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Facile and robust methods for the regioselective acylation of N-acetylneuraminic acid

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Abstract

The stereoselective synthesis of sialic acid glycoconjugates is still a challenge in the field. Surprisingly, little is known on the regioselective O-substitution of sialic acids. Consequently, the effect of O-protecting groups and/or regioselectively protected building blocks in sialylations, remains practically unexplored. O-Picoloyl protecting groups have emerged as novel substituents that have a profound effect on sialylations. Recently, high stereoselectivities were obtained by introducing picoloyl groups at the C-4 and C-7/C-8 positions. However, to understand the relationship between the position of the picoloyl group and its exact effect in sialylations, a convenient access to a wider range of regioselectively picoloylated building blocks is needed. Reported herein is a new method that provides an accessible route to a wide array of regioselectively acylated building blocks. The regioselective introduction of picoloyl groups at various O-positions was achieved either by controlled direct picoloylation or by applying a modified ReSET methodology.

Keywords: sialic acid, N-acetylneuraminic acid, regioselective acylation, protecting groups

Introduction

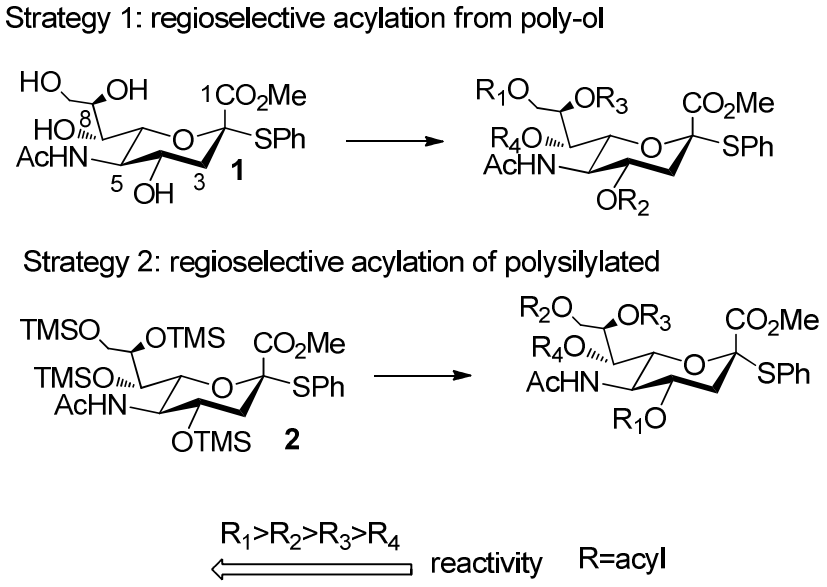
N-Acetylneuraminic acid is the most common member of the sialic acid family, as derivatives of neuraminic acid. The biological functions of this unique carbohydrate are numerous, from being mediator of influenza cell attack, to being essential in the development of embryonic cells.[1,2] The studies of efficient methods to synthesize sialic acid containing glycoconjugates have made a

tremendous improvement for the synthesis of these unique molecules.[3-5] However, when comparing sialic acid chemistry to more investigated monosaccharides such as glucose and galactose, it is clear that sialylations are still very challenging, and not so much is known on the effects of O-protecting groups at C-4 and the glycerol chain. Protecting group manipulations are the essential core of carbohydrate synthesis, with their effect not just limited to 'protect', but also to influence the reactivity and stability of the carbohydrate moiety in glycosylation reactions. Methodologies centered on discoveries or improvements in protecting groups for carbohydrates have been the focus of research for many decades.[6] Development of regioselective methods to protect strategic hydroxyl groups have been having high priority. However, in sialic acid chemistry, the only exploited investigation has been related to the N-5 position.[7,8] The lack of an extensive study of O-protecting groups limits the methodologies offered in sialylation reactions. Recently, we reported the effect of C-4 protecting groups such as tert-butyl dimethyl silyl (TBDMS)[9] and picoloyl (Pico).[10] For the latter, we proved a synergistic effect between Picoloyl group and triflic acid in controlling the stereoselectivity of the reaction. More recently, a C-7,8-di-Pico sialyl donor showed excellent stereocontrol in sialylations.[11] To the growing importance of picoloylated building blocks, there is also a lack of straightforward methodologies for their regioselective introduction. Little is known of how the number of picoloyl groups or their relative positions might influence the general outcome of sialylations. As mentioned above, the field itself lacks a comprehensive investigation of O-protecting group manipulations, in part due to the structural complexity of N-acetylneuraminic acid. Regioselective acetylations have been in general characterized by several synthetic steps and protecting group manipulations[12,13] On the other side, regioselective benzoylations are easier to be performed,[14] however benzoyl groups seem to be deactivating and β-directing.[9] Herein we disclose the synthesis of an array of novel regioselectively functionalized building blocks bearing picoloyl groups at several positions. The methodologies investigated herein can also be extended to other protecting groups.

In order to accomplish this task, we decided to investigate two main strategies depicted in Scheme 1. Following the general trend of relative reactivity of hydroxyl groups, it might be possible to achieve controlled acylation of polyol **1** that would lead to a C-9 monoacylated product, followed by a diacylated product at C-4,9 (Strategy 1, Scheme 1). On the other hand, Gervay's group reported a C4/9 inverted reactivity upon acetylation of persilylated sialyl moiety.[15] The

methodology, called Regioselective Silyl Exchange Technology (ReSET) gave on microwave assisted reaction a reactivity sequence as C4>C9>C8>C7, and moderate yields of 28% (C-4 acetyl); 31% (C-4,9 diacetyl) and 32% (C-4,8,9 triacetyl). Hence, we decided to investigate if a silyl-acetyl exchange reaction from **2** can also be feasible without the assistance of microwave and still lead to similar order of reactivity and yields (Scheme 1, Strategy 2).

Scheme 1. Two major strategies for regioselective acylations



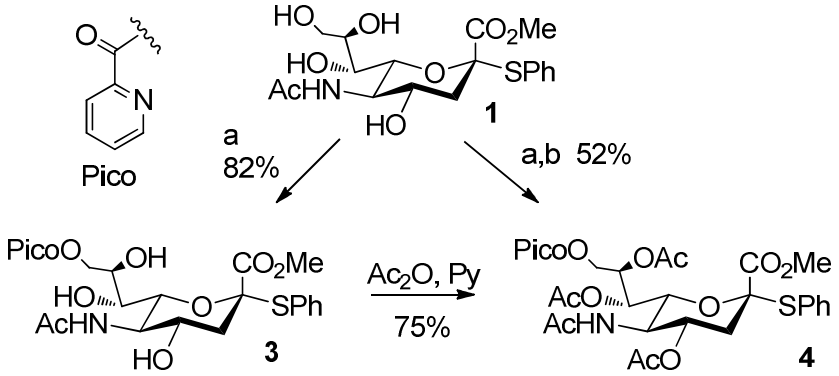
Scheme 1

Results and Discussion

Strategy 1: Synthesis of 9-pico and 4,9 dipico thiosialosides

For our first regioselective picoloylation from polyol **1**, we targeted O-9 mono-picoloylated derivative **3** as product. A one-step acylation directly of C-9 is very appealing, and would dramatically cut many synthetic steps. Our previous picoloylation of sialic acid was done in the presence of N,N'-Dicyclohexylcarbodiimide (DCC) or 1-Ethyl-3-(3-dimethylaminopropyl carbodiimide (EDC), depending on the reactivity of the substrate and the positions targeted, while dichloromethane was the solvent of choice. In the presence of a 4,7-diol, DCC was able to introduce the Pico group at both positions, while EDC only introduced to the most reactive position,

i.e. C-4.[10] Thus, we decided to test both coupling agents, and to use pyridine as the solvent of choice, due to the poor solubility of **1** in dichloromethane. Moreover, using pyridine would offer a better regiocontrol by being an acid scavenger and slowing down the reaction, as well as giving the opportunity to acetylate the picoloylated product **3** *in situ*. Thus, the treatment of polyol **1** with picolinic acid and DCC as the coupling reagent gave a mixture of 9-monopicoloylated product **3** as well as the 4,9-dipico product along with some unreacted starting material. Upon purification, the desired product was isolated with yields ranging from 40 to 50%. Higher yield was observed when EDC was used as the coupling reagent, resulting in a complete consumption of the starting material and a commendable yield of 82% of the desired target **3** (Scheme 2). We believe that the observed difference between the two coupling agents (EDC and DCC) is mainly due the onset of side reaction from DCC that limit this reagent, unless DMAP is used as coactivator of the acyl group. Acetylation of 9-Pico derivative **3** gave sialoside **4** in 75% yield. The synthesis of thiosialoside **4** was therefore accomplished in only two steps. Our previous synthesis of the same building block comprised six steps of protecting groups manipulations, including a C7-to-C9 acyl migration (unpublished results).



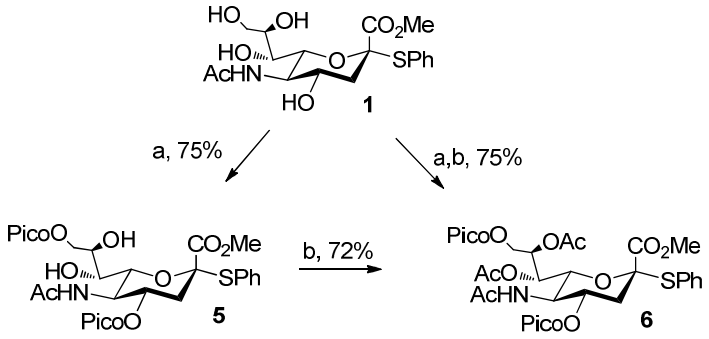
a: Picolinic acid, EDC, py, 45 min; b: Ac_2O, py, 16h

Scheme 2

Encouraged by these results, we proceeded in testing the feasibility of a C-9 picoloylation followed by an *in-situ* acetylation. Thus, upon complete conversion of **1** into product **3**, acetic anhydride was added and the reaction was stirred overnight. TLC analysis of the crude showed completion of the reaction and after column chromatography, the desired product **4** was obtained in 52% yield over two steps (Scheme 2). This yield is comparable with the individual picoloylation

and acetylation previously reported, and therefore can be a valuable option when acetyl or similar group are planned to be introduced.

We then proceeded to investigate whether di-picoloylated target **5** can also be straightforwardly synthesized from the common polyol precursor **1** (Scheme 3). In this case, EDC-mediated reaction resulted in a sluggish, partial addition of picoloyl in position C-4. Conversely, treatment of polyol **1** with picolinic acid in the presence of DCC and DMAP, gave the desired target **5**. Typical for all DCC-mediated reactions, complete removal of side products required the development of several troubleshooting scenarios and combinations of thereof. Finally, we settled upon the precipitation from dichloromethane and filtration that allowed to remove DCC and its side products. The filtrate was then separated by column chromatography to yield **5** in 75% yield. Acetylation of **5** gave thiosialoside **6** in 72% yield (Scheme 3). Similarly to its mono-picoloylated precursor, *in situ* 4,9 di-picoloylation of **1** followed by acetylation was successful, yielding compound **6** in 75% yield (Scheme 3).



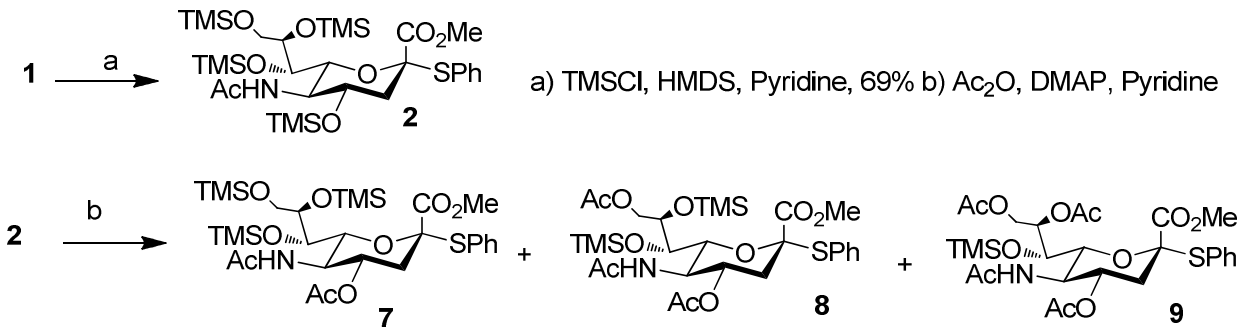
a: Picolinic acid, DDC, DMAP, py, 2.5 h; b: Ac_2O, py, 16h

Scheme 3

Strategy 2: Synthesis of 4-Pico, 7-Pico and 7,8-di-Pico thiosialosides.

As mentioned above, microwave-assisted acetylation of a polysilylated precursor, showed a rather unusual order of regioselectivity, with the C-4 position being more reactive than the C-9.[15] To investigate the suitability of this method for obtaining our targets, we synthetized thiosialoside **2** by per-silylation of polyol **1**. Thus, the treatment of **1** with trimethylsilyl chloride in the presence of hexamethyldisilazane afforded the desired compound **2** in 69% yield (Scheme 4). It should be noted that this yield is associated with a loss of material during the purification step, due to the

lability of the TMS group. To minimize the losses, in subsequent syntheses of this building block, no purification was performed, and crude per-silylated compound **2** was used directly.



Scheme 4

To test the feasibility of regioselective acetylations, and to maximize the yields of each acetylated product, several concentrations of acetic anhydride and DMAP were tested (Table 1, see Scheme 4 for chemical structures). The ratio between the two reagents and the reaction time were critical. Thus, in the presence of 10 equiv. of acetic anhydride and 0.1 equiv. of DMAP, it was possible to obtain C-4 monoacetylated thiosialoside **7** with a yield of 48% (entry 1, Table 1). By decreasing the excess of acetic anhydride while concomitantly increasing the amount of DMAP it was possible to synthesize 4,9-diacetylated compound **8** in 57% yield (entry 2).

Table 1

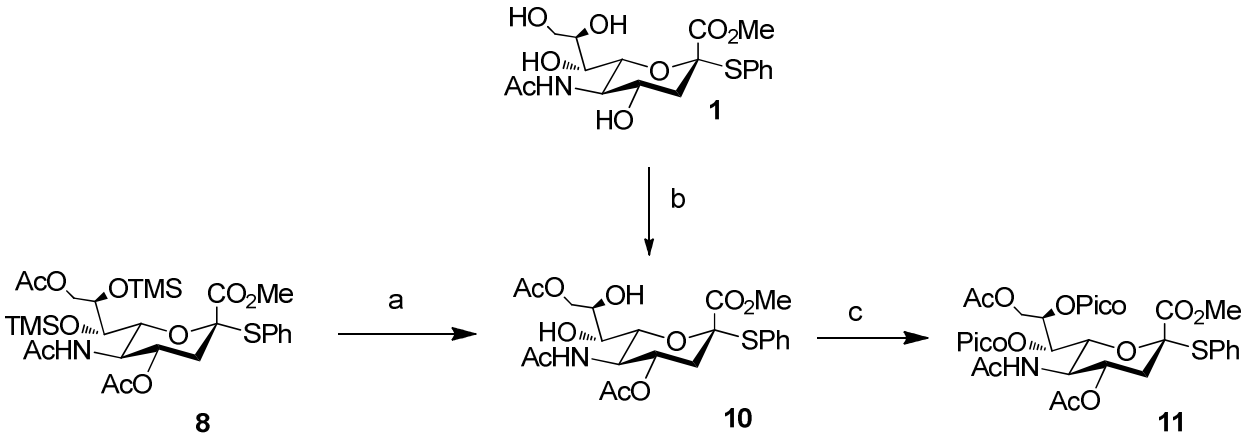
Entry*	Ac_2O eq	DMAP eq.	Yield % **2**±	Yield % **7**	Yield % **8**	Yield % **9**
1	10	0.1	15	**48**	33	-
2	5	0.2	-	7	**57**	33
3	20	0.2	-	-	22	**63**#

* All the reactions were performed at rt, except entry 3, which required higher temperature (40 C);

± recovered starting material

#peracetylated thiosialoside was also recovered in 14% yield.

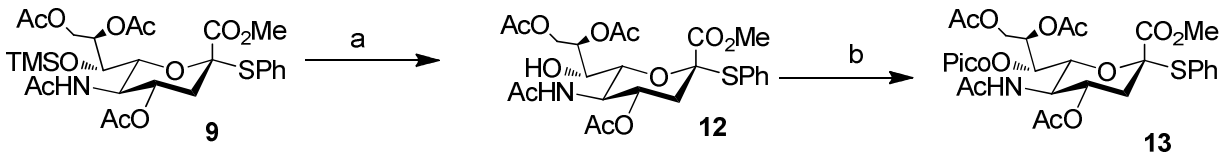
Removal of the remaining TMS groups in 4,9-diacetyl **8** under mildly acidic conditions gave intermediate **10**, which was then picoloylated at positions C-7,8 to yield thiosialoside **11** in 67% yield. The same target can also be obtained in comparable yields by performing regioselective acetylation of **1** at C-4,9 followed by 7,8-picoloylation (Scheme 5).



a) Dowex, H^+, MeOH, 73%; b) Ac_2O, DMAP, py, 63%; c) Picolinic acid, DCC, DMAP, 67%

Scheme 5

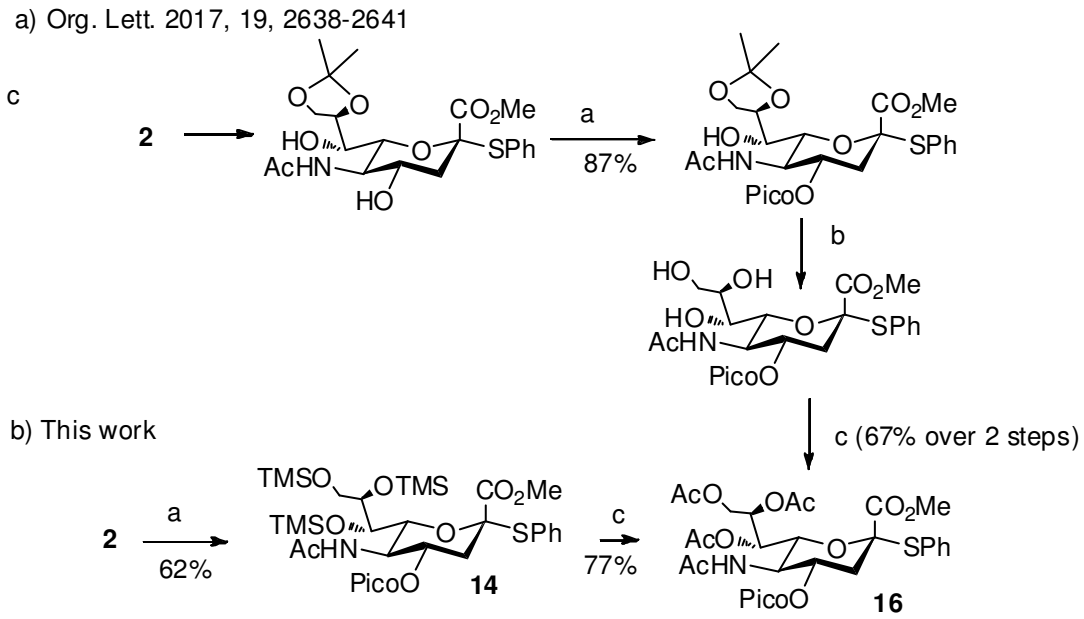
Acetylation of position C-8 to obtain 4,8,9-triacetate **9** from precursor **2** required a much larger amount of acetic anhydride and an increase in temperature (40 °C). As a result of this strategic adjustment, compound **9** was obtained in 63% yield (Table 1, entry 4). Similarly, the removal of the remaining TMS group in **9** gave intermediate **12** in 82% yield. The introduction of picoloyl was then performed, and 7-picoloylated sialoside **13** was obtained in 92% yield (Scheme 6).



a) Dowex H^+ MeOH, 82%, b) Picolinic acid, DCC, DMAP, DCM, 92%

Scheme 6

To expand this methodology to other acyl groups, we tested if it was possible to introduce a picoloyl group at C-4 directly from the persilylated precursor **2**. Our previous synthesis of a C-4 picoloylated thiosialoside is described in Scheme 7a. It required several protecting group manipulations in order to regioselectively introduce picoloyl at C-4. When persilylated compound **2** was reacted with picolinic acid in the presence of EDC as coupling reagent, the C-4 picolylated thiosialoside **14** was successfully obtained in 62% yield (Scheme 7b). Acetylation of **14** gave the direct access to thiosialoside **16** in 77% yield, shortening the number of steps required for its synthesis.[10]



a) Picolinic acid, EDC, py, 62%; b) Dowex, H^+, MeOH; c) Ac_2O, Py, rt

Scheme 7

Conclusions

A series of mono- and di-picoloylated thiosialosides was successfully synthesized by regioselective acylations (picoloylations and acetylations) of polyol and persilylated precursors. The methodology disclosed herein allows for obtaining valuable synthetic targets in a minimal number of steps and, in some cases, in one pot. For instance, C-9 picoloylated thiosialoside **4** was obtained in a two-step reaction, vs. the five-step reaction that was previously needed for this target. The method also allows for the regioselective synthesis of 4,9-diacetylated and dipicolylated building blocks, which are not accessible by other synthetic routes. We are confident that the methodology herein described can be also expanded to other acyl groups.

ACKNOWLEDGMENT

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