Sialylation

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# 1-Picolinyl-5-azido Thiosialosides: Versatile Donors for the Stereoselective Construction of Sialyl Linkages

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***Abstract:*** *With the picolinyl (Pic) group as a C-1 located directing group and $N_3$ as versatile precursor for C5-$NH_2$, a novel 1-Pic-5-$N_3$ thiosialyl donor was designed and synthesized, based on which a new sialylation protocol was established. In comparison to conventional sialylation methods, the new protocol exhibited obvious advantages, including excellent α-stereoselectivity in the absence of a solvent effect, broad substrate scope encompassing the challenging sialyl 8- and 9-hydroxy groups of sialic acid acceptors, flexibility in sialoside derivative synthesis, high temperature tolerance and easy scalability. In particular, the applicability to the synthesis of complex and bioactive N-glycan antennae when combined with the MPEP glycosylation protocol via the "latent-active" strategy has been shown. Mechanistically, the excellent α-stereoselectivity of the novel sialylation protocol could be attributed to the dramatic electron-withdrawing effect of the protonated Pic groups, which was supported by control reactions and DFT calculations.*

## Introduction

Sialic acids are a family of above 50 naturally occurring 2-keto-3-deoxy-nononic acids, found mainly at the terminal position of glycolipids and glycoproteins. Owing to their prominent positions in the glycan chain, sialic acids are involved in a wide spectrum of biological processes, including cell–cell interaction, cell differentiation, pathogen–host recognition, oncogenesis, and metastasis.[1] As the most widespread member of the sialic acid family, *N*-acetyl-neuraminic acid (Neu5Ac) residues are in nature essentially either linked to galactose and galactosamine via α(2,3) and α(2,6) sialic linkages, or they are homopolymerized through α(2,8) and α(2,9), as well as alternating α(2,8)/α(2,9) glycosidic linkages. Although extensively explored, the chemical synthesis of sialosides in a highly efficient manner is still a notable challenge in carbohydrate chemistry.[2] The synthetic difficulty originates from the unique chemical structure of sialic acids. The presence of an electron-withdrawing carboxylic group, the tertiary nature of the anomeric center, and the absence of a C-3 hydroxy group result not only in low chemical sialylation yield and unsatisfactory stereoselectivity but also in the competing elimination side-reaction of the used sialyl donors. To address these issues, different strategies have been devised,[3] amongst which are the use of solvent effect,[4] the application of novel leaving groups,[5] the introduction of new protecting groups (PGs) including participating groups,[6] the development of new promotion systems,[7] the enhancement of acceptor reactivity,[8] as well as the tuning of C-5 $NH_2$ protection pattern strategies.[9] Although equipped with these available strategies, the efficient access of sialosides by chemical synthesis is still by no means a trivial problem as all strategies suffer from seriously limited generality and thus are highly substrate-sensitive.[10] As a result, for each sialyl linkage construction systematic optimizations are required in order to achieve synthetically useful efficiency.[10] Hence, the current chemical syntheses of sialosides can hardly satisfy the modern glycobiology and glycopharmacology demands, calling on novel and efficient sialylation protocols.

The C-5 azido group, first introduced in 1989 by Auge et al., exhibits limited stereocontrol capability in sialoside synthesis even under the assistance of the nitrile effect.[11] However, the azido group is deemed as an ideal precursor to the C-5 amino group of sialyl donor, as the convenient postsialylation modifications can facilitate the diversity-oriented synthesis of sialoside derivatives. Although the C-5 azido group alone cannot efficiently control the stereoselective sialylation, its strong electron-withdrawing effect ($F_{N3} = 0.48$)[12] can be exploited to strengthen the participating effect of the incorporated directing groups via destabilizing the anomeric cation species. Thus, a combination of C5-$N_3$ and a participating group holds the promise to provide satisfactory sialylation selectivity. For the participating group, the picolinyl (Pic) group is considered as the best of choice, as it has been successfully used to stereoselectively forge glycosidic linkages either by neighboring group participation[13] or by intermolecular H-bond formation with acceptors.[14] In addition, the picoloyl (Pico) group, also a potent H-bond acceptor,[14] has been selected as a PG for OHs of sialyl donor, and an intriguing triflic acid effect has been disclosed with 4-

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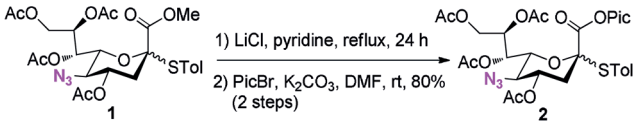
OH picoloylated thiosialoside as donor.[6f,g] The preferred position for directing group attachment is C-1, not only because the directing group introduction does not bring about chirality problems but also because the ester-type linkage can facilitate both the installation and the cleavage of the directing group. Based on the above analysis, a novel sialyl donor featuring the C-5 azido group and the C-1 Pic group was designed. Through systematic investigations, it was demonstrated to be a powerful donor for sialic linkages construction, with excellent α-stereoselectivity, broad substrate scope, high flexibility, high temperature tolerance, and easy scalability. Furthermore, in conjunction with the MPEP (*o*-(*p*-methoxyphenylethynyl)phenyl) glycosylation protocol,[15] highly efficient routes to produce tetrasaccharide antennae, widely spread in biologically relevant glycolipids and glycoproteins, have also been established. Via systematic investigations as well as DFT calculations, the working mechanism of donor **2** was delineated and the beneficial effect of electron-withdrawing PGs at C-1 on the stereoselectivity of sialyl donors is disclosed for the first time.

## Results and Discussion

The introduction of the Pic group to **1**[11] was realized as shown in Scheme 1. Thus, Krapcho demethylation[16] with lithium chloride and pyridine under refluxing conditions afforded the carboxylic acid intermediate, which was successively alkylated with PicBr under basic conditions to afford **2** as a pair of α/β anomers (α/β = 1:3, 80% yield for 2 steps).

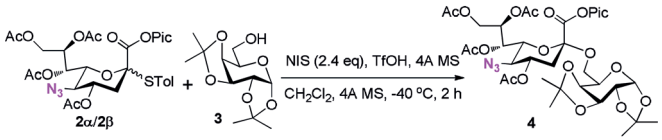
With **2** in hand, its feasibility in sialyl linkage construction was evaluated with the coupling between **2** and **3** as a model




***Scheme 1.*** The synthesis of 1-Pic-5-azido donor **2**.

reaction (Table 1). With the prevailing **2β** as donor, it was revealed that the amounts of triflic acid have a profound effect on the sialylation results. When the triflic acid was used

***Table 1:*** Sialylation conditions optimization with **2α/β** as donors.



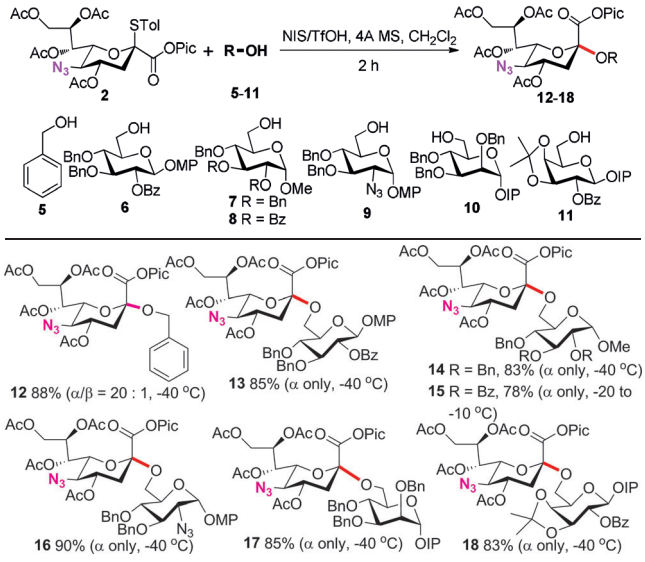

| Entry | Donor | TfOH [eq] | α/β[a] | **4** [%][b] |
|---|---|---|---|---|
| 1 | **2β** | 0.2 | 25/1 | 9 |
| 2 | **2β** | 0.5 | 25/1 | 34 |
| 3 | **2β** | 1.0 | 25/1 | 90 |
| 4 | **2α** | 1.0 | 20/1 | 83 |

[a] Ratios were determined by the combination of isolation and $^1$H NMR. [b] Isolated yield.

in a catalytic amount (0.2 eq), only 9% yield of the product **4** was isolated (entry 1). With the applied amounts of TfOH increased from 0.5 to 1.0 equivalents, the sialylation yields rose from 34% to 90% (entries 2 and 3). Surprisingly, no matter how many equivalents of TfOH were applied, the excellent sialylation stereoselectivity was maintained (α/β = 25:1). To examine the effect of the anomeric chirality of the thiosialoside donor on the sialylation outcome, donor **2α** was also put to react with **3** under conditions of entry 3. Although a slight decrease was observed, the yield and stereoselectivity remained excellent (entry 4, 83%, α/β = 20:1). Of note, the reaction of donor **2α** only required 1 h to go to completion, demonstrating that the reactivity of **2α** is higher than that of the β-counterpart.

With the optimal sialylation conditions established, the substrate scope was then checked (Table 2). With primary hydroxy group as acceptors, including simple benzylic alcohol **5** and glucosides **6–7**, the couplings with **2** proceeded smoothly under the optimized conditions to afford the desired sialosides **12–14** with excellent yields and stereoselectivity

***Table 2:*** Sialylation of primary acceptors with donor **2**.




(above 83% yield and α-isomer only). Influenced by the two electron-withdrawing benzoyl (Bz) groups, the reactivity of acceptor **8** diminished.[17] As a result, elevated reaction temperature (−20 to −10 °C) and extended reaction time (4 h) were required to allow the reaction to reach completion. In contrast to the 5-*N*-4-*O*-oxazolidinone thiosialoside donor developed by Crich and co-workers,[18] which has been shown highly temperature sensitive, donor **2** was proved much less temperature sensitive and delivered **15** with good yield and stereoselectivity even at elevated temperature (78% yield and complete α-selectivity). Furthermore, 2-azido-2-deoxy glucoside **9**, mannoside **10**, as well as galactoside **11**, decorated with different PGs, all proved to be viable substrates for the new sialylation protocol, affording the desired sialosides **16–18** efficiently (above 83% yields). It should be pointed out that the deactivating $N_3$ functionality in

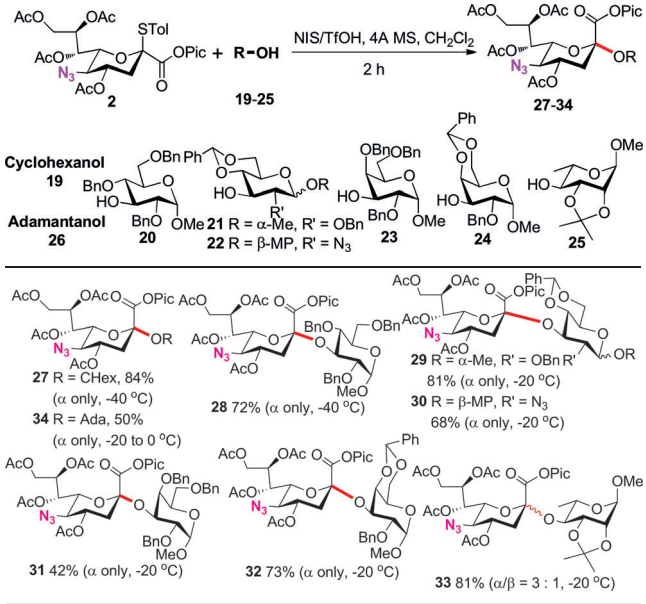
**9** did not undermine the sialylation efficiency, and **16** was isolated in 90% yield. The sialosides **13** and **16** with *p*-methoxyphenyl (MP) groups at the anomeric positions of the reducing-end sugars can be used as building blocks for complex sialosides synthesis after MP group cleavage and leaving group installation; while sialosides **17** and **18** with *ortho*-iodophenyl groups (IPs) at the anomeric positions of the reducing-end sugars can be directly transformed to glycosyl MPEP donors by Sonogashira reaction. With primary alcohols as acceptors, conventional sialylation protocols tend to give low stereoselectivity even in nitrile solvents due to the high reactivity.[10a] In sharp contrast, with **2** as donor, all tested primary acceptors afforded the α-sialosides exclusively in the absence of a solvent effect.

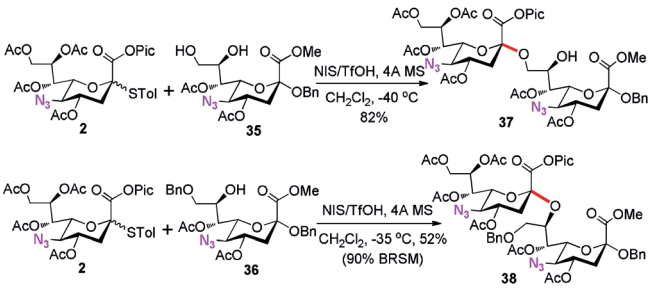
The sialylation of secondary and tertiary acceptors can be more challenging due to the serious steric repulsion and to alleviate the steric hindrance, partially protected acceptors are generally applied.[19] However, the formation of sialylation regioisomers as well as the lactonization byproducts represents the serious drawbacks of using partially protected acceptors.[20] Fully protected secondary acceptors have been employed in sialylation reactions, but these glycosylations suffer from either low chemical yields or unsatisfactory stereoselectivity.[21] To overcome the problems inherent to bulky acceptors and extend the substrate scope of donor **2**, a series of secondary acceptors with only one unprotected OH were selected and subjected to sialylations with **2** in $CH_2Cl_2$ (Table 3). Simple secondary acceptor cyclohexanol **19** was tried first, and under the standard conditions sialoside **27** was obtained as a single anomer in 84% yield. The use of glucoside **20** led to sialoside **28** with comparable efficiency. Glucosyl acceptors **21** and **22** exhibited somewhat diminished reactivity, thus higher reaction temperature (−20 °C) and prolonged reaction time (4 h) were required to get good yields. Again, even at elevated temperature, no erosion of stereoselectivity was observed. Galactosyl 3-OH acceptors are notorious for their low stereoselectivity in sialylation.[22] However, to our pleasure, when **23** was sialylated with **2** at −20 °C only α-**31** was isolated, albeit in moderate yield (42%). The low coupling yield could be remedied by changing the PGs at the C4 and C6 hydroxyls of the acceptor from benzyl groups to a benzylidene acetal, and the glycosylation between **2** and **24** afforded **32** with good yield and α-stereoselectivity (72%, α-isomer only). As a representative of the L-series acceptors, the rhamnoside **25**, which was shown to react in a β-selective fashion in Crich's sialylation protocol,[7a,18] was also examined. Although the stereoselectivity of the reaction dropped dramatically, the desired α-**33** was still isolated as the predominating product (81%, α/β = 3:1). Finally, the feasibility of the tertiary alcohol, adamantanol **26**, as a competent acceptor for the new sialylation protocol was examined, and 50% yield of **34** was isolated at −20 to 0 °C with **2α** as donor. Again, even at the temperature as high as 0 °C, no detrimental effect on the sialylation stereoselectivity was detected, and **34** was isolated as the α-isomer exclusively.

Neu5Ac α(2,9) and α(2,8) linkages, especially the α(2,8) linkages, are among the most challenging sialyl linkages to construct in sialoside synthesis because of the extremely low reactivity of the C8- and C9-OHs of Neu5Ac acceptors.[23] On the other hand, these linkages are widely spread in biologically relevant glycoproteins and gangliosides.[24] Pleasantly, the novel sialylation protocol could be employed for the construction of these challenging linkages. Under the standard sialylation conditions, donor **2** was efficiently coupled with the primary sialyl acceptor **35**, delivering **37** in 82% yield (Scheme 2). Also the weakly nucleophilic C8-OH of **36** was sialylated in good yield to deliver disialoside **38** (52% isolated yield and 90% yield based on recovered starting material (BRSM). Importantly, in both cases, only the α disilosides were obtained from the clean reaction systems.

To function as a practical participating group, the Pic group should not only be installed efficiently but also be cleaved selectively and conveniently. Thus, the selective removal of the Pic group was subsequently investigated (Table 4). Inspired by the selective removal of the Pico group in the presence of Bz groups,[25] conditions using $Cu(OAc)_2$ in $CH_2Cl_2$/MeOH were attempted first. However, with **4** as the model substrate, no reaction was detected. Modification of the conditions by using methanol as the solvent and increasing the reaction temperature to 45–50 °C led to the simultaneous

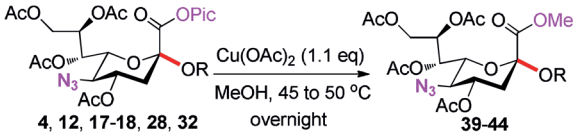
***Table 3:*** Sialylation of secondary and tertiary acceptors with donor **2**.




***Scheme 2.*** The construction of Neu5Ac α(2,9) and α(2,8) linkages with donor **2**.

***Table 4:*** Selective cleavage of the Pic group under the effect of $Cu(OAc)_2$.

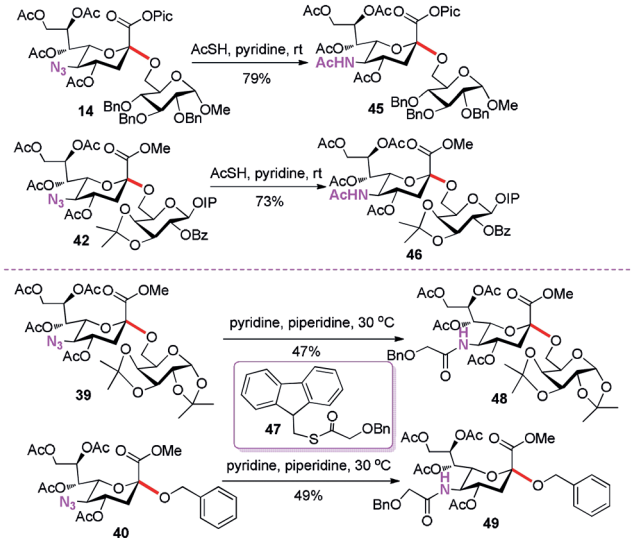



| Entry | Substrate | Product | Yield [%][a] |
|---|---|---|---|
| 1 | **4** | **39** | 82 |
| 2 | **12** | **40** | 88 |
| 3 | **17** | **41** | 93 |
| 4 | **18** | **42** | 90 |
| 5 | **28** | **43** | 65 |
| 6 | **32** | **44** | 75 |

[a] Isolated yield.

Pic group cleavage and methyl ester installation, and the desired methyl ester **39** was isolated in good yield (82%, entry 1) with the base-sensitive acetyl groups intact. The reaction conditions conceded a quite broad substrate scope, and the selected Pic esters including **12**, **17**–**18**, **28**, and **32** were all successfully converted to the corresponding methyl esters **40**–**44** without touching the co-existing functionalities (entries 2–6).

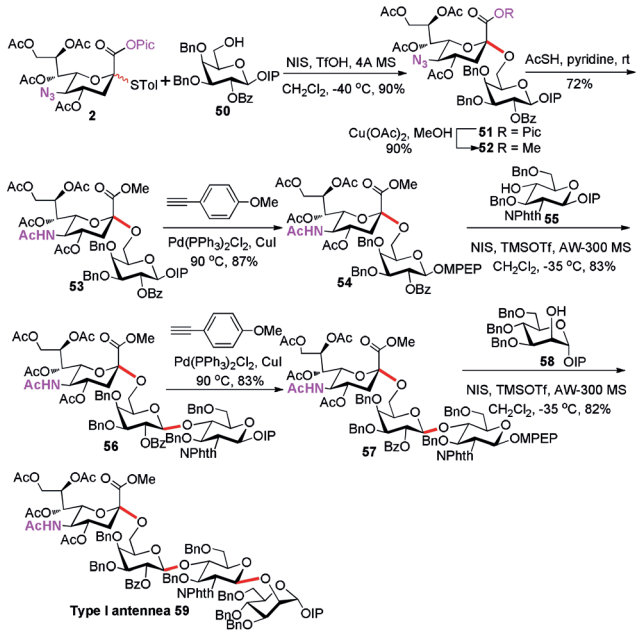
In the donor design stage, the azido group in **2** was envisioned to be a versatile precursor for the amino group, thus the transformations of the azido group to the corresponding acetamido as well as glycolamido groups were investigated (Scheme 3). Under the effect of thioacetic acid,[26] the reduction and simultaneous acetylation of **14** was achieved, affording **45** efficiently (79%). Similarly, methyl ester **42** was converted to **46** with comparable efficiency (73%). The one-step reduction and glycolylation of the 5-$N_3$-sialosides proved to be problematic as the hydroxythioacetic acid derivatives are not readily available. Thus, a stepwise glycolylation procedure, in which the reduction and glycolylation reactions were conducted in separate steps, was explored first. However, severe acetyl group migration was observed in the reduction step.[27] After all attempts to suppress the undesired acetyl group migration uniformly meeting with failure, we finally resorted to reagent **47**, which was introduced by Crich et al. for the simultaneous reduction and glycolylation of isothiocyanato group.[28] Slight modification of the Crich reduction/glycolylation protocol by switching the reaction medium to pyridine led to the successful conversion of **40** to **49** with a respectable 49% yield. For more complex substrate **39**, similar reaction efficiency was obtained (47% yield for **48**). It is worth mentioning that the reaction systems are so clean that the BRSM yields of both reactions could reach as high as above 90%.




***Scheme 3.*** Divergent derivatization of the azido group of sialosides.

Antennae are widely spread in *N*-glycans (asparagine-linked glycans) of glycolipids and glycoproteins. Structurally, the antennae can be divided into type I and type II categories, with the type I antenna containing an terminal α-2,6 linked sialyl residue while the type II antenna possessing an α-2,3 linked sialic acid terminus. Given the crucial roles of *N*-glycans in maintaining the proper functions of glycoproteins as well as in mediating the virus–host recognition processes, chemical synthesis of *N*-glycans with defined structures has been an extensively studied field in carbohydrate chemistry.[29] To secure the high overall synthetic efficiency, a strategy is preferred in which antennae of the same type are simultaneously installed in a single glycosylation step. Suffering from severe steric hindrance of the complex acceptors, a large excess of antennae donors is generally required to guarantee the satisfactory installation efficiency.[30] Hence, to facilitate the *N*-glycan synthesis as well as the following structure–activity relationship studies, the establishment of efficient routes for antennae assembly is urgently needed. The reliable sialylation method based on donor **2** as well as the suitability of the MPEP glycosylation protocol to the "latent-active" strategy of glycoside synthesis provides an excellent opportunity for developing efficient antennae syntheses.

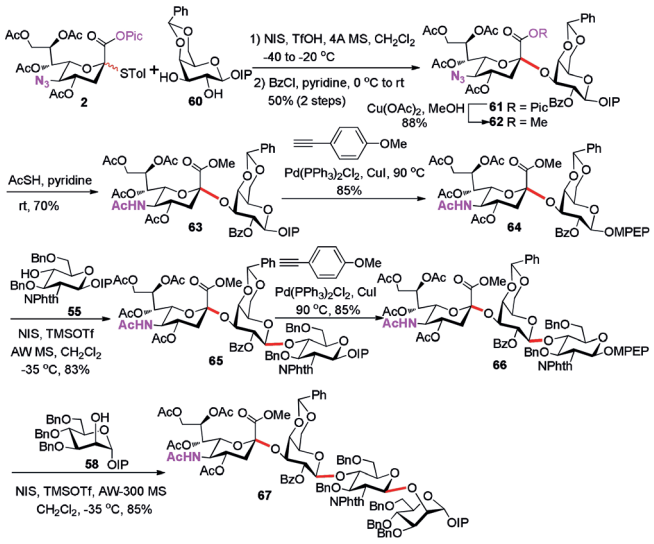
The assembly of type I antenna **59** commenced with the coupling of **2** and primary acceptor **50**, equipped with an IP group at the anomeric position (Scheme 4). Under the effect of NIS/TfOH, the sialylation proceeded without any event to stereoselectively deliver the disaccharide sialoside **51** in 90% yield. Simultaneous Pic-removal and methyl esterification was achieved under the agency of $Cu(OAc)_2$, affording **52** smoothly (90%). The transformation of the azido group in **52** into the corresponding acetamido group was effected by AcSH/pyridine to generate the latent disaccharide donor **53** (72%), which was then converted to the disaccharide MPEP donor **54** through a Sonogashira reaction (87% yield). Under the standard MPEP activation conditions, **54** was efficiently activated to react with **55**, delivering the trisaccharide latent donor **56** in 83% yield. Thus obtained latent donor was then converted to the trisaccharide MPEP donor **57** through a Sonogashira coupling (83%). In the ensuing glycan-chain extension, the trisaccharide MPEP donor **57** was used to glycosylate the axial hydroxy group of **58**. To our pleasure, the latent tetrasaccharide donor **59**, which is ready for type I antenna installation on synthetic *N*-glycans after Sonogashira activation, was obtained in a good 82% yield. Thus, through the combination of the picolinyl ester sialylation method and the MPEP glycosylation protocol, the type I antenna building



***Scheme 4.*** Synthesis of type-I antenna via the latent-active strategy.

block **59** can be obtained from readily accessible monosaccharide building blocks via a seven-step longest linear sequence in 29% overall yield. The synthesis of the corresponding tetrasaccharide fluoride donor has been achieved in 11% overall yield through the same number of steps using a sialyl phosphate donor.[5f]

Encouraged by the successful synthesis of type I antenna **59**, the synthesis of the more challenging type II antenna **67** was then conducted (Scheme 5). For the construction of the pivotal sialic linkage, diol acceptor **60** was selected. The glycosylation between **2** and **60** proceeded uneventfully to exclusively afford **61**, after benzoylation of the free OH (50% for 2 steps). No regioisomeric or lactonization side products were detected. Sialoside **61** was then subjected to the Pic

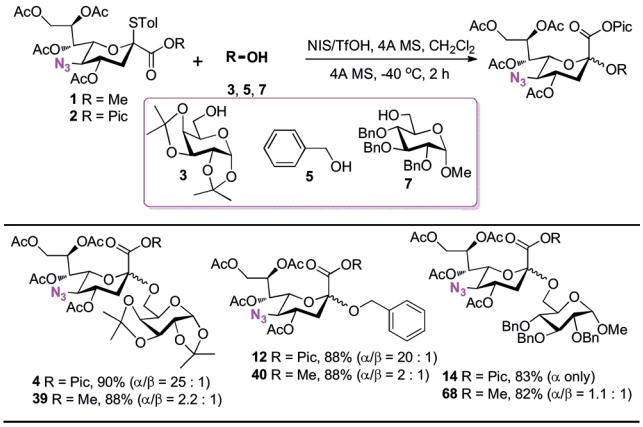


***Scheme 5.*** Synthesis of type-II antenna via the latent-active strategy.

group cleavage and methyl esterification conditions to afford **62** (88%), which was then exposed to the thioacetic acid-mediated reduction/acetylation conditions to provide the latent disaccharide **63** (70%). Activation of **63** via a Sonogashira reaction was followed by sugar chain elongation with acceptor **55** to generate the latent trisaccharide donor **65** (70%, 2 steps). Finally, incorporation of the last sugar residue entailed the activation of **65** to **66** and a subsequent coupling with the mannosyl acceptor **58**. The desired tetrasaccharide **67** was generated in 85% yield and can be used for the incorporation of type II antenna after activation. Overall, with readily accessible monosaccharide building blocks as the starting materials, the type II tetrasaccharide antenna **67** was obtained via an eight-step longest linear sequence in 16% overall yield. The synthesis of a comparable NeuAc-Gal-GlcNAc trisaccharide thioglycoside donor has previously been reported and required 12 successive steps proceeding with an 13% overall yield.[29d]

With the sialylation protocol established and its practical application in the synthesis of *N*-glycan antennae demonstrated, the underlying mechanism responsible for the α-stereoselectivity was investigated. To evaluate the effect of the Pic group on the stereoselectivity of the sialylation reactions, direct comparisons between donors **1** and **2** were made with **3**, **5**, and **7** as acceptors (Table 5). While the glycosylation of donor **2** and primary alcohol acceptor **3** proceeded stereoselectively, the application of **1** in the

***Table 5:*** Evaluation of the stereodirecting effect of the Pic group in sialylation.



corresponding sialylation led to a dramatic drop in stereoselectivity (25:1 vs. 2.2:1). A similar erosion in stereoselectivity was also observed in glycosylations of benzylalcohol **5** with **1** and **2** (2:1 vs. 20:1). When **7** was used as an acceptor, disaccharide **68** was obtained in a non-stereoselectivity manner when combined with donor **1**, while sialoside **14** was isolated as a single anomer when donor **2** was used. These results clearly indicate that the Pic group mounted at C-1 plays a decisive role in the high α-stereoselectivity of donor **2**.

Three possible mechanisms, through which the Pic group exerts its chirality-control effect, are conceivable: the Pic-participation mechanism (route a), the protonated Pic-stabilized sialyl triflate mechanism (route b), and the Pic-

participation initiated protonated Pic-stabilized sialyl triflate mechanism (route c, Figure 1). In the first mechanism, the Pic group directly attacks the anomeric carbon under the assistance of C-5 $N_3$ to form the 6-membered intermediate

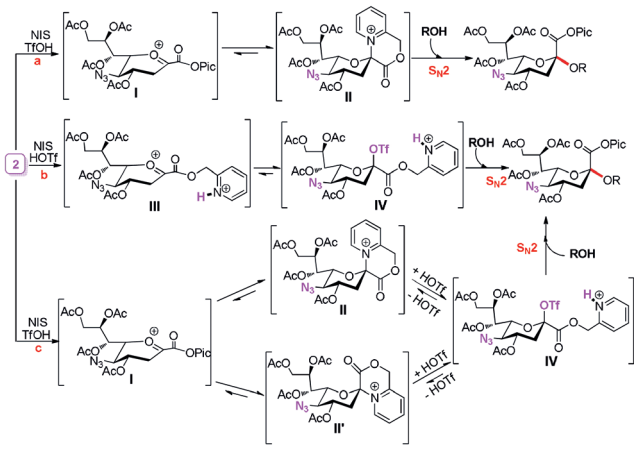
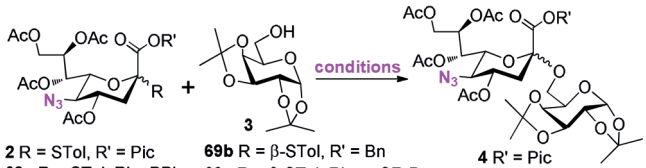


***Figure 1.*** Plausible reaction mechanisms responsible for the excellent stereoselectivity of donor **2**.

**II** via **I**, through which an ensuing $S_N2$ (or $S_N2$-type) substitution by acceptors affords the desired α-sialosides. In the second mechanism, the protonation of the Pic group is followed by thioglycoside activation to afford **III**, which can be β-selectively trapped by the triflate anion to form intermediate **IV**. The formation of intermediate **IV** is promoted by the combined electron-withdrawing effects of the protonated Pic (PicH) group and C5-$N_3$ functionality, which dramatically destabilizes intermediate **III**, while the stereoselectivity of **IV** is dictated by the anomeric effect. The sialyl triflate intermediate **IV** then undergoes $S_N2$ (or $S_N2$-type) substitution with acceptors to deliver α-sialosides. In the third mechanism, the transient intermediate **I** ramifies to intermediates **II** and **II′** by the direct attack of the Pic group to the anomeric carbon. Under the effect of stoichiometric amounts of TfOH intermediates **II** and **II′** converge to **IV**, which then undergoes $S_N2$ substitution to provide α-sialosides.

To probe the proposed reaction mechanisms, systematic control reactions were conducted with **3** as acceptor (Table 6). Donor **69a** containing a *p*-picolinyl (PPic) group at C-1 was coupled with **3** under the standard conditions affording **70a** in a 7:1 α/β stereoselectivity (entry 1); meanwhile sialyl *o*-alkynylbenzoate donors[31] **2′** and **2″** with Pic and PPic groups at C-1, respectively, afforded moderate but almost the same stereoselectivity (entries 2 and 3). These results clearly indicate that route a can be ruled out as a prominent mechanism in **2** sialylation. Otherwise, nonstereoselectivity for **69a** and **2″** with non-participation C1 PPic-ester and excellent α-selectivity for **2′** with participation C1 Pic-ester should be secured. Thus, our attention was then turned to routes b and c. Donors **69b–e** carrying C-1 benzyl esters with different substituents on the benzyl phenyl ring were synthesized[32] and coupled with **3** under the standard conditions. Interestingly, with the increase of the electron-withdrawing

***Table 6:*** Control reactions designed to determine the reaction mechanism of donor **2**.




**2** R = STol, R' = Pic
**69a** R = STol, R' = PPic
**2'** R = β-OABz, R' = Pic
**2''** R = β-OABz, R' = PPic

**69b** R = β-STol, R' = Bn
**69c** R = β-STol, R' = *p*-$CF_3$Bn
**69d** R = β-STol, R' = *p*-$NO_2$Bn
**69e** R = β-STol, R' = *o*-$NO_2$Bn

**4** R' = Pic
**70a** R' = PPic
**70b** R' = Bn
**70c** R' = *p*-$CF_3$Bn
**70d** R' = *p*-$NO_2$Bn
**70e** R' = *o*-$NO_2$Bn

| Entry | Donor | Conditions | Results[a,b] |
|---|---|---|---|
| 1 | **69a** | standard conditions | **70a**, 90% (α/β = 7:1) |
| 2 | **2′** | $Ph_3PAuNTf_2$ (0.2 eq), 4A MS, DCM rt | **4**, 91% (α/β = 2.5:1) |
| 3 | **2″** | $Ph_3PAuNTf_2$ (0.2 eq), 4A MS, DCM rt | **70a**, 80% (α/β = 2:1) |
| 4 | **69b** | standard conditions | **70b**, 85% (α/β = 1.2:1) |
| 5 | **69c** | standard conditions | **70c**, 83% (α/β = 1.8:1) |
| 6 | **69d** | standard conditions | **70d**, 84% (α/β = 2.4:1) |
| 7 | **69e** | standard conditions | **70e**, 83% (α/β = 3:1) |
| 8 | **2β** | standard conditions except for the reversed addition sequence of NIS/TfOH | NR[c] |
| 9 | **2β** | standard conditions except for the reversed addition sequence of NIS/TfOH then HOTf (0.2 eq) | **4**, 83% (α/β > 15:1) |
| 10 | **2α** | standard conditions except for the reversed addition sequence of NIS/HOTf | **4**, 86% (α/β > 15:1) |
| 11 | **2β** | standard conditions except for 1.2 eq HOTf at −60 °C | **4**, 10% (α/β > 15:1) |
| 12 | **2β** | HOTf (1.0 eq) then NIS (2.4 eq) and HOTf (0.2 eq) at −60 °C | **4**, 10% (α/β > 15:1) |
| 13 | **2β** | MeOTf (1.0 eq) then NIS (2.4 eq) and HOTf (1.0 eq) at −40 °C | NR[c] |
| 14 | **2α** | MeOTf (1.0 eq) then NIS (2.4 eq) and HOTf (0.2 eq) at −40 °C | **4**, 60% (α/β > 15:1) |
| 15 | **2′** | TfOH (1.0 eq) then $Ph_3PAuNTf_2$ (0.2 eq), 4A MS, DCM, rt | **4**, 93% (α/β = 7:1) |
| 16 | **2′** | TfOH (1.0 eq) then $Ph_3PAuNTf_2$ (0.2 eq), 4A MS, DCM, −40 °C | **4**, 87% (α/β > 15:1) |
| 17 | **2β** | IBr (2.0 eq), AgOTf (1.5 eq), 4A MS, DCM, −40 °C | **4**, 88% (α/β = 5:1) |

[a] Isolated yield. [b] Determined by $^1H$ NMR. [c] NR = No reaction.

capability of the substituents[12] at the phenyl ring, the α-stereoselectivity of sialylations rose gradually (**70b**–**e**, entries 4–7), verifying the importance of the electronic property of C-1 PGs for the stereoselective sialylation. As only moderate stereoselectivity was achieved with 2-nitrobenzyl group as the C1 PG, which has a comparable electron-withdrawing capability as the Pic group, the electronic property of the free Pic group cannot be invoked to explain the α-selectivity of **2** under the standard conditions. Instead, the protonated Pic group with a dramatically enhanced electron-withdrawing effect should be responsible for the high α-stereoselectivity.

The effect of the protonated Pic on the sialylation of **2** was subsequently evaluated. Reversing the addition sequence of NIS/TfOH, by adding TfOH prior to NIS, halted the sialylation of **2β**, indicating a rapid protonation of **2β** at low temperature (entry 8). Surprisingly, the subsequent addition of catalytic amounts of TfOH (0.2 eq) to the interrupted reaction retriggered the glycosylation, eventually affording **4** with an efficiency almost equal to the standard procedure (entry 9). Differently, for the more reactive **2α** no additional TfOH was required to promote the sialylation to reach completion under the reversed reagent addition conditions (entry 10). These results indicate that Pic group protonation leads to deactivation of the donors due to the strong electron-withdrawing effect of the protonated Pic group. The only difference between **2α** and **2β** is the deactivation extent of the protonated Pic group, which is more profound for **2β** than that for **2α**. This was further substantiated by the fact that a lower reaction temperature (−60 °C) largely invalidated the increase in the amounts of TfOH (1.2 eq) in the sialylations with **2β**, regardless whether the standard or the reversed reagent addition procedure was adopted, leading to poor conversion (entries 11 and 12). Further evidence was provided by the reaction of **2β** and **3** under the combined effect of MeOTf (1.0 eq), NIS (2.4 eq), and HOTf (1.0 eq), which did not lead to any product formation (entry 13). In contrast, methylation of **2α** with MeOTf (1.0 eq) followed by treatment with NIS and HOTf (0.2 eq) led to stereoselective sialylation, albeit with decreased yield (60 %, entry 14). Since the methylation of the Pic group is highly analogous to the pronation process, the results of entries 13 and 14 clearly show that donor **2α** can be activated via route b and the protonation of the Pic group can indeed guarantee the satisfactory α-stereoselectivity. However, this route is not viable for **2β**. Even for the reactive **2α**, its activation through route b at −40 °C is not favored as verified by the decreased sialylation yield.

Taking the privileged activation of the free instead of the protonated form of donor **2** and the beneficial effect of the protonated Pic group on the stereocontrol of sialylation into account, route c, featuring initial formation of **II** and **II′** and subsequent transformation to the more stable **IV** under the effect of stoichiometric amounts of TfOH, is preferred. The formation of **II** and **II′** could potentially facilitate the activation of donor **2** by the participation of the Pic nitrogen atom; while their convergent transformation to **IV** could improve the α-stereoselectivity of sialylation. This mechanistic proposal was supported by donor **2′**, which afforded a 7/1 mixture of α/β-sialosides at room temperature while α-sialoside exclusively at −40 °C under the combined effect of catalytic amounts of $Au^I$ complex and 1.0 equivalent of TfOH (entries 15 vs. 16). The inferior α-stereoselectivity of **2′** at room temperature compared with that obtained at −40 °C may lie in the competing sialylation of intermediates **II** and **II′**. In the absence of TfOH, species **II** and **II′** can serve as the principle intermediates for products formation, hence only moderate stereoselectivity was recorded for **2′** under the catalysis of $Au^I$ complex (entry 2). Route c accommodates the results of all control reactions, including the sialylation reaction of **2β** promoted by IBr/AgOTf[33] (entry 17) and the sialylation of **69a** (entry 1). In the former case the diminished stereoselectivity could be attributed to the inefficient protonation resulting from in situ generated TfOH, while the lowered but still good stereoselectivity for donor **69a** could be credited to the presence of the protonated pyridyl group with attenuated electron-withdrawing effect. More accurately, the sialyl triflate **IV** may exist as a closely related ion pair, thus nullifying all attempts to detect it by low temperature NMR.

To further support the mechanism route c, we investigated several possible reactive intermediates using DFT calculations (Figure 2).[32] The results of these computational studies (conducted on somewhat simplified structures because of computation costs) reveal that the intermediate **IV-β′** is significantly more stable than intermediates **II-β′** and **II-α′** by more than 6 kcal mol$^{-1}$, verifying that the conversion of **II**/**II′** to **IV** is thermodynamically favored. Furthermore, triflate **IV-β′** is also more stable than its α-counterpart **IV-α′** by 5.0 kcal mol$^{-1}$, strongly supporting the covalent β-triflate **IV** or the closely related contact ion pair as the pivotal intermediate in the stereoselective sialylation with **2**. Interestingly, opposite to the notion that the intermediate with a β-oriented participating group at the anomeric carbon should be thermodynamically more favored over the corresponding α-counterpart owing to the anomeric effect in the sialyl scaffold,[6] the **II-α′** proved to be more stable than the **II-β′** by 1.0 kal mol$^{-1}$.

While it has been well documented that the PGs with high electron-withdrawing effect on C5-$NH_2$ can improve the stereoselectivity of sialyl donors,[9] to the best of our knowledge this is the first time that a similar but more profound PG effect at C-1 of sialyl donor is disclosed, and this finding will open a new avenue to access highly efficient sialylation reactions.

Finally we aimed to show the robustness of the current sialylation protocol by performing the reaction at room temperature. Although the above described results have already indicated that the here reported sialylation protocol is less temperature sensitive than existing methods, we were pleased to find that the sialylation between donor **2** and **3** at room temperature led to the desired disaccharide **4** in high yield and with excellent selectivity. Furthermore, the scalability of the present sialylation protocol was also examined showing that **4** can be generated in gram-scale from **2** and **3** with even higher stereoselectivity and yield than those performed on small scale.

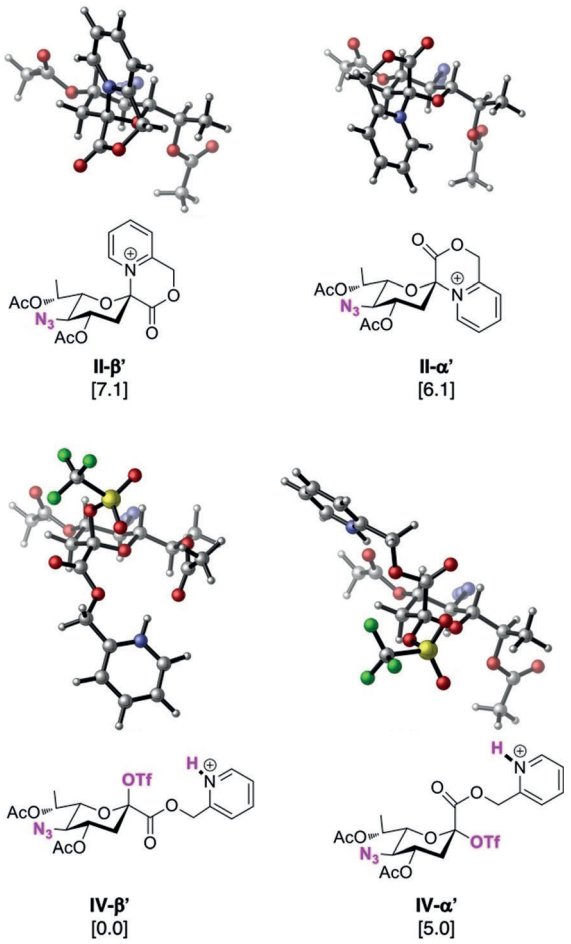
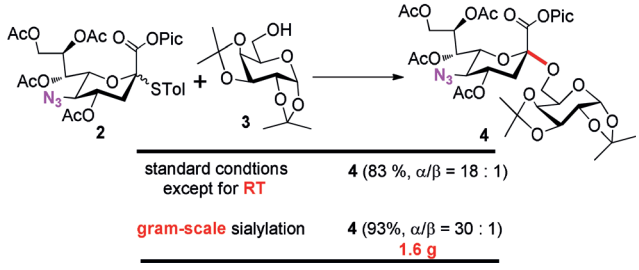



***Figure 2.*** Computed geometries of simplified relevant reactive intermediates for the glycosylation reaction with donor **2**. Entries are calculated at SMD ($CH_2Cl_2$)-M06-2X/6–311 + G(d,p). The corresponding solvated Gibbs free energy in kcal mol$^{-1}$ at $T$ = 253.15 K is denoted in brackets. In the Figure the HOTf is omitted for **II-α'** and **II-β'** for clarity reasons.




***Scheme 6.*** Room temperature and gram-scale sialylation with **2**.

## Conclusion

In summary, the combination of the electron-withdrawing C-5 azide and C-1 picolinyl ester led to the design and synthesis of the 1-Pic-5-$N_3$ sialyl donor, based on which a novel sialylation protocol was established. The novel sialylation method offers broad substrate scope, excellent α-stereoselectivity without resorting to a solvent effect, flexibility in the synthesis of sialoside derivatives with different substituents on the C-5 amino group, high temperature tolerance and easy scalability. It also can be applied in the efficient synthesis of *N*-glycan antennae in combination with the MPEP glycoylation protocol using a "latent-active" strategy. The flexibility of the sialosides synthesis approach is derived from the versatile C5-$N_3$ moiety that can be efficiently transformed into acetamido and glycolamido groups via simultaneous reduction and acylation reactions. The outstanding α-stereoselectivity was shown to originate from the strong electronic effect of the protonated picolinyl ester as revealed in the mechanistic investigations and DFT calculations. The effect of a strongly electron-withdrawing PG at C-1 on the α-stereoselectivity of sialyl donor has been disclosed for the first time, which should find broad applications in highly efficient synthesis of bioactive sialosides.

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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** directing groups · glycan antennae · glycosylation · sialoside · stereoselective sialylation

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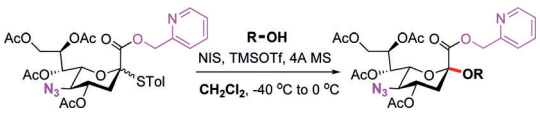
# Research Articles

## Sialylation

J. Chen, T. Hansen, Q.-J. Zhang, D.-Y. Liu, Y. Sun, H. Yan, J. D. C. Codée, R. R. Schmidt,* J.-S. Sun* ____ ▮▮▮▮–▮▮▮▮

1-Picolinyl-5-azido Thiosialosides: Versatile Donors for the Stereoselective Construction of Sialyl Linkages




**Sugar building blocks**: With 1-Picolinyl-5-azido thiosialoside as donor, a robust sialylation protocol, which enjoys broad substrate scope, good to excellent chemical yield, excellent α-stereoselectivity, flexible synthesis of sialosides, applicable in complex sialoside synthesis, as well as high temperature tolerance and easy scalability, was established.