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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 4023–4027

# Simultaneous stereoselective 4-amination with cyclic secondary amines and 2-O-deacetylation of peracetylated sialic acid derivatives

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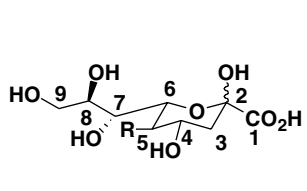
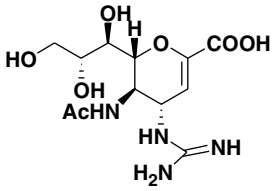
Received 15 January 2007; revised 4 April 2007; accepted 5 April 2007
Available online 11 April 2007

**Abstract**—Treatment of methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (**1**) with cyclic secondary amines in pyridine at room temperature for 24 h afforded unusual products (**2a**–**g**). Related experiments were carried out to explain the formation of 4-amination and 2-O-deacetylation of peracetylated sialic acid derivatives (**2a**–**g**). This reaction may provide a new strategy for the preparation of Zanamivir analogues as neuraminidase inhibitors for anti-H5N1 subtype of avian influenza virus (AIV).


Sialic acids are acidic 9-carbon sugars (numbering shown in Fig. 1), which are intimately involved in a number of important physiological phenomena and disease states.[1,2] *N*-Acetylneuraminic acid (Neu5Ac, Fig. 1),[3] the most commonly found derivative of the 43 different naturally occurring sialic acid derivatives,[4] is a key intermediate for the preparation of anti-influenza drug Zanamivir (Fig. 1). A number of approaches including general chemical synthesis[5–7] and chemoenzymatic synthesis[8,9] toward structurally modified Neu5Ac derivatives starting with Neu5Ac have been published.




R=NHAc(Neu5Ac)




Zanamivir

**Figure 1.**

Recently, a series of C-4 triazole-modified Zanamivir analogues via click chemistry for anti-H5N1 virus was reported by our group.[10] Continuing this study, we are surprised to discover a new reaction involving the modification of Neu5Ac derivatives as depicted in Scheme 1. Treatment of Methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (**1**) with 10 equiv of morpholine in the presence of pyridine (Py) afforded unusual 4-amination with morpholine and 2-O-deacetylation with conserved configuration product (compound **2a**) in high yield. The structure of compound **2a** was confirmed by MS and NMR data, and further secured unequivocally through single crystal X-ray analysis (Fig. 2).[11] This simple and mild reaction can furnish a strategy for the structural modification of sialic acids: 4-amination substitution can provide C-4 modified Neu5Ac derivatives, on the other hand, 2-O-deacetylation can provide free 2-OH which can be selectively derived to 2-O-sialation.[12] With this interesting result, a series of experiments were carried out to provide the proposed mechanism as shown in Scheme 1. The applications of the reaction are also examined in the following text.

Using morpholine as a model cyclic amine, we tested the reaction conditions by testing several parameters, such as different amounts of morpholine, temperature, as well as solvent. The results are summarized in Table 1.

*Keywords*: Sialic acid derivatives; Cyclic secondary amines; Deacetylation; Neu5Ac; Neuraminidase inhibitors; H5N1.
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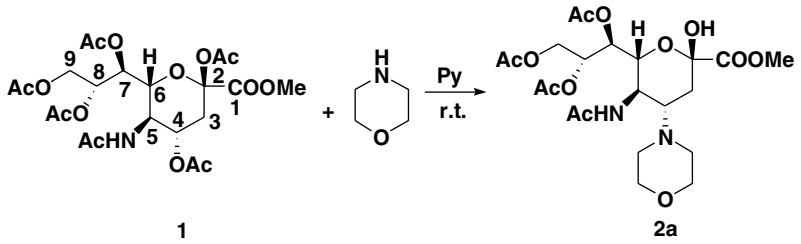
doi:10.1016/j.tetlet.2007.04.023




**Scheme 1.**

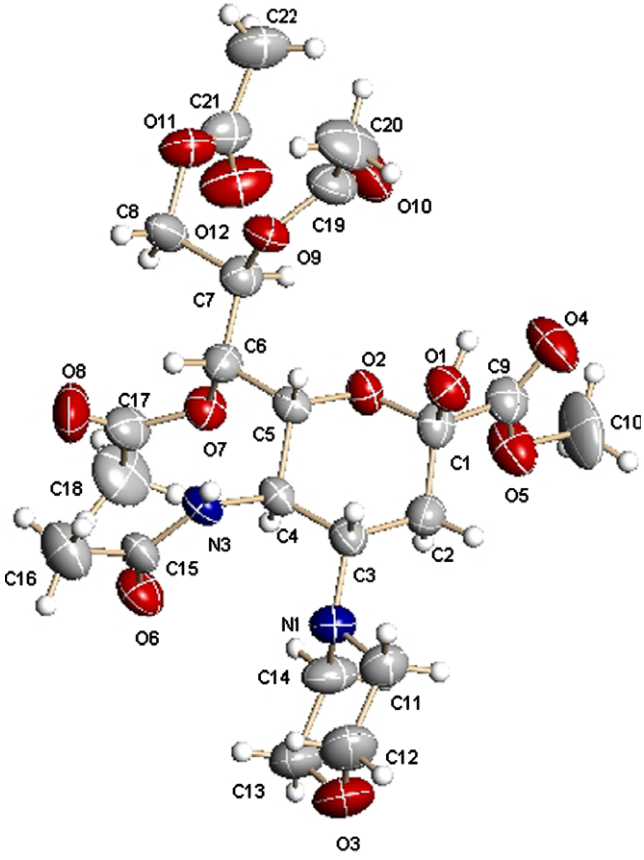



**Figure 2.** X-ray crystal structure of **2a**.

**Table 1.** Procedure of optimizing the reaction using morpholine as cyclic secondary amine

| Entry | Solvent | Morpholine (equiv) | $T$ (°C) | $t$ (h) | Yield[a] (%) |
|---|---|---|---|---|---|
| 1 | Py | 1 | rt | 24 | 18 |
| 2 | Py | 5 | rt | 24 | 31 |
| 3 | Py | 10 | rt | 24 | 82 |
| 4 | Py | 10 | 0 | 24 | 21 |
| 5 | Py | 10 | 40 | 12 | 75 |
| 6 | $CH_2Cl_2$[b] | 10 | rt | 24 | 0 |
| 7 | $CH_2Cl_2$/TEA | 10 | rt | 24 | 5 |
| 8 | DMF | 10 | rt | 36 | 76 |

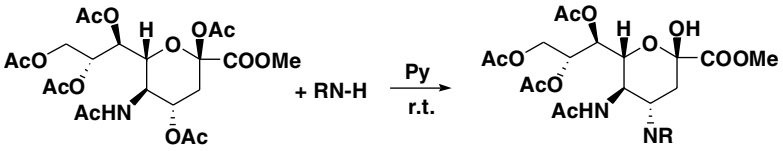
[a] Isolated yield.
[b] The solvent is dry $CH_2Cl_2$.

Compound **1** was separately mixed with 1, 5 and 10 equiv of morpholine at different reaction temperatures in Py for 24 h. The yield of **2** increased with the amount of morpholine (entries 1–3) at rt, and 10 equiv of which can complete the reaction. At 0 °C, only 32% of **1** transformed to **2a** (yield 21%, entry 4), and at 40 °C, the time was shortened to 12 h to complete the reaction but the yield of compound **2a** decreased (75%, entry 5) due to the byproducts. Taking the reaction in different solvents, such as $CH_2Cl_2$, DMF and Py with 10 equiv of morpholine at rt (entries 6, 8, 3), the yield of **2** increased with the polarity of the solvent, and reaction in Py afforded the highest yield (entry 3) due to its polarity.[13] It is interesting that when using $CH_2Cl_2$ combined with triethylamine as solvent, only 5% of yield was given (entry 7), which showed that the basicity of the solvent had no profound effect on the reaction. In general, the optimum results were usually obtained when 100 mg of **1** was allowed to react with 10 equiv of cyclic secondary amines in 1–2 mL of Py at rt for 24 h.

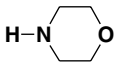
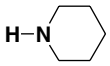
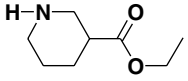
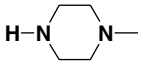
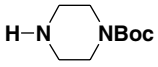
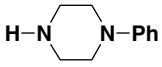
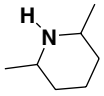
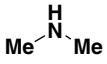
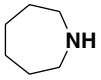
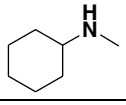
When we applied the reactions to different cyclic secondary amines, different yields of the desired 4-amination and 2-O-deacetylation of peracetylated sialic acid derivatives (**2a**–**g**) were isolated as shown in Table 2.[14] Cyclic secondary amines with six-member ring can proceed smoothly (entries a–f). Pyrrolidine with five-member ring gave **2g** with the yield of 31% after 24 h, and when the reaction time was prolonged to 48 h, the yield only increased to 43% along with byproducts (entry g). There was no transformation of **1** when using pyrrole as the nucleophile due to the pyrrole's lower nucleophilicity (entry h). When applying the reaction to 2,6-dimethylpiperidine, product **2i** was not obtained (entry i) most likely due to the steric hindrance of bulky 2,6-dimethyl with the nitrogen atom. No products were detected when applying the reaction to acyclic amines, cyclic amines with seven-member ring, and cyclic primary amines with different substitutents (entries j–m).

To explain the formation of **2a** in the 4-amination and 2-deacetylation of compound **1**, the effect of 2-acetyloxyl of **1** was investigated. Compound **3** was synthesized from regioselective acetylation of Neu5Ac using iodine in the presence of acetic anhydride[15] and compound **4** was obtained from 2-deacetyloxylation of **1**[12b] and then catalytic reduction with $H_2$ using 10% Pa/C,[16] respectively. As shown in Scheme 2, compound **3** when treated with 10 equiv of morpholine for 12 h was completely converted to compound **2a**. However, when mixing compound **4** with morpholine, no transformation product was detected. These results indicated that the oxygen atom on the C-2 of compound **1** played an important role in the reaction.

**Table 2.** Examples of selective 4-amination and 2-O-deacetylation products



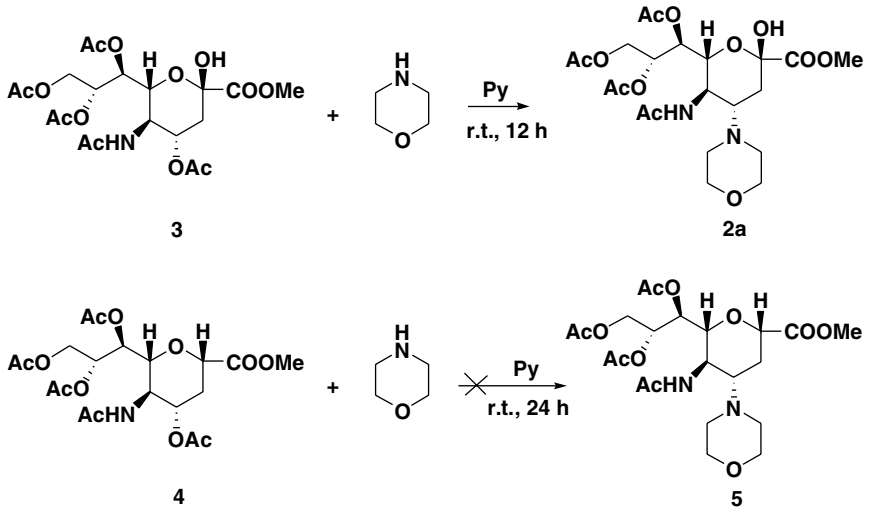

1 2a-i

| Entry | RN–H | *t* (h) | Product **2** (yield, %)[a] |
|---|---|---|---|
| a |  | 24 | 82 |
| b |  | 24 | 79 |
| c |  | 24 | 76 |
| d |  | 24 | 75 |
| e |  | 24 | 80 |
| f |  | 24 | 73 |
| g |  | 24<br>48 | 31<br>43 |
| h |  | 24 | 0 |
| i |  | 24 | 0 |
| j |  | 24 | 0 |
| k |  | 24 | 0 |
| l |  | 24 | 0 |
| m |  | 24 | 0 |

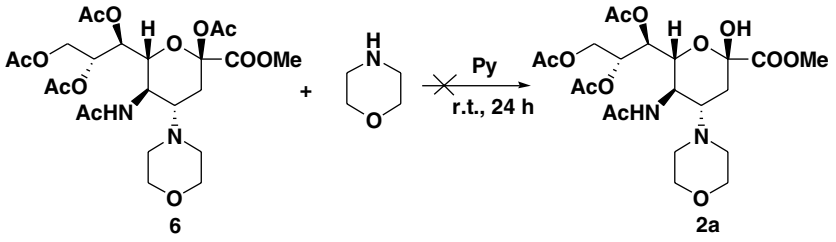
[a] Isolated yield.

Compound **6** was obtained from 2-O-acetylation of **2a**. Treatment of compound **6** with 10 equiv of morpholine in Py for 24 h failed to give product **2a** (Scheme 3), which showed that product **2a** was not formed from the further 2-O-deacetylation of compound **6**.

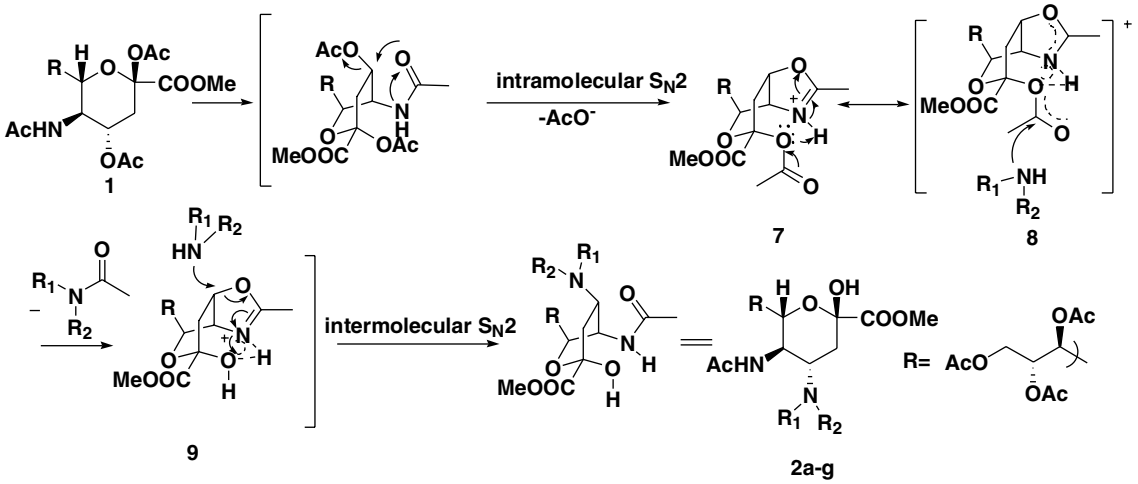
Based on the above experimental results, we proposed a mechanism to explain the formation of the products as depicted in Scheme 4. A literature survey reveals that compounds with *O*-acetyl group trans to adjacent acetamido group can proceed with neighboring-group participation to give the oxazolinium intermediate.[17] Compound **1** was firstly converted into oxazolinium intermediate **7** with invertion configuration via intramolecular $S_N2$ reaction, which might be stabilized by the p orbitals of the oxygen atom on C-2. Under the tautomerization effect, the positive charge of oxazolinium was dispersed to form intermediate **8**. Intermediate **9** was formed through the attack of the cyclic secondary amines to 2-carbonyl group of **8**, at the same time the side products acetamides were detected. Finally, via intermolecular $S_N2$ reaction at C-4 of intermediate **9** and the cyclic secondary amines, the oxazolinium opened to give products **2a**–**g**.




Scheme 2.




Scheme 3.




Scheme 4. Proposed mechanism for compound **1** conversion to the 4-amination and 2-O-deacetylation products **2a**–**g**.

When using compound **3** as the starting material, it was directly converted into intermediate **9**, which made the reaction time shorten to 12 h. It was difficult to form the unstable intermediate oxazolinium without oxygen atom on C-2 of compound **4** for the stabilization. Because morpholine-4-yl is a poor leaving group to $S_N2$ reaction, compound **6** was unable to proceed with neighboring-group participation to give the oxazolinium intermediate, which later blocked 2-O-deacetylation. Considering the whole process in Scheme 4, the configuration of C-4 of compound **2a**–**g** was retained due to the two $S_N2$ reactions, and since there was no bond broken between C-2 and its conjoint oxygen atom, after deacetylation, the configuration of C-2 was also retained. Increasing the amount of amines and the polarity of solvent[18] should accelerate the reaction.

In summary, it has been shown that reactions of peracetylated sialic acid derivatives with cyclic secondary amines in Py at rt afforded stereoselective products with 4-amination and 2-O-deacetylation. This reaction indicates a new route for obtaining Zanamivir analogues

as neuraminidase inhibitors for anti-H5N1 subtype of avian influenza virus (AIV). A plausible reaction mechanism involving the formation of the oxazolinium intermediate was provided. Further studies will be developed to elucidate the scope and limitation of this reaction, and will be reported in due course.

## Acknowledgments

We gratefully acknowledge generous support from the National Natural Science Foundation of China (Grants 20372069, 29725203 and 20472094), the Key Technologies R&D Program from CAS (Grants 2005BA711A01), and the 863 Hi-Tech Program of China (Grants 2006AA020402, 2006AA020602).

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11. *X-ray crystallographic data of* **2a** were solutions at $T = 273(2)$ K: $C_{22}H_{34}N_2O_{12}$, $M_r = 518.51$, monoclinic, space group $P2$ (1), $a = 12.069(2)$ Å, $b = 10.4513(19)$ Å, $c = 21.177(4)$ Å, $\alpha = 90°$, $\beta = 103.400(4)°$, $\gamma = 90°$, $V = 2598.5(8)$ Å$^3$, $Z = 4$, $D_X = 1.325$ mg/m$^3$, $F(000) = 1104$, $\lambda(MoK\alpha) = 0.71073$ Å, $\mu = 0.108$ mm$^{-1}$. CCDC 633149 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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14. *General process for the synthesis of* **2a–i**: 100 mg (0.19 mmol) of **1** and 1.90 mmol of cyclic secondary amine were dissolved in 2 mL of Py, after reaction for 24 h at rt, the solvent was evaporated under vacuum below 40 °C, and the residues were dissolved with 30 mL of ethyl acetate, then washed with water (10 mL × 3) and dried over anhydrous sodium sulfate. On evaporation of the solvent, pure products **2a–i** were obtained by column chromatography with silica gel.
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