, *J* = 12.5, 2.0 Hz, 1 H, H-6), 4.26 (dd, *J* = 12.5, 5.0 Hz, 1 H, H-6), 4.81 (d, *J* = 9.5 Hz, 1 H, H-1), 5.01 (t, *J* = 9.5 Hz, 1 H, H-2), 5.12 (t, *J* = 9.5 Hz, 1 H, H-4), 5.20 (t, *J* = 9.5 Hz, 1 H, H-3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.37, 20.40, 20.6, 61.5, 67.8, 72.4, 74.0, 82.7, 127.1, 169.3, 169.4, 170.2, 170.7 ppm. $C_{15}H_{19}NO_{10}$ (373.3): calcd. C 48.26, H 5.13, N 3.75; found C 48.25, H 5.08, N 3.74.

General Method for the Preparation of the Glucopyranosyl Urea: Glucopyranosyl isocyanate **1** (62 mg, 0.17 mmol, 1.1 equiv.) was added in a single portion to a solution of cyclohexylamine (15 mg, 0.15 mmol) in toluene (1.0 mL). After stirring at room temperature for 2 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (10 μl, 0.08 mmol) and then concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc/hexane, 1:1) to give **5** (66 mg, 92%) as a white solid.

Methyl (2*S*)-*N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)aminocarbonyl)-2-phenylalaninate(8): Glucopyranosyl isocyanate **1** (63 mg, 0.17 mmol, 1.1 equiv.) was added in a single portion to a solution of L-phenylalanine methyl ester hydrochloride (33 mg, 0.15 mmol) and diisopropylethylamine (40 μL, 0.23 mmol, 1.5 equiv.) in toluene (2.0 mL). After stirring at room temperature for 2 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (10 μL, 0.08 mmol) and then concentrated under reduced pressure. Purification of the resulting residue by silica gel chromatography (EtOAc/hexane, 1:1) afforded **8** (76 mg, 90%) as a white solid. M.p. 75- 77 °C. $[\alpha]_D^{21} = +32.1$ (*c* = 1.12, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3385, 1655, 1560, 1231 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.01 (s, 3 H, s, Ac), 2.03 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 3.08 (2H), 3.71 (s, 3 H), 3.79 (ddd, *J* = 10.0, 4.5, 2.0 Hz, 1 H), 4.07 (dd, *J* = 12.5, 2.0 Hz, 1 H), 4.33 (dd, *J* = 12.5, 4.5 Hz, 1 H), 4.74 (q, *J* = 7.0 Hz, 1 H, 6.0 Hz), 4.90 (t, *J* = 9.5 Hz, 1 H), 5.06 (t, *J* = 9.5 Hz, 1 H), 5.12 (t, *J* = 9.5 Hz, 1 H), 5.18- 5.24 (1 H, br), 5.30 (d, *J* = 9.5 Hz, 1 H), 5.42- 5.50 (br., 1 H), 7.08- 7.12 (2H), 7.24- 7.32 (3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.4, 20.6, 38.1, 52.2, 54.0, 61.8, 68.3, 70.3, 72.9, 73.1, 80.0, 127.1, 128.5, 129.3, 136.0, 155.9, 169.7, 170.0, 170.8, 171.0, 172.9 ppm. $C_{25}H_{32}N_2O_{12}$ (552.5): calcd. C 54.34, H 5.84, N 5.07; found C 54.36, H 5.84, N 5.10.

2,3,4,6-Tetra-*O*-acetyl-*N*-(benzylaminocarbonyl)-β-D-glucopyranosylamine (10): M.p. 92−94 °C. $[\alpha]_D^{24} = -19.1$ (*c* = 0.89, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3394, 1670, 1560, 1231 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.02 (s, 3 H, s, Ac), 2.03 (s, 3 H, Ac), 2.04 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 3.84 (ddd, *J* = 9.5, 4.5, 2.0 Hz, 1 H), 4.08 (dd, *J* = 12.5, 2.0 Hz, 1 H), 4.32 (dd, *J* = 12.5, 4.5 Hz, 1 H), 4.94 (t, *J* = 9.5 Hz, 1 H), 5.08 (t, *J* = 9.5 Hz, 1 Hv), 5.28 (dd, 1 H, *J* = 10, 9.5 Hz), 5.32 (d, *J* = 9.5 Hz, 1 H), 6.14 (d, *J* = 9.5 Hz, 1 H), 7.07 (1H), 7.28 (4H), 7.52 (1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.44, 20.47, 20.56, 20.63, 61.9, 68.3, 70.3, 72.9, 73.3, 79.8, 120.5, 124.0, 129.2, 137.9, 154.7, 169.8, 170.0, 170.9, 171.1 ppm. $C_{21}H_{26}N_2O_{10}$ (466.4): calcd. C 54.07, H 5.62, N 6.01; found C 54.08, H 5.55, N 5.73.

General Method for the Preparation of the Glucopyranosyl Carbamate: A solution of geraniol (29 mg, 0.19 mmol) and glucopyranosyl isocyanate **1** (140 mg, 0.38 mmol, 2.0 equiv.) in toluene (1.5 mL) was heated at 100 °C for 2 h. The reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (20 μL, 0.16 mmol) and then concentrated under reduced pressure. Purification of the resulting residue by silica gel chromatography (EtOAc/hexane, 1:2) afforded **11** (97 mg, 98%) as a colorless oil.

2,3,4,6-Tetra-*O*-acetyl-*N*-(geranylcarbonyl)-β-D-glucopyranosylamine (11): $[\alpha]_D^{26} = -0.5$ (*c* = 1.0, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3340, 1752, 1541 1232 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.60-2.10 (13H), 2.02 (s, 3 H), 2.03 (s, 3 H), 2.06 (s, 3 H), 2.09 (s, 3 H), 3.80 (br. d, 1 H), 4.10 (dd, *J* = 12.5, 2.0 Hz, 1 H), 4.32 (dd, *J* = 12.5, 4.5 Hz, 1 H), 4.61 (br. d, 2 H), 4.92 (d, *J* = 9.5 Hz, 1 H), 5.04 (t, *J* = 9.5 Hz, 1 H), 5.08 (t, *J* = 9.5 Hz, 1 H), 5.08 (1H), 5.30 (t, *J* = 9.5 Hz, 1 H), 5.31 (1H), 5.52 (d, *J* = 9.5 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.1, 17.3, 20.2, 20.3, 20.4, 25.4, 26.0, 39.2, 61.5, 62.2, 68.9, 70.0, 72.8, 73.0, 80.5, 118.0, 123.5, 131.6, 142.1, 155.7, 169.4, 169.8, 170.4, 170.5 ppm. $C_{25}H_{37}NO_{11}$: calcd. C 56.92, H 7.07, N 2.65; found C 56.92, H 7.20, N 2.80.

2,3,4,6-Tetra-*O*-acetyl-*N*-[(1*S*,2*R*,5*S*)-(+)-menthylcarbonyl]-β-D-glucopyranosylamine (12): M.p. 130−132 °C. $[\alpha]_D^{28} = +39.6$ (*c* = 1.05, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3357, 1756, 1533 1236 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.75- 1.70 (17H), 2.02 (s, 3 H), 2.04 (s, 6 H), 2.10 (s, 3 H), 3.80 (br. d, 1 H), 4.10 (br. d, 1 H), 4.32 (dd, *J* = 12.5, 4.5 Hz, 1 H), 4.58 (td, *J* = 11.0, 5.0 Hz, 1 H), 4.92 (d, *J* = 9.5 Hz, 1 H), 5.05 (t, *J* = 9.5 Hz, 1 H), 5.08 (t, *J* = 9.5 Hz, 1 H), 5.30 (t, *J* = 9.5 Hz, 1 H), 5.40 (d, *J* = 9.5 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.7, 20.2, 20.3, 20.5, 21.8, 23.7, 26.5, 31.2, 34.0, 41.0, 47.0, 61.5, 68.0, 70.3, 72.7, 73.3, 75.6, 80.7, 155.3, 169.5, 169.9, 170.55, 170.61 ppm. $C_{25}H_{39}NO_{11}$ (529.6): calcd. C 56.70, H 7.42, N 2.64; found C 56.71, H 7.56, N 2.79.

2,3,4,6-Tetra-*O*-acetyl-*N*-(cholesterolylcarbonyl)-β-D-glucopyranosylamine (13): M.p. 182−184 °C. $[\alpha]_D^{23} = -12.7$ (*c* = 1.05, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3431, 1755, 1540, 1234 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.68−2.40 (43 H), 2.02 (s, 3 H, Ac), 2.03

(s, 3 H), 2.07 (s, 3 H), 2.09 (s, 3 H), 3.80 (ddd, J = 12.5, 3.5, 2.0 Hz, 1 H), 4.10 (dd, J = 12.5, 2.0 Hz, 1 H), 4.32 (dd, J = 12.5, 3.5 Hz, 1 H), 4.50 (m, 1 H), 4.92 (d, J = 9.5 Hz, 1 H), 5.02 (dd, J = 10.0, 9.5 Hz, 1 H), 5.07 (t, J = 9.5 Hz, 1 H), 5.30 (t, J = 9.5 Hz, 1 H), 5.36 (1H), 5.44 (d, J = 9.5 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.7, 18.6, 19.1, 20.4, 20.5, 20.6, 20.9, 22.4, 22.7, 23.7, 24.1, 27.8, 27.9, 28.1, 31.7, 31.8, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 50.0, 56.0, 56.6, 61.6, 68.1, 70.2, 72.8, 73.3, 75.5, 80.7, 122.8, 139.5, 155.1, 169.6, 170.0, 170.7 ppm. $C_{42}H_{65}NO_{11}$ (760.0): calcd. C 66.02, H 8.51, N 1.88; found C 66.01, H 8.74, N 1.94.

Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)ureaethyl]amine (14): Glucopyranosyl isocyanate **1** (107 mg, 0.28 mmol) was added in a single portion to a solution of tris(2-aminoethyl)amine (12 mg, 0.08 mmol) in CH_2Cl_2 (4.0 mL). After stirring at room temperature for 2 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (10 μL, 0.08 mmol) and then concentrated under reduced pressure. Purification of the resulting residue by silica gel chromatography (EtOAc/ethanol, 20:1 and 10:1) afforded **14** (97 mg, 90%) as a white solid. M.p. 147−149 °C. $[\alpha]_D^{24}$ = +24.8 (c = 0.86, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3406, 1663, 1559 1233 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.04 (s, 9 H), 2.06 (s, 18 H), 2.08 (s, 9 H), 2.54 (br., 3 H), 2.74 (br., 3 H), 2.93 (br., 3 H), 3.32 (br., 3 H), 3.94 (m, 3 H), 4.18 (6H), 5.05 (t, J = 9.5 Hz, 3 H), 5.25 (t, J = 9.5 Hz, 3 H), 5.32 (t, J = 9.5 Hz, 3 H), 5.40 (t, J = 9.5 Hz, 3 H), 6.00 (6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.48, 20.56, 20.57, 20.7, 39.9, 57.1, 62.4, 68.8, 70.1, 73.0, 73.8, 80.4, 157.2, 169.7, 170.1, 170.7, 170.8 ppm. FAB-MS: m/z = 1266 [M$^+$ + H] ppm. $C_{51}H_{75}N_7O_{30}$ (1266.2): calcd. C 48.38, H 5.97, N 7.74; found C 48.38, H 6.07, N 7.53.

Tris[2-(β-D-glucopyranosyl)ureaethyl]amine (15): A solution of **14** (51 mg) and Et_3N (0.1 mL) in MeOH (1.0 mL) was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in water, and then precipitated by the addition of acetone to give **15** (30 mg, 98%) as a white solid. $[\alpha]_D^{24}$ = −19.9 (c = 0.82, H_2O). IR (KBr): $\tilde{\nu}_{max.}$ = 1653, 1564 1274 cm^{-1}. ^{1}H NMR (300 MHz, D_2O): δ = 2.68 (t, J = 6.5 Hz, 6 H), 3.26 (br., 6 H), 3.35 (t, J = 9.5 Hz, 3 H), 3.38 (t, J = 9.5 Hz, 3 H), 3.50 (m, 3 H), 3.54 (t, J = 9.5 Hz, 3 H), 3.70 (dd, 3 H, J = 12.5, 5.5 Hz), 3.88 (dd, 3 H, J = 12.5, 1.5 Hz), 4.83 (3H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 38.3, 54.3, 61.3, 70.2, 70.8, 72.5, 73.9, 78.7, 160.7 ppm. HRMS (FAB) calcd. for $C_{27}H_{52}N_7O_{18}$ [M + H]$^+$ 762.3369, found 762.3381.

Methyl 2,3-D-Isopropylidene-6-deoxy-6-azido-α-D-mannopyranoside (17b): A solution of **16** (0.50 g, 2.14 mmol), triphenylphosphane (0.84 g, 3.21 mmol, 1.5 equiv.), imidazole (0.44 g, 6.44 mmol, 3.0 equiv.) and iodine (0.76 g, 2.99 mmol, 1.4 equiv.) in toluene (50 mL) was heated at 70 °C for 2.5 h with vigorous stirring. The reaction mixture was washed with aq. $NaHCO_3$, water and brine, dried (Na_2SO_4), and then concentrated. The resulting residue was treated with diethyl ether and the insoluble triphenylphosphane oxide was removed by filtration. Concentration of the filtrate and purification of the residue by silica gel chromatography furnished **17a** (698 mg, 95%) as a colorless oil.

A solution of **17a** (580 mg, 1.7 mmol) and sodium azide (220 mg, 3.4 mmol, 2.0 equiv.) in DMF (20 mL) was heated at 80 °C for 30 min. The reaction mixture was diluted with diethyl ether and then washed with water. The organic layer was dried (Na_2SO_4) and then concentrated under reduced pressure to afford the residue, which was purified by silica gel chromatography to give **17b** (386 mg, 88%) as a colorless oil. $[\alpha]_D^{28}$ = +10.6 (c = 1.00, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 2102 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (s, 3 H), 1.54 (s, 3 H), 3.44 (s, 3 H), 3.50- 3.64 (m, 3 H), 3.73 (ddd, J = 10.5, 6.0, 4.0 Hz, 1 H), 4.11 (t, J = 6.0 Hz, 1 H), 4.15 (d, J = 6.0 Hz, 1 H), 4.94 (s, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.9, 27.8, 51.6, 55.1, 69.2, 70.1, 75.5, 78.4, 98.3, 109.8 ppm. $C_{10}H_{17}N_3O_5$ (259.3): calcd. C 46.33, H 6.61, N 16.21; found C 46.33, H 6.84, N 16.01.

Methyl 2,3-*O*-Isopropylidene-6-deoxy-6-[(*N*′-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)ureido]-α-D-mannnopyranoside (18a): A solution of **17b** (54 mg, 0.21 mmol) and palladium on carbon (20%, 10 mg) in ethanol (3.0 mL) was stirred vigorously for 30 min under an atmospheric pressure of hydrogen gas. Concentration of the mixture afforded the crude amine, which was treated subsequently with a solution of **1** (85 mg, 0.23 mmol, 1.1 equiv.) in CH_2Cl_2 (2.0 mL). After being stirred at room temperature for 2 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (10 μL, 0.08 mmol). The palladium on carbon was removed by filtration, and the filtrate was concentrated under reduced pressure. Purification of the resulting residue by silica gel chromatography (EtOAc/hexane, 2:1) afforded **18a** (107 mg, 85%) as a white solid. M.p. 105−106 °C. $[\alpha]_D^{26}$ = −34.9 (c = 1.00, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 1684, 1560, 1229 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.35 (s, 3 H), 1.48 (s, 3 H), 2.01 (s, 3 H), 2.03 (s, 3 H), 2.04 (s, 3 H), 2.08 (s, 3 H), 3.18 (m, 1 H), 3.35 (s, 3 H), 3.39 (m, 1 H), 3.50 (dt, J = 10.0, 2.5 Hz, 1 H), 3.82 (ddd, J = 10.0, 4.0, 2.0 Hz, 1 H), 3.88 (br., 1 H), 4.08 (dd, J = 12.5, 2.0 Hz, 1 H), 4.13 (d, J = 6.0 Hz, 1 H), 4.15 (dd, J = 7.5, 5.5 Hz, 1 H), 4.33 (dd, J = 12.5, 4.0 Hz, 1 H), 4.86 (s, 1 H), 4.97 (t, J = 9.5 Hz, 1 H), 5.08 (t, J = 9.5 Hz, 1 H), 5.13 (t, J = 9.5 Hz, 1 H), 5.15 (1H), 5.30 (t, J = 9.5 Hz, 1 H), 5.52 (br. d, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.4, 20.5, 20.6, 26.0, 28.0, 40.4, 54.9, 61.7, 68.1, 69.0, 70.3, 73.0, 75.6, 80.0, 98.5, 109.3, 158.3, 169.7, 170.0, 170.8 ppm. $C_{25}H_{38}N_2O_{15}$ (606.6): calcd. C 49.50, H 6.31, N 4.62; found C 49.50, H 6.56, N 4.59.

Methyl 2,3-*O*-Isopropylidene-6-deoxy-6-[(*N*′-β-D-glucopyranosyl)-ureido]-α-D-mannnopyranoside (19): A solution of **18a** (150 mg) and Et_3N (0.5 mL) in MeOH (5 mL) was stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in methanol, and then precipitated by the addition of diethyl ether to give **19** (66 mg, 60%) as a white solid. M.p. 195−197 °C. $[\alpha]_D^{27}$ = −3.9 (c = 1.00, CH_3OH). IR (KBr): $\tilde{\nu}_{max.}$ = 1660, 1566, 1222 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.32 (s, 3 H), 1.46 (s, 3 H), 3.14 (t, J = 9.5 Hz, 1 H), 3.25 (t, J = 9.5 Hz, 1 H), 3.32−3.42 (4H), 3.38 (s, 3 H), 3.47 (m, 1 H), 3.55 (dd, J = 14.0, 3.0 Hz, 1 H), 3.63 (dd, J = 12.0, 5.5 Hz, 1 H), 3.81 (dd, J = 12.0, 2.5 Hz, 1 H), 3.99 (dd, J = 7.5, 5.5 Hz, 1 H), 4.10 (d, J = 5.5 Hz, 1 H), 4.74 (d, J = 9.5 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 26.5, 28.4, 41.7, 55.4, 62.8, 70.3, 71.3, 71.5, 74.3, 77.1, 79.1, 79.2, 79.5, 82.8, 99.6, 110.5, 161.1 ppm. HRMS (FAB) calcd. for $C_{17}H_{31}N_2O_{11}$ [M + H]$^+$ 439.1928, found 439.1941.

Methyl 4-Acetyl-2,3-*O*-isopropylidene-6-deoxy-6-[(*N*′-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)ureido]-α-D-mannnopyranoside (18b): Acetic anhydride (0.5 mL) was added to a solution of **19** (16 mg) in pyridine (0.5 mL). After being stirred at room temperature for 10 h, the reaction mixture was concentrated under reduced pressure. Purification of the resulting residue by silica gel chromatography (EtOAc/hexane, 2:1) afforded **18b** (22 mg, 90%) as a white solid. M.p. 107−109 °C. $[\alpha]_D^{26}$ = +5.4 (c = 1.83, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 1669, 1558, 1229 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.34 (s, 3 H), 1.55 (s, 3 H), 2.01 (s, 3 H), 2.03 (s, 3 H), 2.07 (s, 3 H), 2.07 (s, 3 H), 2.10 (s, 3 H), 3.28 (dt, J = 15.0, 6.0 Hz, 1 H), 3.37 (s, 3 H), 3.46 (ddd, J = 15.0, 6.0, 2.5 Hz, 1 H), 3.70 (ddd, J = 10.5, 5.5, 2.5 Hz, 1 H), 3.80 (ddd, J = 10.0, 4.0, 2.0 Hz, 1 H), 4.08

(dd, J = 12.5, 2.0 Hz, 1 H), 4.12 (d, J = 5.5 Hz, 1 H), 4.18 (dd, J = 7.5, 5.5 Hz, 1 H), 4.31 (dd, J = 12.5, 4.0 Hz, 1 H), 4.90 (dd, J = 10.5, 7.5 Hz, 1 H), 4.90 (t, J = 9.5 Hz, 1 H), 4.94 (s, 1 H), 5.02 (t, J = 6.0 Hz, 1 H), 5.06 (t, J = 9.5 Hz, 1 H), 5.15 (t, J = 9.5 Hz, 1 H), 5.31 (t, J = 9.5 Hz, 1 H), 5.40 (br. d, J = 9.5 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5, 20.7, 20.9, 26.3, 27.6, 40.2, 55.1, 61.8, 66.8, 68.3, 70.1, 70.4, 73.0, 75.7, 75.8, 80.1, 98.0, 109.9, 156.3, 169.6, 169.9, 170.1, 170.6, 171.2 ppm. $C_{27}H_{40}N_2O_{16}$ (648.6): calcd. C 50.00, H 6.22, N 4.32; found C 50.06, H 6.36, N 4.19.

Methyl 4-*O*-Acetyl-2,3-*O*-isopropylidene-6-*O*-[(*N*-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)carbamoyl]-α-D-mannnopyranoside (21): A mixture of **16** (25 mg, 0.11 mmol) and **1** (60 mg, 0.16 mmol, 1.5 equiv.) in toluene (1.0 mL) was heated at 100 °C for 2 h. The reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (20 μL, 0.16 mmol) and then concentrated under reduced pressure to afford the residue, which was dissolved in acetic anhydride (0.5 mL) and pyridine (0.5 mL). After being stirred at room temperature overnight, the reaction mixture was concentrated. Purification by silica gel chromatography (EtOAc/hexane, 1:1) gave **21** (64 mg, 92%) as a white solid. M.p. 86−88 °C. $[\alpha]_D^{26}$ = +6.8 (c = 1.00, $CHCl_3$). IR (KBr): $\tilde{\nu}_{max.}$ = 3343, 1752, 1534, 1233 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.35 (s, 3 H), 1.57 (s, 3 H), 2.00 (s, 3 H), 2.02 (s, 3 H), 2.05 (s, 3 H), 2.07 (s, 3 H), 2.08 (s, 3 H), 3.37 (s, 3 H), 3.77 (1H), 3.79 (ddd, J = 10.5, 5.5, 2.0 Hz, 1 H), 4.09 (dd, J = 12.5, 2.0 Hz, 1 H), 4.04−4.15 (m, 2 H), 4.18 (dd, J = 12, 5 Hz, 1 H), 4.24−4.31 (m, 2 H), 4.89 (t, 1 H, t, J = 9.5 Hz) Hz, 4.93 (s, 1 H), 4.94−5.04 (2H), 5.05 (t, J = 9.5 Hz, 1 H), 5.28 (t, J = 9.5 Hz, 1 H), 5.61 (br. d, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.4, 20.6, 20.8, 20.9, 26.2, 27.5, 55.0, 60.3, 61.6, 63.6, 66.1, 68.0, 69.1, 69.9, 72.8, 73.2, 75.6, 75.8, 80.8, 98.0, 110.0, 155.2, 169.5, 169.7, 170.0, 170.7, 170.8 ppm. $C_{27}H_{39}NO_{17}$ (649.6): calcd. C 49.92, H 6.05, N 2.16; found C 49.84, H 6.19, N 1.91.

Methyl 6-*O*-[(*N*-β-D-Glucopyranosyl)carbamoyl]-2,3-*O*-isopropylidene-α-D-mannnopyranoside (22): A mixture of **21** (90 mg) in MeOH (5 mL) and Et_3N (0.5 mL) was stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in 2-propanol and then precipitated by the addition of diethyl ether to give **22** (35 mg, 57%) as a white solid. M.p. 118−121 °C. $[\alpha]_D^{28}$ = +11.1 (c = 1.00, CH_3OH). IR (KBr): $\tilde{\nu}_{max.}$ = 1713, 1546 1247 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.32 (s, 3 H), 1.48 (s, 3 H), 3.39 (s, 3 H), 3.51 (dd, J = 10.5, 7.0 Hz, 1 H), 3.64 (m, 2 H), 3.81 (br. d, J = 12.0 Hz, 1 H), 4.00 (dd, J = 7.0, 5.5 Hz, 1 H), 4.11 (d, J = 5.5 Hz, 1 H), 4.20 (dd, J = 12.0, 6.0 Hz, 1 H), 4.38 (dd, J = 12.0, 1.5 Hz, 1 H), 4.68 (d, J = 9.5 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $[D_4]CD_3OD$): δ = 26.4, 28.3 55.4, 62.7, 65.4, 69.6, 70.2, 71.4, 73.8, 77.1, 79.0, 79.4, 80.0, 83.8, 99.5, 110.6, 159.0 ppm. HRMS (FAB) calcd for $C_{17}H_{30}NO_{12}$ $[M + H]^+$ 440.1768, found 440.1786.

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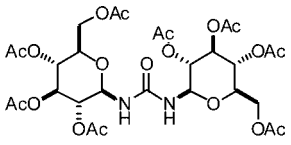

i

[9] This procedure is a dramatic improvement over our previous report in ref.[6b], where many attempts to isolate crystalline **1** failed. In the previous unsuccessful trials, the extracted solution of **1** in dichloromethane was concentrated. The resulting crude glucopyranosyl isocyanate might be contaminated with unreacted triphosgen, which would interfere with crystallization. Similar problems were noted in the synthesis of peptide isocyanates. See: J. S. Nowick, D. L. Holmes, G. Noronha, E. M. Smith, T. M. Nguyen, S.-L. Huang, *J. Org. Chem.* **1996**, *61*, 3929−3934.
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