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Note

Measurement of interglycosidic $^{3}J_{CH}$ coupling constants of selectively ^{13}C labeled oligosaccharides by 2D *J*-resolved ^{1}H NMR spectroscopy

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Abstract

Tri-, tetra-, and penta-saccharide fragments of the O-specific polysaccharide of *Shigella dysenteriae* type 1 have been prepared in which a D-galactose residue of each oligosaccharide methyl glycoside derivative contains a ^{13}C label at C-1. The interglycosidic coupling constants ($^{3}J_{CH}$) of these ^{13}C nuclei with the H-3 nuclei of the adjacent 2-acetamido-2-deoxy-D-glucose residues have been measured by two-dimensional, *J*-resolved ^{1}H NMR spectroscopy. The magnitudes of these coupling constants indicate that the trisaccharide is conformationally different to the higher oligosaccharide homologs, in agreement with previous studies of ^{13}C chemical shifts and $^{1}J_{CH}$ values.

Keywords: Trisaccharide; Tetrasaccharide; Pentasaccharide; Oligosaccharides; Carbon-13 labeling; Carbon-13–proton coupling constants; *Shigella dysenteriae* type 1; 2D *J*-resolved NMR spectroscopy

As part of a project to develop improved saccharide–protein conjugate vaccines [1], we are studying synthetic approaches to fragments of the O-specific polysaccharide (O-SP) of *Shigella dysenteriae* type 1 [2–7], an organism that causes dysentery, an acute invasive disease of the lower intestines, in many parts of the world [8]. High-resolution NMR spectroscopy is being used to define the structural and conformational aspects of the problem [9]. We had previously observed unexpected changes in some ^{13}C chemical shifts and $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants upon chain extension of the trisaccharide fragment **1** of the O-SP to higher fragments, such as **2** and **3** [4,6]. In compound **1**, the Gal unit that bears no glycose residue at C-2 shows a $^{1}J_{C\text{-}1,H\text{-}1}$ value of 171 Hz, which is characteristic of the α configuration [10]. On the other hand, in compounds **2** and **3** the Gal units that have a Rha residue attached to O-2 of this unit display a $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constant that is 3–4 Hz larger.

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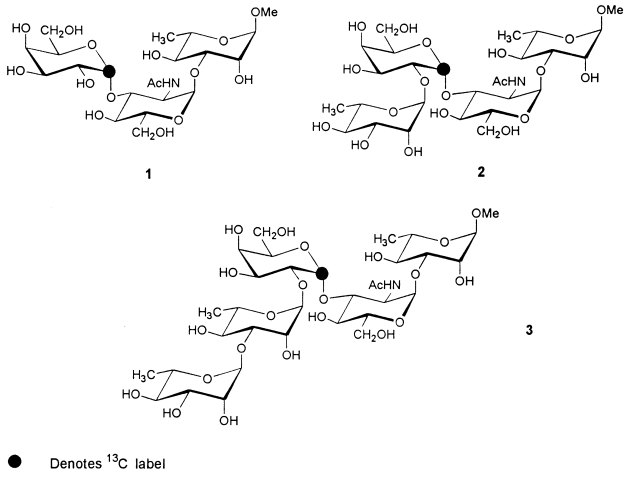
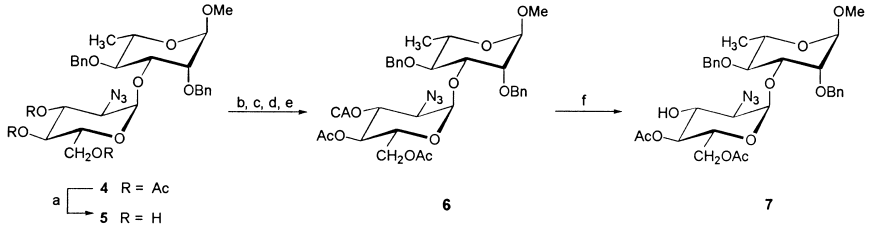
This effect is mirrored in the ^{13}C chemical shift of C-3 of the GlcNAc residue to which the Gal unit is α-(1→3) linked. Addition of a Rha residue at O-2 of the Gal unit causes the chemical shift of C-3 of the GlcNAc moiety to decrease from ~78 ppm to ~75 ppm. A shift in the H-3 signal of 0.07–0.08 ppm was also observed for the GlcNAc residue in **1**, relative to **2** and **3**. These differences have been interpreted as being due to conformational effects, possibly caused by an increase in steric crowding on attachment of a Rha residue to O-2 of the Gal unit [4,6]. This phenomenon has now been investigated further by measurement of the interglycosidic $^{3}J_{CH}$ coupling constants in the vicinity of the nuclei that show the unexpected NMR parameters. Many different methods have been developed for the measurement of long-range ^{13}C–^{1}H coupling constants, and a number of these methods have been applied to carbohydrates [11–27]. Some of the methods are applicable to ^{13}C at natural abundance, but may have the disadvantage of undue complexity, or a requirement for sophisticated instrumentation. We have chosen to measure these coupling constants by two-dimensional (2D) *J*-resolved ^{1}H NMR spectroscopy [28] of selectively ^{13}C labeled oligosaccharides **1**–**3**, firstly, because of the simplicity of the method, and secondly, because it can be used for older spectrometers without good inverse detection capability.

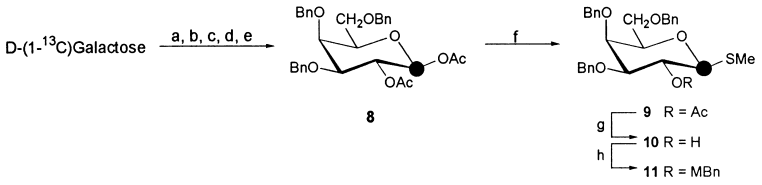


1. Results and discussion

Oligosaccharides **1**–**3** were prepared in a stepwise fashion using compound **4** [6] as the key starting material (Scheme 1). The triol **5** obtained from **4** was converted into the diacetate **7** through a sequence that is shown in Scheme 1, and described in detail in the Experimental part. The ^{13}C-labeled galactose donor **11** (Scheme 2) was prepared from D-(1-^{13}C)galactose, which was first converted into the diacetate **8** in 51%, in analogy with a procedure described for the unlabeled analog [4]. Next, the methylthio group was introduced using Me_3SiSMe as the methanethiol equivalent [29] (→**9**), and then



Scheme 1. Reagents and conditions: (a) NaOMe (cat), MeOH, 23 °C, 4 h, quant; (b) 7 equiv 4-methoxybenzaldehyde dimethylacetal, 10-camphorsulfonic acid (cat), DMF, 23 °C, 1 h; (c) 1.8 equiv $(ClCH_2CO)_2O$, C_5H_5N, 0 °C, 30 min; (d) $HBF_4.Et_2O$, MeOH, 0 °C, 1 h; (e) Ac_2O, C_5H_5N, 0 °C, 1 h, 61% for four steps; (f) 3.3 equiv thiourea, MeOH–C_5H_5N, 23 °C, 72 h, 64%. Bn = benzyl; CA = monochloroacetyl.



Scheme 2. Reagents and conditions: (a) Ac_2O, $HClO_4$ (cat), 30–40 °C, 60 min; (b) HBr, AcOH, 23 °C, 90 min; (c) 4 equiv EtOH, Bu_4NBr, 2 equiv 2,4,6-trimethylpyridine, CH_2Cl_2, 23 °C, 4 d; (d) 4 equiv NaH, DMF, then 4 equiv BnBr; (e) Me_3SiOAc, reflux, 8 h, 51% for five steps; (f) Me_3SiSMe, Me_3SiOSO_2Me (cat), CH_2Cl_2 85%; (g) NaOMe (cat), MeOH, 23 °C, 48 h, quant; (h) 1.2 equiv NaH, DMF, then 1.2 equiv MBnCl, 0 °C, 3 h, 85%. Bn = benzyl; MBn = 4-methoxybenzyl; ● Denotes ^{13}C label.

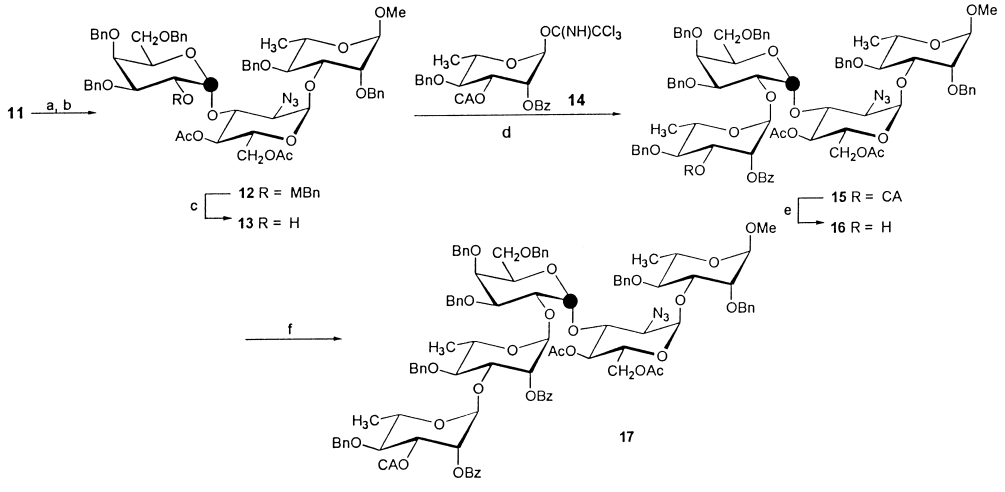
the 4-methoxybenzyl group was attached through the intermediacy of the alcohol **10**, to provide **11** as the source of the α-galactosyl moiety. Thiogalactoside **11** was treated with chlorine, and then the intermediate so obtained was treated, without isolation, with the acceptor **7** under AgOTf activation, to provide the trisaccharide **12** in 66% yield (Scheme 3). Oxidative removal of the 4-methoxybenzyl group [30] afforded the acceptor **13**, which was rhamnosylated by using the selectively protected trichloroacetimidate **14** to give the tetrasaccharide **15** in 89% yield. Next, the mono-chloroacetyl group was removed (→**16**) and then the alcohol so obtained was rhamnosylated with **14**. In this reaction, the target pentasaccharide **17** was obtained in 88% yield. Conversion of the N_3 groups in compounds **13**, **16**, and **17** to acetamido groups ((i) PPh_3, (ii) Ac_2O), and conventional removal of the O-protecting groups ((i) NaOMe, (ii) H_2/Pd-C) afforded the unprotected oligosaccharides **1**–**3**, the properties of which matched those reported for the unlabeled equivalents [6].

The presence of the ^{13}C label in the Gal residue allowed the measurement of a number of coupling constants that are not readily detectable in the unlabeled counterparts. No attempt was made to determine the signs of these constants. A large amount of such data is now available for unprotected saccharides and glycosides [31]. However, comparable data for protected saccharides appears to be limited. For the acetate **8**, both two and three-bond C–H couplings were seen (Table 1). The measurement of the corresponding data for the thiogalactosides **9**–**11** was hampered by the higher order nature of the ^1H NMR spectra of these compounds. Nevertheless, the two-bond, $^2J_{C1,H2}$ coupling constant in **9** could be measured and is significantly smaller than the value reported for the corresponding O-galactoside (4.8 versus 6.1 Hz) [32]. A similar pattern was found for the three-bond $^3J_{C1,SCH_3}$ coupling constants, which are in the 4.0-4.2 Hz range for **9**–**11**, whereas the corresponding value in methyl β-D-galactopyranoside is 4.5 Hz. The intra-residual ^{13}C–^{13}C coupling constants (Table 2) for the glycosides **1**–**3** and **12**–**17** generally agree with those published for unprotected α- and β-D-(1-^{13}C)galactopyranoses [33] except that we did not detect the two bond $^2J_{C1,C5}$ coupling in these compounds that was reported for α-D-(1-^{13}C)galactopyranose. Sensitivity to the terminal substituent of the coupling pathway has been demonstrated previously [31]. Additional examples of this phenomenon are reported here. In the thiogalactosides **9**–**11**, $^1J_{C1,C2}$ is 41–42 Hz and is

Table 1
^{13}C–^1H intra-residue coupling constants (Hz)[a] in galactopyranoses

Compound	$^2J_{C1,H2}$	$^3J_{C1,H3}$	$^4J_{C1,H4}$	$^3J_{C1,H5}$	$^3J_{C1,SCH_3}$
8	6.9	1.5	0.9	2.4	[b]
9	4.8				4.1
10					4.0
11					4.2

[a] The digital resolution was 0.9 Hz or less.
[b] Spaces indicate that the coupling constant was not determined.



Scheme 3. Reagents and conditions: (a) 0.78 equiv **11**, 6.2 equiv 2,6-di-*tert*-butyl-4-methylpyridine, Cl_2 (excess), 23 °C, 2 min, then hex-1-ene (excess); (b) CF_3SO_2OAg, CH_2Cl_2, −78 °C, 3 h, 66% for two steps; (c) 3 equiv $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O, 23 °C, 10 min, then $NaHSO_3$, 80%; (d) 2.5 equiv **14**, 0.28 equiv $BF_3.Et_2O$, CH_2Cl_2, 0 °C, 2 h, 89%; (e) 22 equiv thiourea, DMF–C_5H_5N, 23 °C, 12 h, 91%; (f) 4.8 equiv **14**, 0.28 equiv $BF_3.Et_2O$, CH_2Cl_2, 0 °C, 2 h, 88%. Bn = benzyl; CA = monochloroacetyl; ●, Denotes ^{13}C label.

Table 2
^{13}C–^{13}C intra-residue coupling constants (Hz)[a] in galactopyranoses

Compound	$^{1}J_{C1,C2}$	$^{2}J_{C1,C3}$	$^{2}J_{C1,C5}$	$^{3}J_{C1,C6}$
1	46	[b]		3.6
2	47			3.2
3	47			3.4
8	48	4.7		5.1
9	42	3.4	2.3	5.1
10	41			5.2
11	42	3.5	~1	5.3
12	47			3.9
13	46			4.0
15	48			3.7
16	47			4.0
17	47.5			3.5

[a] The digital resolution was 0.9 Hz or less.
[b] Spaces indicate that the coupling constant was not observed.

significantly smaller by 5–6 Hz than that reported for β-D-(1-^{13}C)galactopyranose [33]. The $^{2}J_{C1,C3}$ values for **9**–**11** are also smaller (3.4, 3.5 versus 4.6 Hz) whereas the opposite was observed for the $^{3}J_{C1,C6}$ couplings (5.1–5.3 versus 4.4 Hz). A global comparison of the observed coupling constants indicates their insensitivity to the environment as long as no direct substitutions occur at the sugar carbon atoms.

We have now found that the differences in the chemical shifts and the one-bond heteronuclear coupling constants observed upon chain elongation of the trisaccharide **1** to **2** and **3** (vide supra) are also reflected in the magnitude of the inter-residue $^{3}J_{C\text{-}1(C),H\text{-}3(B)}$[1] coupling constant. Values of this coupling constant have been measured for **1**–**3** by two-dimensional, *J*-resolved ^{1}H NMR spectroscopy, a technique that rotates ^{1}H–^{1}H splittings into the second dimension, thus separating them from ^{1}H chemical shifts in the first dimension. In this work, the heteronuclear ^{13}C–^{1}H splittings remained in the first dimension, thus distinguishing them from the ^{1}H–^{1}H splittings in the second dimension. All of the oligosaccharides studied gave spectra that were sufficiently well resolved in the first dimension for the $^{3}J_{C\text{-}1(C),H\text{-}3(B)}$ splittings to be detected. For example, the 2D *J*-resolved ^{1}H NMR spectrum of the pentasaccharide derivative **3** shown in Fig. 1, in which the full spectrum appears in (a) and the $^{3}J_{C\text{-}1(C),H\text{-}3(B)}$ splitting is identified in the spectrum expansion (b). The value of this coupling is 4.9 Hz in trisaccharide **1**, but only 4.1–4.2 Hz in the tetra- and penta-saccharides **2** and **3**

[1] Subscript characters in parentheses denote individual monosaccharide residues, with A standing for the potential reducing-end residue.

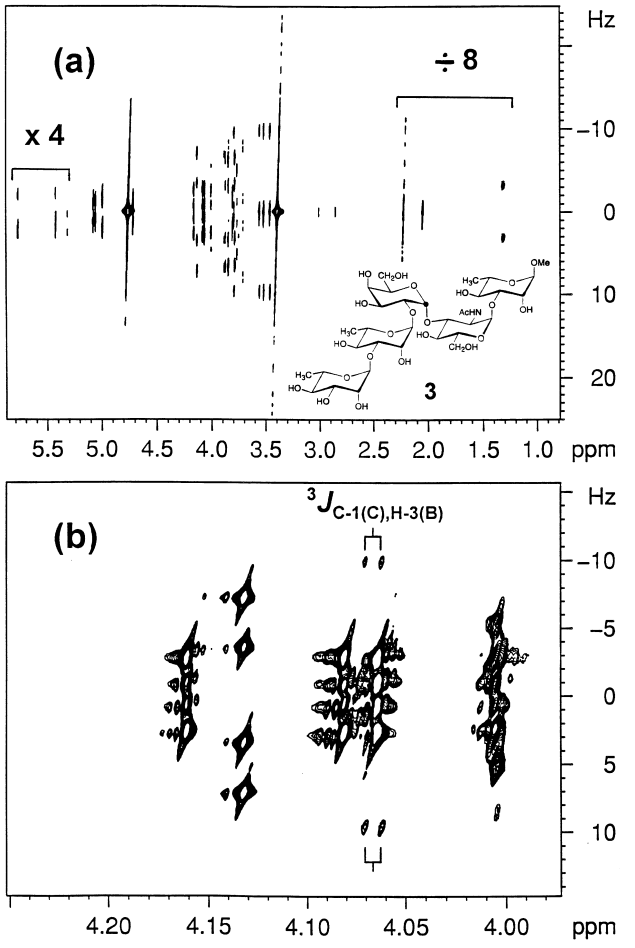


Fig. 1. 2D *J*-resolved ^{1}H NMR spectra of the pentasaccharide **3** in deuterium oxide at 500 MHz. (a) full spectrum; the intensities in two regions of the spectrum have been scaled as indicated, to permit an advantageous display on one chart; ● denotes the ^{13}C label; (b) expansion showing the splitting of the H-3_B multiplet (δ 4.066) by the $^{3}J_{C\text{-}1(C),H\text{-}3(B)}$ coupling constant, in the F_2 dimension.

that bear a rhamnose residue at O-2 of the Gal unit (Table 3). If it is assumed that these values of the coupling constants do not result from ensemble averaging of conformations, but originate from a single conformation, then application of the Karplus equation

$$^{3}J_{C,H} = 5.7\cos^2\psi - 0.6\cos\psi + 0.5 \qquad (1)$$

to the $^{3}J_{C\text{-}1(C),H\text{-}3(B)}$ values yields two sets of interglycosidic dihedral angles $\psi_{C-1(C),H-3(B)}$ [16] (Table 3). Early experience with other oligosaccharides suggests that the smaller set of dihedral angles may be correct [34], although the larger values could also be considered [35]. If the smaller set of values is taken, then the $\psi_{C-1(C),H-3(B)}$ values for the higher oligosaccharide homologues **2** and **3** are ~10–11° larger than the value for the trisaccharide **1**.

Table 3
Interglycosidic coupling constants (Hz) and dihedral angles of selectively ^{13}C labeled oligosaccharides

Oligosaccharide	$^3J_{C\text{-}1(C)\text{-}H\text{-}3(B)}$ (± 0.1 Hz [b])	$\psi_{C-1(C),H\text{-}3(B)}$ [a]
Trisaccharide **1**	4.9	21° or 146°
Tetrasaccharide **2**	4.1	32° or 138°
Pentasaccharide **3**	4.2	31° or 139°

[a] Calculated from eq (1). Absolute values are shown.
[b] Estimated standard uncertainty. According to eq (1), a 0.1 Hz error in the coupling constant results in a 0.9°–1.6° error in the dihedral angle, for the range of angles of interest.

An alternative explanation could be based on a change in conformational equilibrium. In that case, the estimated changes of the average dihedral angle $< \psi >$ would represent the conformational change of a "virtual" conformation [36].

A correlation of the $^1J_{C\text{-}1,H\text{-}1}$ values of **1**–**3** with the dihedral angle ϕ might also be attempted [35]. However, it has been suggested that this type of angular dependence is determined mainly by the orientation of lone pairs of electrons on the glycosidic oxygen atom (O-1) with respect to the C-1–H-1 bond [35], which may not be entirely appropriate for a comparison of **1**, **2**, and **3** that involves changes in other steric factors and the electronegativity of substituents at C-2_C.

We conclude that extension of the trisaccharide **1** by attachment of a rhamnose residue at the 2-position of the Gal unit (→ **2**, **3**) causes a change in the conformation of the Gal–GlcNAc linkage, which is reflected by enlargement of the $\psi_{C-1(C),H-3(B)}$ dihedral angle from ~21° to ~31–32°, and also changes in the ^{13}C-3_B chemical shift and $^1J_{C\text{-}1(C),H\text{-}1(C)}$ coupling constant.

2. Experimental

General methods.—For general methods, see ref. [6]. D-(1-^{13}C)Galactose (^{13}C >99 atom%) was purchased from Isotec Inc. (Miamisburg, OH).[2] Subscript characters $_{A–E}$ refer to the individual monosaccharide residues starting at the reducing end. Ammonia was used as the ionizing gas for the chemical ionization (CI) mass spectra. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

NMR spectroscopy.—The ^1H and the ^{13}C NMR spectra of the intermediates were recorded at 300 and 75.5 MHz, respectively, by using a Varian Associates XL-300 spectrometer. Internal references: Me_4Si (0.000 ppm for ^1H NMR of solutions in $CDCl_3$), acetone (2.225 ppm for ^1H NMR and 31.00 ppm for ^{13}C NMR of solutions in D_2O), and $CDCl_3$ (77.00 ppm for ^{13}C NMR of solutions in $CDCl_3$).

The ^1H NMR spectra of solutions of **1**–**3** (15–21 mg) in deuterium oxide (0.5 mL) were recorded at 500 MHz and 300 K by use of a Bruker Instruments AMX-500 NMR spectrometer. The data were acquired and processed by means of the Bruker XWINNMR program versions 1.2 (Silicon Graphics Indy R4600, 133 MHz) and 1.3 (Silicon Graphics Indigo[2] R4400, 250 MHz), respectively. 2D *J*-resolved ^1H NMR spectroscopy [28] was performed by using spectral widths of 2.5 kHz (F_2) and 50 Hz (F_1), 90° and 180° pulse widths of 9.85 and 19.7 μs, respectively, 80–128 scans per free induction decay (FID), two dummy scans, a pulse sequence recycle time of 4.2 s, and data sizes of 16,384 points (F_2) and 128 points (F_1), zero-filled or linearly predicted to 32,768 and 256 points, respectively. Spectral resolution was enhanced in both dimensions by Gaussian multiplication of the FID, using a line broadening of −0.5 Hz and a Gaussian truncation fraction of 0.3. The digital resolution was 0.08 Hz and 0.2 Hz in the F_2 and F_1 dimensions, respectively. A magnitude mode display was used for the 2D *J*-resolved ^1H NMR spectra. For each of the oligosaccharide derivatives **1**–**3**, the $^3J_{C\text{-}1(C),H\text{-}3(B)}$ coupling constant was measured as the splitting of the H-3 multiplet of the GlcNAc residue (Scheme 1) in the F_2 dimension of the 2D *J*-resolved ^1H NMR spectrum.

Methyl O-*(2-azido-2-deoxy-*α*-*D*-glucopyranosyl)-(1→3)-2,4-di*-O-*benzyl*-α-L-*rhamnopyranoside* (**5**).—A catalytic amount of NaOMe was added to a solution of **4** in MeOH. After 4 h, the solution was treated with Dowex 50×2 (H^+), filtered, and the filtrate concentrated to afford **5** (quant) as an amorphous substance: $[\alpha]_D$ +47° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H, δ 4.98 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1 of GlcN), 4.79, 4.76, 4.68, 4.61 (4 d, 4 H, J~ 12 Hz, H_2C of 2 Bn), 4.67 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 of Rha), 3.29 (s, 3 H, CH_3O), 3.20 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2 of GlcN), 1.29 (d, 1 H, $J_{5,6}$ 5.6 Hz, H-6 of Rha); ^{13}C, δ 98.1 (C-1 of Rha), 93.7 (C-1 of GlcN), 79.3, 74.6, 73.3, 71.8, 70.9, 70.7, 67.9, 62.6

[2] Certain commercial equipment, instruments, or material are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply that the materials are necessarily the best available for the purpose.

(C-2,3,4,5 of Rha, C-2,3,4,5 of GlcN), 75.4, 72.7 (CH_2 of Bn), 61.4 (C-6 of GlcN), 54.8 (CH_3O), 17.9 (C-6 of Rha); CI-MS: m/z 563 [$(M+NH_4)^+$]. Anal. Calcd for $C_{27}H_{35}N_3O_9$: C, 59.44; H, 6.47. Found: C, 59.34; H, 6.50.

Methyl O-*(4,6-di*-O-*acetyl-2-azido-3*-O-*chloroacetyl-2-deoxy*-α-D-*glucopyranosyl)-(1→3)-2,4-di*-O-*benzyl*-α-L-*rhamnopyranoside* (**6**).—A solution of **5** (19 g, 35 mmol) in DMF (40 mL) and 4-methoxybenzaldehyde dimethyl acetal (30 mL, 205 mmol) was treated with 10-camphorsulfonic acid (~ 0.5 g) at 23 °C. After 1 h, the solution was treated with Et_3N (2 mL). The solution was vigorously stirred with hexane (3×200 mL) and the supernatant decanted. The residue was equilibrated with $CHCl_3$ and H_2O, the organic phase was dried (Na_2SO_4), and then concentrated. A solution of the residue in pyridine (50 mL) was treated with monochloroacetic anhydride (11 g, 64 mmol) at 0 °C. After 30 min, the solution was treated with MeOH (20 mL) and then concentrated. A solution of the residue in MeOH (150 mL) was treated with HBF_4 (54% in ether, 1 mL) at 0 °C. After 1 h, the solution was treated with aq $NaHCO_3$ until pH ~5 was reached (indicator paper). The solution was concentrated to ~100 mL, and then the residue was equilibrated between $CHCl_3$ and H_2O. The organic phase was dried (Na_2SO_4) and concentrated. A solution of the residue in pyridine (30 mL) was treated with Ac_2O (20 mL) at 0 °C. The usual work-up followed by column chromatography (2:1 hexanes–EtOAc) of the residue afforded **6** (15.0 g, 61% for four steps) as a colorless syrup: $[\alpha]_D$ +105° (*c* 0.2, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 5.57 (t, 1 H, *J* 9.8 Hz, H-3 of GlcN), 3.36 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2 of GlcN), 3.31 (s, 3 H, CH_3O), 2.03, 1.90 (2 s, 6 H, 2 CH_3CO), 1.37 (d, 1 H, $J_{5,6}$ 6.2 Hz, H-6 of Rha); ^{13}C, δ 98.1 (C-1 of Rha), 93.0 (C-1 of GlcN), 79.1, 75.0, 72.7, 72.5, 68.0, 67.8, 67.2, 60.6 (C-2,3,4,5 of Rha, C-2,3,4,5 of GlcN), 75.4, 72.5 (CH_2 of 2 Bn), 61.3 (C-6 of GlcN), 54.8 (CH_3O), 40.4 (CH_2Cl), 20.6, 20.5 (2 CH_3CO), 17.9 (C-6 of Rha). Anal. Calcd for $C_{33}H_{40}ClN_3O_{12}$: C, 56.13; H, 5.71. Found: C, 56.23; H, 5.79.

Methyl O-*(4,6-di*-O-*acetyl-2-azido-2-deoxy*-α-D-*glucopyranosyl)-(1→3)-2,4-di*-O-*benzyl*-α-L-*rhamnopyranoside* (**7**).—A solution of **6** (19 g, 20 mmol) in MeOH (100 mL) and pyridine (2 mL) was treated with thiourea (5 g, 66 mmol) at 23 °C. After 72 h, the mixture was concentrated at < 30 °C. The residue was stirred with $CHCl_3$ (100 mL) and was filtered. Concentration of the mother liquor followed by column chromatographic purification of the residue (2:1 hexane–EtOAc) gave **7** (8.0 g, 64%) as an amorphous solid: $[\alpha]_D$ +67° (*c* 0.4, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 4.99 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1 of GlcN), 4.83 (dd, 1 H, *J* 9.1 Hz, *J* 10.2 Hz, H-4 of GlcN), 4.80, 4.78, 4.71, 4.68 (4 d, *J*~ 11 Hz, H_2C of 2 Bn), 4.70 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1 of Rha), 3.81 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2 of Rha), 3.32 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2 of GlcN), 3.30 (s, 3 H, CH_3O), 2.03, 1.99 (2 s, 6 H, 2 CH_3CO), 1.33 (d, 1 H, $J_{5,6}$ 6.2 Hz, H-6 of Rha); ^{13}C, δ 98.3 (C-1 of Rha), 93.0 (C-1 of GlcN), 79.2, 74.4, 72.9, 71.0, 70.6, 68.0, 67.2, 63.1, (C-2,3,4,5 of Rha, C-2,3,4,5 of GlcN), 75.1, 72.6 (CH_2 of 2 Bn), 61.6 (C-6 of GlcN), 54.7 (CH_3O), 20.7, 20.6 (2 CH_3CO), 17.9 (C-6 of Rha); CI-MS: m/z 647 [$(M+NH_4)^+$]. Anal. Calcd for $C_{31}H_{39}N_3O_{11}$: C, 59.13; H, 6.24. Found: C, 59.04; H, 6.27.

1,2-Di-O-*acetyl-3,4,6-tri*-O-*benzyl*-β-D-*(1-*^{13}*C)galactopyranose* (**8**).—To a stirred solution of $HClO_4$ (40 μL) in Ac_2O (15 mL) was added D-(1-^{13}C) galactose (3 g, 16.6 mmol) in portions at 30–40 °C over a period of 60 min. The solution was stirred for another 10 min then was concentrated at < 30 °C. To a solution of the residual syrup in AcOH (5 mL) was added a solution of HBr in AcOH (15 mL of a 33% solution) at 23 °C. After 90 min, the solution was diluted with $CHCl_3$ (100 mL) and was then extracted with H_2O (3×200 mL). The organic phase was dried (Na_2SO_4) and then concentrated. To a solution of the residue in anhydrous CH_2Cl_2 (30 mL) were added 2,4,6-trimethylpyridine (4 mL, 30 mmol), EtOH (4 mL, 65 mmol), and Bu_4NBr (0.4 g) at 23 °C. After 4 days, the mixture was concentrated, and the residue treated with ether. The solids were removed by filtration, and the residue obtained after concentration of the filtrate was purified by column chromatography (2:1 hexane–EtOAc). The fractions containing the product were pooled and concentrated. Residual trimethylpyridine was removed at ~0.7 Pa (5 mtorr) to give 4.4 g of a syrup that was dissolved in dry DMF (40 mL). To this solution was added under stirring NaH (2.5 g of a 60% suspension in oil, ~ 63 mmol) in portions at 0 °C, followed by benzyl bromide (7.2 mL, 61 mmol). The mixture was stirred for 4 h then processed as usual, followed by column chromatographic purification (9:1 hexane–EtOAc) to give a syrupy residue. A solution of the syrup so obtained in Me_3SiOAc (90 mL) was stirred under reflux for 8 h. Concentration of the solution followed by

equilibration of the residue between $CHCl_3$ and H_2O and crystallization ($^iPr_2O$) afforded **8** (4.5 g, 51% for 5 steps) which had physical properties identical to those of the unlabeled preparation [37]. NMR ($CDCl_3$): ^1H δ 5.57 (dd, 1 H, $J_{H-1,H-2}$ 8.1 Hz, $J_{H-1,C-1}$ 166 Hz, H-1), 5.48 (ddd, 1 H, $J_{H-2,H-3}$ 10 Hz, $J_{H-2,H-1}$ 6.9 Hz, H-2), 4.95, 4.69, 4.61, 4.52, 4.46, 4.35 (6 d, 6 H, CH_2 of 3 Bn), 4.00 (dd, 1 H, $J_{H-3,H-4}$ 2.8 Hz, $J_{H-4,C-1}$ 0.9 Hz, H-4), 3.71 (dddd, 1 H, $J_{H-5,H-6}$ 5.8 Hz, $J_{H-5,H-6'}$ 7.1 Hz, $J_{H-5,C-1}$ 2.4 Hz, H-5), 3.66–3.57 (m, 2 H, H-6,6′), 3.58 (ddd, 1 H, $J_{H-3,C-1}$ 1.5 Hz, H-3), 2.1, 2.0 (2 s, 6 H, 2 CH_3CO); ^{13}C, δ 92.6 (C-1), 80.0 (d, $J_{C-1,C-3}$ 4.7 Hz, C-3), 74.6, 73.5, 72.0 (CH_2 of 3 Bn), 74.3 (C-5), 72.1 (C-4), 70.3 (d, $J_{C-1,C-2}$ 48 Hz, C-2), 67.8 (d, $J_{C-1,C-6}$ 5.1 Hz, C-6), 20.9, 20.8 (2 CH_3CO); CI-MS: m/z 553 $[(M+NH_4)^+]$.

*Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-*β*-*D*-(1-^{13}C)galactopyranoside* (**9**).—Treatment of **8** with $MeSSiMe_3$ as described for the unlabeled compound afforded **9** which had physical properties identical to those of the unlabeled preparation [4]. NMR ($CDCl_3$): ^1H δ 4.23 (dd, 1 H, $J_{H-1,H-2}$ 9.8 Hz, $J_{H-1,C-1}$ 152 Hz, H-1), 5.44 (dt, 1 H, $J_{H-2,H-3}$ 9.8 Hz, $J_{H-2,C-1}$ 4.8 Hz, H-2), 4.95, 4.68, 4.58, 4.54, 4.46, 4.41 (6 d, 6 H, CH_2 of 3 Bn), 4.00 (br d, 1 H, $J_{H-3,H-4}$ 2.8 Hz, H-4), 2.14 (d, 3 H, J_{C-1,SCH_3} 4.1 Hz, CH_3S); ^{13}C, δ 83.0 (C-1), 81.3 (d, $J_{C-1,C-3}$ 3.4 Hz, C-3), 77.4 (d, $J_{C-1,C-5}$ 2.3 Hz, C-5), 74.4, 73.5, 71.9 (CH_2 of 3 Bn), 73.0 (C-4), 68.8 (d, $J_{C-1,C-2}$ 42 Hz, C-2), 68.3 (d, $J_{C-1,C-6}$ 5.1 Hz, C-6), 21.0 (CH_3CO), 10.9 (CH_3S); CI-MS: m/z 541 $[(M+NH_4)^+]$.

*Methyl 3,4,6-tri-*O*-benzyl-1-thio-*β*-*D*-(1-^{13}C)-galactopyranoside* (**10**).—Treatment of **9** with NaOMe–MeOH as described for the preparation of **5** afforded **10** (95%) that had physical properties identical with those of the unlabeled equivalent [4]. NMR ($CDCl_3$): ^1H δ 2.19 (d, 3 H, J_{C-1,SCH_3} 4.0 Hz, CH_3S), ^{13}C δ 86.0 (C-1), 83.1 (C-3), 77.5 (C-5), 74.5, 73.5, 72.3 (CH_2 of 3 Bn), 73.2 (C-4), 69.0 (d, $J_{C-1,C-2}$ 41 Hz, C-2), 68.5 (d, $J_{C-1,C-6}$ 5.2 Hz, C-6), 11.6 (CH_3S); CI-MS: m/z 499 $[(M+NH_4)^+]$.

*Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-1-thio-*β*-*D*-(1-^{13}C)galactopyranoside* (**11**).—Treatment of **10** with NaH then with 4-methoxybenzyl chloride, as described for the unlabeled counterpart afforded **11** which had physical properties identical to those of the unlabeled analog [4]. NMR ($CDCl_3$): ^1H δ 4.32 (dd, 1 H, $J_{H-1,H-2}$ 9.6 Hz, $J_{H-1,C-1}$ 152 Hz, H-1), 2.20 (d, 3 H, J_{C-1,SCH_3} 4.2 Hz, CH_3S); ^{13}C, δ 85.7 (C-1), 84.0 (d, $J_{C-1,C-3}$ 3.5 Hz, C-3), 77.6 (d, $J_{C-1,C-2}$ 42 Hz, C-2), 77.2 (d, $J_{C-1,C-5}$ ~1 Hz, C-5), 75.3, 74.4, 73.5, 72.6 (CH_2 of 3 Bn, MBn), 73.6 (C-4), 68.6 (d, $J_{C-1,C-6}$ 5.3 Hz, C-6), 55.3 (CH_3O), 12.8 (CH_3S); CI-MS: m/z 619 $[(M+NH_4)^+]$.

Methyl O*-[3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-*α*-*D*-(1-^{13}C)galactopyranosyl]-(1→3)-*O*-(4,6-di-*O*-acetyl-2-azido-2-deoxy-*α*-*D*-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-*α*-*L*-rhamnopyranoside* (**12**).—To a solution of **11** (150 mg, 0.25 mmol), and 2,6-di-tert-butyl-4-methylpyridine (300 mg, 1.5 mmol) in CH_2Cl_2 (5 mL) was added at 23 °C a solution of Cl_2 in CCl_4 (excess). After 2 min, 1-hexene was added until the yellow color disappeared. This solution was transferred by syringe to a stirred mixture of **7** (200 mg, 0.32 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (100 mg, 0.5 mmol), 4Å molecular sieves (100 mg), and CH_2Cl_2 (2 mL). The mixture was stirred at 23 °C for 15 min and then cooled to −78 °C and treated with CF_3SO_2OAg (126 mg, 0.49 mmol). After 3 h, the mixture was processed as usual followed by column-chromatographic purification (2:1 hexane–EtOAc) to afford **12** (194 mg, 66%) as a syrup: $[\alpha]_D$ +65° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.15 (dd, 1 H, *J* 10.3 Hz, *J* 8.8 Hz, H-4 of GlcN), 5.07 (dd, 1 H, $J_{H-1,H-2}$ 3.4 Hz, $J_{H-1,C-1}$ 171 Hz, H-1 of Gal), 5.05 (d, 1 H, *J* 3.6 Hz, H-1 of GlcN), 3.74 (CH_3O of MBn), 3.37 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2 of GlcN), 5.15 (dd, 1H, *J* 10.3 Hz, *J* 8.8 Hz, H-4 of GlcN), 3.27 [CH_3O (aglycon)], 2.03, 1.75 (2 CH_3CO), 1.33 (d, 1 H, $J_{5,6}$ 6.2 Hz, H-6 of Rha); ^{13}C, δ 98.6 (C-1 of Gal), 92.8 (C-1 of GlcN), 79.1, 79.0, 74.8, 74.2, 74.0, 72.7, 70.2, 70.0, 67.9, 67.2, 62.6 (C-2,3,4,5 of Rha, C-2,3,4,5 of GlcN, C-3,4,5 of Gal), 75.2, 74.9, 73.3, 73.2, 72.8, 72.5 (CH_2 of 5 Bn, MBn), 74.8 (d, $J_{C-1,C-2}$ 47 Hz, C-2 of Gal), 68.5 (d, $J_{C-1,C-6}$ 3.9 Hz, C-6 of Gal), 61.6 (C-6 of GlcN), 55.1, 54.8 (2 CH_3O), 20.8, 20.6 (2 CH_3CO), 17.8 (C-6 of Rha). FAB-MS: m/z 1156 $[(M-N_2+H)^+]$. Anal. Calcd for $C_{66}H_{75}N_3O_{17}$·EtOAc: C, 65.96; H, 6.50. Found: C, 65.45; H, 6.21.

Methyl O*-[3,4,6-tri-*O*-benzyl-*α*-*D*-(1-^{13}C)galactopyranosyl]-(1→3)-*O*-(4,6-di-*O*-acetyl-2-azido-2-deoxy-*α*-*D*-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-*α*-*L*-rhamnopyranoside* (**13**).—To a solution of **12** (5 g, 4.2 mmol) in MeCN (10 mL) and H_2O (10 mL) was added $(NH_4)_2Ce(NO_3)_6$ (7 g, 12.8 mmol) at 23 °C. After 10 min, the mixture was treated with aq $NaHSO_3$ then was concentrated. Equilibration of the residue between $CHCl_3$ and H_2O, followed by concentration of the organic layer and column chromatographic purification of the residue

afforded **13** as a syrup (3.6 g, 80%): $[\alpha]_D$ +124° (*c* 0.1, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 4.97 (dd, 1 H, $J_{1,2}$ 3.9 Hz, $J_{H\text{-}1,C\text{-}1}$ 172 Hz, H-1 of Gal), 5.06 (d, 1 H, *J* 3.6 Hz, H-1 of GlcN), 4.97 (dd, 1 H, *J* 10.0 Hz, *J* 9.2 Hz, H-4 of GlcN), 3.30 (CH_3O), 2.04, 1.92 (2 CH_3CO), 1.35 (d, 1 H, $J_{5,6}$ 6.2 Hz, H-6 of Rha); ^{13}C, δ 101.5 (C-1 of Gal), 98.2 (C-1 of Rha), 93.3 (C-1 of GlcN), 79.2 (2 C), 76.1, 73.8, 73.7, 72.36, 70.3, 70.2, 67.9, 67.2, 61.3 (C-2,3,4,5 of Rha, C-2,3,4,5 of GlcN, C-3,4,5 of Gal), 75.2, 74.9, 73.5, 72.40, 72.2 (CH_2 of 5 Bn), 68.6 (d, $J_{C\text{-}1,C\text{-}2}$ 46 Hz, C-2 of Gal), 68.3 (d, $J_{C\text{-}1,C\text{-}6}$ 4.0 Hz, C-6 of Gal), 61.5 (C-6 of GlcN), 54.9 (CH_3O), 20.9, 20.6 (2 CH_3CO), 17.9 (C-6 of Rha). FAB-MS: *m/z* 1035.47 [$(M\text{-}N_2+H)^+$], 1061.39 [$(M\text{-}H_2+H)^+$]. Anal. Calcd for $C_{58}H_{67}N_3O_{16}$: C, 65.59; H, 6.36. Found: C, 65.17; H, 6.36.

Methyl O-*(2*-O-*benzoyl-4*-O-*benzyl-3*-O-*chloroacetyl*-α-L-*rhamnopyranosyl)-(1→2)*-O-*[3,4,6-tri*-O-*benzyl*-α-D-*(1-^{13}C)galactopyranosyl]-(1→3)*-O-*(4,6-di*-O-*acetyl-2-azido-2-deoxy*-α-D-*glucopyranosyl)-(1→3)-2,4-di*-O-*benzyl*-α-L-*rhamnopyranoside* (**15**).—To a stirred mixture of **13** (3.40 g, 3.2 mmol), **14** (4.60 g, 7.9 mmol), 4Å molecular sieves (2 g), and CH_2Cl_2 (33 mL) was added at 0 °C $BF_3{\cdot}Et_2O$ (270 μL, 2.2 mmol). After 2 h, the mixture was filtered and concentrated. Column-chromatographic purification of the residue (3:2 hexane–EtOAc) afforded **15** (4.20 g, 89%) as an amorphous solid: $[\alpha]_D$ +75° (*c* 0.2, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.64 (dd, 1 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.3 Hz, H-2 of Rha_D), 5.40 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3 of Rha_D), 5.25 (dd, 1 H, $J_{1,2}$ 3.4 Hz, $J_{H\text{-}1,C\text{-}1}$ 170.5 Hz, H-1 of Gal), 5.30 (d, 1 H, H-1 of Rha_D), 5.15 (dd, 1 H, *J* 10.0 Hz, *J* 9.2 Hz, H-4 of GlcN), 4.99 (d, 1 H, *J* 4.0 Hz, H-1 of GlcN), 4.71 (d, 1 H, H-1 of Rha_A), 3.30 (s, 3 H, CH_3O), 1.99, 1.96 (2 CH_3CO), 1.33, 1.29 (2 d, 6 H, $J_{5,6}$ ~ 6 Hz, H-6 of 2 Rha); ^{13}C, δ 96.8 (C-1 of Gal), 93.0 (C-1 of GlcN), 79.0, 78.8, 78.4, 74.6, 74.4, 74.0, 73.6, 70.2 (2 C), 70.0, 69.6, 68.1, 68.0, 66.9, 62.7 (C-2,3,4,5 of 2 Rha, C-2,3,4,5 of GlcN, C-3,4,5 of Gal), 75.1, 74.9, 74.8, 73.3, 72.9, 72.7 (CH_2 of 6 Bn), 73.7 (d, $J_{C\text{-}1,C\text{-}2}$ 48 Hz, C-2 of Gal), 68.9 (d, $J_{C\text{-}1,C\text{-}6}$ 3.7 Hz, C-6 of Gal), 61.5 (C-6 of GlcN), 54.8 (CH_3O), 20.9, 20.6 (2 CH_3CO), 18.0, 17.9 (C-6 of 2 Rha). FAB-MS: *m/z* 1451.53 [$(M\text{-}N_2+H)^+$]. Anal. Calcd for $C_{80}H_{88}ClN_3O_{22}$: C, 65.06; H, 6.18. Found: C, 64.80; H, 6.03.

Methyl O-*(2*-O-*benzoyl-4*-O-*benzyl*-α-L-*rhamnopyranosyl)-(1→2)*-O-*[3,4,6-tri*-O-*benzyl*-α-D-*(1-^{13}C)galactopyranosyl]-(1→3)*-O-*(4,6-di*-O-*acetyl-2-azido-2-deoxy*-α-D-*glucopyranosyl)-(1→3)-2,4-di*-O-*benzyl*-α-L-*rhamnopyranoside* (**16**).—To a solution of **15** (4.15 g, 2.8 mmol) in pyridine (2 mL) and DMF (20 mL) was added thiourea (5 g, 63 mmol) at 23 °C. After 12 h, the solution was concentrated at <30 °C. To the residue was added $CHCl_3$ (50 mL). The insoluble material was removed by filtration and the filtrate was extracted with H_2O (3×50 mL). Concentration of the solution, followed by column chromatographic purification of the residue (3:2 hexane–EtOAc) afforded **16** as an amorphous solid (3.58 g, 91%): $[\alpha]_D$ +57° (*c* 0.2, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.46 (dd, 1 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.4 Hz, H-2 of Rha_D), 4.71 (dd, 1 H, $J_{1,2}$ 3.4 Hz, $J_{H\text{-}1,C\text{-}1}$ 170.5 Hz, H-1 of Gal), 5.36 (d, 1 H, H-1 of Rha_D), 5.10 (dd, 1 H, *J* 10.4 Hz, *J* 8.9 Hz, H-4 of GlcN), 4.99 (d, 1 H, *J* 3.9 Hz, H-1 of GlcN), 3.29 (CH_3O), 2.01, 1.95 (2 CH_3CO), 1.37, 1.28 (2 d, 6 H, $J_{5,6}$ ~ 6 Hz, H-6 of 2 Rha); ^{13}C, δ 96.8 (C-1 of Gal), 93.0 (C-1 of GlcN), 81.4, 79.0 (2 C), 74.7, 74.2 (2 C), 72.7 (2 C), 70.4, 70.1, 69.4, 68.3, 67.9, 66.8, 63.3 (C-2,3,4,5 of 2 Rha, C-2,3,4,5 of GlcN, C-3,4,5 of Gal), 75.2, 75.0, 74.8, 73.3, 72.9, 72.7 (CH_2 of 6 Bn), 71.9 (d, $J_{C\text{-}1,C\text{-}2}$ 47 Hz, C-2 of Gal), 68.8 (d, $J_{C\text{-}1,C\text{-}6}$ 4.0 Hz, C-6 of Gal), 61.5 (C-6 of GlcN), 54.7 (CH_3O), 20.9, 20.6 (2 CH_3CO), 18.2, 17.8 (C-6 of 2 Rha). FAB-MS: *m/z* 1375.60 [$(M\text{-}N_2+H)^+$]. Anal. Calcd for $C_{78}H_{87}N_3O_{21}$: C, 66.80; H, 6.25. Found: C, 66.54; H, 6.27.

Methyl O-*(2*-O-*benzoyl-4*-O-*benzyl-3*-O-*chloroacetyl*-α-L-*rhamnopyranosyl)-(1→3)*-O-*(2*-O-*benzoyl-4*-O-*benzyl*-α-L-*rhamnopyranosyl)-(1→2)*-O-*[3,4,6-tri*-O-*benzyl*-α-D-*(1-^{13}C)galactopyranosyl]-(1→3)*-O-*(4,6-di*-O-*acetyl-2-azido-2-deoxy*-α-D-*glucopyranosyl)-(1→3)-2,4-di*-O-*benzyl*-α-L-*rhamnopyranoside* (**17**).—Reaction of **16** (490 mg, 0.35 mmol) and **14** (1 g, 1.7 mmol) as described for the preparation of **15** afforded, after column-chromatographic purification (3:2 hexane–EtOAc), **17** (560 mg, 88%) as an amorphous solid: $[\alpha]_D$ +59° (*c* 0.2, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.61, 5.55 (2 dd, 2 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.3 Hz, H-2 of Rha_D, Rha_E), 5.46 (d, 1 H, H-1 of Rha_E), 5.37 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3 of Rha_E), 5.21 (d, 1 H, H-1 of Rha_D), 5.17 (dd, 1 H, $J_{1,2}$ 3.7 Hz, $J_{H\text{-}1,C\text{-}1}$ 171 Hz, H-1 of Gal), 5.10 (dd, 1 H, *J* 10.9 Hz, *J* 8.9 Hz, H-4 of GlcN), 4.98 (d, 1 H, *J* 3.8 Hz, H-1 of GlcN), 4.70 (d, 1 H, H-1 of Rha_A), 3.29 (s, 3 H, CH_3O), 1.82, 1.80 (2 CH_3CO), 1.30, 1.29, 1.23 (3 d, 9 H, $J_{5,6}$ ~6 Hz, H-6 of 3 Rha); ^{13}C, δ 96.8 (C-1 of Gal), 92.7 (C-1 of GlcN), 79.8, 79.0, 78.9, 78.5, 78.1,

74.6, 74.4, 73.6, 72.7, 72.5, 72.3, 70.4, 70.2, 70.0, 68.4, 68.3, 68.0, 67.2, 62.6 (C-2,3,4,5 of 3 Rha, C-2,3,4,5 of GlcN, C-3,4,5 of Gal), 75.4, 75.1, 74.8, 73.9, 73.3, 73.0 (2 C) (CH_2 of 7 Bn), 71.9 (d, $J_{C-1,C-2}$ 47.5 Hz, C-2 of Gal), 68.8 (d, $J_{C-1,C-6}$ 3.5 Hz, C-6 of Gal), 61.4 (C-6 of GlcN), 54.8 (CH_3O), 20.8, 20.4 (2 CH_3CO), 18.2, 17.9 (2 C) (C-6 of 3 Rha). FAB-MS: m/z 1791.82 [$(M\text{-}N_2+H)^+$]. Anal. Calcd for $C_{100}H_{108}ClN_3O_{27}$: C, 66.02; H, 5.98. Found: C, 66.13; H, 6.05.

Methyl O-α-D-*(1-^{13}C)galactopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)*-O-α-L-*rhamnopyranoside* (**1**).—A solution of **13** (650 mg, 0.55 mmol) and Ph_3P (600 mg, 2.3 mmol) in CH_2Cl_2 (6 mL) was stirred at 23 °C for 24 h, and was then treated with H_2O (0.5 mL). After another 36 h, the solution was cooled to 0 °C and treated with Ac_2O (0.5 mL). The solution was extracted with H_2O, and was then concentrated. Column chromatographic purification of the residue (2:1 hexane–EtOAc) gave an amorphous solid (463 mg). A solution of the intermediate in dry MeOH (5 mL) was treated at 23 °C with NaOMe (~10 mg). After 72 h, the solution was treated with Dowex 50×8–100 (H^+), and then concentrated. The residue was dissolved in a mixture of EtOH (10 mL) and AcOH (1 mL) and was hydrogenolyzed over Pd-C (10%, ~0.2 g) at 1.4 MPa (200 psi) for 24 h. The mixture was filtered and the filtrate concentrated. The residue was purified through Biogel P-2 using 0.05 N pyridine–AcOH as the eluant. The fractions containing the product were pooled and the combined solution was freeze-dried to afford **1** as an amorphous solid (220 mg) having $[\alpha]_D$ identical with that of the unlabeled equivalent [6]; NMR (D_2O): ^{13}C, δ 77.9 (br, C-3 of GlcN), 71.5 (br, C-5 of Gal), 69.2 (d, $J_{C-1,C-2}$ 46 Hz, C-2 of Gal), (d, $J_{C-1,C-6}$ 3.6 Hz, C-6 of Gal).

Methyl O-α-L-*rhamnopyranosyl-(1→2)*-O-α-D-*(1-^{13}C)galactopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)*-O-α-L-*rhamnopyranoside* (**2**).—Transformations of compound **16** as described for the preparation of **1** afforded **2** as an amorphous substance having $[\alpha]_D$ identical to that of the unlabeled equivalent [6]; NMR (D_2O): ^{1}H δ 5.59 (dd, 1 H, $J_{1,2}$ 3.6 Hz, $J_{H-1,C-1}$ 174 Hz; ^{13}C, δ 74.4 (d, $J_{C-1,C-2}$ 47 Hz, C-2 of Gal), 61.5 (d, $J_{C-1,C-6}$ 3.2 Hz, C-6 of Gal).

Methyl O-α-L-*rhamnopyranosyl-(1→3)*-O-α-L-*rhamnopyranosyl-(1→2)*-O-α-D-*(1-^{13}C)galactopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)*-O-α-L *rhamnopyranoside* (**3**).—Transformations of compound **17** as described for the preparation of **1** afforded **3** as an amorphous substance having $[\alpha]_D$ identical to that of the unlabeled counterpart [6]; NMR (D_2O): ^{1}H δ 5.59 (dd, 1 H, $J_{1,2}$ 3.6 Hz, $J_{H-1,C-1}$ 174 Hz; ^{13}C, δ 74.5 (d, $J_{C-1,C-2}$ 47 Hz, C-2 of Gal), 61.5 (d, $J_{C-1,C-6}$ 3.4 Hz, C-6 of Gal).

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