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Practical synthesis of [1-^{13}C]- and [6-^{13}C]-D-galactose

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Abstract—The chemical synthesis of ^{13}C-labeled D-galactose as useful molecular probes for studying the conformation of oligosaccharides attached to proteins was performed. The method for synthesizing the title labeled compounds was newly developed via the corresponding 1-ene and 5-ene compounds derived from 1,2:5,6-di-*O*-isoproppylidene-α-D-galactofuranose by considering the efficient introduction of the atom. All protons of galactose from H-1 to H-6 were observed by the HMQC–HOHAHA technique using 1:1 mixtures of methyl [1-^{13}C]- and [6-^{13}C]-β-D-galactopyranoside, which were prepared from the title compounds.

Sialyl glycoconjugates are important cell surface components active in a variety of intercellular recognition events. Therefore, a study of the conformation and dynamics of these sialyloligosaccharides and their analogs should provide useful insights into how these cell surface oligosaccharides interact with the corresponding receptor molecules. However, although the conformational properties[1,2,3a–c] of sialyloligosaccharide analogs of low molecular weight have been reported by many research groups, the conformation and dynamics of sialyloligo-saccharides and their analogs attached to glycoproteins have not yet been fully analyzed. To address this problem, ^{13}C-labeled sialic acid (NeuAc) has been utilized for the conformational analysis of sialyloligosaccharides on artificial membrane surfaces[4a,b] and TRNOE experiments.[5] Recently, novel NMR techniques, HSQC–TOCSY–NOESY–TOCSY, for observation of all protons of NeuAc, H-3 to H-9, even with only a single ^{13}C-atom at the 3-position of NeuAc, bound to a glycoprotein are reported.[2] However, since combined multi-pulse techniques generally suffer from low sensitivity, the authors have synthesized minimal [3,9-^{13}C]-labeled NeuAc for convenient observation of all protons of NeuAc from H-3 to H-9 by the HMQC–HOHAHA technique. The authors have also synthesized [3-^{13}C]- and [9-^{13}C]-labeled NeuAc, respectively, and demonstrated that identical results are obtained by NMR for [3,9-^{13}C]-NeuAc and 1:1 mixtures of [3-^{13}C]- and [9-^{13}C]-NeuAc.[6]

It is generally accepted that it is rather difficult to detect ^{1}H information from [U–^{13}C]-sugars, in high sensitivity, because of ^{13}C–^{13}C couplings and long-range couplings. This minimal and efficient ^{13}C-labeling method promised to enable us to prepare practical amounts of important ^{13}C-labeled component monosaccharides, such as NeuAc, KDN, galactose, mannose, mannosamine, and fucose for studying how the functions and roles of cell surface sialyl and KDN oligosaccharides intact with the corresponding receptor molecules.

In this paper, a practical synthesis of [1-^{13}C]- and [6-^{13}C]-D-galactose and the labeling effect of 1:1 mixtures of the galactoses by the HMQC–HOHAHA technique are described. ^{13}C is preferably introduced at the last stage for saving the labeled compound, $^{13}CH_3I$, that was commercially available. The introduction of ^{13}C was performed by extension of either end of pentoses derived from the same intermediate, 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose with phosphonium salt ($Ph_3P^{13}CH_3I$).

Synthesis of [1-^{13}C]-D-galactose from D-glucose

[1-^{13}C]-D-Galactose was synthesized from D-glucose as follows (Scheme 1). 1,2:5,6-Di-*O*-isopropyridene-α-D-galctofuranose (**3**) was synthesized by the modified Weygand method.[7] That is, the starting compound 3-*O*-triflate **1** instead of 3-*O*-tosylate was derived from 1,2:5,6-di-*O*-isopropyridene-α-D-glucofuranose (**1**) quantitatively. Compound **1** was treated with DBU/DMSO to give the elimination compound **2** in 98% yield. The hydroboration–oxidation reaction of **2** gave

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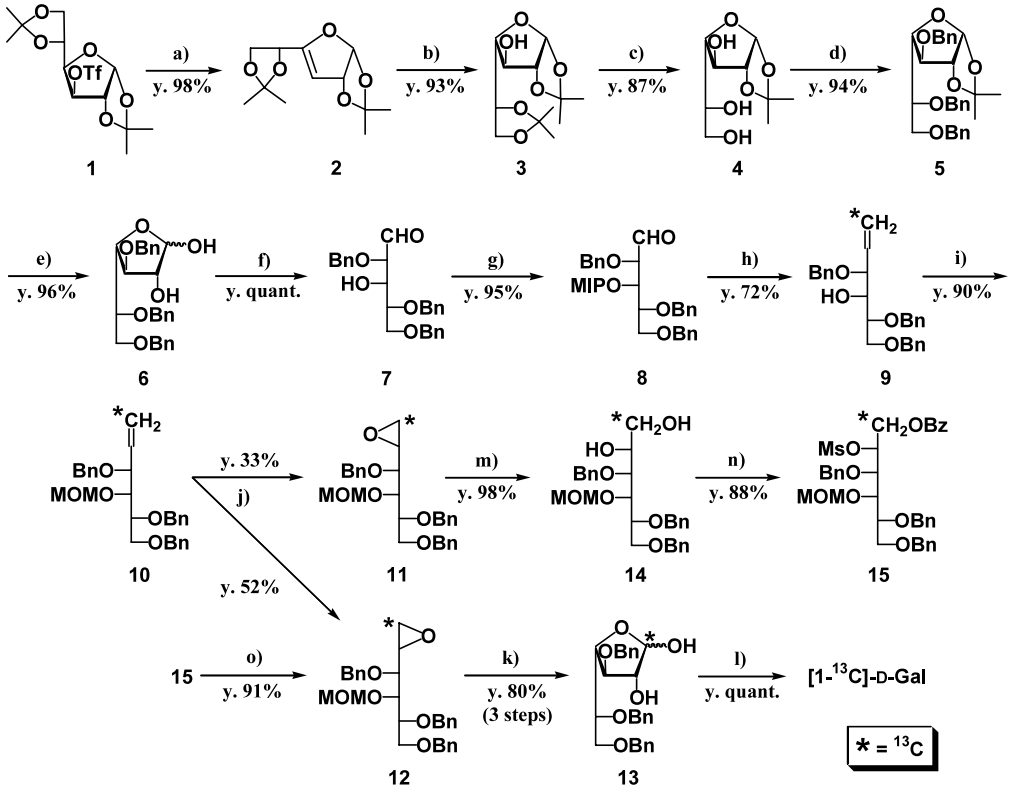

Scheme 1. *Reagents and conditions*: (a) DBU/DMSO; (b) BH_3–THF, then NaOH aq., H_2O_2/THF; (c) 70% AcOH aq.; (d) NaH, BnBr/DMF; (e) 85% AcOH aq.; (f) $NaIO_4$/MeOH–H_2O, then 1 M HCl aq.; (g) isopropenylmethylether, PPTS; (h) $Ph_3P^{13}CH_3I$, *n*-BuLi/THF, then AcOH aq.; (i) NaH, MOMCl/DMF; (j) *m*-CPBA/$C_2H_4Cl_2$; (k) (i) 1 M KOH aq./DMSO; (ii) TEMPO, trichloroisocyanuric acid/CH_2Cl_2, (iii) CSA/CH_2Cl_2; (l) 10% Pd–C, H_2/EtOH–H_2O; (m) 1 M KOH aq./DMSO; (n) BzCl/Py., then MsCl; (o) 1 M KOH aq./MeOH.

the corresponding **3** in 93% yield. The partial hydrolysis of **3** at rt gave 1,2-*O*-isopropylidene-α-D-galactofuranose (**4**) in 87% yield. Compound **4** was then benzylated with NaH, BnBr in DMF to give the tri-*O*-benzylated compound **5** in 94% yield. The hydrolysis of **5** at 70°C gave **6** in 96% yield. Periodate oxidation of **6** with $NaIO_4$ and 1 M HCl in MeOH–H_2O gave the corresponding pentose derivative **7** quantitatively. The hydroxyl group of the unstable carbonyl compound **7** was protected with MIP (methoxyisopropyl) by using isopropenyl methyl ether and a catalytic amount of PPTS at 0°C to give **8** in 95% yield. The Wittig reaction of **8** with labeled phosphonium salt $Ph_3P^{13}CH_3I$ (1.0 equiv.), which was quantitatively synthesized from $^{13}CH_3I$, and treating with 30% AcOH aq. gave labeled hexose derivative **9** in 72% yield. The MIP protecting group proved to be suitable for this carbonyl compound and the reaction conditions. The product was too unstable, even to a column of silica gel. We treated the resulting mixture of the Wittig reaction with acid (30% AcOH aq.) to obtain a single deprotected (MIP) compound **9**. Re-protection of **9** with an MOM group, which could not be introduced to **7**, gave **10** in 90% yield. Peracid oxidation of **10** with *m*-CPBA in 1,2-dichloroethane at 60°C gave the corresponding epimeric epoxides **11** and **12** in 33 and 52% yields, respectively. The required epoxide **12** was hydrolyzed with 1 M KOH aq. in DMSO to give the 1,2-di-ol compound in 99% yield. The primary hydroxyl group of the product was then selectively oxidized with 2,2,6,6-tetramethyl-1-piperidinyloxy, a free radical (TEMPO[7]) and trichloroisocyanuric acid in CH_2Cl_2 to give the galactose derivative in 90% yield, which, in turn, afforded the corresponding galactofuranose derivative **13** in 90% yield by treatment with (±)-10-camphorsulfonic acid (CSA) in CH_2Cl_2 (three steps, 80% yield). Removal of the benzyl group of **13** by catalytic reduction with 10% Pd–C, H_2 in EtOH–H_2O gave [1-^{13}C]-galactose quantitatively. It is also possible to obtain **13** from **12** directly by treatment with BF_3-Et_2O, DMSO,[8] albeit in lower yield (68%). On the other hand, conversion of the diastereomeric epoxide **11** to **12** was possible for saving the labeling compound as follows. Compound **11** was hydrolyzed to give the corresponding 1,2-di-ol derivative **14**, in 98% yield, by treatment with 1 M KOH aq./DMSO at 70°C. Selective benzoylation and mesylation of each hydroxyl group of **14** gave **15** in 88% yield. Compound **15** was then treated with 1 M KOH aq./MeOH to give epoxide **12** in 91% yield. A direct acylation and mesylation of **11** gave unsatisfactory results. As a result of this synthesis, the percentage of the utilization of $^{13}CH_3I$ was 40%.

Synthesis of [6-^{13}C]-D-galactose from D-glucose

[6-^{13}C]-D-Galactose was synthesized from D-glucose as follows (Scheme 2). The same intermediate **3** used for the synthesis of [1-^{13}C]-D-galactose was benzylated to give **16** quantitatively. Compound **16** was then partially hydrolyzed to give di-ol compound **17**, which was quantitatively converted into the corresponding aldehyde **18** by oxidation with $NaIO_4$. The introduction of ^{13}C was achieved by the Wittig reaction of **18** and $Ph_3P^{13}CH_3I$ (1.0 equiv.) to give the labeled olefin compound **19** in 74% yield. Peracid oxidation of **19** with *m*-CPBA in a similar manner as mentioned above gave epimeric epoxides **20** and **21** in 58 and 25% yields, respectively. Hydrolysis of an epoxide **21** with 1 M KOH aq./DMSO gave the corresponding di-ol **22** in 90% yield. Galactofuranose derivative **22** was then hydrogenolyzed and hydrolyzed to obtain [6-^{13}C]-D-galactose. The yield was 96% (two steps). On the other hand, the undesired epoxide **20** can be converted into the desired **21** as follows.

Compound **20** was hydrolyzed to give the corresponding 1,2-di-ol derivative **23**, in 89% yield, by treatment with 1 M KOH aq./DMSO at 70°C. Selective benzoylation and mesylation of each hydroxyl group of **23** gave **24** in 78% yield. Compound **24** was then treated with 1 M KOH aq./MeOH to give the epoxide **21** in 95% yield. This procedure brings the percentage of utilization of $^{13}CH_3I$ to 40%. Compounds **20** and **21** should also be useful ^{13}C-labeled precursors for synthesizing analogs to study the functional properties of oligosaccharides.

^{1}H and ^{13}C NMR data for each ^{13}C-labeled compound, [1-^{13}C]- and [6-^{13}C]-D-galactose, are given in Ref. 9. ^{1}H NMR data for the non-labeled and ^{13}C-labeled galactoses are shown in Figure 1. In order to measure the HMQC–HOHAHA spectra of a 1:1 mixture of [1-^{13}C]- and [6-^{13}C]-D-galactose as a single isomer, both ^{13}C-labeled D-galactoses were converted into its corresponding methyl β-D-galactopyranoside via 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate.[10a,b] NMR data for the HMQC–HOHAHA spectra of a 1:1 mixture of methyl [1-^{13}C]- and [6-^{13}C]-β-D-galactopyranoside are shown in Figure 2, which were prepared from ^{13}C-labeled galactoses for convenient measurement as a single compound.

As described above, a practical synthesis of minimally ^{13}C-labeled D-galactose should facilitate studies on the conformational properties and dynamic behavior of oligosaccharides that contain galactose. This short and efficient ^{13}C-labeling method may also be extended to other important component monosaccharides and the oligosaccharides derived from these labeled monosaccharides reacted with the corresponding receptor molecules to determine the mechanism of interaction. [1-^{13}C]- and [6-^{13}C]-D-galactose were synthesized from α-D-glucofuranose derivative **1** in 17 steps (total yield, 27%) and 13 steps (total yield, 32%), respectively. Our new work promises to be an effective method to obtain gram-scale ^{13}C-labeled galactoses.

Acknowledgements

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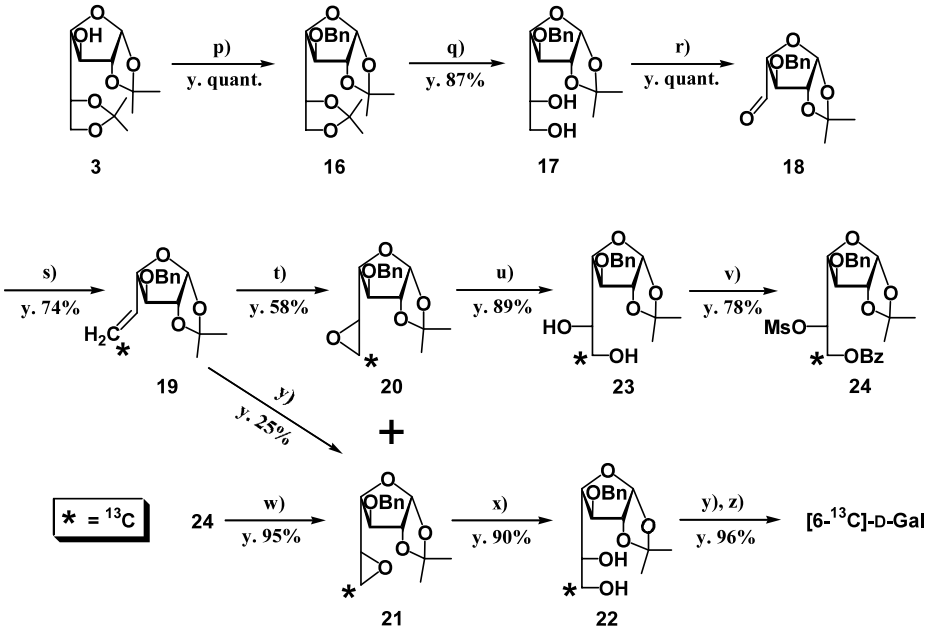

Scheme 2. *Reagents and conditions*: (p) BnBr, NaH/DMF; (q) 70% AcOH aq.; (r) $NaIO_4$/MeOH–H_2O; (s) $Ph_3P^{13}CH_3I$, *n*-BuLi/THF; (t) *m*-CPBA/$C_2H_4Cl_2$; (u) 1 M KOH aq./DMSO; (v) BzCl, Py., then MsCl; (w) *n*-Bu_4NOH/THF; (x) 1 M KOH aq./DMSO; (y) 10% Pd–C, H_2/EtOH; (z) Dowex H^+ form/H_2O.

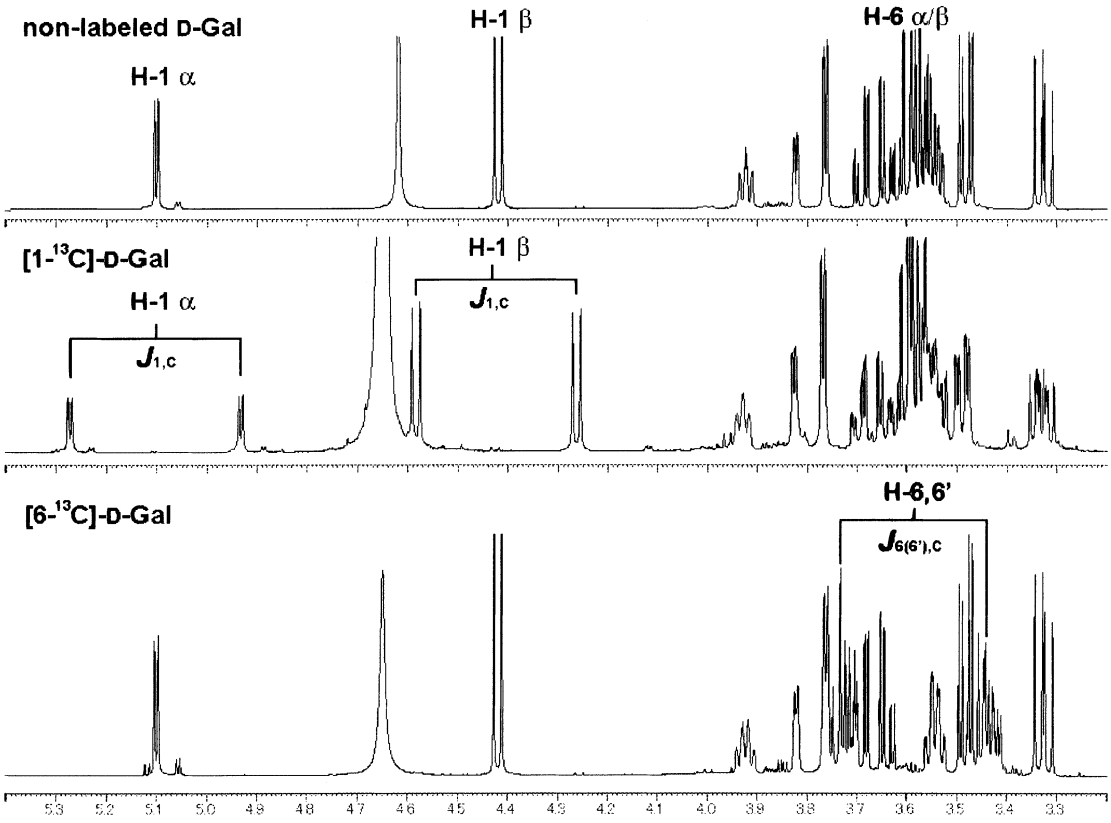

Figure 1. ^{1}H NMR data of non-labeled and ^{13}C-labeled D-galactoses (α:β = 35:65).

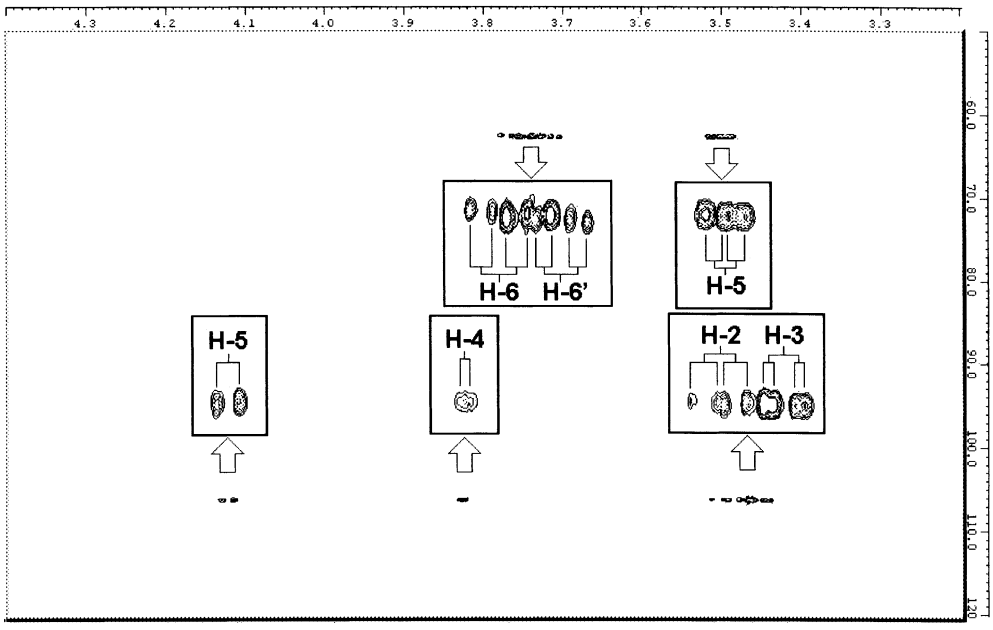

Figure 2. HMQC-HOHAHA spectra of a 1:1 mixture of methyl [1-^{13}C]- and [6-^{13}C]-β-D-galactopyranoside.

References

1. Miyazaki, T.; Sakakibara, T.; Sato, H.; Kajihara, Y. *J. Am. Chem. Soc.* **1999**, *121*, 1411–1412.
2. Miyazaki, T.; Sato, H.; Sakakibara, T.; Kajihara, Y. *J. Am. Chem. Soc.* **2000**, *122*, 5678–5694 see also references cited therein.
3. (a) Weis, W.; Brown, J. H.; Cusack, S.; Paulson, J. C.; Skehel, J. J.; Wiely, D. C. *Nature* **1988**, *333*, 426–431; (b) Ng, K. K.-S.; Weis, W. I. *Biochemistry* **1997**, *36*, 979–988; (c) Henrichsen, D.; Ernst, B.; Magnani, J. L.; Wang, W.-T.; Meyer, B.; Peters, T. *Angew. Chem., Int. Ed.* **1999**, *38*, 98–102.
4. (a) Aubin, Y.; Prestegard, J. H. *Biochemistry* **1993**, *32*, 3422–3428; (b) Salvatore, B. A.; Ghose, R.; Prestegard, J. H. *J. Am. Chem. Soc.* **1996**, *118*, 4001–4008.

5. Harris, R.; Kiddle, G. R.; Field, R. A.; Milton, M. J.; Ernst, B.; Magnani, J. L.; Homans, S. W. *J. Am. Chem. Soc.* **1999**, *121*, 2546–2551.
6. Sato, K.; Akai, S.; Hiroshima, T.; Aoki, H.; Sakuma, M.; Suzuki, K. *Tetrahedron Lett.* **2003**, *44*, 3513–3516.
7. Luca, L. D.; Giacomelli, G.; Porcheddu, A. *Org. Lett.* **2001**, *3*, 3041–3043.
8. Santosusso, T. M.; Swern, D. *J. Org. Chem.* **1975**, *40*, 2764–2769.
9. ^{1}H NMR data for [1-^{13}C]- and [6-^{13}C]-D-galactose.
[1-^{13}C]-D-Galactose: ^{1}H NMR (500 MHz, D_2O) (α:β = 35:65) δ 5.10 (1H, dd, $J_{1,2}$ = 3.4 Hz, $J_{1,C}$ = 169.5 Hz, H-1α), 4.42 (1H, dd, $J_{1,2}$ = 8.0 Hz, $J_{1,C}$ = 161.0 Hz, H-1β), 3.93 (1H, br dd, $J_{5,6}$ = 8.0 Hz, $J_{5,6'}$ = 4.6 Hz, H-5α), 3.83 (1H, br d, H-4α), 3.77 (1H, dd, $J_{4,5}$ = 1.1 Hz, H-4β), 3.70 (1H, dd, $J_{2,3}$ = 10.3 Hz, H-2α), 3.64 (1H, dd, $J_{3,4}$ = 3.4 Hz, H-3α), 3.61 (1H, dd, $J_{6,5}$ = 8.0 Hz, $J_{6,6'}$ = 11.5 Hz, H-6β), 3.58 (1H, dd, $J_{6,6'}$ = 9.7 Hz, H-6α), 3.56 (1H, dd, H-6′α), 3.56 (1H, dd, $J_{6',5}$ = 4.6 Hz, H-6′β), 3.55 (1H, ddd, H-5β), 3.49 (1H, ddd, $J_{3,4}$ = 3.4 Hz, $J_{3,C}$ = 1.1 Hz, H-3β), 3.33 (1H, ddd, $J_{2,3}$ = 9.7 Hz, $J_{2,C}$ = 5.7 Hz, H-2β); ^{13}C NMR (125 MHz, D_2O) δ 92.26 (C-1α), 96.43 (C-1β)
[6-^{13}C]-D-Galactose: ^{1}H NMR (500 MHz, D_2O) (α:β = 35:65) δ 5.10 (1H, d, $J_{1,2}$ = 3.7 Hz, H-1α), 4.42 (1H, d, $J_{1,2}$ = 7.9 Hz, H-1β), 3.92 (1H, br ddd, $J_{5,6}$ = 7.2 Hz, $J_{5,6'}$ = 4.0 Hz, $J_{5,C}$ = 5.4 Hz, H-5α), 3.82 (1H, br dd, $J_{4,C}$ = 1.2 Hz, H-4α), 3.76 (1H, ddd, $J_{4,5}$ = 1.0 Hz, $J_{4,C}$ = 1.2 Hz, H-4β), 3.69 (1H, dd, $J_{2,3}$ = 10.3 Hz, H-2α), 3.64 (1H, dd, $J_{3,4}$ = 3.2 Hz, H-3α), 3.59 (1H, ddd, $J_{6,6'}$ = 10.5 Hz, $J_{6,C}$ = 145.6 Hz, H-6β), 3.59 (1H, ddd, $J_{6',C}$ = 145.2 Hz, H-6′β), 3.58 (1H, ddd, $J_{6,6'}$ = 8.3 Hz, $J_{6,C}$ = 144.4 Hz, H-6α), 3.58 (1H, ddd, $J_{6,C}$ = 144.4 Hz, H-6′α), 3.54 (1H, dddd, $J_{5,6}$ = 7.0 Hz, $J_{5,6'}$ = 5.3 Hz, $J_{5,C}$ = 6.1 Hz, H-5β), 3.48 (1H, dd, $J_{3,4}$ = 3.4 Hz, H-3β), 3.33 (1H, dd, $J_{2,3}$ = 10.0 Hz, H-2β); ^{13}C NMR (125 MHz, D_2O) δ 61.15 (C-6α), 60.95 (C-6β).
10. (a) D-.Hornebeck, B.; Joly, J.-P.; Coulon, J.; Chapleur, Y. *Carbohydr. Res.* **1999**, *320*, 147–160; (b) Manning, D. D.; Bertozzi, C. R.; Rosen, S. D.; Kiessling, L. L. *Tetrahedron Lett.* **1996**, *37*, 1953–1956.