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Synthesis of Betaglycan-type Tetraosyl Hexapeptide: A Possible Precursor Regulating Enzymatic Elongation Toward Heparin

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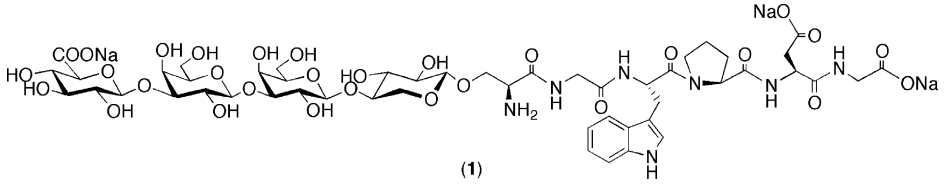
Abstract—Tetraosyl hexapeptide, a part of the sequence of betaglycan: β-D-GlcA-(1→3)-β-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Xyl-(1→*O*-SerGlyTrpProAspGly (**1**), which was designed as a probe for glycan elongation toward heparin, was synthesized in a stereocontrolled manner.

Glycosaminoglycans (GAGs) are classified into two categories based on the type of hexosamine residue in the repeating region; heparin and chondroitin, which contain α-GlcNAc and β-GalNAc, respectively. GAGs are thought to be synthesized by the stepwise additions of monosaccharides with the help of the corresponding UDP-sugars and glycosyltransferases. However, the detailed mechanisms of the first addition of the hexosamine have been ambiguous. This important elongation step sorts the GAG into heparin and chondroitin. So far as we know, the factors regulating the sorting step have been hypothesized to exist in the glycan or core-peptide regions. The former, the tetrasaccharides comprising the linkage regions of GAG often have sulfates at specific positions.[1] Interestingly, the GAGs of the heparin-type have no sulfate in the linkage region, different from the chondroitin type.[2] It seems the sulfate might orient the GAG to the chondroitin type elongation. On the other hand, the heparin-type GAGs often link to hydrophobic and acidic regions in the core-peptide.[3] Esko et al.[4] demonstrated the glycan elongation by using xylosides having hydrophobic aglycons to produce the heparin-type GAG. Although these results showed important evidence for the relationship between heparin and the character of the aglycon part, the mechanism remains unclear just in the first hexosaminyl transfer to the tetrasaccharide of GAG.

Clarifying the sorting mechanism in detail for biomedical purposes is urgently needed. These facts prompted us to synthesize the required part of the proteoglycan. We have selected and synthesized the tetraosyl hexapeptide (**1**) as described below. The targeted compound is a part of betaglycan[5] and is expected to be a precursor for the heparin biosynthesis. Some glycosyl peptides at the linkage region of GAG have already been synthesized.[6,7]

Based on the retrosynthetic analysis, the targeted compound was divided into the glycan, serylglycine and tetrapeptide parts, and each of them was synthesized as follows. The glycan part was synthesized from the reducing terminus as depicted in Scheme 1. The galactosyl donor (**2**) was synthesized from the corresponding *p*-methoxyphenyl glycoside[6i] in two steps: (1) CAN/CH_3CN–H_2O, and (2) CCl_3CN, DBU/CH_2Cl_2 in 79 and 75% yield, respectively. The xylosyl acceptor (**3**) was converted from the known peracetate[8] in three



(**1**)

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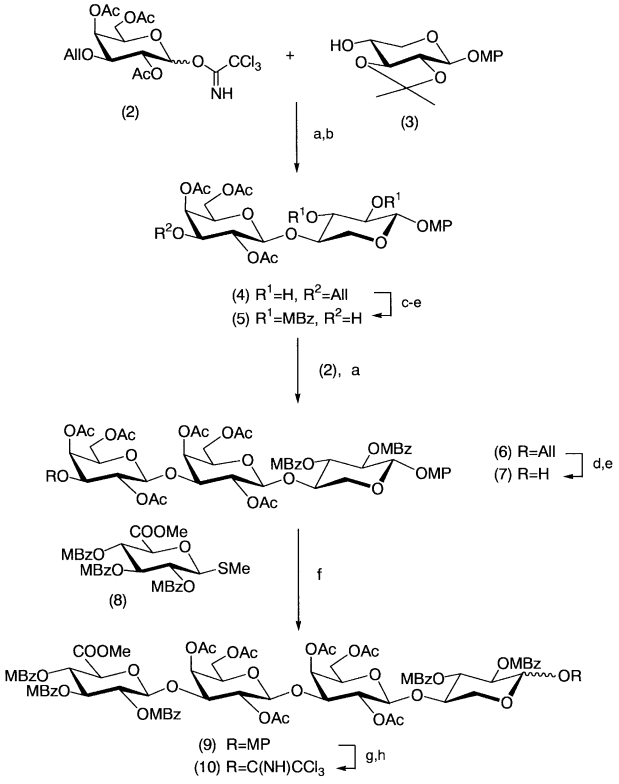
steps: (1) *p*-MPOH, TMSOTf, MSAW300/CH_2Cl_2, (2) Et_3N–MeOH–H_2O, and (3) 2-methoxypropene, camphorsulfonic acid/DMF, 60 °C (48%, three steps). We coupled **2** with **3** by a TMSOTf mediated manner at −20 °C. Removal of the isopropylidene acetal with camphorsulfonic acid gave the desired disaccharide **4** in 78% yield (from **3**) without the formation of the α-glycoside.

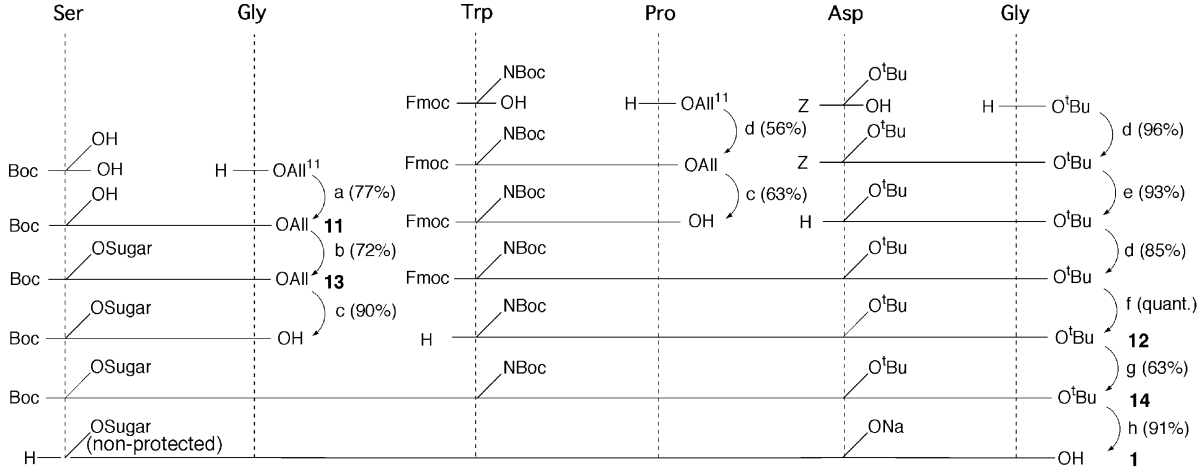


Scheme 1. Reagents and conditions: (a) TMSOTf, MSAW300/CH_2Cl_2; (b) camphorsulfonic acid/MeOH–CH_2Cl_2; (c) MBzCl/pyridine; (d) [Ir(COD)($PMePh_2$)$_2$$PF_6$], H_2/THF; (e) I_2, $NaHCO_3$/THF–H_2O; (f) AgOTf, $CuBr_2$, *n*-Bu_4NBr, MS4A/CH_3Cl_2; (g) CAN/CH_3CN–H_2O; (h) CCl_3CN, DBU/CH_2Cl_2. MP, *p*-$MeOC_6H_4$; MBz, *p*-MeC_6H_4CO.

Two hydroxyl groups of **4** were masked as the 4-methylbenzoyl ester in 65% yield and the allyl group was removed to yield the acceptor **5** in 97%. The same glycosylation procedure as above with **2** and **5** exclusively afforded the β-linked trisaccharide **6** in 86% yield. The repetitive removal of the allyl ether at the non-reducing terminal of **6** was effectively executed to yield **7** in 85%. We coupled the glucuronyl thio glycoside **8**[7] to **7** by the reported method[9] in 31% yield which should be improved. The tetrasaccharide **9**[10] was converted into the imidate **10** via the corresponding hemiacetal as the synthesis of **2** in 79% yield (two steps).

The synthesis of the peptide parts and the following condensations are shown in Scheme 2. Starting from the commercially available or known[11] amino acid derivatives, we obtained the suitably protected SerGly (**11**) and TrpProAspGly (**12**) sequences in high yields. The coupling of **10** and **11** was performed in the same manner as the synthesis of **4** to yield the desired glycosyl serylglycine (**13**)[10] in 72%. The allyl ester of **13** was changed to the carboxylic acid in 90% yield and coupled with **12** via the HBTU method to give **14** in 63% yield. Finally, careful deprotection procedures with TFA cocktail[12] removed the *tert*-Bu and Boc groups, and then saponification with NaOMe was performed to afford the targeted compound **1**[10] in 91% yield (two steps). The product was purified by columns of LH-20, Mono Q and Sephasil C18. The assigned structure of **1** was confirmed by ^1H NMR and FABMS. No β-elimination product was observed.

In summary, we have synthesized for the first time the tetraosyl hexapeptide—a partial sequence of betaglycan—in a stereocontrolled manner. The probe **1** is under enzymatic glycan elongation and will elucidate the biological mechanisms for the sorting of GAG. This tetraosyl hexapeptide will contribute to the development of medicines composed of GAG, especially of heparin and heparan sulfate.



Scheme 2. Reagents and conditions: (a) HOBt, DCC/CH_2Cl_2, 0 °C; (b) TMSOTf, MSAW300/CH_2Cl_2, −20 °C; (c) $Pd(PPh_3)_4$, *N*-methylaniline/THF; (d) HOBt, WSCD·HCl/CH_2Cl_2, −20 °C; (e) Pd/C, H_2/EtOH; (f) morpholine/CH_2Cl_2; (g) HBTU, HOBt, *i*Pr_2EtN/DMF, −20 °C; (h) TFA cocktail,[12] then NaOMe/aqMeOH. HOBt, *N*-hydroxybenzotriazole; WSCD, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide; HBTU, *O*-benzotriazol-1-yl-*N*, *N*, *N*′, *N*′-tetramethyluronium hexafluorophosphate.

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10. ^{1}H NMR data for key compounds are given below, values of δ_H were measured at 25 °C. Chemical shifts are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$, and for the solutions in D_2O, in ppm downfield from the signal for Me_4Si, by reference to internal *tert*-BuOH (1.23). Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue 3 from the reducing terminal. **9**: δ_H (500 MHz, $CDCl_3$) 7.97–7.93 (Ph), 7.81–7.70 (Ph), 7.23–7.07 (Ph), 6.96 (m, MeOPh), 6.81 (m, MeOPh), 5.80 (t, $J_{2,3}=J_{3,4}=9.3$ Hz, H-3^4), 5.65 (t, $J_{2,3}=J_{3,4}=6.1$ Hz, H-3^1), 5.64 (brt, $J=9.8$ Hz, H-4^4), 5.47 (d, $J_{3,4}=3.5$ Hz, H-4^3), 5.41 (dd, $J_{1,2}=4.9$ Hz, H-2^1), 5.37 (dd, $J_{1,2}=7.6$ Hz, H-2^4), 5.29 (d, H-1^1), 5.22 (d, $J_{3,4}=3.4$ Hz, H-4^2), 5.08 (dd, $J_{1,2}=8.1$ Hz, $J_{2,3}=10.0$ Hz, H-2^2), 5.02 (dd, $J_{1,2}=8.1$ Hz, $J_{2,3}=10.2$ Hz, H-2^3), 4.91 (d, H-1^4), 4.51 (d, H-1^2), 4.37 (d, H-1^3), 4.26 (d, $J_{4,5}=10.0$ Hz, H-5^4), 4.21 (dd, $J_{4,5e}=3.9$ Hz, $J_{gem}=12.2$ Hz, H-5e^1), 4.16 (dd, $J_{5,6b}=5.9$ Hz, $J_{gem}=11.7$ Hz, H-6b^3), 4.03 (dd, $J_{5,6a}=6.3$ Hz, H-6a^3), 3.93 (m, H-4^1), 3.85–3.81 (m, H-6b^2, 3^3), 3.79–3.73 (m, H-3^2, 5^2, 3^3, 5^3), 3.76 (s, MeOPh), 3.70 (s, COOMe), 3.61 (dd, $J_{4,5a}=5.6$ Hz, H-5a^1), 3.51 (dd, $J_{5,6a}=7.1$ Hz, $J_{gem}=11.5$ Hz, H-6a^2), 2.40, 2.37, 2.37, 2.36, 2.30 (5s, 5MePh), 2.16, 2.09, 2.02, 1.93, 1.89, 1.65 (6s, 6Ac). **13**: δ_H (500 MHz, $CDCl_3$) 7.80–7.62 (Ph), 7.12–7.00 (Ph), 6.82 [m, NH(Gly)], 5.80 (m, =CH), 5.72 (dd, $J_{2,3}=9.0$ Hz, $J_{3,4}=9.8$ Hz, H-3^4), 5.57 (t, H-4^4), 5.49 (t, $J_{2,3}=J_{3,4}=7.8$ Hz, H-3^1), 5.40 (d, $J_{3,4}=3.2$ Hz, H-4^3), 5.36 [m, NH(Ser)], 5.28 (dd, $J_{1,2}=7.1$ Hz, H-2^4), 5.20 (m, =CH_2), 5.14 (d, $J_{3,4}=1.7$ Hz, H-4^2), 5.12 (d, $J_{1,2}=6.1$ Hz, H-2^1), 4.95 (dd, $J_{1,2}=8.1$ Hz, $J_{2,3}=7.8$ Hz, H-2^2), 4.92 (dd, $J_{1,2}=7.8$ Hz, $J_{2,3}=10.5$ Hz, H-2^3), 4.84 (d, H-1^4), 4.65 (d, H-1^1), 4.46 (m, OCH_2), 4.37 (d, H-1^2), 4.29 (d, H-1^3), 4.19 (d, H-5^4), 4.15 (m, Serα), 4.09 (dd, $J_{5,6b}=6.1$ Hz, $J_{gem}=11.0$ Hz, H-6b^3), 4.06 (dd, $J_{4,5e}=5.6$ Hz, $J_{gem}=13.2$ Hz, H-5e^1), 3.97 (dd, $J_{5,6a}=5.9$ Hz, H-6a^3), 3.89 (m, H-4^1), 3.89 (dd, $J_{\alpha,NH}=5.4$ Hz, $J_{gem}=18.5$ Hz, Glyαa), 3.79 (dd, Glyαb), 3.76 (dd, H-3^3), 3.73 (dd, $J_{5,6b}=5.9$ Hz, $J_{gem}=11.2$ Hz, H-6b^2), 3.68 (brt, $J=6.1$ Hz, H-5^3), 3.63 (m, H-3^2), 3.63 (s, COOMe), 3.57 (brt, $J=6.1$ Hz, H-5^2), 3.48 (m, Serβ), 3.45 (d, H-5a^1, 6a^2), 2.29, 2.28 (2s, 4 MBz), 2.22 (s, MBz), 2.07, 2.01, 1.94, 1.85, 1.83, 1.59 (6s, 6Ac), 1.36 (s, *tert*-Bu). **14**: δ_H (500 MHz, $CDCl_3$) 8.2–8.6 (Ph), 7.45 [d, $J=8.3$ Hz, NH(Asp)], 7.3–7.0 (Ph), 7.18 [m, NH(Gly)], 6.94 [br, NH(Gly)], 6.78 [br, NH(Trp)], 5.80 (t, $J_{2,3}=J_{3,4}=9.4$ Hz, H-3^4), 5.64 (brt, $J=9.6$ Hz, H-4^4), 5.53 (dd, $J_{2,3}=7.8$ Hz, $J_{3,4}=8.3$ Hz, H-3^1), 5.47 (d, $J_{3,4}=2.8$ Hz, H-4^3), 5.37 [m, NH(Ser)], 5.36 (dd, $J_{1,2}=7.1$ Hz, H-2^4), 5.20 (d, $J_{3,4}=3.2$ Hz, H-4^2), 5.17 (d, $J_{1,2}=6.2$ Hz, H-2^1), 5.06 (m, Trpα), 4.99 (m, 2H, H-2^2, 2^3), 4.92 (d, H-1^4), 4.79 (m, Aspα), 4.66 (d, H-1^1), 4.49 (t, $J=6.4$ Hz, Proα), 4.40 (d, $J_{1,2}=8.0$ Hz, H-1^{3or2}), 4.37 (d, $J_{1,2}=8.0$ Hz, H-1^{2or3}), 4.28 (m, Serα), 4.27 (d, $J_{4,5}=9.9$ Hz, H-5^4), 4.16 (dd, $J_{5,6b}=5.7$ Hz, $J_{gem}=11.6$ Hz, H-6b^3), 4.06 (m, H-5e^1), 4.03 (dd, $J_{5,6a}=5.3$ Hz, H-6a^3), 4.00 (m, Glya), 3.92 (m, H-4^1), 3.83 (dd, $J_{2,3}=10.3$ Hz, H-3^3), 3.81 (m, Glyb), 3.8.5 (m, 2H, H-6^2), 3.76 (brt, $J=6.6$ Hz, H-5^3), 3.75 (m, Gly), 3.70 (s, COOMe), 3.69 (m, H-3^2), 3.68 (m, Proγb), 3.61 (brt, $J=7.1$ Hz, H-5^2), 3.55 (m, Serβ), 3.47 (dd, $J_{4,5a}=8.0$ Hz, $J_{gem}=12.1$ Hz, H-5a^1), 3.37 (m, Proγa), 3.24, 3.15 (m, Trpβ), 2.90 (dd, $J_{\alpha,\beta b}=4.8$ Hz, $J_{gem}=17.0$ Hz, Aspβa), 2.62 (dd, $J_{\alpha,\beta a}=6.0$ Hz, Aspβb), 2.36, 2.35, 2.34 (3s, 4 MBz), 2.29 (s, MBz), 2.15 (s, 2Ac), 2.09, 2.02, 1.93, 1.91 (4s, each 3H, 4Ac), 1.66, 1.44, 1.40, 1.39 (4s, each 9H, 4*tert*-Bu). **1**: δ_H (500 MHz, D_2O) (selected) 7.71, 7.59, 7.53, 7.27, 7.20 (m, Ph), 4.96 (t, $J=6.9$ Hz, Trpα), 4.75 (dd, $J=6.0$, 7.0 Hz, Aspα), 4.70 (d, $J_{1,2}=7.9$ Hz, H-1^4), 4.66 (dd, $J=5.8$, 7.8 Hz, Aspα′), 4.59 (d, $J_{1,2}=7.9$ Hz, H-1^3), 4.57 (m, Trpα′), 4.56 (d, $J_{1,2}=7.5$ Hz, H-1^2), 4.46 (d, $J_{1,2}=7.8$ Hz, H-1^1), 4.42 (d, $J_{1,2}=7.3$ Hz, H-$1'^1$), 4.41 (m, Proα, Serα), 4.33 (dd, $J=5.0$, 11.0 Hz, Serβa), 4.30 (t, $J=4.1$ Hz, Serα′), 4.24 (m, Serβa′), 4.14 (s, H-4^{2or3}), 4.10 (s, H-4^{3or2}), 4.10 (m, Serβb), 4.04 (m, Serβb′), 3.78 (m, H-3^{2or3}), 3.73 (m, H-2^2, 2^3, Proδa), 3.63 (m, H-3^{3or2}), 3.42 (m, H-2^4), 3.37 (m, H-2^1, Proδb), 3.33 (m, H-$2^{1'}$), 3.31 (m, Trpβa), 3.30 (m, Proδb′), 3.14 (m, Trpβb), 2.94 (dd, $J_{gem}=19.1$ Hz, Aspβa), 2.90 (dd, $J_{gem}=19.1$ Hz, Aspβa′), 2.79 (dd, Aspβb), 2.77 (dd, Aspβb′), 2.22 (m, Proβa), 1.92 (m, Proβb, Proγ), 1.64, 1.44 (m, Proβ′, Proγa′), 0.97 (m, Proγb′); FAB-MS (positive) *m/z*: 1316.3 (calcd for $C_{50}H_{69}N_7Na_3O_{30}$ 1316.38, $[M+H]^+$), 1338.4 (calcd for $C_{50}H_{68}N_7Na_4O_{30}$ 1338.36, $[M+Na]^+$).

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