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SHORT COMMUNICATION

Synthesis of a novel glycosaminoglycan pentasaccharide serine having an *N*-acetylgalactosamine residue α-linked to the core linkage tetrasaccharide

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A novel pentaosyl serine; GalNAcα(1–4)GlcAβ(1–3)Galβ(1–3)Galβ(1–4)Xylβ(1–3)Ser (**2**), a putative intermediate of chondroitin sulfate and/or heparan sulfate biosynthesis, was synthesized.

Keywords: glycosaminoglycan, pentasaccharide serine, synthesis

It is generally established that specific glycosyltransferases are used *in vivo* to construct glycosaminoglycan chains by using the corresponding uridine diphosphate sugars as glycosyl donors [1–3]. However, little information is available about the *N*-acetylgalactosaminyltransferase which transfers an *N*-acetylgalactosamine (GalNAc) residue to the core tetrasaccharide region of proteoglycans. In 1985, Rohrmann *et al.* [4] reported the purification of this transferase, by which β-GalNAc was linked to GlcAβ(1–3)Galβ(1–3)Gal (GlcA: glucuronic acid). In contrast to their result two remarkable discoveries were reported very recently. Freeze's group found a novel pentasaccharide; GalNAcα(1–4)GlcAβ(1–3)Galβ(1–3)Galβ(1–4)Xylβ-MU (**1**) (Xyl: xylose, MU: 4-methylumbelliferyl), using several types of cell lines [5]. Their assignment by ^{1}H-NMR gave evidence for the above pentasaccharide structure. Sugahara's group also reported a similar pentasaccharide sequence **2** (Fig. 1) after treatment of a synthetic tetrasaccharide serine precursor with foetal bovine serum. They enzymatically determined the structure of **2**. It resisted digestion by chondoroitinase ACII and β-N-acetylhexosaminidase but was sensitive to α-N-acetylgalactosaminidase. Compound **2** was formed presumably by the action of α-GalNAc transferase in the serum [6]. The formation of this α-linked pentasaccharide may suggest an alternative route for the biosynthetic pathway of glycosaminoglycans, especially of chondroitin sulfate and/or heparan sulfate. Therefore, these results prompted us to synthesize pentasaccharide (**2**) not only to verify the structure through an unambigious synthetic sequence but also to provide a key substrate for the biosynthetic study of glycosaminoglycans. We report the synthesis and ^{1}H-NMR assignment at 600 MHz of this novel pentasaccharide linked to a serine residue.

Retrosynthetic analysis of target compound (**2**) led us to design a synthesis of **2** by the coupling of disaccharide donor composed of GalNAcα(1–4)GlcA and trisaccharide acceptor having Galβ(1–3)Galβ(1–4)Xyl moiety as shown in Fig. 1.

The disaccharide donor was synthesized as follows. As depicted in Fig. 2, suitably protected donor (**3**) [7] and acceptor (**4**) [7] were subjected to coupling using 0.1 equivalent of TMSOTf as a promotor in the presence of MS4A in toluene at −50 °C to afford an inseparable mixture of stereoisomeric disaccharides (**5**α and **5**β) with

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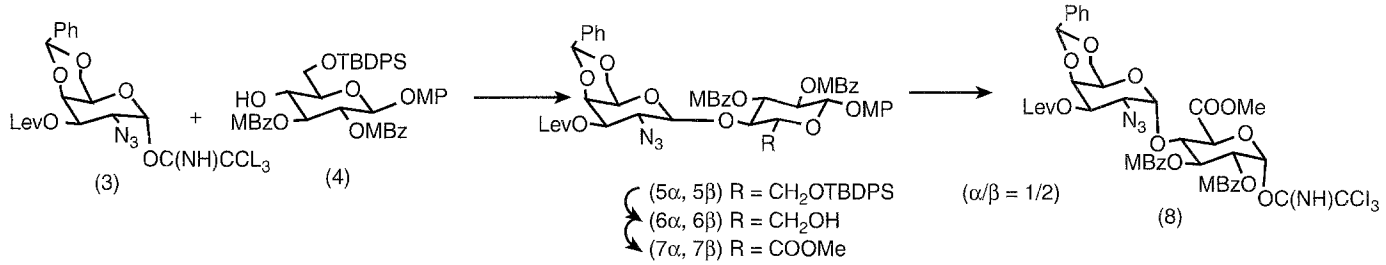

Figure 2. Coupling of protected donor (**3**) and acceptor (**4**).

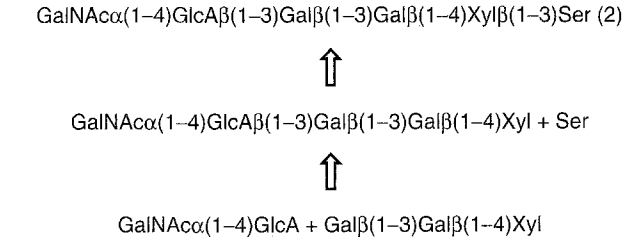

Figure 1. Retrosynthesis.

α:β ratio of 1:2 (as estimated from ^{1}H-NMR). The successive removal of the TBDPS group was carried out with *n*-Bu_4NF and AcOH in THF in 77% yield for two steps. The inseparable mixture of alcohols (**6**α and **6**β) was subjected to the Swern oxidation [$(COCl)_2$, DMSO/CH_2Cl_2, then *i*-Pr_2EtN]. The resultant aldehyde was converted to carboxylic acid by the use of $NaClO_2$ and NaH_2PO_4 in *t*-BuOH-H_2O in the presence of 2-methyl-2-butene and final esterification with CH_2N_2 gave methyl esters (**7**α and **7**β [7]) in 88% yield (three steps). Column chromatography on silica gel allowed the partial separation of both stereoisomers and only the α-glycoside (**7**α) was used for a further reaction. Selective removal of MP group by the use of $(NH_4)_2Ce(NO_3)_6$ followed by the reaction with CCl_3CN-DBU converted (**7**α) into imidate (**8**) *via* the corresponding hemiacetal (67%).

The pentasaccharide serine (**2**) was synthesized as shown in Fig. 3. The glycosylation of **8** with the trisaccharide acceptor (**9**) [8] was performed by the action of BF_3·OEt_2 as a promotor in the presence of MS4A in toluene to give the desired β-linked pentasaccharide (**10**)[a] in 50% yield together with the pentasaccharide (**11**) (15%) having orthoester linkage. The levulynoyl group of **10** could be removed with H_2NNH_2·AcOH to yield **12** quantitatively. Hydrogenolysis of **12** by using Lindlar catalyst and successive acetylation were carried out to give **13** in 98% yield in two steps. By the use of palladium on charcoal, **13** was hydrogenolized and the product was completely acetylated (77% yield in two steps). Anomeric acetate was selectively removed with H_2NNH_2·AcOH and the corresponding hemiacetal was converted to the imidate (**14**) as above in 91 and 55% yield, respectively.

The serine acceptor (**15**) [9] was obtained from commercially available Fmoc-Ser-OH with $CsCO_3$ and allyl bromide in 95% yield. The glycosylation of **15** with **14** was carried out by using BF_3·OEt_2 as a promotor in CH_2Cl_2 at 0 °C to room temperature to afford pentaosyl serine (**16**)[a] in 27% yield. The complete deprotection of **16** ((1) $Pd(PPh_3)_4$-PhNHMe/THF, (2) LiOH/H_2O-THF, (3) NaOH/MeOH-H_2O) and purification of the product by gel permeation (LH-20, H_2O) gave compound **2** in 66%

[a] Physical data for key compounds are given below, values of δ_H were measured at 25 °C. Chemical shifts are expressed in p.p.m. downfield from the signal for internal Me_4Si for solutions in $CDCl_3$. Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue *3*. **7**α: $[\alpha]_D$ + 134.8° (*c* 0.873, $CHCl_3$), ^{1}H δ 2.09 (s, 3H, $COCH_3$), 2.36 (s, 6H, 2PhCH_3), 2.55–2.75 (m, 4H, $2CH_2$), 3.67 (bs, H-5^2), 3.75 (s, 3H, COOMe), 3.77 (s, 3H, OMe), 3.83 (dd, H-2^2, $J_{1,2}$ = 3.63, $J_{2,3}$ = 11.22 Hz), 4.00 (dd, 1H, H-6^2a, $J_{5,6a}$ = 1.32, $J_{6a,6b}$ = 12.54 Hz), 4.22 (dd, H-6^2b, $J_{5,6b}$ = 0.99 Hz), 4.24 (d, H-5^1, $J_{4,5}$ = 9.24 Hz), 4.38 (bd, H-4^2, J = 2.64 Hz), 4.51 (t, H-4^1, $J_{3,4}$ = 9.24 Hz), 5.14 (dd, H-3^2, $J_{3,4}$ = 3.30 Hz), 5.21 (d, H-1^1, $J_{1,2}$ = 7.26 Hz), 5.26 (d, H-1^2), 5.48 (s, 1H, PhC*H*), 5.56 (dd, H-2^1, $J_{2,3}$ = 9.24 Hz), 5.78 (t, H-3^1), 6.73–6.80 (m, 2H, aromatic H), 6.87–6.92 (m, 2H, aromatic H), 7.09–7.20 (m, 4H, aromatic H), 7.32–7.47 (m, 5H, aromatic H), 7.81–7.87 (m, 4H, aromatic H). Anal. Calcd. for $C_{48}H_{49}N_3O_{16}$: C 62.39, H 5.36, N 4.55. Found: C 62.38, H 5.37, N 4.51. **10**: $[\alpha]_D$-33.7° (*c* 0.887, $CHCl_3$), ^{1}H δ 1.13 (s, 9H, *t*-Bu), 2.09 (s, 3H, $COCH_3$), 2.27 and 2.32 (2s, 2X3H, 2PhCH_3), 2.58–2.75 (m, 4H, $2CH_2$), 3.61 (m, H-3^1), 3.76 (m, H-2^5), 3.78 (s, 3H, COOMe), 4.14 (d, H-5^4, $J_{4,5}$ = 9.90 Hz), 4.35 (m, H-4^5), 4.45 (bt, H-4^4, J = 8.91 Hz), 4.49 (d, H-1^1, $J_{1,2}$ = 6.26 Hz), 5.02 (dd, H-2^1, $J_{2,3}$ = 7.59 Hz), 5.13 (dd, H-3^5, $J_{2,3}$ = 10.89, $J_{3,4}$ = 3.30 Hz), 5.15 (d, H-1^5, $J_{1,2}$ = 3.30 Hz), 5.31 (d, H-1^4, $J_{1,2}$ = 7.26 Hz), 5.45 (dd, H-2^4, J = 8.58 Hz), 5.48 and 5.54 (2s, 2X1H, 2PhC*H*), 5.66 (bt, H-3^4, J = 8.74 Hz), 6.97 (d, 2H, J = 8.25 Hz, aromatic H), 7.09–7.42 (m, 38H, aromatic H), 7.43–7.57 (m, 4H, aromatic H), 7.69 (d, 2H, aromatic H, J = 7.91 Hz), 7.78 (d, 2H, aromatic H, J = 8.25 Hz). Anal. Calcd. for $C_{112}H_{119}N_3O_{30}$: C 67.68, H 6.05, N 2.11. Found: C 67.69, H 6.11, N 2.08. **16**: ^{1}H δ (selected 5.55 (d, 1H, SerNH, J = 8.30 Hz), 5.30 (m, H-1^5), 4.86 (d, H-1^4, $J_{1,2}$ = 7.32 Hz), 4.51 (m, NH^5), 4.39 (d, H-1^1, $J_{1,2}$ = 7.31 Hz), 4.37 (d, H-1^3, $J_{1,2}$ = 7.80 Hz), 4.32 (d, H-1^2, $J_{1,2}$ = 9.26 Hz), 4.23 (m, 1H, SerβCH), 3.91 (dd, H-5^1eq, J_{gem} = 11.71, $J_{4,5eq}$ = 4.83 Hz), 3.27 (dd, H-5^1ax, $J_{4,5ax}$ = 8.79 Hz).

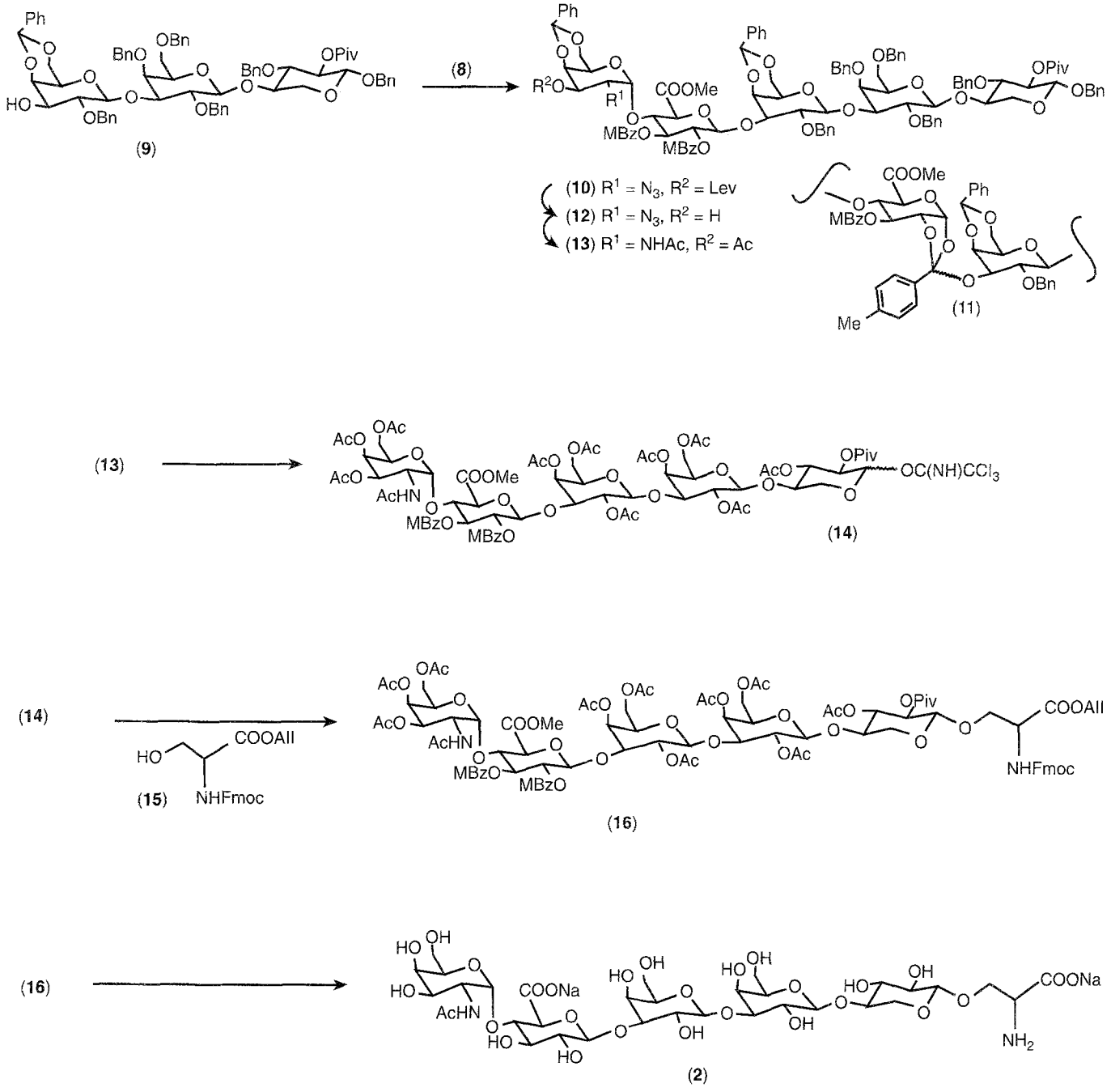

Figure 3. Synthesized pentasaccharide serine.

yield in three steps. ^{1}H-NMR assignments by 1D selective TOCSY at 600 MHz are in good agreement with the data reported for the compound **1** by Freeze *et al.* [5] and are given in Table 1.

It is not known whether this pentasaccharide might be a key intermediate in the chondroitin sulfate and/or heparan sulfate biosynthesis. Development of a reasonable synthetic route could give a way to support the elucidation of

Table 1. ^{1}H-Chemical shifts of 1 and 2.

	2[a]	1[b] [ref 5]		2[a]	1[b] [ref 5]
Xyl[1] H-1	4.44	5.243			
-2	3.35	3.655	GlcA[4] H-1	4.66	4.780
-3	3.61	3.922	-2	3.43	3.369
-4	3.87	3.735	-3	3.78	3.729
-5ax	3.41	3.922	-4	3.70–3.75	3.860
-5eq	4.12	4.210	-5	3.70–3.75	ND[c]
Gal[2] H-1	4.54	4.598			
-2	3.68	3.721	GalNAc[5] H-1	5.45	5.480
-3	3.83	3.831	-2	4.17	4.173
-4	4.19	4.224	-3	3.88	3.897
-5	3.70–3.80	ND[c]	-4	3.99	4.016
-6a,b	3.70–3.80	ND[c]	-5	3.70–3.80	3.705
			-6a,b	3.70–3.80	ND[c]
			NAc	2.05	2.080
Gal[3] H-1	4.67	4.701			
-2	3.74	3.746			
-3	3.79	3.785			
-4	4.16	4.180			
-5	3.70–3.80	ND[c]			
-6a,b	3.70–3.80	ND[c]			

[a] Sample was dissolved in D_2O (~1.5 mM). Chemical shifts are given in ppm. t-BuOH was referenced as 1.23 ppm.
[b] Chemical shifts are given in ppm downfield from DSS at 25 °C.
[c] ND, not determined.

the biosynthetic pathway of proteoglycan by providing a putative intermediate **2** for the enzymatic study.

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