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Glycosylation

Harnessing Free Sulfate Groups in Glycosylation Reactions

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Abstract: General strategies for synthesizing sulfated oligosaccharides employ the protection of sulfate groups for glycosylation or post-sulfation after the synthesis of oligosaccharides. However, the chemical behavior of free sulfate groups in glycosylation reactions has not been thoroughly studied. We examined several glycosyl donors with free sulfate groups, but neither glycosyl imidates nor thioglycosides achieved products. Conversely, activating 6-sulfated GalNAc donors with either a 2-NTroc group or 2-O-acetyl group using an ortho-hexynylbenzoate group at the anomeric position yielded β-glycosides in good yield. These results indicate that glycosylations with a free sulfate group can be performed and that the neighboring group participation of 2-NHTroc and 2-O-acetyl groups works even with an unprotected sulfate group at the 6-position. Ab initio calculations supported the formation of acyloxonium-cation via 2-O-acyl participation. Additionally, glycosylation reactions under various counter cations of the sulfate group, such as Na^+, Li^+, K^+, Ba^{2+}, and pyridinium were examined. Results showed that sodium and lithium salt donors yielded the products in good yield. These results were also supported by ab initio calculations. Practical glycosylation reactions between disaccharide donor-acceptor pairs with free sulfate groups successfully yielded the sulfated tetrasaccharides. This study also discusses how a sodium salt acts as a protecting group during glycosylation.

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Proteoglycans consist of polysaccharides (GAGs) attached to serine residues of a core protein. GAGs have repeating units and are classified based on their disaccharide structure such as chondroitin sulfate, heparan sulfate, and keratan sulfate.[1,2] Sulfated glycans are further divided by their sulfation patterns. In the case of chondroitin sulfate (CS), CS–A, CS–C, CS–D, and CS–E are the major sulfation patterns in nature.[3–5]

GAGs have three main functions: acting as cell surface receptor, co-receptors/modulators, and signaling molecules.[5] Mizoguchi et al. reported that chondroitin was required for embryonic cytokinesis of *Caenorthabditis elegans (C. elegans)*.[6] Chondroitin sulfate A inhibits the neurite elongation, whereas chondroitin sulfate E stimulates the elongation of it.[7] The biological activities of chondroitin sulfate are modulated by their sulfation patterns.

Sulfated glycans without a core protein also show many important biological activities. Heparin is widely used as an anticoagulant drug.[8,9] Not only heparin, but also chondroitin sulfate exhibits various bioactivities, such as anti-inflammation,[10–12] anti-oxidation,[13,14] anti-coagulant[13,14] and so on.

Sulfation patterns of GAGs are diverse, varying depending on tissue type and species. However, this diversity has made difficult to investigate the relationship between the sulfation patterns of glycan and their bioactivity.

In order to understand which sulfation pattern is essential for specific biological events, a series of homogeneous sulfated oligosaccharides have been synthesized.[15,16] In these syntheses, the sulfation of hydroxy groups was generally performed after the synthesis of the oligosaccharide scaffolds.[7,17–19] The Boons and Hung groups have demonstrated robust and efficient GAG syntheses to generate well-diverse glycans with a homogeneous sulfation pattern. The synthesis of hexasaccharide chondroitin sulfate was developed by combining liquid phase sulfation with an automated glycan synthesis.[20] In addition to total chemical synthesis, semi-synthesis of chondroitin sulfate was achieved by the Vibert group.[21] To enable more flexible synthesis routes of heparin oligosaccharide, the Huang group was the first to intorduce the protected sulfate group in glycosyl donors.[22] The Tamura group also reported the synthesis of CS–D disaccharide using 2,2,2-trichloroethoxy (TCE) sulfate groups.[23] Although these protection protocols gave some byproducts, efficient syntheses have been demonstrated.[24,25]

The methods of both post-sulfation and sulfate protection groups have been extensively studied, but the chemical

behaviors of free sulfate groups in glycosylation reactions have not been well-studied. If free sulfate groups can be utilized in glycosylations, these donors and acceptors could contribute to the synthesis of additional diverse GAGs.

For making an interesting new strategy using glycosyl donors and acceptors with free sulfate groups, we began a study of the chemistry of sulfate group in glycosylation reactions. As shown in Figure 1, the unprotected sulfate group has a lower pKa value, around −9.0.[26] On the other hand, protonated esters and hydronium ions show higher pKa values, ranging from −2.0 to −8.0,[26] and −1.74,[26] respectively. These physicochemical values enabled us to hypothesize that the hydroxy group is a more highly nucleophilic functional group than that of sulfate group, indicating that glycosylation reactions can proceed in the presence of free sulfate groups.

In this paper, we will report glycosylations with free sulfate groups and the chemical behavior of free sulfate group.

In order to study how a free sulfate group affects glycosylation reactions, we synthesized three kinds of glycosyl donor, thioglycoside, imidate and ortho-hexynylbenzoate donors. However, thioglycoside donor (Scheme S8) gave only a trace amount of glycosylation products under the several conditions. The imidate donor (Scheme S9) could not be isolated owing to its instability. Therefore, we selected the donor with an ortho-hexynylbenzoate group[27] developed by the Yu group. We selected a galactoside (Gal) scaffold and installed either O-acety group or NHTroc group to 2-position for optimization (Table 1), because chondroitin sulfate includes GalNAc form. Therefore, donors **5** and **10** with an ortho-hexynylbenzoate group[27] were prepared from substrates **1** and **6**, respectively, by the standard protection/deprotection protocols including treatment with SO_3/Py for sulfation reaction (Table 1). The details of these syntheses are shown in the Supporting Information (Scheme S1).

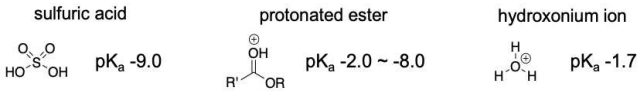
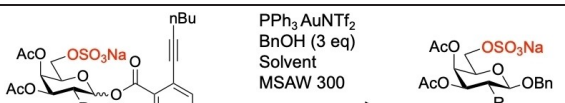


Figure 1. pKa values of several functional groups.

Table 1: Glycosylation in the presence of a free sulfate group.



Entry	Activation condition	Donor	Concentration	Solvent	Result	Yield*
1	PPh_3AuNTf_2 (0.2 eq)	**5**	100 mM	DCM	β-product	83%
2		**5**		THF	β-product	46%
3		**5**		MeCN	β-product	27% (45%**)
4		**5**		DMF	orthoester	42%**
5		**10**		DCM	β-product	79%
6		**10**		MeCN	β-product	72%

*Determined by NMR, **orthoester

Glycosyl donors **5** and **10** were prepared from **1** and **6**. A detail of the synthesis is shown in Supporting Information.

Table 1 shows glycosylation reactions toward benzyl alcohol in the presence of 6-sodium sulfate group. The anomeric position was activated with an ortho-hexynylbenzoate functional group and Ph_3AuNTf_2.[27] The experiments with galactoside donors clearly showed that the glycosyl product formed the β linkage in good yield. The glycosylation reactions in DCM gave a product in good yield (83 %), whereas the same reaction in THF (46 %) and MeCN (27 %) were less efficient. Above all experiments showed that the products were β selective, indicating that the acyloxonium ion is likely an intermediate (Figure 2, B'). We also evaluated the reaction velocity of two glycosyl donors: one with 6-sulfate (Na salt) group and the other with 6-O-acetate (Figure S3). The results indicated that the reaction velocities of these two donors were similar, suggesting that the 6-sulfate group did not significantly affect the glycosylation reaction. Additionally, glycosylations in MeCN and DMF were found to give a byproduct, orthoester (about 40 %). In terms of Gal-2-NHTroc donor, these glycosylations gave β product in both DCM and MeCN. These results indicated that 2-NHTroc seemed to regulate β glycosylation reactions more significantly than the solvent effect by the acetonitrilium ion formation.

Regarding the 6-sulfate, the intermediate C' (Figure 2) can form, but the inversion of pyranoside ring is required. The results shown in Table 1 suggested that the neighboring group participation of *O*-acetyl group and NHTroc at the 2-position worked even in the presence of unprotected sulfate groups at the 6-position, although the 6-sulfate group was expected to interact with the anomeric cation intermediate C' (Figure 2).

Because there are three representative intermediates that can modulate the stereoselectivity of glycosylation reactions, the energies of three possible intermediates A', B', and C' were calculated to evaluate which species are stable. All the calculations were carried out using the Gausian09 program package and B3LYP hybrid functional. 6-31 + G(d) basis sets were used for all atoms. Their energy

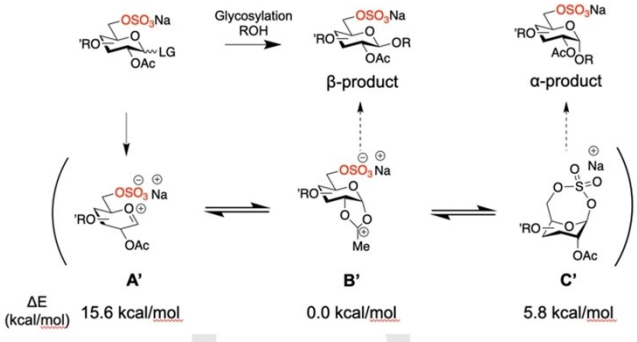


Figure 2. Plausible intermediates on glycosylation with a free sulfate group at 6-position.

profiles were obtained by summing the total energy and zero-point energy. As shown in Figure 2, the calculation clearly showed that acyloxonium cation B', formed by the 2-O-acyl neighboring group participation, is the most stable intermediate, being consistent with the β selectivity (Table 1, Tables S1–6, Figure S4).

In addition to acyloxonium ion intermediates, we studied which counter cation species stabilize the sulfate group in glycosylation reactions. In order to prepare several donors with a counter cation such as Na^+, Li^+, K^+, Ba^{2+}, and pyridinium cations, 6-sodium sulfated galactosyl donor **5** was passed through the proton-form resin (Dowex 50Wx8) once and then passed through a corresponding salt form of Dowex 50Wx8. Mass analyses indicated that original sodium salt was successfully substituted with all corresponding salt forms (Figure S1). Using these donors, glycosylation reactions were conducted. As shown in Table 2, the sodium salt and lithium salt donors gave the products in good yield, 95% and 86% respectively. However, in the case of other salts, the yields decreased.

In order to understand the effect of counter cation on glycosylation reactions, the stability of individual salt forms was estimated by the Gaussian program. We estimated energies of both salt and non-salt forms of a sulfated group. As shown in Table S7, lithium and sodium salts of sulfated sugars showed low energy, whereas other salts showed high energy. These results indicated that sodium and lithium cations stabilize salt forms through strong interactions with sulfate anions. On the other hand, the interaction between other counter cations and sulfate groups is weak. Therefore, a strong interaction between counter cations and a sulfate group appears to be critical for the glycosylation reactions in the presence of the free sulfate group.

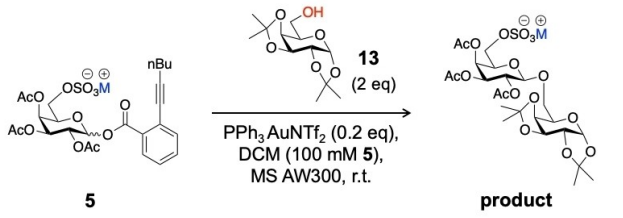
As mentioned, we gained insight into the effect of a free sulfate group on glycosylation reactions, including the stability of counter cations, and neighboring group participation at 2-position in the presence of a 6-sufate group. We then examined a practical synthesis of CS-oligosaccharides. In these experiments, we examined the synthesis of tetrasaccharide forms.

The common building block of chondrosin **14** was obtained through several steps from commercially available chondroitin sulfate sodium salts[28] (Scheme 1). The hydroxy group at C-6 position of GalNAc residue of **16** was selectively protected by a trityl group. Subsequently, the other four free hydroxy groups were protected by acetyl groups. Thioglycoside **17** was converted into its hemiacetal form with *N*-bromosuccinimide in acetone/H_2O and the resultant hemiacetal derivative was then condensed with ortho-hexynyl benzoic acid to give donor form **18**. Finally, the 6-trityl group was converted to a sulfate group by acid treatment with TFA, followed by treatment with sulfur trioxide pyridine complex, yielding donor **19**.

The preparation of glycosyl acceptor **24** was also performed from chondrosin **14** as a starting material (Scheme 2). Detailed individual conversions are shown in Scheme S3.

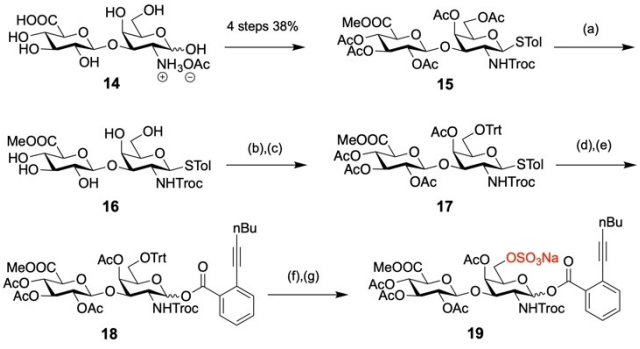
Next, we examined glycosylation reaction between chondroitin sulfate donor **19**, which has a free sulfate group at 6-position, and a nonsulfated thioglycoside acceptor with 4-hydroxy group. The thioglycoside acceptor was prepared from **22** (Scheme 2) with $H_2NNH_2 \cdot H_2O$ in AcOH/pyridine. Although glycosylation with the low-reactive 4-hydroxy group was successfully performed in 28% yield (isolated), our structural analyses revealed that the low yield was due to an aglycon transfer reaction.[29] To avoid aglycon transfer reactions, extensive optimization was attempted by changing

Table 2: Glycosylation in the presence of several cation species.

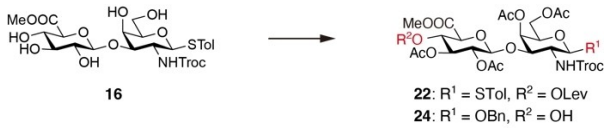


Entry	Counter cation	Yield	Side product
1	Li^+	86%	—
2	Na^+	95%	—
3	K^+	40%	SM (40%)
4	PyH^+	25%	Sulfate migration (62%)
5	Tetrabutylammonium	36%	—
6	Ba^{2+}	0%	Decomposed

SM indicates the recover of starting material. The hyphen indicates no detection.



Scheme 1. Synthesis of disaccharide donor with a sulfate group. (a) MeOH, MeONa, r.t., 90 min, 61%. (b) TrtCl, DMAP, pyridine, 50 °C, overnight. (c) Ac_2O, pyridine, r.t., overnight, 77% (2 steps). (d) NBS, acetone, H_2O, 0 °C, 90 min. (e) DMAP, DIC, DCM, o-hexynylbenzoic acid, overnight, r.t., 61% (2 steps). (f) TFA, TIPS, DCM, r.t., 3 min. (g) $SO_3 \cdot$py, DMF, r.t., 2 hrs, 85% (2 steps).



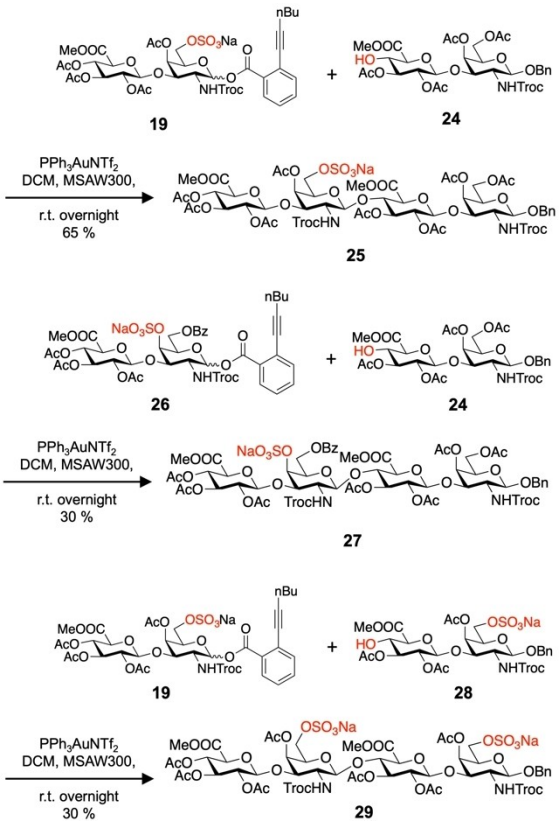
Scheme 2. Preparation of glycosyl acceptors **22** and **24**. Detailed synthetic conditions are shown in Scheme S3.

the reaction solvent, reaction time, and temperature, but the yield did not improve.

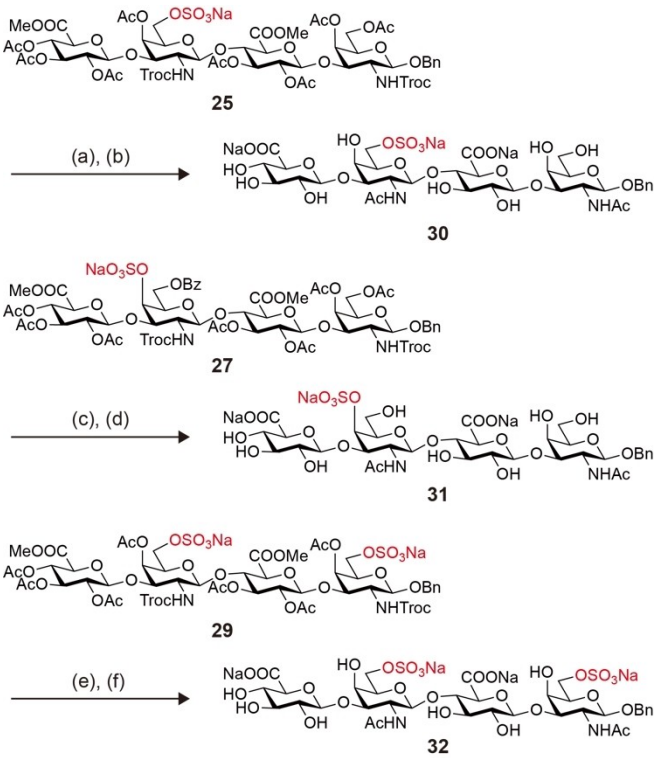
In order to avoid the aglycon transfer and enhance the isolated yield, the anomeric group of the acceptor was changed from thioglycoside to the benzyl group **24**. The preparation of benzly acceptor was carried out by conventional protocols (Scheme 2 and S3).

Using the benzyl-protected acceptor **24**, additional glycosylation reactions were performed. As expected, the yield of glycosylation was successfully increased. Scheme 3 shows various results of glycosylation reactions by altering the positions and numbers of sulfate groups. The glycosylation reaction between donor **19** and acceptor **24** resulted in 65 % isolated yield. Regarding sulfation isomers, glycosylation reactions with 4-sulfated donor **26** and acceptor **24** proceeded with moderate yield (30 % isolated yield). Additionally, a glycosylation reaction between sulfated donor **19** and sulfated acceptor **28** was performed. We found that the presence of two sulfate groups in both the donor and acceptor did not interfere with the glycosylation reaction (30 % isolated yield). The structures of all products were confirmed by NMR and HRMS.

Finally, deprotection of glycosylation products was examined to investigate the stability of a free sulfate group (Scheme 4). First, the Troc group of tetrasaccharide **25** was converted to acetamide group by treatment with zinc powder in THF:AcOH:acetic anhydride (3:2:1). Zinc



Scheme 3. Synthesis of tetrasaccharides in the presence of sulfate groups.



Scheme 4. Deprotection of tetrasaccharides with sulfate groups.(a) Zn, THF/AcOH/Ac_2O (3:2:1), 3 h, r.t., (b) 1 M aq NaOH, MeOH, r.t., overnight, 77% (2 steps), (c) Zn, THF/AcOH/Ac_2O (3:2:1), 5 h, r.t., (d) 1 M aq NaOH, MeOH, 17 h, r.t., 30% (2 steps), (e) Zn, THF/AcOH/Ac_2O (3:2:1), 7 h, r.t., (d) 1 M aq NaOH, MeOH, 6 h, r.t., 40% (2 steps).

powder was removed by filtration, and then the filtrate was evaporated. The resulting residue was suspended in 500 mM NaOH in MeOH to remove all ester protecting groups. As the result of deprotection, the unprotected chondroitin sulfate tetrasaccharide **30** was obtained in 77 % yield (2 steps). Deprotection of other tetrasaccharides **27** and **29** was also performed with the similar conditions as for the preparation of **30**. Tetrasaccharide **31** and **32** were successufully obtained in 30 % and 40 % yields, respectively. These data clearly indicated that a free sulfate group installed was stable under the conventional deprotection conditions.

We have an interest into the behavior of a free sulfate group on glycosylation reactions for the synthesis of chondroitin oligosaccharide derivatives, therefore, we studied the function of a sulfate group, including its stability, salt effect, and neighouring effect.

Because chondroitin sulfate has β-glycosyl linkage, β-glycosylation needs to be performed through acyloxonium participation at the 2-position in the presence of the 6-sulfate group. As shown in Table 1 and Figure 2, acyloxonium participation is the major intermediate. According to ab initio calculation, intermediate C‘ (Figure 3) is not stable, indicationg the nucleophilic attack of oxygen of acyl group at the C2 position is more likely or its product is thermodynamically more stable than the product formed by the attack of the oxygen from 6-sulfate group.

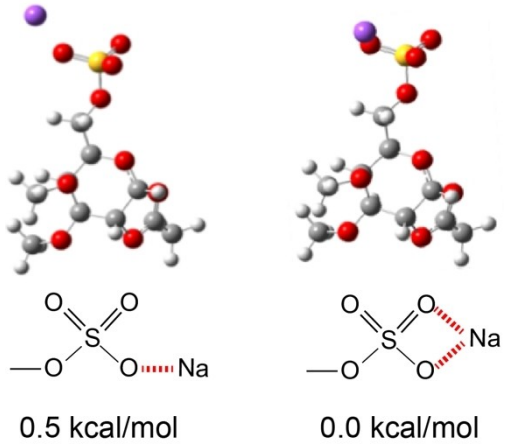


Figure 3. Plausible salt form of sodium sulfate in glycosylation. Left: Sodium is fixed with an oxygen. Right: Sodium interacts with two oxygen alternatively.

In addition to acyloxonium intermediate, the participation of the oxygen of free 6-sulfate was also evaluated. The corresponding bicyclic intermediate (Figure 3, C') involves a seven-membered ring, which is less stable due to the ring distortion. According to the literature,[30] the strain energy of seven-membered cycloalkanes is generally unstable, exceeding 6.3 kcal/mol. Furthermore, for the formation of sulfate-participated intermediate (Figure 2, C'), a ring flip is required from the $^4C_1$ chair form to $^1C_4$ chair form. The ring flip of cyclohexane typically requires 10.8 kcal/mol as the activation energy.[26] In addition to this energy barrier, the size of substituent groups, such as protecting groups, also increases the energy barrier during the ring flip. Therefore, glycosyl donors require more than 10 kcal/mol for the formation of 6-sulfate-participated intermediate via their ring flip (Figure 2, C'). According to the above reports, intermediate B' is more favorable than the C'. We also studied energy calculation to validate the above discussion. Our calculations clearly support that acyloxonium ion is a stable intermediate (Figure 2 and Table S5).

Next, the effect of counter cations was evaluated based on the calculation results with 6-31+G(d). Figure S4 and Table S3–S7 show the stability of glycosyl donor with a free sulfate group, varying its salt form. Because the sodium and lithium salts showed the low energy values of −10.3 and −11.3 kcal/mol, respectively (Table S7C), indicating that sodium and lithium cations strongly interact with the sulfate anion. On the other hand, the interaction between other counter cations, such as potassium and pyridinium cations, and sulfate groups is weak. In particular, the tetrabutylammonium cation shows 7.0 kcal/mol (Table S7). This result is opposite to the interaction observed in water. In aqueous solutions, the interaction between potassium cation and sulfate anion is stronger than that of lithium or sodium cation. The pK values of $LiSO_4^-$, $NaSO_4^-$, and KSO_4^- are 0.77, 0.82, and 0.85, respectively.[31] These values suggest that the interaction between potassium cation and sulfate anion is stronger than that of sodium and lithium in water.[32] This difference arises from the hydration of cations because lithium/sodium cations are more strongly hydrated than potassium cation. However cations do not form hydrates in the organic solvent such as DCM. In this case, Coulomb force is the dominant interaction between cation and anion and is related to the distance between two ions, indicating that smaller ions can approach each other more closely than larger ions. The radii of these cations are 0.071 nm (Li^+),[32,33] 0.098 nm (Na^+),[32,33] and 0.133 nm (K^+).[32,33] Therefore, lithium and sodium cations could interact strongly with the sulfate anion in DCM. This strong interaction between counter cations and sulfate groups appears to act as a protecting group of sulfate group. In our deprotection steps of tetrasaccharides, we intentionally added NaCl to the solution. Without this addition, the tetrasaccharides gradually decompose.

Several groups have reported the stability of sulfate groups depending on their salt forms and the degree of interaction with various counter cations. The Meldal group found that the tetrabutylammonium salt of peptide-sulfate exhibited greater stability compared to their sodium and barium salts during ionization in mass spectrometric analysis and HPLC analysis.[34] Desulfation under acidic conditions was also discussed as being dependent on the salt forms by the Futaki group.[35] The Linhardt group reported that the guanidine group in arginine can bind sulfate groups more effectively than the amine group in lysine.[36] However, we observed that the sulfate group might be partially removed when sulfated sugars with a sulfate-Na salt, such as **31** and **32**, were left in water for an extended period.

We also studied the coordination of a sodium ion to a sulfate group. As shown in Figure 3, there are two possible modes for fixing a sodium position. In Figure 3 (left), sodium binds tightly with one of oxygen atoms. The other interaction mode is shown in Figure 3 (right), where the sodium ion is located between two oxygen atoms and may interact with either one of two oxygens alternatively. We estimated which form is more likely by means of ab initio calculations. As shown in Figure 3 (right), sodium is more prone to be fixed between two oxygens rather than interacting with an oxygen (Figure 3, left).

These data indicate that a sodium ion can tightly bind to one of oxygen atoms, acting like a protecting group and stabilizing the sulfate anion during glycosylation reactions. Therefore the interaction of the sulfate group with anomeric position (Figure 2, C') seems to be unfavorable. Based on the above data, glycosylation reactions using sulfated glycosyl donors are concluded to proceed via acyloxonium intermediate (Figure 2, B') to generate β selective glycosyl product. Because of these two reasons, glycosylation reaction can be performed even in the presence of an unprotected sulfate group. Although we did not examined the NMR study of the oxocarbenium ion intermediate (Figure 2 A') that has been extensively studied by the Jiménez-Barbero group,[37] we presume that an acyloxonium ion intermediate may form immediately upon the activation of glycosyl donor with an ortho-hexynylbenzoate group. Considering the pKa values of unprotected sulfate groups (−9.0) and hydronium ions (−1.74) under acidic conditions,[26] the acceptor alcohol was expected to have

somewhat higher nucleophilic ability toward the acyloxonium ion intermediate compared to the unprotected sulfate group. Our experiment also showed that the relative nucleophilicity of the alcohol is higher than that of the sulfate under conditions closer to neutral pH in the presence of gold catalysis.

In conclusion, glycosylation reaction can be performed even in the presence of unprotected sulfate groups. Acyl protecting groups, such as acetyl group or Troc group at C2 position, can regulate the stereoselectivity at anomeric position during the glycosylation reaction via neighboring group participation. Investigations into the effect of counter cations revealed that a sodium cation strongly interact with a sulfate group in DCM. Due to this strong interaction, lithium and sodium cations act as a protecting group for free sulfate groups during the glycosylation reaction.

Based on these findings, we could synthesize sulfated tetrasaccharides via disaccharide-donor and -acceptor with unprotected sulfate groups. This strategy is efficient for the synthesis of di-, tri- and tetrasaccharides. For the synthesis of larger oligosaccharides such as hexa- or octa-saccharides with their multiple sulfation patterns, optimization is essential. Especially, the solubility of those oligosaccharides in the glycosylation solvent should be optimized. This strategy will be useful as an additional approach in combination with current strategies for the synthesis of homogeneous sulfated oligosaccharides.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: glycosylation · free sulfate group · glycosaminoglycan · chondroitin sulfate

[1] B. Chakrabarti, J. W. Park, E. S. Stevens, *Crit. Rev. Biochem. Mol. Biol.* **1980**, *8*, 225–313.
[2] K. Lena, J. Staffan, *Annu. Rev. Biochem.* **1984**, *53*, 847–869.
[3] M. Ly, F. E. Leach, T. N. Laremore, T. Toida, I. J. Amster, R. J. Linhardt, *Nat. Chem. Biol.* **2011**, *7*, 827–833.
[4] J. E. Silbert, G. Sugumaran, *IUBMB Life* **2002**, *54*, 177–186.
[5] T. Mikami, H. Kitagawa, *Biochim. Biophys. Acta Gen. Subj.* **2013**, *1830*, 4719–4733.
[6] M. Souhei, U. Toru, K. Hiroshi, H. N. Kazuo, D. Katsufumi, G.-A. Keiko, M. Shohei, S. Kazuyuki, N. Kazuya, *Nature* **2003**, *423*, 443–448.
[7] C. I. Gama, S. E. Tully, N. Sotogaku, P. M. Clark, M. Rawat, N. Vaidehi, W. A. Goddard, A. Nishi, L. C. Hsieh-Wilson, *Nat. Chem. Biol.* **2006**, *2*, 467–473.
[8] I. Björk, U. Lindahl, *Mol. Cell. Biochem.* **1982**, *48*, 161–182.
[9] M. Höök, I. Björk, J. Hopwood, U. Lindahl, *FEBS Lett.* **1976**, *66*, 90–93.
[10] P. Melgar-Lesmes, F. Garcia-Polite, P. Del-Rey-Puech, E. Rosas, J. L. Dreyfuss, E. Montell, J. Vergés, E. R. Edelman, M. Balcells, *Atherosclerosis* **2016**, *245*, 82–87.
[11] F. Ronca, L. Palmieri, P. Panicucci, G. Ronca, *Osteoarthr. Cartilage* **1998**, *6*, 14–21.
[12] F. Legendre, C. Baugé, R. Roche, A. S. Saurel, J. P. Pujol, *Osteoarthr. Cartilage* **2008**, *16*, 105–114.
[13] C. H. Xue, Y. Fang, H. Lin, L. Chen, Z. J. Li, D. Deng, C. X. Lu, *J. Appl. Phycol.* **2001**, *13*, 67–70.
[14] G. M. Campo, A. Avenoso, S. Campo, G. Nastasi, P. Traina, A. D'Ascola, C. A. Rugolo, A. Calatroni, *Br. J. Pharmacol.* **2008**, *155*, 945–956.
[15] S. B. Dulaney, X. Huang, *Adv. Carbohydr. Chem. Biochem.* **2012**, *67*, 95–136.
[16] M. Mende, C. Bednarek, M. Wawryszyn, P. Sauter, M. B. Biskup, U. Schepers, S. Bräse, *Chem. Rev.* **2016**, *116*, 8193–8255.
[17] a) S. Arungundram, K. Al-Mafraji, J. Asong, F. E. Leach III, J. Amster, A. Venot, J. E. T. Turnbull, G.-J. Boons, *J. Am. Chem. Soc.* **2009**, *131*, 17394–17405; b) P. Chopra, A. Joshi, J. Wu, W. Lu, T. Yadavalli, M. A. Wolfert, D. Shukla, J. Zaia, G. J. Boons, *Proc. Nat. Acad. Sci.* **2021**, *118*; c) L. Sun, P. Chopra, G. J. Boons, *J. Org. Chem.* **2020**, *85*, 16082–16098; d) R. Karlsson, P. Chopra, A. Joshi, Z. Yang, S. Y. Vakhrushev, T. M. Clausen, C. D. Painter, G. P. Szekeres, Y.-H. Chen, D. R. Sandoval, L. Hansen, J. D. Esko, K. Pagel, D. P. Dyer, J. E. Turnbull, H. Clausen, G.-J. Boons, R. L. Miller, *Sci. Adv.* **2021**, *7*, eabl6026.
[18] a) T. Y. Huang, D. Irene, M. M. Zulueta, T. J. Tai, S. H. Lain, C. P. Cheng, P. X. Tsai, S. Y. Lin, Z. G. Chen, C. C. Ku, C. D. Hsiao, C. L. Chyan, S. C. Hung, *Angew. Chem. Int. Ed.* **2017**, *56*, 4192–4196; b) Y. P. Hu, Y. Q. Zhong, Z. G. Chen, C. Y. Chen, Z. Shi, M. M. Zulueta, C. C. Ku, P. Y. Lee, C. C. Wang, S. C. Hung, *J. Am. Chem. Soc.* **2012**, *134*, 20722–20727; c) Y. P. Hu, S. Y. Lin, C. Y. Huang, M. M. Zulueta, J. Y. Liu, W. Chang, S. C. Hung, *Nat. Chem.* **2011**, *3*, 557–563; d) K. Sakamoto, T. Ozaki, Y. C. Ko, C. F. Tsai, Y. Gong, M. Morozumi, Y. Ishikawa, K. Uchimura, S. Nadanaka, H. Kitagawa, M. M. L. Zulueta, A. Bandaru, J. I. Tamura, S. C. Hung, K. Kadomatsu, *Nat. Chem. Biol.* **2019**, *15*, 699–709.
[19] a) J. Zhang, L. Liang, W. Yang, S. Ramadan, K. Baryal, C. X. Huo, J. J. Bernard, J. Liu, L. Hsieh-Wilson, F. Zhang, R. J. Linhardt, X. Huang, *Angew. Chem. Int. Ed.* **2022**, *61*, e202209730; b) Z. Wang, Y. Xu, B. Yang, G. Tiruchinapally, B. Sun, R. Liu, S. Dulaney, J. Liu, X. Huang, *Chem. Eur. J.* **2010**, *16*, 8365–8375; c) S. Ramadan, T. Li, W. Yang, J. Zhang, Z. Rashidijahanabad, Z. Tan, N. Parameswaran, X. Huang, *ACS Cent. Sci.* **2020**, *6*, 913–920.
[20] S. Eller, M. Collot, J. Yin, H. S. Hahm, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2013**, *52*, 5858–5861.
[21] A. Vibert, C. Lopin-Bon, J. C. Jacquinet, *Chem. Eur. J.* **2009**, *15*, 9561–9578.
[22] G. Tiruchinapally, Z. Yin, M. El-Dakdouki, Z. Wang, X. Huang, *Chem. Eur. J.* **2011**, *17*, 10106–10112.
[23] K. Matsushita, T. Nakata, J. Tamura *Carbohydr. Res.* **2015**, *406*, 76–85.
[24] K. Matsushita, Y. Sato, S. Funamoto, J. I. Tamura, *Carbohydr. Res.* **2014**, *396*, 14–24.

[25] J. I. Tamura, K. W. Neumann, S. Kurono, T. Ogawa, *Carbohydr. Res.* **1997**, *305*, 43–63.
[26] E. V. Amslyn, D. A. Dougherty, in *Modern Physical Organic Chemistry*, **2005**. Univ. Science Books.
[27] B. Yu, *Acc. Chem. Res.* **2018**, *51*, 507–516.
[28] C. Lopin, J. C. Jacquinet, *Angew. Chem. Int. Ed.* **2006**, *45*, 2574.
[29] Z. Li, J. C. Gildersleeve, *J. Am. Chem. Soc.* **2006**, *128*, 11612–11619.
[30] K. B. Wiberg, *Angew. Chem. Int. Ed.* **1986**, *25*, 312–322.
[31] E. J. Reardon, *J. Phys. Chem.* **1975**, *79*, 422–425.
[32] Y. Marcus, *Ion Properties*, **1997**. CRC press.
[33] C. E. Housecroft, H. D. Brooke Jenkins, *RSC Adv.* **2017**, *7*, 27881–27894.
[34] S. V, Campos, L. P. Miranda, M. Meldal, *J. Chem. Soc. Perkin Trans. 1* **2002**, 682–686.
[35] T. Yagami, K. Kitagawa, C. Aida, H. Fujiwara, S. Futaki, *J. Pept. Res.* **2000**, *56*, 239–249.
[36] J. R. Fromm, R. E. Hileman, E. E. O. Caldwell, J. M. Weiler, R. J. Linhardt, Arch, *Biochem. Biophys* **1995**, *323*, 279–287.
[37] A. Franconetti, A. Ardá, J. L. Asensio, Y. Blériot, S. Thibaudeau, J. Jiménez-Barbero, *Acc. Chem. Res.* **2021**, *54*, 2552–2564.

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Communication

Glycosylation

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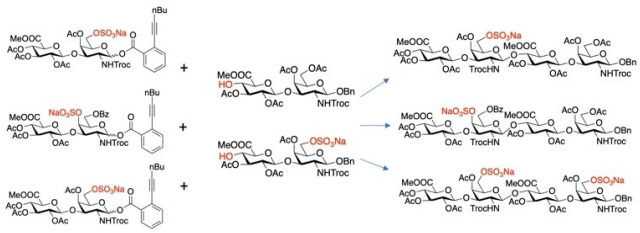
Harnessing Free Sulfate Groups in Glycosylation Reactions



The chemical nature of sodium sulfate groups in glycosylation reactions was studied. The findings enabled us to develop alternative synthesis routes for sulfated oligosaccharides. Experiments and calculations suggested that the sodium cation acts as a protecting group in glycosylation reactions.