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STEREOSELECTIVE SYNTHESES OF PHOSPHORYLATED AND SULFATED GLYCOSYL SERINES IN GLYCOSAMINOGLYCAN FOR BIOLOGICAL PROBES

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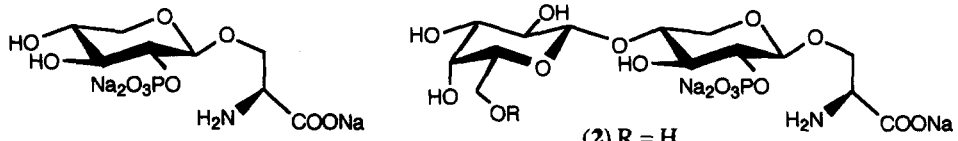
Abstract Phosphorylated glycosyl serines of glycosaminoglycan with/without sulfate: β-D-Xyl(2P)-Ser (**1**) and β-D-Gal(±6S)-(1→4)-β-D-Xyl(2P)-Ser (**2**, **3**) were suitably designed for biological probes. These oligosaccharides were synthesized in a stereocontrolled manner.

Glycosaminoglycans (GAGs) are classified into two categories based on a type of hexosamine residue in repeating disaccharide region. Heparan sulfate type GAG has α-*N*-acetyl glucosamine (α-GlcNAc), whereas chondroitin/dermatan sulfate have β-*N*-acetyl galactosamine (β-GalNAc) as the hexosamine residue, respectively. Biological sortings are taken place in a transfer of the first hexosamine residue to a non-reducing end of linkage tetrasaccharide (GlcA^4-Gal^3-Gal^2-Xyl^1) (Xyl: xylose, Gal: galactose, GlcA: glucuronic acid), of which mechanisms have been ambiguous so far. However, recent studies have suggested influences of substituents on the linkage tetrasaccharide[1-3] or core-peptide[4] in the biological sorting. For example, Sugahara's group[1] isolated GAG oligosaccharides possessing sulfate(s) on Gal moieties [e.g. Gal^3(4S), Gal^3(6S) and Gal^2(6S)]. These sulfates exist only in chondroitin/dermatan sulfates but not in heparan sulfate. On the other hand, phosphate at *O*-2 of Xyl has been found both in heparan sulfate[2] and in chondroitin sulfates.[3] Furthermore, Fransson et al.[5] reported that degree of phosphorylation varied along with glycan elongation very recently. These acidic substituents surely relate to the glycan elongation and might also control the sorting mechanisms.



(**1**)

(**2**) R = H
(**3**) R = SO_3Na

Fig. 1.

Oligosaccharide probes at the reducing end of GAG having specific sulfate and phosphate will be able to elucidate the problems. Whereas, enough amount of these labile oligosaccharides can hardly be obtained biologically due to difficulties in isolation. These facts prompted us to synthesize such acidic GAG oligosaccha-

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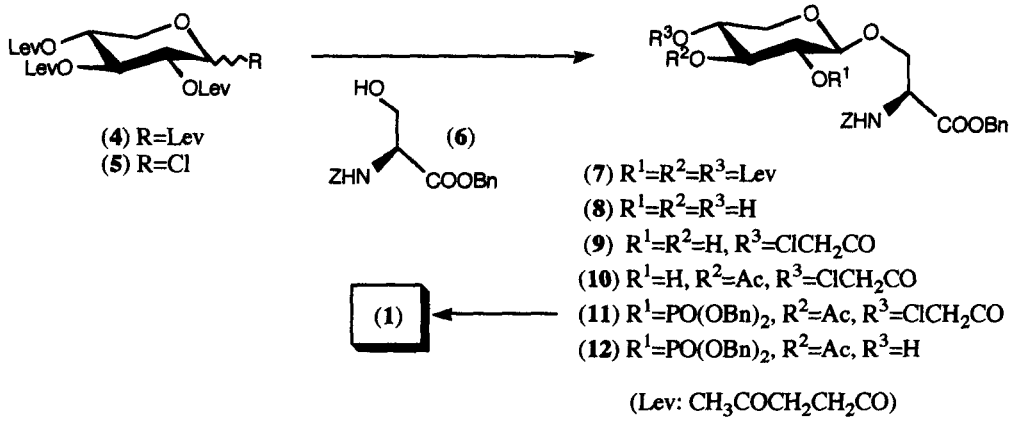
rides. We thus selected GAG glycosyl serines (**1**~**3**) as target compounds which are required for dynamic biological studies (Fig. 1).

It is noted that Goto and Ogawa[6] have reported syntheses of tetra- and hexaosyl serine having sulfate at *O*-4 of Gal[3] in 1992. Di-, tri- and tetrasaccharides of GAG at the reducing end containing phosphate at *O*-2 on Xyl have been synthesized by Nillson's[7] group as methyl glycosides in 1993. In the following year, Jacquinet et al.[8] reported syntheses of corresponding di- and tetraosyl dipeptides having phosphate at *O*-2 on Xyl. However, complicated oligosaccharide having phosphate, sulfate and carboxylic acid simultaneously, such as **3**, has never been synthesized to our knowledge.

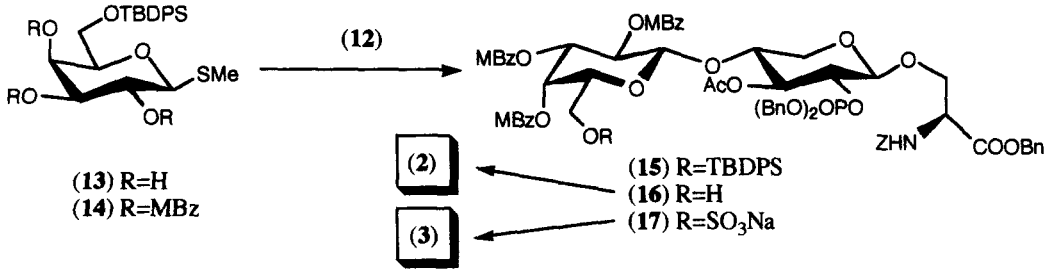
We adopted a newly arranged synthetic strategy by which Xyl-Ser bond was formed prior to glycan elongation and phosphorylation. As shown in Scheme 1, levulinoyl ester was employed as temporary protecting group of Xyl; D-xylose was per-levulinated with levulinic anhydride in the presence of DMAP to afford **4** (86%). Then, we converted **4** to a corresponding chloride (**5**) with titanium (IV) chloride in CH_2Cl_2 in 81% yield. The chloride **5** was immediately applied to coupling reaction with known N-Z-serine benzyl ester (**6**)[9] in the presence of AgOTf in 1,2-dichloroethane at 0 °C. Thus, desired β-linked xylosyl serine moiety (**7**) was exclusively obtained in 66% yield. This result shows a superiority of levulinoyl ester exhibiting a better neighboring group effect at *O*-2 of Xyl. Because, similar coupling reaction of 2,3,4-tri-*O*-chloroacetyl-D-xylosyl chloride and hydroxyl group of L-seryl-glycine gave anomeric mixture.[8] Then, all the levulinoyl groups of **7** were removed with H_2NNH_2•AcOH[10] to give triol (**8**) (95%). Nilsson's group reported a regiospecific chloroacetylation at *O*-4 of methyl xyloside via dibutylstannylene acetal.[7] We have proved that the method was applicable also for xylosyl serine (**8**). Thus, chloroacetate (**9**) was obtained regioselectively [1) Bu_2SnO /dioxane, reflux, 2) $ClCH_2COCl/CH_2Cl_2$, (62%, 2 steps)]. Regioselective acetylation at *O*-3 of **9** was performed with acetyl chloride to give **10** (80%). Resided *OH*-2 of **10** was phosphorylated with commercially available dibenzyl *N*,*N*-diisopropylphosphoramidite followed by oxidation with mCPBA[8] to give desired dibenzylphosphate (**11**) (88%, 2 steps). Chemoselective removal of the chloroacetyl group of **11** with H_2NNH_2•AcOH gave an acceptor (**12**) for further glycosylation in 88% yield.



Scheme 1.

In order to obtain phosphorylated Gal-Xyl-Ser moieties with/without sulfate, we have synthesized a galactosyl donor suitably designed for alternative purpose. Scheme 2 shows a synthesis of the glycosyl donor

and a coupling procedure to Gal-Xyl-Ser sequence. Primary hydroxyl group of commercially available methyl 1-thio-β-D-galactopyranoside was regioselectively protected with TBDPS ether to give triol (**13**) (92%). In order to avoid undesired acyl migration during later desilylation procedure, we employed a steric hindered *p*-methylbenzoyl ester as protecting group of the resided hydroxyl groups.[11] Thus, acylation with *p*-methylbenzoyl chloride in pyridine afforded a pivotal galactosyl donor (**14**) in 86% yield. Final coupling of **12** and **14** by the action of NIS-TfOH yielded a β-linked disaccharide (**15**) in 52% accompanied by α-isomer in 18%. The silyl group was removed with tetrabutylammonium fluoride in the presence of acetic acid to give **16** in 85% yield. No acyl migration was observed.



Scheme 2. (TBDPS: *t*-$BuPh_2Si$, MBz: *p*-MeC_6H_4CO)

Having protected Xyl-Ser and Gal-Xyl-Ser sequences in hand, we first exposed **11** to hydrogenolytic condition (Pd/C) and saponification (Et_3N-MeOH-H_2O). The crude materials were purified through a column of gel-permeation (LH-20, 1% ammonium carbonate) and ion-exchange resin [AG® 50W-X8(Na^+)] to give Xyl(2P)-Ser (**1**)[12] in 78% yield (2 steps). Next, we converted **16** to Gal-Xyl(2P)-Ser (**2**)[12] in 82% yield by hydrogenolysis (Pd/C), saponification [1) Et_3N-MeOH-H_2O, 2) aq NaOH] and following purification procedures as desribed above. On the other hand, free hydroxyl group at *O*-6 of **16** was sulfated [1) SO_3•Me_3N/DMF 50~60 °C, 2) AG® 50W-X8(Na^+)] to give **17** in 90% yield. Deprotection was achieved in a same manner as the case of **2**. We purified the crude materials through a column of gel-permeation (LH-20, 1% AcOH) followed by the same ion-exchange resin as above and, finally, obtained Gal(6S)-Xyl(2P)-Ser (**3**)[12] in 59% yield (2 steps). All the compounds obtained were identified the structures by measurement of ^1H NMR spectra at 400 MHz and ESI-MS.[12,13] Enzymatic elongation with these compounds are under investigation.

In summary, we have designed GAG glycosyl serines (**1**~**3**) having phosphate with/without sulfate in order to use for biological probes. They were synthesized successfully in stereocontrolled manners. We also showed a first synthesis of **3**, which is a novel oligosaccharide simultaneously containing phosphate, sulfate and carboxylic acid.

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10. Nakano, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1991**, *32*, 1569.
11. Sterically hindered pivaloyl group was introduced only at *O*-3 of **13** in 82 %.
12. Physical data for target compounds are given below, values of δ_H were measured at 20°C. Chemical shifts are expressed in p.p.m. downfield from the signal for Me_4Si, by reference to internal DHO (4.65) for the solutions in D_2O. Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue *3*. **1**: δ_H (D_2O) 3.20 (dd, $J_{4,5ax}$ = 10.0 Hz, J_{gem} = 11.46 Hz, $5ax^1$), 3.43 (dd, $J_{2,3}$ = 8.78 Hz, $J_{3,4}$ = 9.03 Hz, 3^1), 3.52 (ddd, $J_{4,5eq}$ = 5.37 Hz, 4^1), 3.67 (dt, $J_{1,2}$ = 7.32 Hz, $J_{2,3}$ = $J_{2,P}$ = 8.78 Hz, 2^1), 3.82 (dd, $5eq^1$), 3.84 (dd, $J_{\alpha,\beta1}$ = 5.12 Hz, J_{gem} = 3.66 Hz, Serβ1), 3.87 (dd, $J_{\alpha,\beta2}$ = 10.25 Hz, Serβ2), 4.07 (dd, Serα), 4.42 (d, 1^1); ESI-MS (positive) *m/z*: 340.1 $[M-2Na+H]^+$, 362.1 $[M-Na+2H]^+$, 384.0 $[M+H]^+$, 657.4 $[2M-5Na+6H]^+$, 679.1 $[2M-4Na+5H]^+$, 701.3 $[2M-3Na+4H]^+$, 723.2 $[2M-2Na+3H]^+$, 745.3 $[2M-Na+2H]^+$, (negative) *m/z*: 316.0 $[M-3Na+2H]^-$, 337.9 $[M-2Na+H]^-$, 633.2 $[2M-6Na+5H]^-$, 655.2 $[2M-5Na+4H]^-$, 677.0 $[2M-4Na+3H]^-$, 699.3 $[2M-3Na+2H]^-$. **2**: δ_H (D_2O) 3.23 (dd, $J_{4,5ax}$ = 9.51 Hz, J_{gem} = 11.95 Hz, $5ax^1$), 3.29 (dd, $J_{1,2}$ = 7.81 Hz, $J_{2,3}$ = 9.75 Hz, 2^2), 3.42 (dd, $J_{3,4}$ = 3.42 Hz, 3^2), 3.48 (ddd, $J_{4,5}$ < 0.5 Hz, $J_{5,6a}$ = 3.91 Hz, $J_{5,6b}$ = 8.05 Hz, 5^2), 3.51 (dd, J_{gem} = 11.71 Hz, $6a^2$), 3.53 (dd, $J_{2,3}$ = 8.76 Hz, $J_{3,4}$ = 9.04 Hz, 3^1), 3.60 (dd, $6b^2$), 3.65 (dt, $J_{1,2}$ = 7.08 Hz, $J_{2,3}$ = $J_{2,P}$ = 8.76 Hz, 2^1), 3.69 (bd, 4^2), ~3.7 (m, 4^1), 3.78 (dd, $J_{\alpha,\beta1}$ = 5.85 Hz, J_{gem} = 3.41 Hz, Serβ1), 3.81 (dd, $J_{\alpha,\beta2}$ = 10.73 Hz, Serβ2), 3.90 (dd, $J_{4,5eq}$ = 5.12 Hz, $5eq^1$), 4.01 (dd, Serα), 4.26 (d, 1^2), 4.39 (d, 1^1); ESI-MS (positive) *m/z*: 502.2 $[M-2Na+3H]^+$, 524.3 $[M-Na+2H]^+$, 546.4 $[M+H]^+$, 981.3 $[2M-5Na+6H]^+$, 1025.5 $[2M-3Na+4H]^+$, 1047.5 $[2M-2Na+3H]^+$, 1069.5 $[2M-Na+2H]^+$, (negative) *m/z*: 478.3 $[M-3Na+2H]^-$, 500.2 $[M-2Na+H]^-$, 522.0 $[M-Na+2H]^-$, 957.4 $[2M-6Na+5H]^-$, 979.5 $[2M-5Na+4H]^-$, 1001.2 $[2M-4Na+3H]^-$, 1023.4 $[2M-3Na+2H]^-$. **3**: δ_H (D_2O) 3.30 (dd, $J_{4,5ax}$ = 9.76 Hz, J_{gem} = 11.95 Hz, $5ax^1$), 3.36 (dd, $J_{1,2}$ = 7.81 Hz, $J_{2,3}$ = 10.00 Hz, 2^2), 3.51 (dd, $J_{3,4}$ = 3.42 Hz, 3^2), 3.58 (t, $J_{2,3}$ = $J_{3,4}$ = 8.78 Hz, 3^1), 3.72 (dt, $J_{1,2}$ = 7.08 Hz, $J_{2,3}$ = $J_{2,P}$ = 8.78 Hz, 2^1), 3.77 (m, 4^1), 3.78 (m, 5^2), 3.82 (d, 4^2), 3.89 (dd, $J_{\alpha,\beta1}$ = 11.22 Hz, J_{gem} = 3.42 Hz, Serβ1), 3.96 (dd, $J_{4,5eq}$ = 4.88 Hz, $5eq^1$), 3.98 (m, Serβ2), 4.04 (bd, 2H, J = 6.34 Hz, 6^2), 4.12 (dd, $J_{\alpha,\beta2}$ = 5.37 Hz, Serα), 4.35 (d, 1^2), 4.45 (d, 1^1); ESI-MS (positive) *m/z*: 604.3 $[M-2Na+3H]^+$, 626.3 $[M-Na+2H]^+$, 669.9 $[M+Na]^+$, 1229.2 $[2M-3Na+4H]^+$, 1251.4 $[2M-2Na+3H]^+$, (negative) *m/z*: 558.2 $[M-4Na+3H]^-$, 580.1 $[M-3Na+2H]^-$, 601.9 $[M-2Na+H]^-$, 1139.3 $[2M-7Na+6H]^-$, 1161.3 $[2M-6Na+5H]^-$, 1183.2 $[2M-5Na+4H]^-$, 1205.2 $[2M-4Na+3H]^-$.
13. It was difficult to identify by mass spectrum whether all the acidic groups were converted to sodium salt.