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SYNTHESES OF SIALIC ACID ISOMERS WITH INHIBITORY ACTIVITY AGAINST NEURAMINIDASE

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Keyword: sialic acid isomers with 6-acetylamino group; *N*-acetylneuraminic acid; aldol condensation; neuraminidase; influenza virus.

ABSTRACT: The isomers of sialic acid (*N*-acetylneuraminic acid) with 6-acetylamino group, **1a** and **1b** were synthesized through aldol condensation of D-glucose with oxalacetic acid. These compounds inhibit neuraminidases of influenza virus A, A_2, and B remarkably.

N-Acetylneuraminic acid (sialic acid) of a nonulosonic acid with 5-acetylamino group is known as an essential component of gangliosides (sialoglycosphingolipids) which are involved in cellular interactions, differentiation, and growth.[1,2] We exploited a convenient chemical synthesis of *N*-acetylneuraminic acid through aldol condensation of D-glucose with oxalacetic acid [3] as shown in Fig. 1. The pyranose isomer **2** was converted to *N*-acetylneuraminic acid in relatively short steps *via* the key intermediate 1,5-lactone derivative.

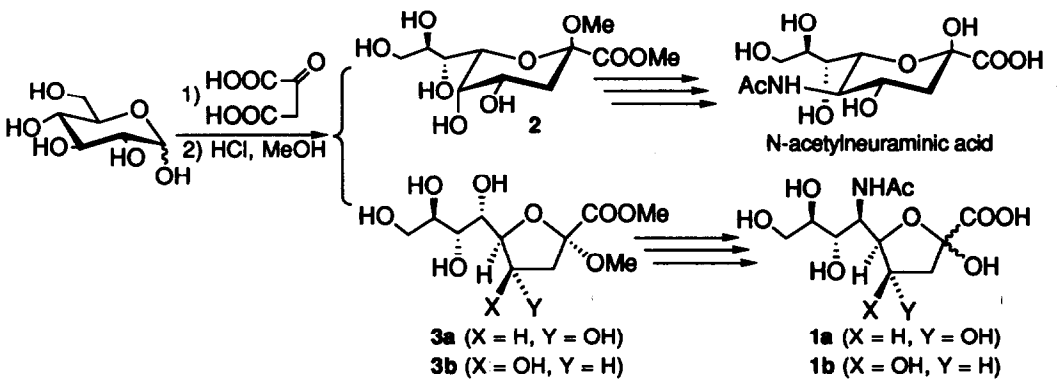
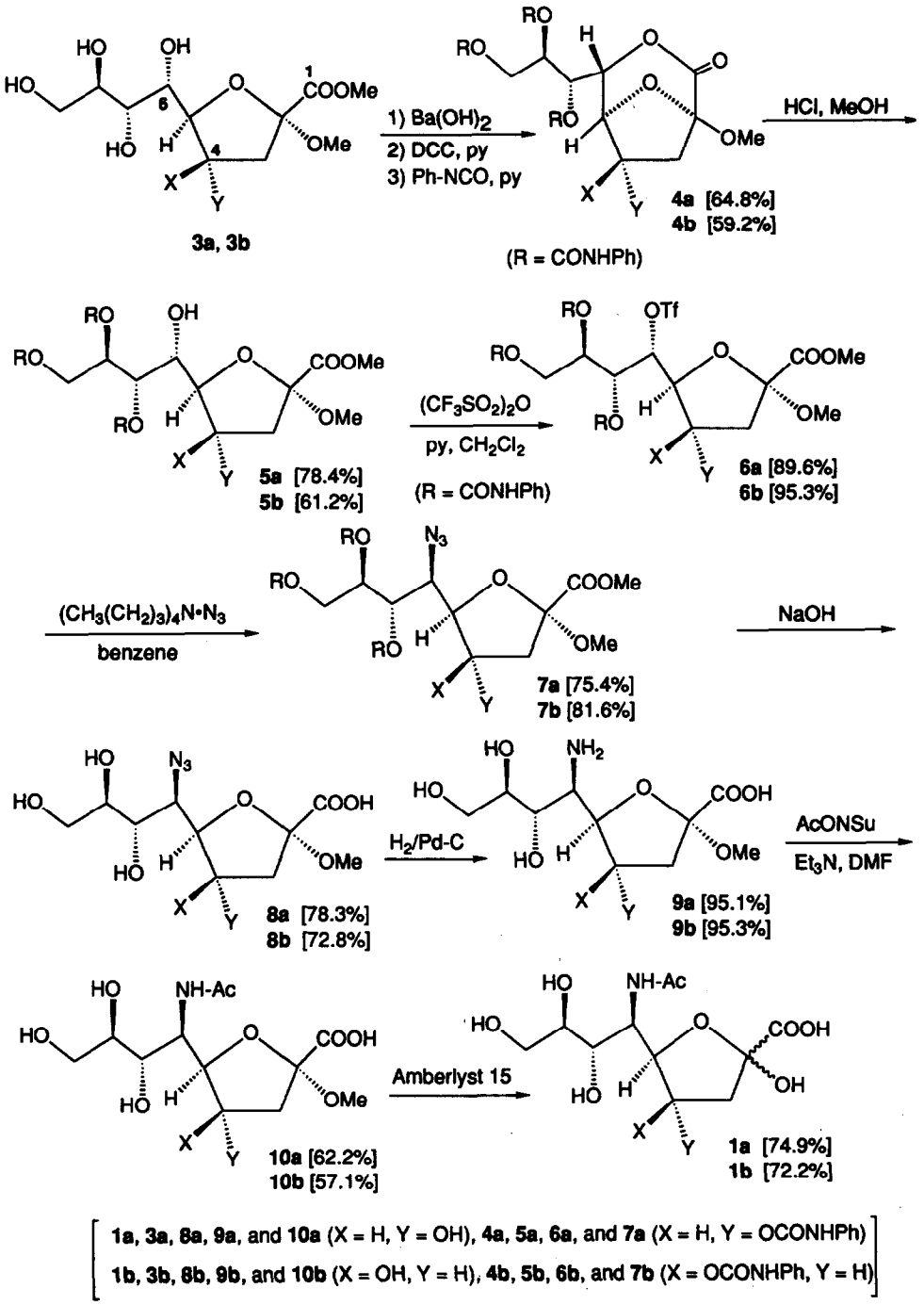


Fig. 1

Through the enzymatic studies on influenza virus during the recent decade, neuraminidases of influenza virus were suggested to play an important role in viral infection.[4,5] Although a number of analogs of sialic acid were synthesized for neuraminidase inhibitors with expectation for development to anti-influenza drugs, those could not be clinically used so far. We performed the syntheses of *N*-acetylneuraminic acid isomers **1a** and **1b** with 6-acetylamino group from the furanose isomers **3a** and **3b**, products in aldol condensation respectively, in order to search for a new compound with neuraminidase inhibitory activity.



[**1a**, **3a**, **8a**, **9a**, and **10a** (X = H, Y = OH), **4a**, **5a**, **6a**, and **7a** (X = H, Y = OCONHPh)
1b, **3b**, **8b**, **9b**, and **10b** (X = OH, Y = H), **4b**, **5b**, **6b**, and **7b** (X = OCONHPh, Y = H)]

Fig. 2

The aldol condensation of D-glucose with oxalacetic acid followed by treatment with HCl in methanol gave a mixture of five isomers including compounds **2**, **3a**, and **3b** in 30% yield.[6] The isomers were separated each other by use of HPLC. The stereochemistry at C4 in furanose isomers **3a** and **3b** were concluded to be *S* and *R* respectively by chemical conversions.[7] Namely compounds **3a** and **3b** are epimers each other on the C4 position. Methyl ester **3a** was saponified with barium hydroxide, and then lactonized with dicyclohexylcarbodiimide (DCC) in pyridine selectively between C1-carboxyl and C6-hydroxyl groups as shown in Fig. 2. Without separation of the product, C4, C7, C8, and C9-hydroxyl groups in the lactone were protected as phenylcarbamate to differentiate them from C6-hydroxyl group as shown in **4a**.

The key intermediate, 1,6-lactone **4a** thus obtained was converted to 6-hydroxyl methyl ester **5a** with HCl in methanol. The compound **5a** possessing only one free hydroxyl group at C6 was sulfonylated with trifluoromethanesulfonic anhydride to give compound **6a**. The triflate **6a** was converted to azide **7a** with tetrabutylammonium azide, and then methyl ester and all of phenylcarbamoyl groups in the compound **7a** were cleaved with sodium hydroxide to give compound **8a** with all free hydroxyl groups.[8] Catalytic hydrogenation of the azide **8a** was smoothly carried out in high yield. The amine **9a** was then acetylated with *N*-acetoxysuccinimide (AcONSu) and triethylamine to form compound **10a**. Finally, the methyl glycoside **10a** was hydrolyzed to give compound **1a**.[9]

The compound **1b** was also synthesized from another furanose isomer **3b** through the same route described above for **1a**.[9]

Table 1. Inhibitory activities of the isomers **1a**, **1b**, and DDNA against neuraminidases of influenza virus A, A_2, and B.

origins of neuraminidases	**1a**	**1b**	DDNA
influenza virus A	84.9%	67.6%	94.9%
A_2	70.3	41.4	84.6
B	68.5	31.2	77.5

The concentration of test compounds **1a**, **1b**, and DDNA : 100 μM

Results of the preliminary tests for inhibitory activities of the isomers **1a** and **1b** against neuraminidases of influenza virus are shown in Table 1 in comparison of those of DDNA (2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid) [10] which is one of the strongest inhibitors against neuraminidases *in vitro*, but not in practical use due to invalidity *in vivo*. These furanose isomers in Table 1, especially compound **1a** with the same stereochemistry at C4 as that of *N*-acetylneuraminic acid, exhibit the remarkable inhibitory activity comparable to DDNA. Therefore, the isomer **1a** or **1b** with 6-acetylamino group in this study is expected to be developed to a promising new anti-influenza drug.

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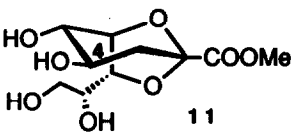
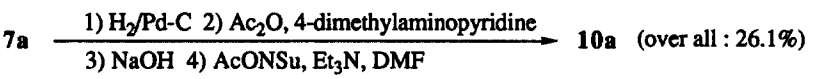
6) Yields of the individual isomers are as follows: compound **2** (3.1%), **3a** (10.0%), and **3b** (5.1%).
7) The furanose rings of **3a** and **3b** were confirmed by the observation of H5 at higher field than those of the other methine protons in ^{1}H-NMR spectra of peracetylated **3a** and **3b**. The furanose **3a** was convertible into pyranose **2** (4*S*) by saponification, cleavage of methyl glycoside, and the treatment with HCl in methanol successively. On the other hand, with the same treatment, the furanose **3b** was converted to bicyclic compound **11** (4*R*) whose structure was determined by the analysis of ^{1}H-NMR spectra of **11** itself and its peracetate.

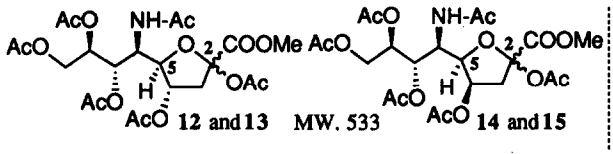


11

8) For the transformation of protected azide **7a** into compound **10a**, we first attempted the alternative route which was applied for synthesis of sialic acid as shown below.[3] However, the catalytic hydrogenation in this case, did not proceed smoothly perhaps due to the steric hindrance of phenylcarbamoyl groups. Moreover, the free amine formed by partial cleavage of *N*-acetyl group during the deprotection, had to be reacetylated. The changing order of hydrogenation, acetylation, and deprotection as shown on the text, increased the total yield of compound **10a** from **7a** about twice.



9) Compounds **1a** and **1b** were obtained as mixtures (3:2) at C2, although the stereochemistry at C2 of the predominant anomers were not determined. Moreover, in order to confirm the structures of compounds **1a** and **1b**, these were respectively acetylated and then treated with diazomethane to give corresponding two pairs of anomers **12**, **13** (from **1a**) and **14**, **15** (from **1b**). According to the data (Table 2) the structures of four isomers **12**-**15** were determined except the stereochemistry at C2. The presence of the amide proton at C6 and the observation of H5 at higher field than those of the other methine protons revealed that **12**-**15** were not converted to piperidine ring compound like in **17**. Consequently compounds **1a** and **1b** can be unambiguously assigned to the furanose isomers, and the possibility of structure **16** could be ruled out.



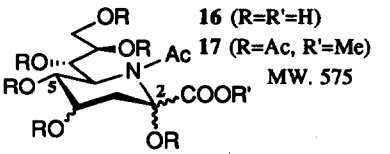


Table 2. Data in ^{1}H-NMR spectra (270 MHz, in $CDCl_3$, δ<ppm> and J<Hz>) and plasma desorption mass spectra (PD-MS, m/z ($[M+H]^+$, $[M+Na]^+$)) of compounds **12**, **13**, **14**, and **15**.

compounds	H3	H3'	H4	H 5	H6	H7	H8	H9	H9'	N H	COOMe	acetyl groups (18H)
12	2.60	2.80	5.16	**4.24**	4.47	5.25	5.25	4.16	4.41	**6.42**	3.79	2.06-2.15
13	2.51	2.92	5.18	**4.37**	4.37	5.30	5.24	4.17	4.38	**6.49**	3.82	2.05-2.10
14	2.64	2.64	5.45	**4.28**	4.75	5.28	5.28	4.19	4.47	**6.10**	3.80	1.98-2.09
15	2.42	2.67	5.46	**4.14**	4.77	5.13	5.28	4.21	4.43	**6.06**	3.77	1.97-2.14

compounds	J(3,3')	J(3,4)	J(3',4)	J(4,5)	J(5,6)	J(6,7)	J(7,8)	J(8,9)	J(8,9')	J(9,9')	J(6,NH)	PD-MS
12	15.1	3.7	7.0	*	*	*	*	6.0	2.4	12.2	9.8	534, 556
13	15.6	1.2	7.1	*	*	3.9	6.1	5.4	2.6	12.5	8.3	534, 556
14	*	*	*	3.7	8.8	5.6	*	7.0	2.0	12.2	10.3	534, 556
15	15.3	<1.0	5.6	3.7	9.5	5.3	5.3	6.7	2.4	12.5	10.3	534, 556

* These coupling constants could not be determined due to overlapping of the signals.

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