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Chemoenzymatic synthesis of CMP-*N*-acetyl-7-fluoro-7-deoxy-neuraminic acid

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Abstract—7-Fluoro sialic acid was prepared and activated as cytidine monophosphate (CMP) ester. The synthesis started with D-glucose, which was efficiently converted into *N*-acetyl-4-fluoro-4-deoxy-D-mannosamine. Aldolase catalyzed transformation yielded the corresponding fluorinated sialic acid which was activated as CMP ester using three different synthetases in the presence as well as in the absence of pyrophosphatase which possesses inhibitory properties. Finally, conditions were optimized to perform a one-pot reaction starting from fluorinated mannosamine, which yielded the 7-fluoro-7-deoxy-CMP-sialic acid by incubation with three enzymes.

Keywords: CMP-7-F-sialic acid; Chemoenzymatic synthesis; Sugar nucleotide

1. Introduction

Sialic acids are a widely distributed class of negatively charged 9-carbon sugars with more than 40 natural occurring derivatives in vertebrates and bacteria.[1] The most important member of this family is *N*-acetyl-neuraminic acid. The synthesis of sialic acid and analogues has been extensively studied.[2,3] The enzymatic synthesis of *N*-acetyl-neuraminic acid (sialic acid, **1**) from *N*-acetyl-D-mannosamine or *N*-acetyl-D-glucosamine followed by activation as CMP-ester is well established.[4–6] Multienzyme systems have been optimized for this sequence,[7–9] which includes the regeneration of CMP to CTP.[10] To access new derivatives, every position of sialic acid was addressed during the past years[1–3] and several variations at position 7 of sialic acid were synthesized.[3,11,12] Several approaches follow chemoenzymatic routes using derivates of *N*-acetyl-D-mannosamine and pyruvate or pyruvate derivatives.[2,3] Also, several total synthesis approaches of native and artificial sialic acids were published.[2,3] Many sialic acid recognizing proteins exhibit strong interactions to the glycerol side chain of sialic acid, which makes this position important for modifications.[3]

2. Results and discussion

Here, we report an improved synthesis of CMP-*N*-acetyl-7-fluoro-7-deoxy-neuraminic acid (**1**)[12] starting from D-glucose. The synthesis of **1** (Scheme 1) starts with commercially available methyl glycoside **2**. After activation as triflate, azide introduction was readily accomplished using sodium azide in DMF following an approach published by Moravcova and coworkers[13] and Pipik and coworkers.[14] In our hands, the isolated

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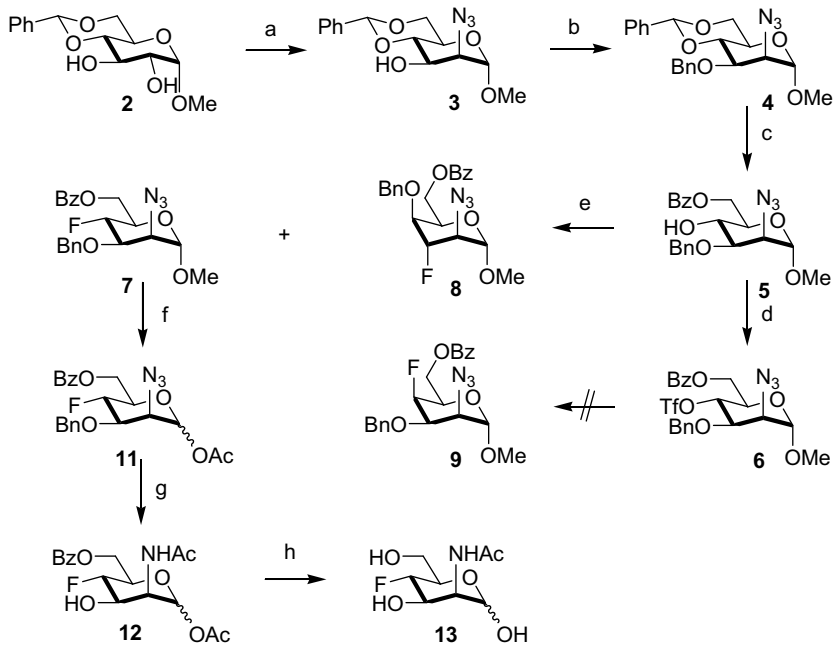
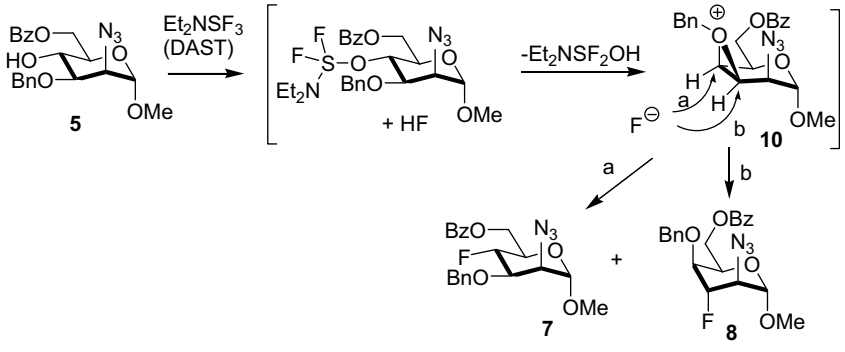


Scheme 1. Reagents and conditions: (a) Tf_2O, pyridine, CH_2Cl_2, −20 °C, 2 h; then NaN_3, 15-crown-5, DMF, 50 °C, 24 h, 93%; (b) BnBr, TBAI, NaH, DMF, 0 °C→20 °C, 14 h, 93%; (c) *p*-TSA, MeOH, 20 °C, 3 h; then BzCl, 2,6-lutidine, CH_2Cl_2, 0 °C, 2 h, 99% (d) Tf_2O, pyridine, CH_2Cl_2, −20 °C→−8 °C, 3 h; (e) DAST, CH_2Cl_2, −78 °C→20 °C, 4 d, 63% for **7**; 13% for **8**, 11% for **5**; (f) Ac_2O/H_2SO_4 (3v%), 0 °C, 1 h, α-anomer 63%, β-anomer 26%; (g) $Pd(OAc)_2$, EtOAc, Ac_2O, 20 °C, 5.6 bar, 4 × 24 h, 79%; (h) NaOMe, MeOH, 20 °C, 3 h.

yield of *manno* azide **3** could be improved from 53% to 93% by the addition of crown ether (15-c-5), which also allowed us to reduce the amount of sodium azide and lower the reaction temperature.

To selectively address position 4 of the hexose, benzyl ether formation at 3-OH was carried out first. Hydrolysis of the benzylidene acetal **4** under acidic conditions (*p*TsOH in methanol) yielded the 4,6-diol, which could be selectively converted into benzoate **5** in 99% overall yield. Any attempts to fluorinate triflate **6** failed; however, the reaction of alcohol **5** with DAST in dichloromethane gave fluorinated hexose **7** in 63% yield. Surprisingly, product **9** which results from S_N2 inversion of configuration was not detected. In fact, we could only isolate the 3-fluoro sugar **8**. We tentatively assume that a DAST-promoted removal of the hydroxyl group in **5** furnishes the intermediate O-benzylated oxirane **10**, which was ring-opened by fluoride to the corresponding *manno*- and *ido*-configured hexoses **7** and **8**, respectively (Scheme 2). DAST-induced oxirane formation was also encountered during the synthesis of fluorinated furanosides by Mikhailopulo and Sivets.[15] Anomeric deprotection (H_2SO_4 in acetic acid anhydride) gave acetate **11** as anomeric mixture (89%; $\alpha/\beta = 2.4{:}1$), which was debenzylated at elevated pressure to yield compound **12**. Final transesterification was performed with MeOH/NaOMe and the resulting *N*-acetyl-4-deoxy-4-fluoro-D-mannosamine (**13**) was formed and could be employed for enzymatic syntheses without further purification.

Studies on the enzymatic synthesis of 7-deoxy-7-fluoro sialic acid (**14**) were initiated using commercially available sialic acid aldolase (Fluka). Reaction conditions[16] found for complete transformation of *N*-acetyl-mannosamine into *N*-acetyl-neuraminic acid were



Scheme 2. Proposed mechanism for the benzyloxy migration and formation of **7** and **8**.