Efficient Synthesis of Two HNK-1 Related Pentasaccharides

Frederic Belot,[a] Albin Otter,[b] Minoru Fukuda,[a] Ole Hindsgaul*[a,b]

[a] The Burnham Institute, 10901 North Torrey Pines Rd., La Jolla, CA, USA 92122

[b] University of Alberta, Department of Chemistry, Edmonton, Alberta, Canada T6G 2G2
Fax +1(780)4927705; E-mail: ole.hindsgaul@ualberta.ca

Received 23 January 2002

Dedicated to the memory of Professor Raymond U. Lemieux.

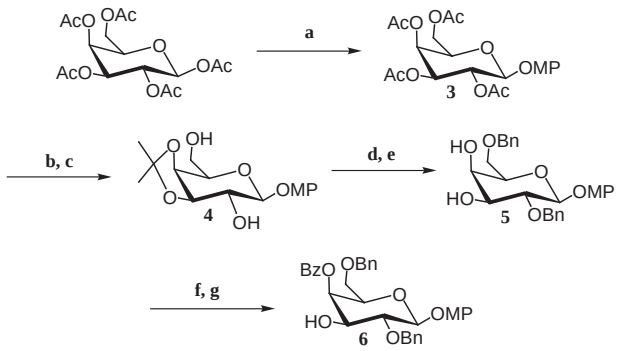
Abstract: Two pentasaccharides, representative of those found on complex *N*-glycans, were synthesized for use as potential substrates for sulfotransferases. The synthesis was achieved by the addition of a disaccharide donor β-D-GlcA(1→3)α-D-Gal-trichloroacetimidate to the two acceptor trisaccharides β-D-GlcNAc(1→6)α-D-Man(1→6)β-D-Man-*O*-octyl (**15**) and β-D-GlcNAc(1→2)α-D-Man(1→6)β-D-Man-*O*-octyl (**14**). After deprotection, the two pentasaccharides **1** and **2** were characterized by ^{1}H NMR spectroscopy.

Key words: pentassacharides, HNK-1, *N*-glycans

Complex carbohydrates, such as the HNK-1 glycan (sulfo→3GlcAβ1→3Galβ1→4GlcNAc→R) can mediate important cellular functions. The HNK-1 epitope is expressed on adhesion molecules in the nervous system where it seems to play a role in cell-cell and cell-substratum interactions.[1] This structure can be present on different branches of *N*-linked glycans, and it is not clear whether there is a branch-specificity for the sulfotransferase that completes the epitope. We therefore decided to synthesize pentasaccharides **1** and **2**, representing two of the branches of *N*-linked oligosaccharides for use in the study of sulfotransferase specificity.

In our synthetic scheme, we envisaged building the pentasaccharide backbone by using as the key step the glycosylation reaction between the imidate donor **9** and the acceptor trisaccharides **14** and **15**, respectively. Compound **9** was prepared by the condensation between the *glucuronic* acid donor **7** and the *galacto* derivative **6**. Compounds **14** and **15** in turn, were prepared by the addition of the *glucosamine N*-trichloroacetyl donor **10** to two different acceptor disaccharides, with either OH-2 (**12**) or OH-6 (**13**) of the α Man (1→6)β Man sequence groups free (Figure 1).

Starting from β-D-galactose pentaacetate, the anomeric center was protected as the paramethoxyphenylether to afford compound **3** (90%). Removal of the acetate esters (MeOH, NaOMe, quant.), followed by selective protection of OH-3 and OH-4 with 2,2-dimethoxy propane and CSA in DMF gave compound **4** (79%). The hydroxyl groups at the 2 and 6 positions were protected as benzyl ethers [BnBr, NaH, DMF (77%)]. Acetal hydrolysis afforded compound **5** (73%). Selective benzoylation of OH-4 using trimethylorthobenzoate (quant.) afforded compound **6** following the hydrolysis of the intermediate 3,4-orthobenzoate (Scheme 1). ^{1}H NMR ($CDCl_3$) confirmed that O-4 was benzoylated (H-4: 5.7 ppm, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$<1.0 Hz, H-3: 3.7 ppm, $J_{2,3}$ = 10.0 Hz). Coupling of **6** and **7**[2] (1.2 equiv), using TMSOTf, provided the target disaccharide **8** in 84% yield. The new glycosidic linkage was confirmed by ^{1}H NMR ($CDCl_3$)-: H-1′: 5.4 ppm $J_{1',2'}$ = 7.5 Hz. Four more steps afforded **9**: catalytic hydrogenation [$Pd(OH)_2$, MeOH, 95%], benzoylation (BzCl, pyridine, 70%) and activation to provide the trichloroacetimidate derivative (CAN, toluene, acetonitrile, water, (75%) then CCl_3CN, DBU, CH_2Cl_2, 80%) (Scheme 2).



Scheme 1 Synthesis of **6**: a) MPOH, CH_2Cl_2, TfOH, molecular sieves 4 Å, 1 h (90%); b) MeOH, NaOMe, overnight (quant.); c) 2,2-dimethoxy propane, CSA, DMF, overnight (79%); d) BnBr, NaH, DMF, 5 h, (77%); e) AcOH, H_2O, 2 h, (73%); f) trimethylorthobenzoate, *p*-TsOH, $PhCH_3$, 1 h, g) AcOH, H_2O, 10 min (2 steps, quant.).

Compound **11**, a GlcNAc derivative having a temporary protecting group on OH-4 and a thioethyl anomeric-blocking group, was selected as the β-hexosamine donor. The use of *N*-trichloroacetyl group was preferred to the more commonly used phthalimido group because the *N*-trichloroacetyl group can be easily converted to the *N*-acetyl group at the end of the synthesis in a single free radical reduction step.[3]

The known compound **10**[2] was deacetylated (MeOH, NaOMe, quant.) and a benzylidene group was introduced to mask the 4 and 6 positions (PhCHO, TFA, 83%). After protecting OH-3 as the benzyl ether (NaH, BnBr, DMF, 78%), regioselective reductive opening of the benzylidene acetal using Et_3SiH / $BF_3{\cdot}Et_2O$,[4] followed by acetylation, afforded **11** [partial ^{1}H NMR ($CDCl_3$) δ 7.02 (N-H), 5.05

Synlett 2003, No. 9, Print: 11 07 2003.
Art Id.1437-2096,E;2003,0,09,1315,1318,ftx,en;S00802ST.pdf.

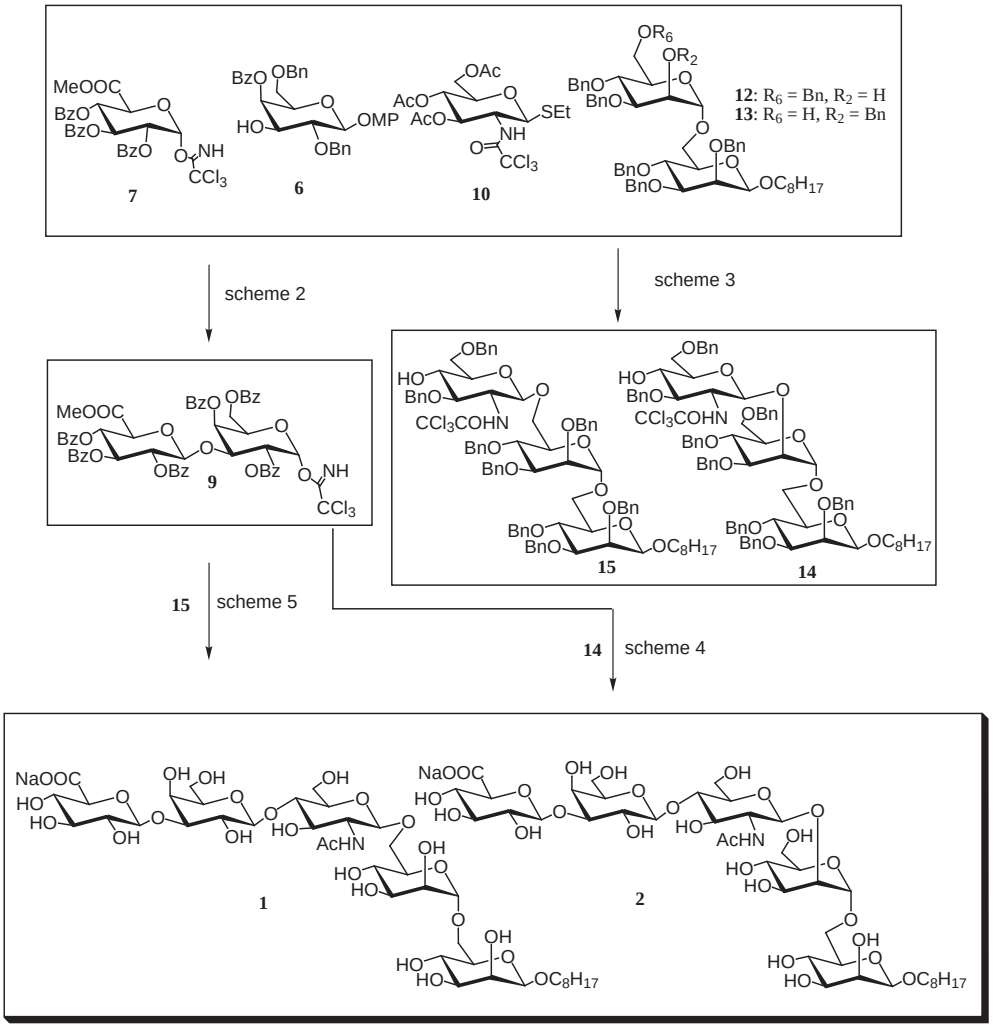
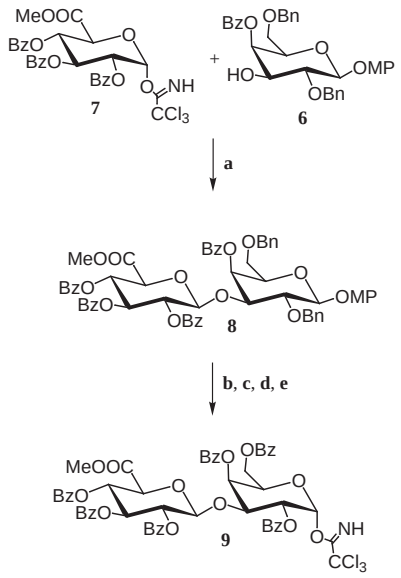


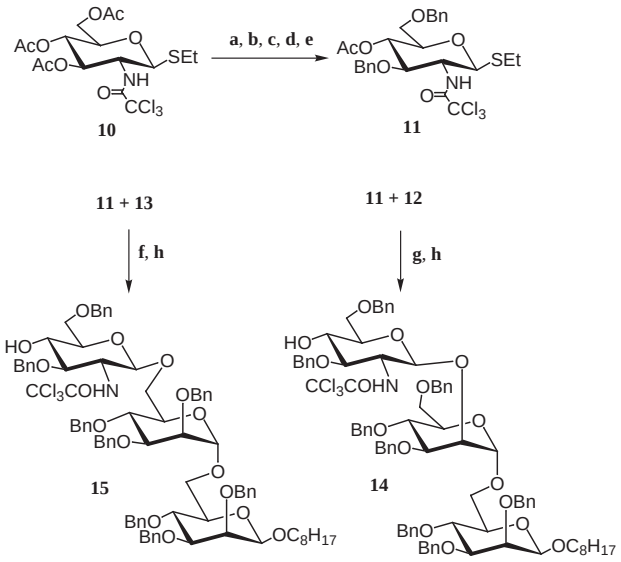
Figure 1



Scheme 2 Synthesis of **9**; a) TMSOTf, molecular sieves 4 Å, CH_2Cl_2, 0 °C to r.t. (84%); b) C/Pd(OH)$_2$, H_2, MeOH, r.t., 20 h, (95%); c) BzCl, pyridine, r.t., 20 h, (70%); d) CAN, $PhCH_3$, CH_3CN, H_2O, r.t., 90 min., (75%); e) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 1 h, (80%).

(1 H, t, $J_{3.4} = J_{4.5} = 9.5$ Hz, H-4), 5.02 (1 H, d, $J_{1.2} = 10.0$ Hz), 4.2 ppm (1 H, t, $J_{2.3} = J_{3.4} = 9.5$ Hz, H-3)].

Compound **12** was obtained by condensation between 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α,β-D-mannopyranosyl trichloroacetimidate[5] and octyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranoside[6] using catalytic TMSOTf, followed by deacetylation (95%, 2 steps). Characteristic proton and carbon signals in the ^1H and ^{13}C NMR ($CDCl_3$) spectra [δ 5.04 (s, $J_{1,2} = 1.5$ Hz, αMan H-1), 4.38 (s, $J_{1,2}<1$ Hz, βMan H-1), 2.22 (s, OH); ^{13}C NMR: δ 101.7, 99.6] confirmed the structure of **12**. Condensation of **11** and **12** and subsequent deacetylation provided the trisaccharide **14** in 63% yield (2 steps) (Scheme 3). Following the same procedure, compounds **11** and **13**[7] were coupled and the trisaccharide was deacetylated in order to give compound **15** in 75% yield (2 steps) (Scheme 3). The observed chemical shifts and coupling constants in $CDCl_3$ [**14**: δ 7.08 (d, $J_{2,NH} = 7.2$ Hz, GlcNAc N-H), 5.10 (s, $J_{1,2} = 1.5$ Hz, αMan H-1), 4.97 (d, $J_{1,2} = 7.5$ Hz, GlcNAc H-1), 4.24 (s, $J_{1,2}<1$ Hz, βMan H-1); **15**: δ 5.19 (d, $J_{1,2} = 8.5$ Hz, GlcNAc H-1), 4.90 (s, $J_{1,2} = 1.5$ Hz, α-Man H-1), 4.36 (s, $J_{1,2}<1$ Hz, β-Man H-1)] unambiguously established the expected stereochemistry in both trisaccharides **14** and **15**.

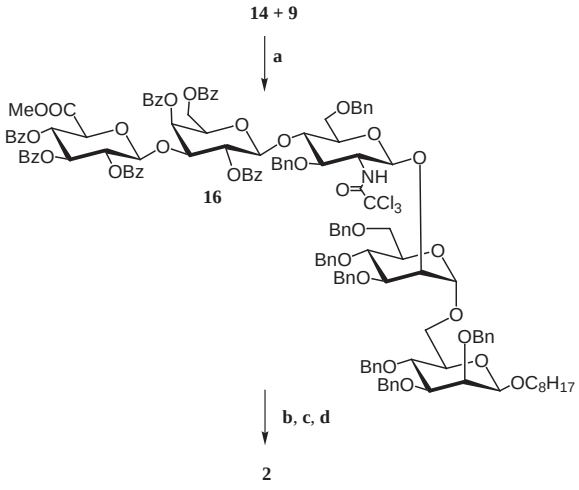


Scheme 3 Synthesis of trisaccharides **14** and **15**: a) NaOMe, MeOH, r.t., 2 h (quant.); b) PhCHO, TFA, r.t., 3 h, 90 min., (83%); c) BnBr, NaH, DMF, 0°C, 2 h, (78%), d) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to r.t., 90 min., (70%); e) Ac_2O, pyridine, r.t., overnight (96%); f) NIS, TMSOTf, molecular sieves 4 Å, 0 °C to r.t., 2 h (75%); g) NIS, TMSOTf, molecular sieves 4 Å, 0 °C to r.t., 3h (63%); h) MeOH, NaOMe, overnight, (quant.).

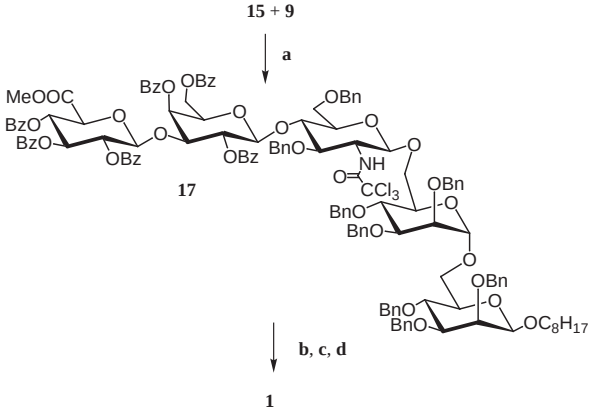
Trisaccharides **14** and **15** were each condensed with trichloroacetimidate **9** to give pentasaccharides **16** and **17** in 71% and 96% yield, respectively (Schemes 4 and 5). Chemical shifts and coupling constants in their ^1H and ^{13}C NMR ($CDCl_3$) spectra [**17**: δ 5.84 (dd, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1 Hz, Gal H-4), 5.77 (m, $J_{2,3} = J_{3,4} = J_{4,5}$ = 10 Hz, GlcA H-3 and H-4), 5.58 (dd, $J_{1,2}$ = 8.0 Hz, GlcA, H-2), 5.30 (dd, $J_{1,2}$ = 7.5 Hz, Gal H-2), 5.07 (d, $J_{1,2}$ = 1.5 Hz, α-Man H-1), 4.37 (s, J<1.0 Hz, β-Man H-1); ^{13}C: δ 101.7, 100.6, 99.9, 99.3, 98.4; **16**: δ 5.86 (dd, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 1 Hz, Gal H-4), 5.70 (m, $J_{2,3} = J_{3,4} = J_{4,5}$ = 10 Hz, GlcA H-3 and H-4), 5.58 (dd, $J_{1,2}$ = 8.2 Hz, GlcA, H-2), 5.30 (dd, $J_{1,2}$ = 7.0 Hz, Gal H-2); ^{13}C: δ 101.7, 100.6, 100.0, 98.5, 97.3] confirmed the stereoselective glycosylation.

The *N*-trichloroacetyl protecting groups were readily transformed into *N*-acetyl on treatment with tributylstannane and azo-bis-isobutyronitrile (AIBN) in refluxing benzene (77% and 80%). Hydrogenation [$Pd(OH)_2$, H_2, MeOH] and careful saponification (LiOH, H_2O_2,[8] THF/H_2O overnight, then NaOH 4M, MeOH, 8 h) led to **2** and **1** in 65% and 43% overall yield, respectively (Schemes 4 and 5). In order to confirm the desired stereochemistry a total assignment by NMR ^1H was carried out[9] (Table 1). The NMR data confirmed the stereochemistry at each interglycosidic linkage as well as the expected regiochemistry.[10] The molecular weights of **1** and **2** were confirmed using high resolution mass spectrometry.[11]

Pentasaccharides **1** and **2** differ only in the position of linkage of the terminal trisaccharide to either O-2 or O-6



Scheme 4 Synthesis of **2**: TMSOTf, CH_2Cl_2, molecular sieves 4 Å, 0 °C, 5 h, (71%); b) Bu_3SnH, PhH, AIBN, 90 °C, 90 min., (77%), c) C/$Pd(OH)_2$, H_2, MeOH (quant.); d) LiOH, H_2O_2, THF/H_2O, 20 h, then NaOH/MeOH, 8 h, (65%).



Scheme 5 Synthesis of **1**: TMSOTf, CH_2Cl_2, molecular sieves 4 Å, 0 °C, 2 h, (96%); b) Bu_3SnH, PhH, AIBN, 90 °C, 90 min., (80%), c) C/$Pd(OH)_2$, H_2, MeOH (quant.); d) LiOH, H_2O_2, THF/H_2O, 20 h, then NaOH/MeOH, 8 h, (43%).

of the αMan (1→6)βMan reducing disaccharide. This could in principle affect the rotameric distribution around C5-C6 of the β-Man residue resulting in a very different presentation of the HNK-1 epitope. The ^1H NMR coupling constants ($J_{5,6a}$ and $J_{5,6b}$) for **1** and **2** were however, almost identical (5.3 and 1.9/2.0) suggesting similar, if not identical rotameric populations for these two compounds. This distribution would be similar to the statistical distribution of 60 (GG): 40 (GT) reported by Bock and Duus.[12] The two pentasaccharides **1** and **2** are currently being evaluated in biological assays. The results of these studies will be reported elsewhere in due course.

Acknowledgment

This work was supported by National Institutes of Health Grants PO1 CA 71932 and CA 33895.

Table 1 Proton-NMR[9] Chemical Shifts and Coupling Constants for **1** and **2**

Ring	β-GlcA		β-Gal		β-GlcNAc		α-Man		β-Man	
Molecule	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
H-1 ($J_{1,2}$)	4.678 (7.8)	4.678 (7.8)	4.533 (7.9)	4.528 (7.9)	4.586 (8.3)	4.610 (7.8)	4.894 (1.5)	4.930 (1.6)	4.666 (<1)	4.666 (<1)
H-2 ($J_{2,3}$)	3.417 (9.1)	3.418 (9.3)	3.697 (9.9)	3.700 (9.8)	3.784 (10.5)	3.750[c]	**3.976**[d] (3.5)	**4.129**[d] (3.5)	3.983 (3.2)	3.988 (3.3)
H-3 ($J_{3,4}$)	3.514[b] (ca.9)	3.516[b] (ca.9)	3.816 (3.3)	3.818 (3.3)	3.740[c]	3.750[c]	3.808 (9.5)	3.854 (9.5)	3.628 (9.4)	3.630 (9.3)
H-4 ($J_{4,5}$)	3.532[b] (8.8)	3.534[b] (9.0)	4.195 (ca. 1)	4.196 (ca. 1)	3.746 (9.2)	3.750 (9.2)	3.651 (9.5)	3.518 (9.5)	3.720 (9.6)	3.720 (9.6)
H-5	3.725	3.725	3.730	3.760	3.608	3.581	**4.168**[d]	**3.652**[d]	3.497	3.495
H-6a ($J_{5,6a}$)[a]	–	–	3.720[c]	3.740[c]	4.008 (2.2)	3.990 (2.4)	**3.790**[d,c]	**3.914**[d] (1.8)	3.914 (5.4)	3.964 (5.4)
H-6b ($J_{5,6b}$)[a]	–	–	3.720[c]	3.740[c]	3.848 (4.9)	3.854 (5.0)	**3.790**[d,c]	**3.660**[d,c]	3.785 (1.9)	3.793 (2.0)
AcNH (CH_3)					2.06	2.06				

[a] When two distinct signals were observed, the greater chemical shift was assigned to H-6a.
[b] Assignments might be interchanged.
[c] Unavailable due to extensive spectral overlap.
[d] Chemical shifts in bold are most affected by the different substitution at the α-Man residue.

References

(1) Voshol, H.; van Zuylen, C. W. E. M.; Orberger, G.; Vliegenthart, J. F. G.; Schachner, M. *J. Biol. Chem.* **1996**, *271*, 22957.
(2) Coutant, C.; Jacquinet, J.-C. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1573.
(3) Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189.
(4) Debenham, S. D.; Toone, E. J. *Tetrahedron: Asymmetry* **2000**, *11*, 385.
(5) Li, Z.-J.; Li, H.; Cai, M.-S. *Carbohydr. Res.* **1999**, *320*, 1.
(6) McAuliffe, J. C.; Fukuda, M.; Hindsgaul, O. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2855.
(7) Srivastava, G.; Hindsgaul, O. *Carbohydr. Res.* **1992**, *224*, 83.
(8) Lucas, H.; Basten, J. E. M.; Van Dinther, T. G.; Meulemenn, D. G.; Van Aelst, S. F.; Van Boeckel, C. A. A. *Tetrahedron* **1990**, *46*, 8207.
(9) Data recorded in D_2O at 600 MHz, and 27.0±0.1 °C; concentration ca. 8 mM; chemical shifts are relative to external 0.1% acetone set at 2.225 ppm measured in a separate sample under identical experimental conditions. Even under strict temperature control, chemical shifts are not reproducible to three decimals, however, within a given experiment at 600 MHz, signals can be distinguished accurately to this precision.
(10) Correct glycosidic linkages are confirmed by the following *inter*-residue NOEs (s for strong, m for medium and w for weak interactions). **1**: H1βGlcA-H3βGal (s) and H1βGlcA-H4Gal (m); H1βGal-H4βGlcNAc (likely s, overlaps with H1/H5βGal); H1βGlcNAc-H6a/bαMan (s) and H1βGlcNAc-H5αMan (w); H1αMan-H6bβMan (s) and H1αMan-H6aβMan (w) and H1αMan-H5βMan (w). **2**: H1βGlcA-H3βGal (s) and H1βGlcA-H4βGal (m); H1βGal-H4βGlcNAc (likely s, overlaps with H1/H5βGal); H1βGlcNAc-H2αMan (s) and H1βGlcNAc-H1αMan (s); H1αMan-H6bβMan (s) and H1αMan-H6aβMan (w) and H1αMan-H5βMan (w).
Furthermore, typical H1-H3 and H1-H5 *intra*-residue NOEs were observed for all β-glycosides. Taken together with the coupling constants, this confirms that all pyranose rings adopt the normal chair form.
(11) HRMS calcd for $C_{40}H_{69}NNaO_{27}$ (**1** and **2**): 1018.3955; found **1**: 1018.3961; **2**:1018.3959
(12) Bock, K.; Duus, J. O. *J. Carbohydr. Chem.* **1994**, *13*, 513.