Versatile Functionalization of Carbohydrate Hydroxyl Groups through Their *O*-Cyanomethyl Ethers

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O-Cyanomethyl ethers of carbohydrates are shown to be versatile intermediates for the preparation of sugar amines, carboxylic acids, amides, and amidine salts. This methodology for the functionalization of carbohydrates can thus provide a new array of analogs for the study of carbohydrate binding proteins. In addition, the resulting *O*-aminoethyl and *O*-carboxymethyl carbohydrates can be coupled to amino acids under standard conditions used in solid-phase peptide synthesis, providing a method for the construction of glycopeptides in which the carbohydrate moiety can be linked through any of its hydroxyl groups to the *C*- or the *N*-terminus of a given peptide.

Introduction

The surface of animal cells is covered with glycoproteins and glycolipids that are directly involved in intercellular recognition. They can be sites of attachment for bacteria, toxins, viruses, essential ligands in fertilization, antigenic determinants, and ligands in cell−cell adhesion.[1] The oligosaccharides controlling these events can be large, but the actual size of the epitope that is recognized is usually 2−4 sugar residues in size. A notable feature is that binding is often weak, with dissociation constants rarely below the micromolar range and much more commonly in the millimolar range. They can nevertheless function as efficient adhesion sites since the interactions can be polyvalent, leading to a net high-affinity interaction.[1]

Inhibitors of carbohydrate−protein binding clearly have potential as pharmaceutical agents since in principle they could inhibit the binding events that lead to disease. Because the association constants for monovalent carbohydrate−protein binding may be lower than desired, however, this situation presents a major challenge to medicinal chemists: the affinity of a useful inhibitor must be improved by orders of magnitude over the natural and supposedly already near-optimum oligosaccharide ligand.

Many groups have worked in the area of oligosaccharide analog synthesis with the hopes of improving affinities, and although an enormous amount of very difficult and very elegant synthesis has been performed, usually only marginal (*ca.* 10-fold) improvements were achieved.[2] Exceptions include a 100-fold improvement in the affinity of a trisaccharide binding to an antibody[3] and the rational design of a sialic acid based inhibitor for influenza hemaglutinin[4] (10000-fold improvement) based on a crystal structure containing bound ligand. In the more common situations where such structural information is unavailable, the development of inhibitors of carbohydrate binding proteins still relies on the synthetic preparation of a large number of analogs of the natural cell surface glycoconjugates.

A general characteristic of the mode of binding of oligosaccharides to proteins is summarized schematically in Figure 1, part A. Many protein-combining sites are cleft or groove-like and bind the oligosaccharide on one "side".[5] Critical hydrogen-bonding interactions involve several of the sugar OH groups which become involved in intricate H-bonding networks that can include water molecules. van der Waals contacts with non-hydroxylated surfaces on the oligosaccharides also frequently occur. The purpose of showing Figure 1, however, is to illustrate that there must be protein structure adjacent to the combining site that is not in contact with the carbohydrate ligand, as well as ligand hydroxyl groups that are not involved in binding. In principle, molecular structure could be added to the ligand through these OH groups, and if this structure formed favorable interactions with the protein surface, then the affinity of the ligand should be enhanced.

Since the features of the protein structure adjacent to the combining site are generally not known, a large number of oligosaccharide derivatizations will likely be necessary to discover added structures that will enhance affinity. Any systematic attempt to prepare such "enhanced" oligosaccharide ligands must therefore have, as its base, a versatile and robust methodology for the functionalization and modification of a given carbohydrate structure. In this paper we propose that *O*-cyanomethyl derivatives of oligosaccharides can provide such a base.

Results and Discussion

The reason for selecting the *O*-cyanomethyl group for the functionalization of sugar hydroxyl groups is based on the rich chemistry of nitriles, key elements of which are summarized in Figure 2. This single group should be amenable to facile reduction to yield primary amines and hydrolysis to yield carboxylic acids. Amide bond

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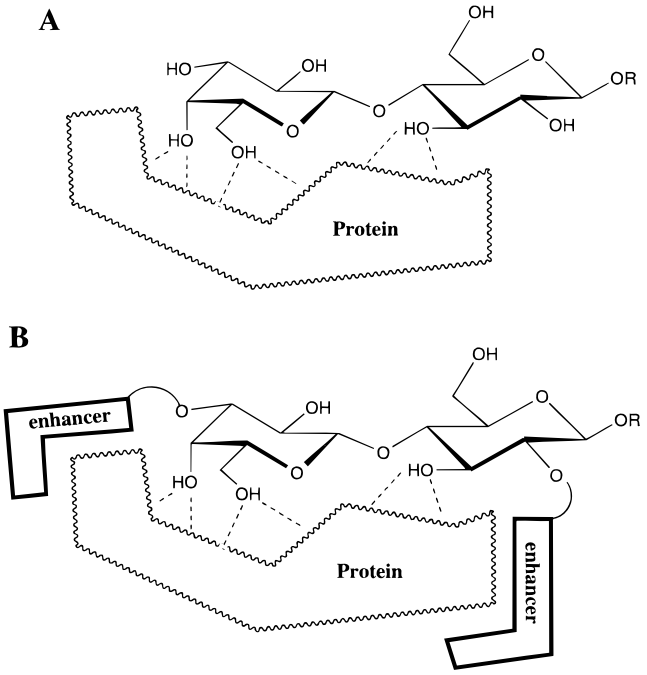

Figure 1. (A) Schematic representation of a disaccharide bound to a protein indicating the OH groups that are directly involved in complex formation. (B) Molecular structure has been added to the ligand to enhance the affinity of the complex by interaction with protein structure adjacent to the combining site.

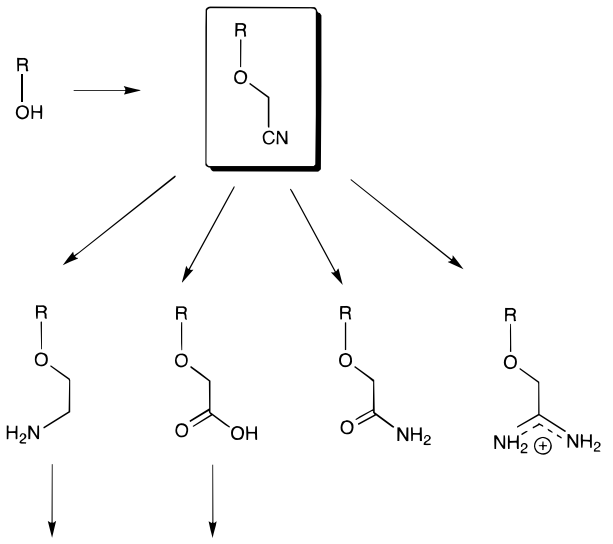

Figure 2. Versatile transformations of the *O*-cyanomethyl group.

formation with commercial acids and amines, including protected amino acids, could therefore yield large arrays of analogs for testing. In addition, the cyano group can be converted to amide derivatives and amidines. To evaluate whether such functional group manipulations are compatible with the general chemical manipulations of carbohydrate synthesis, and the presence of polyhydroxylated species, two monosaccharides (the acetylated galactopyranoside **2** and the benzylated glucopyranoside **10**) carrying the most common *O*-protecting groups in carbohydrate chemistry have been chosen at this stage as initial models.

Introduction of the *O*-Cyanomethyl Group. 2,4,6-Tri-*O*-acetyl-3-*O*-(cyanomethyl)galactopyranoside **2** (Scheme 1) was prepared in 51% yield by *O*-alkylation of regioselectively stannylated galactopyranoside **1**[6] with bromoacetonitrile, followed by acetylation with Ac_2O/py. 2,3,6-Tri-*O*-benzyl-4-*O*-(cyanomethyl)-β-D-glucopyranoside **10** (Scheme 2) was obtained by *O*-alkylation of the sodium salt of octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**9**)[7] in acetonitrile at −20 °C in 82% yield. Other combinations of solvents (DMF, THF) or higher temperatures produced a drastic decrease in the yield.

Conversion of Cyanomethyl Ethers to Amines and Carboxylic Acids. The borane−methyl sulfide complex is one of the most efficient reagents for the reduction of nitriles[8] and has been recently used in the carbohydrate field for the reduction of 6-deoxy-6-cyanocyclodextrins.[9] In our hands, BH_3·Me_2S reduced acetylated nitrile **2** to amino ethyl sugar **3** in 72% yield, with simultaneous cleavage of the *O*-acetyl protecting groups. Hydrolysis of the cyano group with simultaneous saponification of the three acetates of **2** using refluxing 25% NaOH in MeOH, followed by neutralization, yielded 3-*O*-(carboxymethyl)galactopyranoside derivative **4** in 92% yield. Similarly, benzylated glucopyranoside **10** yielded 4-*O*-aminoethyl derivative **11** upon reduction with BH_3·Me_2S (73%) and 4-*O*-(carboxymethyl)glucopyranoside **13** after basic hydrolysis (98%). Removal of the benzyl groups of **11** and **13** by hydrogenolysis with 10% Pd−C afforded fully deprotected amine **12** and carboxylic acid **14**.

Conversion of Cyanomethyl Ethers to Amides and Amidine Salts. As Schaefer and Peters reported in the early 1960s[10] aliphatic nitriles react with alcohols under basic catalysis to yield the corresponding alkyl imidates. The ratio of nitrile to alkyl imidate in the equilibrium is strongly dependent on the presence of electron-withdrawing substituents on the α carbon of the nitrile. This reaction has attracted little attention in the carbohydrate field with the exception of the use cyanomethyl 1-thioglycosides for the amidination of proteins.[11] In the present case, ^{1}H-NMR monitoring of the reaction of benzylated 4-*O*-(cyanomethyl)glucopyranoside **10** with 100 mM CD_3ONa in CD_3OD at 25 °C showed a characteristic upfield shift in the resonance of the methylene protons of the initial 4-*O*-cyanomethyl group from δ 4.41 and 4.45 (AB pattern, J_{gem} = 16 Hz) to δ 3.97 and 4.14 (AB pattern, J_{gem} = 15 Hz). The equilibrium mixture after 24 h contained *ca.* 90% of the putative methyl imidate, which was not isolated but directly converted to the amide **15** (61%) or the amidine hydrochloride **17** (74%) by basic hydrolysis or by treatment with NH_4Cl, respectively. Finally, removal of the benzyl groups by catalytic hydrogenation (10% Pd−C) afforded free sugar amide **16** and sugar amidine **18**. Similarly, treatment of acetylated 3-*O*-cyanomethyl derivative **2** with anhydrous sodium methoxide in MeOH, followed by mild hydrolysis, yielded the corresponding deprotected sugar

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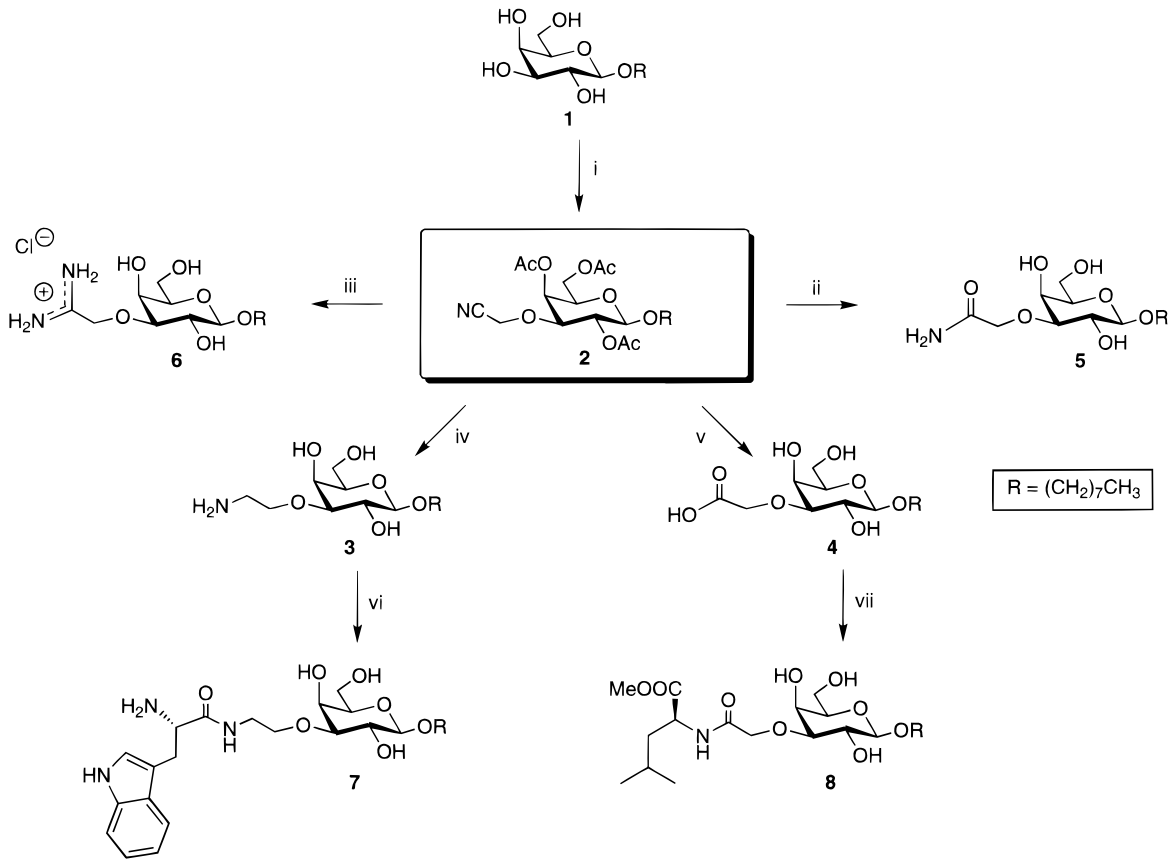
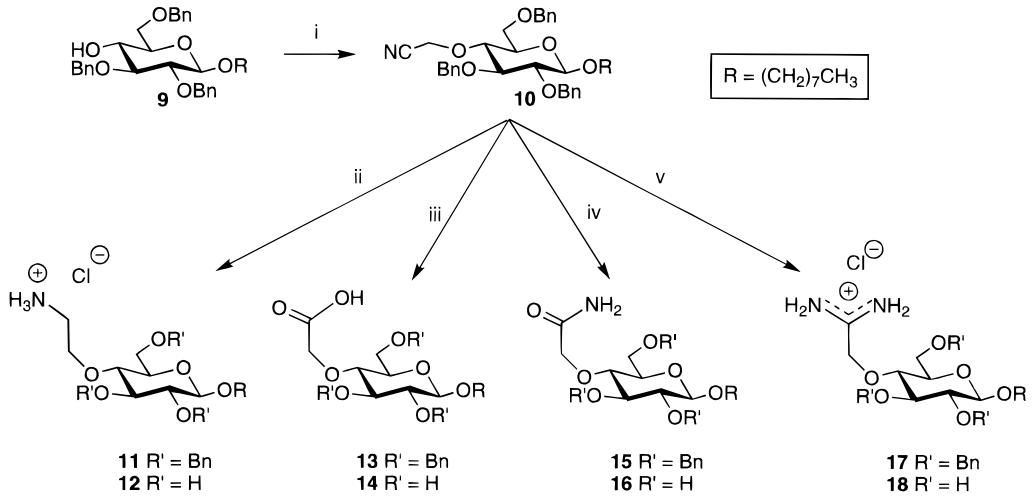
Scheme 1[a]



[a] Key: (i) (a) Bu_2SnO, Bz, reflux, 16 h; (b) $BrCH_2CN$, Bu_4NI, reflux, 90 min; (c) Ac_2O, py, rt, 51% (three steps); (ii) 100 mM NaOMe in MeOH, rt, 6 h, then 5% $NaHCO_3$(aq), 81%; (iii) 100 mM NaOMe in MeOH, rt, 6 h, then NH_3(g), rt, 12 h, 50%; (iv) BH_3·Me_2S, THF, reflux, 12 h, 72%; (v) 25% NaOH(aq) in MeOH, reflux, 12 h, 92%; (vi) (a) Fmoc-L-Trp-OPfp, DMF, rt, 3 h; (b) morpholine, rt, 2 h, 89% (two steps); (vii) L-Leu-OMe·HCl, py, DCC, 40 °C, 48 h, 62%.

Scheme 2[a]



[a] Key: (i) NaH, CH_3CN, rt, then $BrCH_2CN$, −20 °C to rt, 82%; (ii) **11**, BH_3·Me_2S, THF reflux, 12 h, 73%; **12**, H_2, 10% Pd−C, THF−H_2O−HCl, 76%; (iii) **13**, 25% NaOH(aq) in MeOH, reflux, 12 h, 98%; **14**, H_2, 10% Pd−C, THF−H_2O, 79%; (iv) **15**, 100 mM NaOMe in MeOH, 12 h, rt, then 5% $NaHCO_3$, 50 °C, 5 days, 61%; **16**, H_2, 10% Pd−C, MeOH, 95%; (v) **17**, 100 mM NaOMe in MeOH, 12 h, rt, then anhydrous NH_4Cl (5 equiv), 50 °C, 1 h, 74%; **18**, H_2, 10% Pd−C, MeOH, 89%.

amide **5** (81%), while treatment with NH_3(g) of the intermediate imidate gave the corresponding amidine isolated in 50% yield as its hydrochloride salt **6**.

Neutral sugar amides of type **5** and **16**, carboxylic acids of type **4** and **14**, and basic sugar amines or amidines (**3**, **6**, **12**, **18**) might be the first candidates to be tested in the early stages of the development of inhibitors of a given host protein, since they may potentially induce new electrostatic and/or hydrogen-bonding carbohydrate−protein interactions. Moreover, the availability of both sugar amines and sugar acids from a common *O*-cyanomethyl intermediate represents a powerful approach for the construction of glycopeptides in which the carbohydrate moiety can be attached through any of its

hydroxyl groups to either the *N*- or *C*-terminus of a given amino acid or peptide. Two examples are reported in Scheme 1. The 3-*O*-(aminoethyl)galactopyranoside **3** was coupled to the commercially available *N*-Fmoc-L-tryptophan pentafluorophenyl ester to yield sugar amino acid **7** in 89% after removal of the Fmoc group. On the other hand, sugar acid **4** was coupled to L-leucine methyl ester hydrochloride using DCC in pyridine to yield glycoconjugate **8** in 62% yield. The *O*-cyanomethyl group is thus shown to yield carbohydrate amine and acid derivatives which are compatible with chemistry of solid-phase peptide synthesis.

In the examples reported here, the manipulation of the cyanomethyl group is shown to be compatible with some of the most common carbohydrate protecting groups. A clear limitation of the method is that the cyanomethyl group has been introduced on the carbohydrate moiety only under basic conditions, either by regioselective alkylation of dibutyltin acetals of polyhydroxylic compounds or by alkylation of a hydroxyl group when the rest of OH groups are protected as benzyl ethers. If acyl groups are necessary for temporary protection or for anchimeric assistance in glycosidation reactions, they have to be introduced after *O*-cyanomethylation. Although it is possible, in principle, to devise strategies for the introduction of the cyanomethyl group in any OH group of a given carbohydrate, milder *O*-cyanomethylation methods will have to be developped to increase the versatility of protecting and deprotecting strategies.

The extension of this "*O*-cyanomethyl" methodology to the synthesis of *O*-functionalized oligosaccharides and their corresponding glycopeptides, and its application for the preparation of inhibitors of carbohydrate−protein binding, is currently in progress. In addition to this stated objective, it is expected that this *O*-cyanomethyl group may also find application in the preparation of carbohydrates as scaffolds[12] for pharmacophore presentation.

Experimental Section

General. TLC was performed on silica gel 60-F_{254} with detection by charring with a 10% solution of H_2SO_4 in ethanol. Column chromatography was performed on silica gel 60 (230−400 mesh), or with 10% C_{18}-SiO_2. Optical rotations were measured at the sodium D line at 22 ± 2 °C. ^{1}H NMR spectra were recorded at 360 or 500 MHz as indicated. ^{13}C-NMR spectra were recorded at 75 MHz. FAB mass spectra were recorded on samples suspended in a matrix of glycerol with Xe as the bombarding gas.

Octyl 2,4,6-Tri-*O*-acetyl-3-*O*-(cyanomethyl)-β-D-galactopyranoside (2). Octyl β-D-galactopyranoside[6] (10.0 g, 34.2 mmol) and dibutyltin oxide (9.3 g, 37.6 mol) in benzene (200 mL) were heated under reflux with azeotropic removal of water for 16 h. To the resulting yellow solution were added Bu_4NI (12.6 g, 34.1 mol) and bromoacetonitrile (9.1 mL, 137 mol), and stirring under reflux was continued for 90 min. The solvent was evaporated, and the residue was redissolved in $CHCl_3$ and extracted with saturated aqueous $Na_2S_2O_3$. After the solution was dried (Na_2SO_4) and evaporated, the residual brown oil was purified by flash chromatography ($CHCl_3$−MeOH 20:1 and then 5:1) to yield 5.5 g of a hygroscopic material that was directly treated with 50 mL of anhydrous pyridine and 50 mL of Ac_2O. Evaporation of the solvent after 12 h and flash chromatography of the residue (toluene−AcOEt 4:1) yielded 7.4 g (51%) of **2** as a colorless oil: $[\alpha]_D = +9.3°$ (*c* 1.6, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.85 (t, $J = 7.0$ Hz, 3H), 1.20−1.35 (m, 10H), 1.48−1.60 (m, 2H), 2.05, 2.10, 2.11 (3 s, 9H), 3.44 (dt, $J = 9.6$, 6.9 Hz, 1H), 3.70 (dd, $J = 9.9$, 3.6 Hz, 1H), 3.81 (dt, $J = 6.7$, 1.2 Hz, 1H), 3.85 (dt, $J = 9.6$, 6.2 Hz, 1H), 4.13 (dd, $J = 11.2$, 6.9 Hz, 1H), 4.18 (dd, $J = 11.2$, 6.5 Hz, 1H), 4.28, 4.31 (AB system, $J = 16.8$ Hz, 2H), 4.41 (d, $J = 8.0$ Hz, 1H), 5.05 (dd, $J = 9.9$, 8.0 Hz, 1H), 5.34 (dd, $J = 3.6$, 1.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 20.6, 20.7, 22.6, 25.7, 29.2, 29.3, 31.7, 54.7, 61.4, 64.9, 69.5, 70.2, 70.4, 78.1, 101.1, 115.6, 169.5, 170.3, 170.6. Anal. Calcd for $C_{22}H_{35}O_9N$: C, 57.75; H, 7.71; N, 3.06. Found: C, 57.74; H, 8.01; N, 3.05.

Octyl 3-*O*-(Aminoethyl)-β-D-galactopyranoside (3). Compound **2** (1.83 g, 4.0 mmol) was dissolved in anhydrous THF (25 mL), and the solution was heated to reflux under nitrogen. A 2.0 M solution of BH_3·Me_2S in THF (15 mL) was added dropwise with stirring under reflux. After 12 h, the resulting solution was cooled to room temperature, MeOH (15 mL) was added, and stirring was continued for an additional 12 h. After evaporation of the solvent, the crude product was purified by column chromatography using a short SiO_2-C_{18} column (30 g, gradient H_2O−MeOH 1:0 to 1:1) to give compound **3** as a white amorphous solid (0.96 g, 72%). The free amino sugar was converted to the corresponding hydrochloride for analytical characterization (a sample of the free amine was dissolved in H_2O, neutralized with 1 N HCl and freeze-dried): $[\alpha]_D = +17.4°$ (*c* 1.5, H_2O); mp 124−127 °C (from MeOH−Et_2O); ^{1}H NMR (500 MHz, D_2O) δ 0.87 (t, $J = 7.0$ Hz, 3H), 1.24−1.38 (m, 10H), 1.60−1.68 (m, 2H), 3.22−3.33 (m, 2H), 3.51 (dd, $J = 9.8$, 3.2 Hz, 1H), 3.59 (dd, $J = 9.8$, 7.7 Hz, 1H), 3.61−3.67 (m, 2H), 3.75−3.81 (m, 3H), 3.90 (dt, $J = 9.9$, 6.9 Hz, 1H), 3.99 (m, 1H), 4.20 (d, $J = 3.2$ Hz, 1H), 4.39 (d, $J = 7.7$ Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 14.4, 23.1, 26.2, 29.6, 29.7, 29.8, 32.2, 40.3, 61.4, 65.7, 70.7, 71.4, 75.5, 81.8, 103.5; FAB-MS *m*/*z* 336 ($(M - Cl^-)^+$). Anal. Calcd for $C_{16}H_{34}O_6NCl$·$^1/_2H_2O$: C, 50.45; H, 9.26; N, 3.68; Cl, 9.31. Found: C, 50.41; H, 9.33; N, 3.62; Cl, 9.70.

Octyl 3-*O*-(Carboxymethyl)-β-D-galactopyranoside (4). Compound **2** (1.74 g, 3.80 mmol) was dissolved in MeOH (50 mL). A solution of 25% NaOH in H_2O (12 mL) was added, and the mixture was stirred under reflux for 12 h. MeOH was carefully evaporated from the reaction at reduced pressure, and the residue was diluted with H_2O (300 mL) and treated with excess Amberlite IR-120 (H^+). After filtration of the resin, the aqueous solution was directly loaded on a small SiO_2-C_{18} column (30 g), washed with H_2O, and eluted with 40% CH_3-CN in H_2O. Concentration under reduced pressure and freeze-drying yielded **4** as a white solid (1.22 g, 92%): $[\alpha]_D = +4.9°$ (*c* 1.5, H_2O); mp 80−82 °C (from AcOEt−Et_2O); ^{1}H NMR (500 MHz, D_2O) δ 0.87 (t, $J = 7.0$ Hz, 3H), 1.22−1.40 (m, 10H), 1.60−1.68 (m, 2H), 3.51 (dd, $J = 9.8$, 3.4 Hz, 1H), 3.60−3.67 (m, 3H), 3.76 (d, $J = 6.2$ Hz, 2H), 3.90 (dt, $J = 9.9$, 7.0 Hz, 1H), 4.16 (d, $J = 3.4$ Hz, 1H), 4.32, 4.37 (AB system, $J = 16.9$ Hz, 2H), 4.37 (d, $J = 7.9$ Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 14.6, 23.4, 26.6, 30.1, 30.2, 32.6, 61.1, 65.9, 67.5, 70.7, 71.1, 75.1, 83.0, 101.8, 175.5; FAB-MS *m*/*z* 351 ($(M + H)^+$); FT-IR 1730, 1690 cm^{-1} (C=O st). Anal. Calcd for $C_{16}H_{30}O_8$·H_2O: C, 52.16; H, 8.75. Found: C, 52.13; H, 8.46.

Octyl 3-*O*-(Carbamoylmethyl)-β-D-galactopyranoside (5). Compound **2** (144 mg, 0.315 mmol) was dissolved in 100 mM NaOMe in MeOH (2 mL), and the solution was stirred at room temperature for 6 h. Aqueous 5% $NaHCO_3$ (2 mL) was then added, and stirring at room temperature was continued overnight. After evaporation to dryness, the residue was suspended in MeOH and the insoluble salts were removed by filtration. Evaporation of the solvent and purification by column chromatography (CH_2Cl_2−MeOH 7:1) yielded **5** (89 mg, 81%): $[\alpha]_D = +12.0°$ (*c* 1, MeOH); mp 148−150 °C (from MeOH−AcOEt); ^{1}H NMR (360 MHz, CD_3OD) δ 0.89 (t, $J = 7.0$ Hz, 3H), 1.24−1.43 (m, 10H), 1.61 (m, 2H), 3.32 (dd, $J = 9.7$, 3.3 Hz, 1H), 3.47 (t, $J = 5.7$ Hz, 1H), 3.54 (dt, $J = 9.5$, 6.7 Hz, 1H), 3.64 (dd, $J = 7.7$, 9.7 Hz, 1H), 3.70−3.79 (m, 2H), 3.89 (dt, $J = 9.5$, 6.7 Hz, 1H), 4.01 (d, $J = 3.3$ Hz, 1H), 4.09, 4.12 (AB system, $J = 16.1$ Hz, 2H), 4.22 (d, $J = 7.7$ Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.4, 23.7, 27.1, 30.4, 30.5, 30.8,

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33.0, 62.4, 66.9, 69.3, 70.9, 71.4, 76.2, 84.2, 104.8, 176.3; FAB-MS *m/z* 350 ($(M + H)^+$); FT-IR (MeOH) 1670 cm^{-1} (C=O st). Anal. Calcd for $C_{16}H_{31}O_7N$: C, 55.00; H, 8.94; N 4.01. Found: C, 54.74; H, 9.16; N, 3.92.

Octyl 3-*O*-(Amidinomethyl)-β-D-galactopyranoside Hydrochloride (6). Compound **2** (210 mg, 0.459 mmol) was dissolved in 100 mM NaOMe in MeOH (2 mL), and the solution was stirred at room temperature for 6 h. The solution was then saturated with NH_3(g) and stirred at room temperature overnight. After evaporation of the solvent, the residue was resuspended in H_2O and neutralized with 1 N HCl, and the resulting solution was applied to a column of SiO_2-C_{18} and eluted with H_2O and then H_2O−MeOH 95:5. After freeze-drying, **6** was obtained as a white solid (89 mg, 50%): $[\alpha]_D$ = +18.9° (*c* 1, H_2O); mp 158−160 °C (from MeOH−Et_2O); ^{1}H NMR (500 MHz, D_2O) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.24−1.40 (m, 10H), 1.63 (m, 2H), 3.61 (dd, *J* = 9.8, 3.1 Hz, 1H), 3.64−3.70 (m, 3H), 3.75 (dd, *J* = 11.6, 4.7 Hz, 1H), 3.80 (dd, *J* = 11.6, 7.6 Hz, 1H), 3.93 (dt, *J* = 9.8, 6.8 Hz, 1H), 4.18 (d, *J* = 3.2 Hz, 1H), 4.42 (d, *J* = 7.6 Hz, 1H), 4.55, 4.65 (AB system, *J* = 16.3 Hz, 2H); ^{13}C NMR (75 MHz, D_2O) δ 14.5, 23.2, 26.4, 29.8, 29.9, 30.0, 32.4, 61.2, 65.2, 65.8, 70.6, 71.3, 75.2, 83.1, 103.6, 168.6; FAB-MS *m/z* 349 ($(M - Cl^-)^+$); FT-IR 1710 cm^{-1} (C=N st). Anal. Calcd for $C_{16}H_{33}O_6N_2Cl \cdot {}^1/_2H_2O$: C, 48.79; H, 8.70; N, 7.11; Cl, 9.00. Found: C, 48.94; H, 8.88; N, 6.99; Cl, 9.29.

Octyl 3-*O*-[*N*-(L-Trp)aminoethyl]-β-D-galactopyranoside (7). Compound **3** (29 mg, 0.086 mmol) and 4 Å molecular sieves (300 mg) in dry DMF (5 mL) were stirred under vacuum for 30 min to remove traces of volatile amines. *N*-Fmoc-L-tryptophan pentafluorophenyl esther (205 mg, 0.346 mmol) was added, and the mixture was stirred at room temperature for 3 h. Filtration, evaporation of the solvent, and purification by column chromatography ($CHCl_3$−MeOH 10:1) yielded 64 mg of a white solid that was dissolved in morpholine (5mL) and stirred at room temperature for 2 h. Evaporation of the solvent followed by column chromatography (Iatrobeads SiO_2, $CHCl_3$−MeOH−5% NH_3(aq) 50:10:1) afforded **7** (40 mg, 89%): $[\alpha]_D$ = +14.2° (*c* 0.7, MeOH); ^{1}H NMR (360 MHz, CD_3-OD) δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.25−1.42 (m, 10H), 1.55−1.65 (m, 2H), 2.82 (m, 2H), 2.98−3.90 (m, 13H), 4.08 (d, *J* = 7.8 Hz, 1H), 7.00 (ddd, *J* = 8.0, 8.0, 1.0, 1H), 7.09 (ddd, *J* = 8.0, 8.0, 1.0, 1H), 7.10 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.4, 23.7, 27.1, 30.4, 30.5, 30.8, 32.2, 33.0, 40.6, 57.3, 62.4, 66.9, 69.3, 70.8, 71.5, 76.2, 83.6, 104.8, 111.3, 112.3, 119.6, 119.8, 122.5, 124.8, 129.0, 138.0, 177.0.

***N*-[[(1-*O*-Octyl-β-D-galactopyranos-3-yl)methyl]carbonyl]-L-Leucine Methyl Ester (8).** Compound **4** (72 mg, 0.21 mmol), L-leucine methyl ester hydrochloride (187 mg, 1.03 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (84 mg, 0.41 mmol) were dissolved in pyridine (2 mL), and the solution was stirred under Ar at 40 °C for 48 h. Evaporation of the solvent and purification by column chromatography over SiO_2 (CH_2Cl_2−MeOH 20:1) followed by filtration over C_{18}-SiO_2 (MeOH−H_2O 1:1 and then 1.2:1) afforded **8** (61 mg, 62%): $[\alpha]_D$ = +5.0° (*c* 1, MeOH); ^{1}H NMR (360 MHz, CD_3OD) δ 0.89 (t, *J* = 7.0 Hz, 3H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 1.24−1.42 (m, 10H), 1.56−1.76 (m, 5H), 3.32 (1H, overlapped with solvent signal), 3.46 (t, *J* = 6.2 Hz, 1H), 3.53 (dt, *J* = 9.5, 6.7 Hz, 1H), 3.65 (dd, *J* = 7.9, 9.8 Hz, 1H), 3.70−3.79 (m, 2H), 3.71 (s, 3H), 3.88 (dt, *J* = 9.5, 6.7 Hz, 1H), 4.03 (d, *J* = 3.0 Hz, 1H), 4.12, 4.18 (AB system, *J* = 16.4 Hz, 2H), 4.19 (d, *J* = 7.8 Hz, 1H), 4.52 (dd, *J* = 9.7, 5.0 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.4, 21.8, 23.3, 23.7, 25.9, 27.0, 30.4, 30.5, 30.7, 33.0, 41.3, 51.8, 52.8, 62.4, 66.7, 69.1, 70.8, 71.1, 76.2, 84.3, 105.0, 173.2, 174.4.

Octyl 2,3,6-Tri-*O*-benzyl-4-*O*-(cyanomethyl)-β-D-glucopyranoside (10). Octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[7] (**9**, 2.04 g, 3.64 mmol) and NaH (0.54 g, 80% suspension in mineral oil, 18 mmol) in anhydrous CH_3CN (10 mL) were stirred at room temperature under Ar for 1 h. The resulting brownish suspension was cooled to −20 °C, and $BrCH_2CN$ (1.3 mL, 20 mmol) was added dropwise. The reaction was stirred at −20 °C for 5 h and then allowed to reach room temperature overnight. The resulting brown mixture was concentrated by evaporation, diluted with CH_2Cl_2 (200 mL), and filtered. The filtrate was concentrated and purified by flash chromatography (hexane−AcOEt 8:1 and then 4:1) to yield **10** (1.80 g, 82%): $[\alpha]_D$ = +21.5° (*c* 1, $CHCl_3$); mp 52−53 °C (from MeOH); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.0 Hz, 3H), 1.20−1.40 (m, 10H), 1.63 (m, 2H), 3.36 (ddd, *J* = 8.8, 4.3, 2.1 Hz, 1H), 3.40 (dd, *J* = 7.8, 9.0 Hz, 1H), 3.47 (t, *J* = 8.8 Hz, 1H), 3.50 (dt, *J* = 9.5, 6.7 Hz, 1H), 3.57 (t, *J* = 8.9 Hz, 1H), 3.69 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.74 (dd, *J* = 10.9, 2.1 Hz, 1H), 3.92 (dt, *J* = 9.5, 6.7 Hz, 1H), 4.24, 4.28 (AB system, *J* = 15.7 Hz, 2H), 4.34 (d, *J* = 7.8 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.66 (d, *J* = 11.1 Hz, 1H), 4.67 (d, *J* = 11.0 Hz, 1H), 4.92 (d, *J* = 11.1 Hz, 1H), 4.94 (d, *J* = 11.0 Hz, 1H), 7.25−7.35 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 26.1, 29.2, 29.4, 29.7, 31.8, 57.3, 68.6, 70.2, 73.5, 73.8, 74.6, 75.5, 78.6, 82.1, 84.0, 103.6, 116.1, 127.7, 127.8, 128.0, 128.1, 128.4, 128.5, 138.0, 138.1, 138.3. Anal. Calcd for $C_{37}H_{47}O_6N$: C, 73.85; H, 7.87; N 2.33. Found: C, 73.93; H, 7.93; N, 2.34.

Octyl 4-*O*-(Aminoethyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11). Compound **10** (294 mg, 0.489 mmol) was dissolved in anhydrous THF (5 mL), and the solution was heated to reflux under nitrogen. A 2.0 M solution of $BH_3 \cdot Me_2S$ in THF (0.5 mL) was added dropwise, and stirring under reflux was continued for 12 h. The resulting solution was cooled to room temperature, MeOH was added (0.5 mL), and stirring was continued for an additional 12 h. After evaporation of the solvent, the crude product was purified by column chromatography ($CHCl_3$−MeOH−concd aqueous NH_3 300:10:1) to yield 217 mg of a white solid (73%): $[\alpha]_D$ = +10.3° (*c* 1, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.0 Hz, 3H), 1.20−1.40 (m, 10H), 1.63 (m, 2H), 2.60−2.74 (m, 2H), 3.30−3.77 (m, 9H), 3.92 (dt, *J* = 9.5, 6.7 Hz, 1H), 4.35 (d, *J* = 7.8 Hz, 1H), 4.55 (d, *J* = 12.1 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.67 (d, *J* = 11.0 Hz, 1H), 4.71 (d, *J* = 11.0 Hz, 1H), 4.89 (d, *J* = 11.0 Hz, 1H), 4.92 (d, *J* = 11.0 Hz, 1H), 7.25−7.35 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 26.0, 29.1, 29.2, 29.6, 31.6, 42.3, 68.8, 69.9, 73.3, 74.5, 74.8, 74.9, 75.3, 78.0, 82.1, 84.3, 103.5, 127.4, 127.6, 127.9, 128.1, 138.1, 138.4, 138.5. Anal. Calcd for $C_{37}H_{51}O_6N$: C, 73.36; H, 8.49; N 2.31. Found: C, 73.19; H, 8.54; N, 2.24.

Octyl 2,3,6-Tri-*O*-benzyl-4-*O*-(carboxymethyl)-β-D-glucopyranoside (13). Compound **10** (305 mg, 0.507 mmol) was dissolved in MeOH (6 mL), a 25% solution of NaOH(aq) (1.5 mL) was added, and the mixture was heated under reflux for 12 h. After evaporation of the solvent, the residue was diluted with H_2O (50 mL), acidified with 10% HCl, and extracted twice with CH_2Cl_2 (50 mL). After the solvent was dried (Na_2SO_4) and evaporated, the residue was purified by column chromatography ($CHCl_3$−MeOH 15:1) to yield 308 mg of **13** (98%): $[\alpha]_D$ = +11.9° (*c* 1, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.0 Hz, 3H), 1.20−1.40 (m, 10H), 1.63 (m, 2H), 3.36 (m, 1H), 3.41−3.53 (m, 3H), 3.60 (t, *J* = 9.0 Hz, 1H), 3.68 (d, *J* = 3.3 Hz, 2H), 3.90 (dt, *J* = 9.5, 6.7 Hz, 1H), 4.17, 4.25 (AB system, *J* = 17.1 Hz, 2H), 4.36 (d, *J* = 7.6 Hz, 1H), 4.53 (d, *J* = 12.1 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.65 (d, *J* = 10.9 Hz, 1H), 4.74 (d, *J* = 11.1 Hz, 1H), 4.96 (d, *J* = 10.9 Hz, 1H), 5.00 (d, *J* = 11.1 Hz, 1H), 7.25−7.35 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 26.0, 29.1, 29.2, 29.6, 31.6, 68.6, 69.2, 70.0, 73.4, 73.8, 74.4, 75.3, 78.8, 82.1, 83.1, 103.5, 127.6, 127.8, 127.9, 128.3, 137.5, 138.1, 173.3; FT-IR (CH_2Cl_2) 1730, 1760 cm^{-1} (C=O st). Anal. Calcd for $C_{37}H_{48}O_8$: C, 71.59; H, 7.79. Found: C, 71.69; H, 7.84.

Octyl 2,3,6-Tri-*O*-benzyl-4-*O*-(carbamoylmethyl)-β-D-glucopyranoside (15). Compound **10** (242 mg, 0.402 mmol) was dissolved in 100 mM NaOMe in MeOH (3 mL), and the solution was stirred at room temperature for 12 h. The resulting solution was then heated to 50 °C, aqueous saturated $NaHCO_3$ was added dropwise until turbidity (*ca.* 0.4 mL), and stirring at 50 °C was continued for 5 days. The crude was diluted with CH_2Cl_2 (50 mL), washed with H_2O, dried (Na_2SO_4), and evaporated. Column chromatography (CH_2Cl_2−MeOH 100:1) afforded **15** as a white solid (153 mg, 61%): $[\alpha]_D$ = +10.4° (*c* 1, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.0 Hz, 3H), 1.20−1.40 (m, 10H), 1.63 (m, 2H), 3.36 (m, 1H), 3.40−3.60 (m, 4H), 3.65 (dd, *J* = 11.0, 3.6 Hz, 1H), 3.70 (dd, *J* = 11.0, 2.8 Hz, 1H), 3.92 (dt, *J* = 9.5, 6.7 Hz, 1H), 4.05, 4.19

(AB system, J = 16.0 Hz, 2H), 4.37 (d, J = 7.7 Hz, 1H), 4.54 (d, J = 12.0 Hz, 1H), 4.60 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 10.9 Hz, 1H), 4.68 (d, J = 11.0 Hz, 1H), 4.95 (d, J = 11.0 Hz, 1H), 4.98 (d, J = 10.9 Hz, 1H), 7.25−7.35 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 26.1, 29.1, 29.3, 29.7, 31.7, 68.8, 70.1, 71.1, 73.6, 74.4, 74.5, 74.4, 78.7, 82.2, 83.3, 103.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 137.6, 138.2, 172.6; FT-IR (CH_2Cl_2) 1700, 1660 cm^{-1} (amide bands I and II). Anal. Calcd for $C_{37}H_{49}O_7N$: C, 71.70; H, 7.97; N, 2.26. Found: C, 71.49; H, 8.23; N, 2.21.

Octyl 4-*O*-(Amidinomethyl)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside Hydrochloride (17). Compound **10** (270 mg, 0.402 mmol) was dissolved in 100 mM NaOMe in MeOH (3 mL), and the solution was stirred at room temperature for 12 h. Anhydrous NH_4Cl was then added (120 mg, 2.24 mmol), and the solution was heated at 50 °C for 1 h. The mixture was evaporated, and the residue was dissolved in MeOH−H_2O 3:7 and loaded onto a SiO_2-C_{18} column. After an extensive H_2O wash, compound **17** was eluted using MeOH−H_2O 7:3 (219 mg, 74%): $[\alpha]_D$ = +13.5° (*c* 1, MeOH); ^{1}H NMR (360 MHz, CD_3OD) δ 0.86 (t, J = 7.0 Hz, 3H), 1.20−1.45 (m, 10H), 1.63 (m, 2H), 3.38 (dd, J = 9.0, 7.8 Hz, 1H), 3.50−3.78 (m, 6H), 3.92 (dt, J = 9.5, 6.7 Hz, 1H), 4.34, 4.49 (AB system, J = 16.2 Hz, 2H), 4.46 (d, J = 7.8 Hz, 1H), 4.53 (d, J = 11.8 Hz, 1H), 4.64 (d, J = 11.8 Hz, 2H), 4.68 (d, J = 11.2 Hz, 1H), 4.95 (d, J = 11.4 Hz, 1H), 4.96 (d, J = 11.2 Hz, 1H), 7.22−7.38 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.4, 25.9, 29.0, 29.1, 29.5, 31.6, 66.3, 68.2, 69.8, 73.2, 73.7, 74.2, 75.1, 78.7, 82.1, 103.4, 127.5, 127.6, 127.8, 127.9, 128.1, 128.2, 128.5, 136.8, 137.2, 137.9, 168.9. Anal. Calcd for $C_{37}H_{51}O_6N_2Cl\cdot H_2O$: C, 66.01; H, 7.93; N, 4.16; Cl: 5.27. Found: C, 65.74; H, 8.08; N, 4.06; Cl, 5.44.

Octyl 4-*O*-(Aminoethyl)-*β*-D-glucopyranoside Hydrochloride (12). Compound **11** (30 mg, 0.050 mmol) was dissolved in a mixture of THF (0.5 mL), H_2O (0.5 mL), and 5% HCl(aq) (0.05 mL), and the solution was stirred under a stream of H_2 in the presence of 10% Pd−C (30 mg) for 8 h. The catalyst was removed by filtration and washed with MeOH and H_2O, and the resulting solution was passed through a C_{18}-SiO_2 Sep-Pak which was washed with MeOH−H_2O 1:1. Evaporation of the solvent and freeze-drying yielded 14 mg of **12** (76%): $[\alpha]_D$ = −27.8° (*c* 0.6, MeOH); ^{1}H NMR (360 MHz, D_2O) δ 0.87 (t, J = 7.0 Hz, 3H), 1.24−1.40 (m, 10H), 1.58−1.67 (m, 2H), 3.16−3.31 (m, 3H), 3.36 (t, J = 9.2 Hz, 1H), 3.51 (m, 1H), 3.63 (t, J = 9.2 Hz, 1H), 3.67 (dt, J = 9.9, 7.0 Hz, 1H), 3.77 (dd, J = 12.3, 5.1 Hz, 1H), 3.87−4.04 (m, 4H), 4.44 (d, J = 8.0 Hz, 1H); FAB-MS m/z 336 ((M − Cl$^-$)$^+$).

Octyl 4-*O*-(Carboxymethyl)-*β*-D-glucopyranoside (14). Compound **13** (38 mg, 0.062 mmol) was dissolved in 1:1 THF−H_2O (1 mL), and the solution was stirred under a stream of H_2 in the presence of 10% Pd−C (30 mg) for 6 h. The reaction mixture was processed as described for the preparation of **12** to yield 17 mg of **14** (79%): $[\alpha]_D$ = −20.1° (*c* 0.7, H_2O); ^{1}H NMR (360 MHz, D_2O) δ 0.87 (t, J = 7.0 Hz, 3H), 1.24−1.40 (m, 10H), 1.58−1.67 (m, 2H), 3.27 (dd, J = 9.2, 7.9 Hz, 1H), 3.37 (t, J = 9.2 Hz, 1H), 3.51 (m, 1H), 3.66 (t, J = 9.2 Hz, 1H), 3.67 (dt, J = 9.9, 7.0 Hz, 1H), 3.77 (dd, J = 12.4, 5.1 Hz, 1H), 3.87−3.95 (m, 2H), 4.29, 4.35 (AB system, J = 16.6 Hz, 2H), 4.45 (d, J = 7.9 Hz, 1H); FAB-MS m/z 373 ((M + Na)$^+$); FT-IR 1725 cm^{-1} (C=O st).

Octyl 4-*O*-(Carbamoylmethyl)-*β*-D-glucopyranoside (16). Compound **15** (39 mg, 0.063 mmol) in MeOH (3 mL) was warmed until a clear solution was obtained and then stirred under a stream of H_2 in the presence of 10% Pd−C (30 mg) at room temperature for 24 h. The reaction mixture was processed as described for the preparation of **12** to yield 21 mg of **16** (95%): $[\alpha]_D$ = −28.5° (*c* 0.4, H_2O); ^{1}H NMR (360 MHz, CD_3OD) δ 0.89 (t, J = 7.0 Hz, 3H), 1.24−1.42 (m, 10H), 1.55−1.65 (m, 2H), 3.18 (dd, J = 9.2, 7.8 Hz, 1H), 3.30 (m, 1H), 3.36 (t, J = 9.2 Hz, 1H), 3.51 (dt, J = 9.9, 7.0 Hz, 1H), 3.53 (t, J = 9.2 Hz, 1H), 3.73 (dd, J = 12.2, 3.7 Hz, 1H), 3.82 (dd, J = 12.2, 2.1 Hz, 1H), 3.86 (dt, J = 9.9, 7.0 Hz, 1H), 4.20, 4.27 (AB system, J = 16.1 Hz, 2H), 4.24 (d, J = 7.8 Hz, 1H); FAB-MS m/z 372 ((M + Na)$^+$); FT-IR 1710, 1655 cm^{-1} (amide bands I and II).

Octyl 4-*O*-(Amidinomethyl)-*β*-D-glucopyranoside Hydrochloride (18). Compound **17** (48 mg, 0.073 mmol) was dissolved in MeOH (3 mL), and the solution was stirred under a stream of H_2 in the presence of 10% Pd−C (40 mg) for 6 h. The reaction mixture was processed as described for the preparation of **12** to yield 25 mg of **18** (89%): $[\alpha]_D$ = −25.9° (*c* 0.5, MeOH); ^{1}H NMR (360 MHz, CD_3OD) δ 0.89 (t, J = 7.0 Hz, 3H), 1.24−1.42 (m, 10H), 1.55−1.65 (m, 2H), 3.18 (dd, J = 9.1, 7.8 Hz, 1H), 3.35 (m, 1H), 3.46 (t, J = 9.1 Hz, 1H), 3.52 (dt, J = 9.9, 7.0 Hz, 1H), 3.58 (t, J = 9.1 Hz, 1H), 3.74 (dd, J = 12.3, 3.5 Hz, 1H), 3.83 (dd, J = 12.3, 2.4 Hz, 1H), 3.86 (dt, J = 9.9, 7.0 Hz, 1H), 4.25 (d, J = 7.8 Hz, 1H), 4.63, 4.68 (AB system, J = 16.7 Hz, 2H); FAB-MS m/z 349 ((M - Cl$^-$)$^+$); FT-IR 1700, 1690 cm^{-1} (C=N st).

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Supporting Information Available: ^{1}H and ^{13}C NMR assignments for all compounds and ^{1}H NMR spectra for compounds **3**, **4**, **5**, **6**, **7**, **8**, **12**, **14**, **16**, and **18** (39 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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