Chinese Chemical Letters 36 (2025) 109972

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Stereoselective synthesis of α-3-deoxy-D-manno–oct-2-ulosonic acid (α-Kdo) derivatives using a C3-*p*-tolylthio-substituted Kdo fluoride donor

Ao Sun[a], Zipeng Li[a], Shuchun Li[a], Xiangbao Meng[a], Zhongtang Li[a], Zhongjun Li[a,b,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China
[b] Ningbo Institute of Marine Medicine, Peking University, Ningbo 315832, China

ARTICLE INFO

Article history:
Received 7 February 2024
Revised 22 April 2024
Accepted 7 May 2024
Available online 8 May 2024

Keywords:
Glycosylation
Kdo glycosides
Stereoselectivity
Synthetic method
Glycoderivatives

ABSTRACT

3-Deoxy-D-manno–oct-2-ulosonic acid (Kdo) is widely distributed in bacteria, and the synthesis of Kdo-containing oligosaccharides is important for the development of novel antibiotics and immunological agents. We have recently developed a strategy to achieve α-stereocontrolled glycosylation using a C3-*p*-tolylthio-substituted Kdo phosphite donor. The wide substrate scope and high reactivity of the donors enabled the efficient synthesis of a series of Kdo-containing glycosides with complete α-stereoselectivity and without the formation of 2,3-ene byproducts. In this study, we improved the method by replacing the leaving group diethyl phosphite with fluoride, which enhanced the stability of the donor and led to cleaner reaction. Furthermore, the substrate range was expanded by synthesizing a series of Kdo *O*/*C*/*S*/*N*-glycosides, which also opened up a new avenue for the synthesis of CMP-Kdo synthase inhibitors.

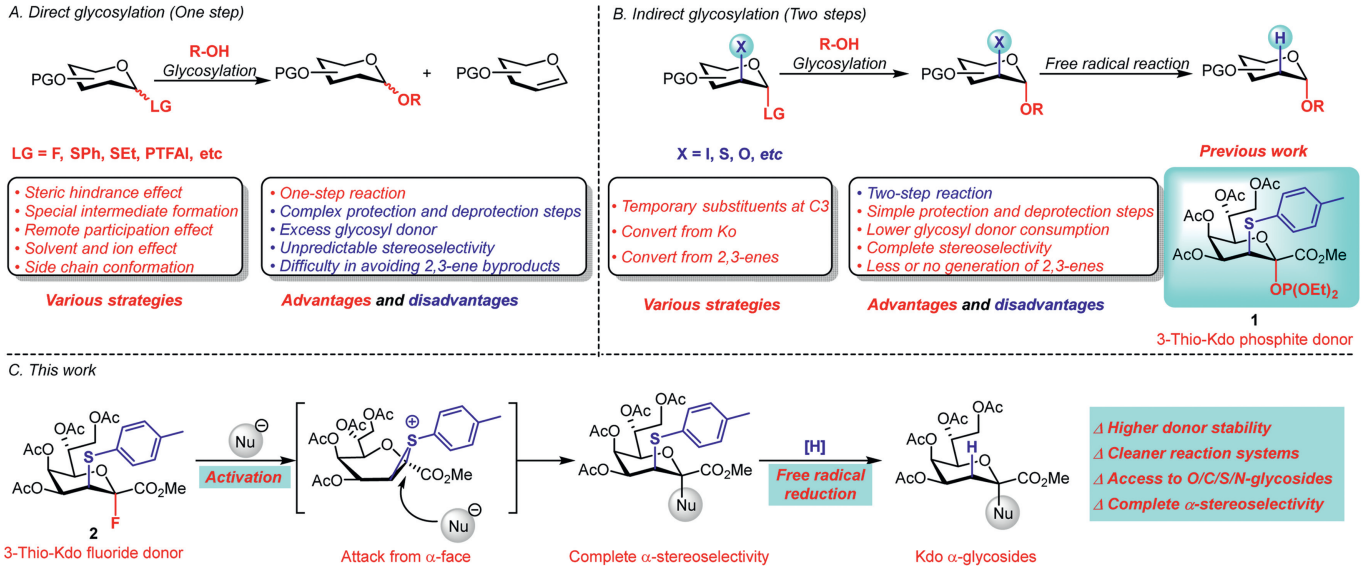
The overuse of antibiotic has led to the emergence of drug-resistant bacteria [1]. As a result, the development of new antimicrobial drugs and vaccines has attracted the increasing attention of scientists [2]. 3-Deoxy-D-manno–oct-2-ulosonic acid (Kdo) is an eight-carbon monosaccharide widely distributed in bacterial lipopolysaccharides (LPS) and capsule polysaccharides (CPS) [3,4]. In the biosynthesis of LPS, several sequential enzymatic reactions are necessary for the synthesis of Kdo from D-ribulose-5-phosphate and attaching it to lipid A [5]. In this biosynthetic pathway, CMP-Kdo plays a crucial role as a key intermediate [5]. Its derivatives have the potential to act as inhibitors of CMP-Kdo synthase (CKS), thereby obstructing the LPS biosynthesis pathway and ultimately disrupting the production of the bacterial outer membrane [6]. Thus, analogs of Kdo have the potential to act as CKS inhibitors and serve as novel antimicrobial agents.

As a deoxy sugar [7,8], Kdo glycosylation is one of the most challenging glycosylation reactions because of the low reactivity, the uncontrolled stereoselectivity and the formation of 2,3-ene byproducts [9-14]. Therefore, the highly efficient and stereoselective synthesis of Kdo glycosides remains an unmet need in glycosylation methodology, which limits the investigation of the biological activities of Kdo-containing oligosaccharides. Currently, Kdo glycosylation methods can be broadly divided into direct and indirect methods [15]. Using the direct methods, the target glycosidic bonds can be achieved in a single step by utilizing steric hinderance effect [16-31], special intermediate formation [32-34], remote participation effect [35], solvent and ion effect [36,37] and side chain conformation (Scheme 1A) [38]. The selection of protective groups in the direct methods is intricate, often resulting in the formation of additional 2,3-ene byproducts. Using the indirect methods, an auxiliary group (such as S [39], I [40-43], O [44-45]) is added at Kdo's C3 position to modulate the anomeric stereoselectivity (Scheme 1B). After glycosylation, the auxiliary group can be removed by additional steps. Compared with direct methods, indirect methods simplify protecting group operations and enhance reaction efficiency while preserving complete stereoselectivity and generating less 2,3-enes [15].

Our team has previously developed a strategy for Kdo α-glycosylation utilizing a C3-*p*-tolylthio-substituted Kdo phosphite donor **1** *via* the classical neighboring group participation effect (Scheme 1B) [15]. The sulfur-positive tricyclic intermediate restricted the acceptor to attacking from the α side of the sugar ring only. Consequently, single α-stereoselective Kdo glycosides were produced without the formation of 2,3-enes. However, the high reactivity of the modified phosphite donor **1**, which must be used immediately after preparation, is a drawback of this strategy. In this study, we investigated the reaction of the C3-*p*-tolylthio-

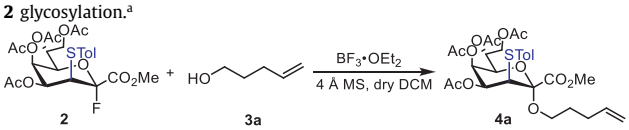
* Corresponding author.
E-mail address: zjli@bjmu.edu.cn (Z. Li).

https://doi.org/10.1016/j.cclet.2024.109972
1001-8417/



Scheme 1. Kdo glycosylation methods developed in previous studies and the present study.

Table 1
Effect of temperature, donor consumption and concentration on Kdo fluoride donor **2** glycosylation.[a]



Entry	T (°C)	Donor consumption (equiv.)	Donor concentration (mol/L)	Yield (%)[b]	Ratio of α/β[c]
1	−20	1.5	0.10	Trace	α only
2	0 to r.t.	1.5	0.10	42	α only
3	0 to r.t.	1.5	0.05	97	α only
4	0 to r.t.	1.5	0.03	75	α only
5	0 to r.t.	1.2	0.05	96	α only

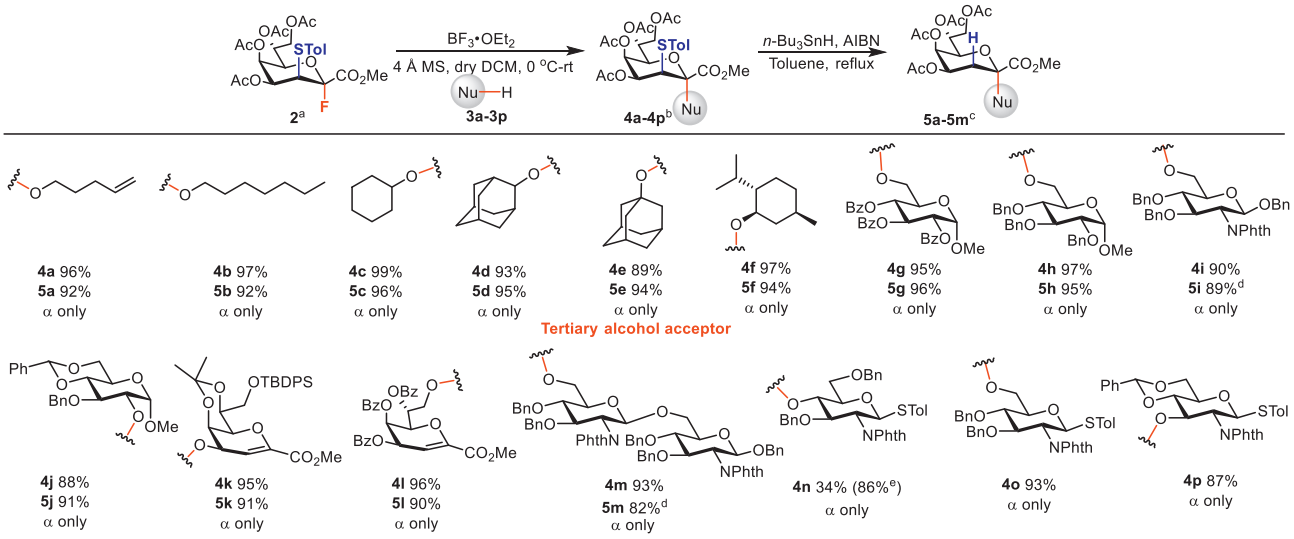
[a] Glycosylations were conducted with Kdo fluoride donor **2** and acceptor **3a** (1.0 equiv.) under the activation of $BF_3{\cdot}OEt_2$ (2.0 equiv.) in anhydrous CH_2Cl_2 (Concentrations were calculated based on donor) for overnight.
[b] Isolated yield.
[c] The α/β ratio was determined by analysis of ^{1}H NMR.

substituted Kdo fluoride donor **2** and demonstrated its increased stability (Scheme 1C). The complete α-stereoselectivity and broad substrate scope proved the superiority of introducing a thioether at the C3 position of Kdo donors. In addition, we synthesized a series of Kdo *O*/*C*/*S*/*N*-glycosides *via* fluoride donor **2**, which have the potential to act as important precursors of candidate molecules for CKS inhibition.

According to the previous work [15], the C3-*p*-tolylthio-substituted Kdo hydroxy sugar **S1** was synthesized with 2,3-ene by a two-step reaction of addition and hydrolysis. Subsequently, the α-Kdo fluoride donor **2** was prepared by fluorination of **S1** in a satisfactory yield of 89% (Scheme S1 in Supporting information). Notably, donor **2** maintained stability for more than one year at room temperature, which was considerably more stable than donor **1**.

Under the classical conditions for the activation of glycosyl fluoride [41-43], the glycosylation of donor **2** (1.2 equiv.) was conducted with 4-penten-1-ol **3a** (1.0 equiv.) in the presence of $BF_3{\cdot}OEt_2$ (2.0 equiv.), 4 Å molecular sieves (MS) at 0 °C to room temperature in anhydrous CH_2Cl_2 (Table 1, entry 2, 0.10 mol/L, calculated based on donor). Surprisingly, the coupling reaction between **2** and **3a** provided the Kdo glycoside **4a** in a moderate 42% yield as the sole α-isomer without the formation of 2,3-ene byproduct. Similar to the phosphite donor **1**, the axial orientation of the thio group at C3 of the fluoride donor **2** prevented the elimination reactions, while efficiently enhanced α-stereoselectivity *via* neighboring group participation effects [15]. The stereoconfigurations of Kdo glycosides was confirmed *via* the non-decoupling ^{13}C NMR ($^3J_{C1,\,H3ax} < 1.0$ Hz) [27]. Considering that the adsorption of hydrogen fluoride formed during the reaction by molecular sieves can drive this reaction [41-43], we adjusted the ratio of molecular sieve and donor by modifying the concentration of the reaction solution (MS: 100 mg/mL). The experiment demonstrated that the reaction afforded glycoside **4a** in 96% yield with outstanding α-stereoselectivity when the donor concentration reached 0.05 mol/L (Table 1, entry 5).

Subsequently, we investigated the *O*-glycosylation scope of donor **2** (1.2 equiv.) with various acceptors (**3a**-**3p**) under the optimal conditions (Scheme 2, donor: 1.2 equiv., acceptor: 1.0 equiv., $BF_3{\cdot}OEt_2$: 2.0 equiv., CH_2Cl_2: 0.05 mol/L calculated based on donor, 4 Å MS: 100 mg/mL calculated based on solvent, 0 °C→r.t.). To our satisfaction, we were able to synthesize the fully α-stereoselective Kdo glycosides **4a**-**4f** in excellent yields of 89%–99% without the formation of 2,3-enes through glycosylation of donor **2** with simple alcohols, including primary alcohols (**3a**: 4-penten-1-ol, **3b**: *n*-heptanol), secondary alcohols (**3c**: cyclohexanol, **3d**: 2-adamantanol, **3f**: L-menthol), and tertiary alcohol (**3e**: 1-adamantanol). For the reacions with glycosyl acceptors, the glycosylation process between donor **2** and acceptor **3g**-**3m** produced sole α-isomer Kdo oligosaccharides **4g**-**4m** with yields ranging from 88% to 97%. It was noteworthy that the fluoride donor **2** reacted with Kdo glycal acceptors **3k** and **3l**, resulting in the successful formation of di-Kdo saccharides **4k** (95%) and **4l** (96%) in excellent yields through α-(2→4)- and α-(2→8) linkages. In particular, the Kdo-α-(2→6)-GlcN-β-(1→6)-GlcN trisaccharide **4m** is the essential component of several bacterial LPS structures [19,22]. Under the optimized free radical reduction conditions developed by our team [15], the thioether groups of the glycosides **4a**-**4m** were successfully removed, leading to the formation of **5a**-**5m** in high yields of 82%–96%. In addition, the glucosamine thioglycosides acceptors **3n**-**3p** coupled with donor **2** and afforded the desired Kdo-containing disaccharides **4n** (34%), **4o** (93%) and **4p** (87%) without activation of the thioglycosides. Among them, the reaction between inactive 4-OH acceptor **3n** and **2** was incomplete with reac-



Scheme 2. Substrate scope of the Kdo *O*-glycosylation. [a] Glycosylations were conducted with Kdo fluoride donor **2** (1.2 equiv.) and acceptor **3a**-**3p** (1.0 equiv.) under the activation of $BF_3{\cdot}OEt_2$ (2.0 equiv.) in anhydrous CH_2Cl_2 (0.05 mol/L, calculated based on donor) for overnight. [b] Isolated yield, and the α/β ratio was determined by analysis of ^1H NMR. [c] Free radical reduction reactions were conducted with glycosyl thioether (**4a**-**4h**; **4j**-**4l**, 1.0 equiv.), initiator (AIBN, 4.0 equiv.) and reducing agent (*n*-Bu_3SnH, 6.0 equiv.) under oxygen-free environment in anhydrous toluene at 110 °C. [d] Free radical reduction reactions were conducted with glycosyl thioether (**4i** and **4m**, 1.0 equiv.), initiator (AIBN, 4.0 equiv.) and reducing agent (Ph_3SnH, 6.0 equiv.) under oxygen-free environment in anhydrous toluene at 110 °C. [e] Yields based on the consumed acceptors. TBDPS = *tert*–butyldiphenylsilyl, NPhth = phthalimido, AIBN = 2,2′-azoisobutyronitrile.

tants remained, and only 34% of the disaccharide **4n** was obtained (86% yield based on the consumed acceptors). Overall, the glycosylation efficiency of fluoride donor **2** with acceptors that are moderately or highly active is comparable to that of phosphite donor **1** [15]. For inactive acceptors, reaction yields of donor **2** can be improved through recycling reactants. In another experiment, acceptor **3h** was coupled with donor **1** and **2** separately, and the reaction solutions were filtered to remove molecular sieves, concentrated without column chromatography separation, and analyzed by NMR (Fig. 1). The ^1H NMR spectra of the crude products reveals that the reaction system of fluoride glycosylation was very clean (Fig. 1b).

CMP-Kdo is a key intermediate in LPS biosynthesis [4]. Therefore, Kdo derivatives have the potential to be developed as CKS inhibitors, which can block bacterial cell wall synthesis. Additionally, *C*/*S*/*N*-glycosides possess distinct properties compared to *O*-glycosides, and exhibit resistance to enzymatic degradation while preserving the structural integrity of the glycosides [46]. No-

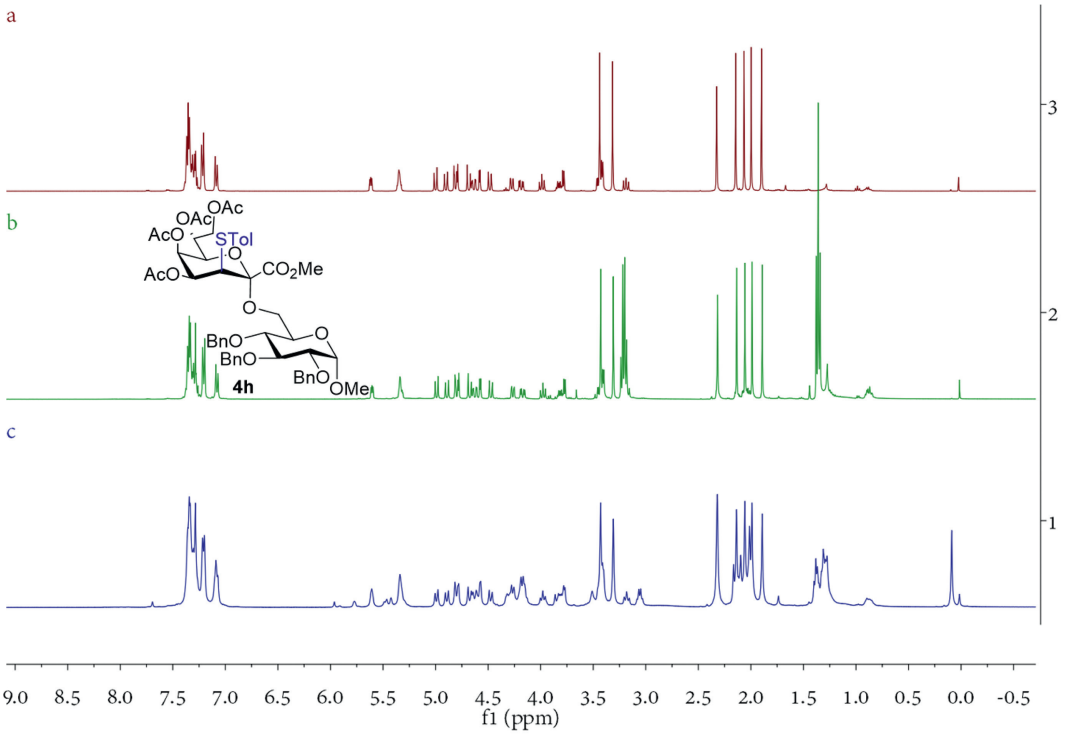
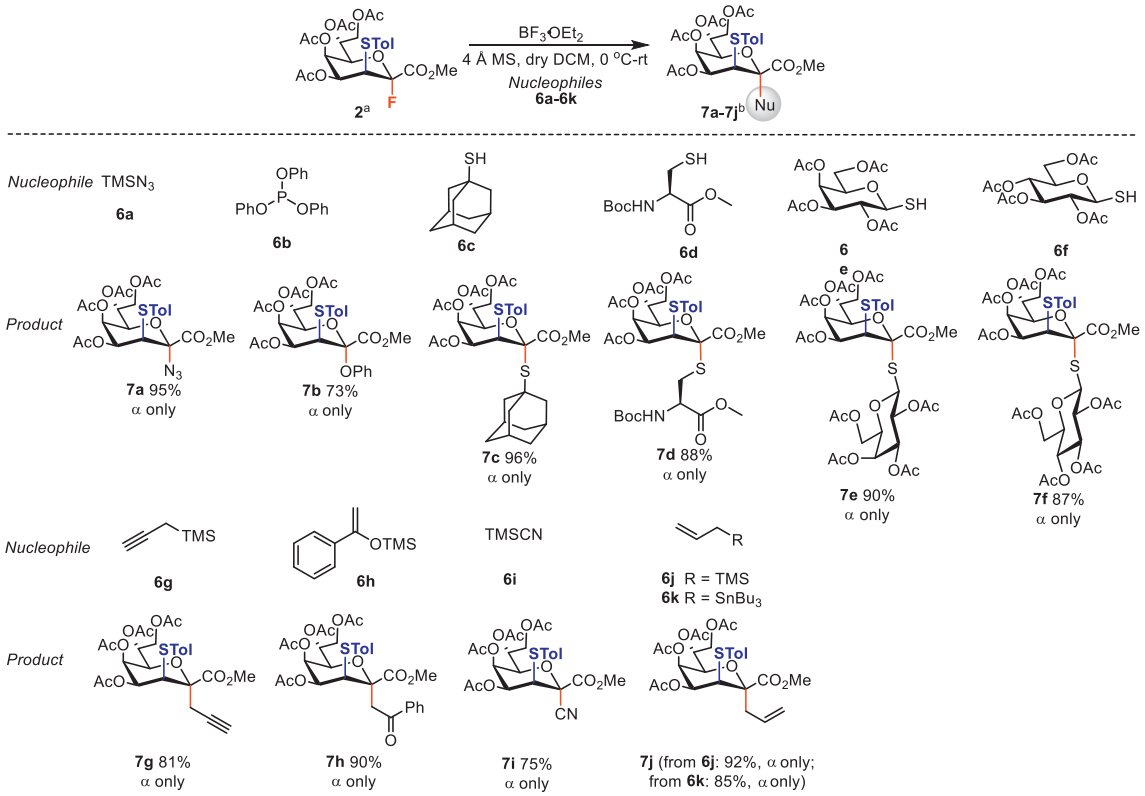
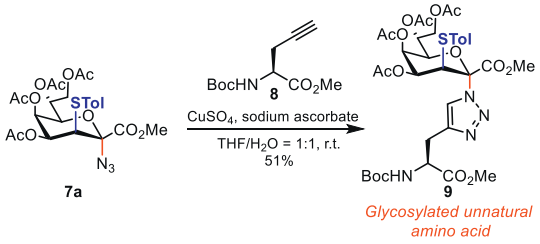


Fig. 1. ^1H NMR spectrum of the reaction crude of disaccharide **4h**. (a) ^1H NMR spectrum of disaccharide **4h** purified by column chromatography. (b) ^1H NMR spectrum of the reaction crude of fluoride donor **2** and acceptor **3h**. (c) ^1H NMR spectrum of the reaction crude of phosphite donor **1** and acceptor **3h**.



Scheme 3. Substrate scope of the Kdo *S*/*C*/*N*-glycosylation. (a) Glycosylations were conducted with Kdo fluoride donor **2** (1.0 equiv.) and nuclephiles **6a**-**6k** under the activation of $BF_3{\cdot}OEt_2$ (2.0 equiv.) in anhydrous CH_2Cl_2 (0.05 mol/L, calculated based on donor) for overnight. (b) Isolated yield, and the α/β ratio was determined by analysis of ^1H NMR. TMS = trimethylsilyl.



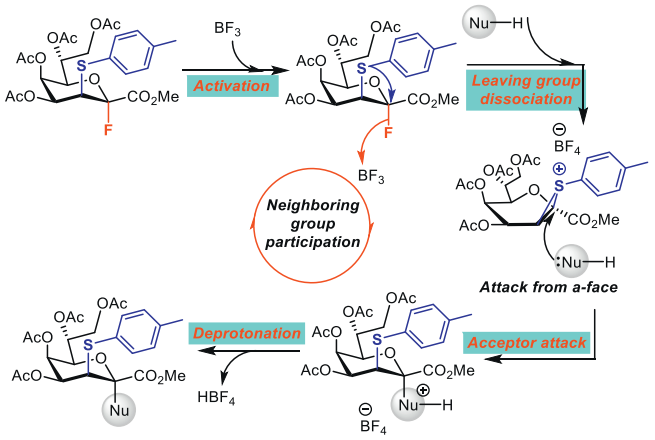
Scheme 4. Synthesis of glycosylated unnatural amino acid **9.** Boc = *t*-butyloxy carbonyl.

tably, some of the CKS inhibitors being investigated are Kdo carboglycoside or thioglycoside derivatives [6]. Thus, we attempted to broaden the structural type of Kdo derivatives by coupling Kdo fluoride donor **2** with common nucleophilic reagents **6a**-**6k** (Scheme 3).

Under standard glycosylation conditions, donor **2** was coupled with $TMSN_3$, resulting in 95% yield of Kdo azide **7a**. Subsequently, protected L-propargylglycine **8** was reacted with **7a** under click reaction conditions to produce glycosylated unnatural amino acid **9** in 51% yield (Scheme 4). Compound **9** has the potential to be used in glycopeptide synthesis and serves as an intermediate for Kdo derivatization and bioactivity research [47-49]. Interestingly, donor **2** reacted with **6b** to yield phenolic glycoside **7b**, which is different from the phosphoglycoside products obtained from the reaction of fluoride with **6b** as reported [50]. It is possible that the reaction underwent the following process. Under acidic conditions, triphenyl phosphite **6b** is easily hydrolyzed to form phosphoric acid and phenol. Molecular sieves can absorb the resulting phosphoric acid and the fluoride **2** is subsequently activated in the presence of boron trifluoride ether and preferentially undergoes glycosylation process with the phenol to produce **7b** rather than Arbuzov reaction. In addition, donor **2** reacted with several sulfur nucleophilic reagents (**6c**-**6f**) to produce the corresponding thioglycoside derivatives **7c**-**7f** in yields ranging from 87% to 96%. The carboglycoside products **7g**-**7j** were obtained in high yields (75%–92%) by using carbo nucleophilic reagents **6g**-**6k** and donor **2**. Moreover, the alkyne group in compound **7g** could undergo further derivatization *via* click reaction. It is worth noting that all of the aforementioned Kdo derivatives exhibit complete α-stereoselectivity. The construction of compound libraries containing Kdo derivatives and the evaluation of their bioactivity are currently in progress.

Scheme 5 shows the proposed reaction mechanism of glycosylation with C3-*p*-tolylthio-substituted Kdo fluoride donor **2** promoted by $BF_3{\cdot}OEt_2$. Boron trifluoride activates the fluoride donor **2**, breaking the carbon-fluorine bond and forming a ternary cyclic episulfonium intermediate. Due to the neighboring group participation effect, the episulfonium blocks the β-side of the Kdo sugar ring, allowing the nucleophilic reagent to attack from the α-side only. After proton dissociation, complete α-stereoselective of Kdo glycosides are formed.

In summary, we synthesized C3-*p*-tolylthio-substituted fluoride donor **2** to increase the stability of the Kdo phosphite donor **1** and investigated the reactions of donor **2** in Kdo α-glycosylation. The results of this study along with previous reports support that the Kdo glycosylation reaction can be treated as two categories: (1) For acceptors with medium to high reactivity, the glycosylation reaction can be performed more conveniently by using the more stable fluoride donor **2**, resulting in cleaner reactions. (2) For low-reactivity acceptors, more reactive phosphite donor **1** can be used for efficient glycosylation reactions. These two methods demon-



Scheme 5. Proposed mechanism of $BF_3{\cdot}OEt_2$ promoted glycosylation with C3-*p*-tolylthio-substituted Kdo fluoride donor **2**.

strated the superiority of introducing a thioether at the C3 position of the Kdo donors. Furthermore, the substrate range was further expanded by utilizing donor **2** to react with various *C*/*S*/*N*-nucleophilic reagents, resulting in high yields and complete α-stereoselectivity of Kdo derivatives. These Kdo derivatives represent new structural types for designing CKS inhibitors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ao Sun: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zipeng Li:** Validation, Investigation. **Shuchun Li:** Writing – review & editing, Project administration. **Xiangbao Meng:** Writing – review & editing. **Zhongtang Li:** Writing – review & editing. **Zhongjun Li:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Acknowledgments

This project was supported by the National Key R&D Program of China (Nos. 2022YFF1203005, 2022YFC2303700), and supported by the National Natural Science Foundation of China (Nos. 81930097, 82151223). We thank Dr. De-Cai Xiong, Peking University, for helpful discussion.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2024.109972.

References

[1] N. Mobarki, B. Almerabi, A. Hattan, Int. J. Med. Dev. Ctries. 40 (2019) 561–564.
[2] H.S. Gold, R.C. Moellering Jr, N. Engl. J. Med. 335 (1996) 1445–1453.
[3] F.M. Unger, Adv. Carbohydr. Chem. Biochem. 38 (1981) 323–388.
[4] L. Cipolla, L. Gabrielli, D. Bini, L. Russo, N. Shaikh, Nat. Prod. Rep. 27 (2010) 1618–1629.
[5] J. Lodowska, D. Wolny, L. Węglarz, Can. J. Microbiol. 59 (2013) 645–655.
[6] L. Cipolla, A. Polissi, C. Airoldi, et al., Curr. Med. Chem. 18 (2011) 830–852.
[7] C.L. Bennett, M.C. Galan, Chem. Rev. 118 (2018) 7931–7985.
[8] S. Meng, W. Zhong, W. Yao, Z. Li, Org. Lett. 22 (2020) 2981–2986.
[9] J. Hansson, S. Oscarson, Curr. Org. Chem. 4 (2000) 535–546.
[10] S. Oscarson, Carbohydr. Chem. 45 (2012) 40–60.
[11] T.K. Pradhan, K.K.T. Mong, Isr. J. Chem. 55 (2015) 285–296.
[12] P. Kosma, Tetrahedron Lett. 57 (2016) 2133–2142.
[13] P. Kosma, Carbohydr. Chem. 42 (2017) 116–164.
[14] T.K. Pradhan, Eur. J. Org. Chem. 26 (2023) e202300146.
[15] A. Sun, L.Z.Y. Wang, et al., Angew. Chem. Int. Ed. 63 (2024) e202313985.
[16] H. Yoshizaki, N. Fukuda, K. Sato, et al., Angew. Chem. Int. Ed. 40 (2001) 1475–1480.
[17] Y. Fujimoto, M. Iwata, N. Imakita, et al., Tetrahedron Lett. 48 (2007) 6577–6581.
[18] K. Mannerstedt, K. Ekelçf, S. Oscarson, Carbohydr. Res. 342 (2007) 631–637.
[19] Y. Zhang, J. Gaekwad, M.A. Wolfert, G.J. Boons, Chem. Eur. J. 14 (2008) 558–569.
[20] T. Ichiyanagi, M. Fukunaga, R. Tagashira, et al., Tetrahedron 67 (2011) 5964–5971.
[21] A. Shimoyama, Y. Fujimoto, K. Fukase, Synlett 2011 (2011) 2359–2362.
[22] A. Shimoyama, A. Saeki, N. Tanimura, et al., Chem. Eur. J. 17 (2011) 14464–14474.
[23] T.J. Boltje, W. Zhong, J. Park, et al., J. Am. Chem. Soc. 134 (2012) 14255–14262.
[24] R. Yi, A. Ogaki, M. Fukunaga, H. Nakajima, T. Ichiyanagi, Tetrahedron 70 (2014) 3675–3682.
[25] E. Mancuso, C. Romanò, N. Trattnig, et al., Chem. Eur. J. 27 (2021) 7099–7102.
[26] T. Ichiyanagi, H. Narimoto, N. Ohtani, Tetrahedron 96 (2021) 132397.
[27] J.S. Huang, W. Huang, X. Meng, et al., Angew. Chem. Int. Ed. 54 (2015) 10894–10898.
[28] X.Y. Zhou, P. Yang, S. Luo, J.S. Yang, Chem. Asian J. 14 (2019) 454–461.
[29] X.Y. Zhou, L.X. Li, Z. Zhang, et al., Angew. Chem. Int. Ed. 61 (2022) e202204420.
[30] S. Hamajima, N. Komura, H.N. Tanaka, et al., Org. Lett. 24 (2022) 8672–8676.
[31] S. Hamajima, N. Komura, H.N. Tanaka, et al., Molecules 28 (2023) 102.
[32] X. Mi, Q. Lou, W. Fan, L. Zhuang, Y. Yang, Carbohydr. Res. 448 (2017) 161–165.
[33] Q. Lou, Q. Hua, L. Zhang, Y. Yang, Org. Lett. 22 (2020) 981–985.
[34] J. Zhang, X. Gao, S. Liu, et al., Org. Lett. 25 (2023) 4150–4155.
[35] W. Huang, Y.Y. Zhou, X.L. Pan, et al., J. Am. Chem. Soc. 140 (2018) 3574–3582.
[36] R.R. Schmidt, A. Esswein, Angew. Chem. Int. Ed. 27 (1988) 1178–1180.
[37] A. Esswein, H. Rembold, R.R. Schmidt, Carbohydr. Res. 200 (1990) 287–305.
[38] P. Ngoje, D. Crich, J. Am. Chem. Soc. 142 (2020) 7760–7764.
[39] K. Ikeda, S. Akamatsu, K. Achiwa, Chem. Pharm. Bull. 38 (1990) 279–281.
[40] P. Kosma, H. Sekljic, G.J. Balint, J. Carbohydr. Chem. 15 (1996) 701–714.
[41] B. Pokorny, P. Kosma, Chem. Eur. J. 21 (2015) 305–313.
[42] B. Pokorny, P. Kosma, ChemOpen 4 (2015) 722–728.
[43] B. Pokorny, P. Kosma, Org. Lett. 17 (2015) 110–113.
[44] M. Reiner, R.R. Schmidt, Tetrahedron Asymmetry 11 (2000) 319–335.
[45] K.K.T. Mong, T.K. Pradhan, C.H. Chiu, et al., Org. Chem. Front. 7 (2020) 2179–2186.
[46] Y. Yang, B. Yu, Chem. Rev. 117 (2017) 12281–12356.
[47] G.Z. Tian, X.L. Wang, J. Hu, et al., Chin. Chem. Lett. 26 (2015) 922–930.
[48] D. Liu, S. Liu, F. Hu, Z. Li, Z. Li, Chin. Chem. Lett. 35 (2024) 108762.
[49] K. Wang, T. Zhang, M. Liu, et al., Chin. Chem. Lett. 34 (2023) 108065.
[50] X. Zhou, H. Ding, P. Chen, et al., Angew. Chem. Int. Ed. 59 (2020) 4138–4144.