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SYNTHESIS OF THE EPIMERIC PAIR OF 4-DEOXY-4-(R)- AND 4-DEOXY-4-(S)-C-METHYL-N-ACETYLNEURAMINIC ACID[1)]

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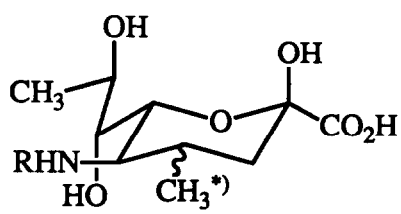
Summary: A 4-C-methylene sialic acid derivative **3** was obtained by the reaction of the corresponding 4-oxo-compound **2** with $CH_2I_2/Zn/Cp_2ZrCl_2$. The product was transformed into a mixture of the 4-deoxy-4-(R)-methyl- and 4-(S)-methyl derivatives **4** and **5**. Sialic acids **6** and **7** were obtained after the removal of protective groups.

N-Acetylneuraminic acid (Neu5Ac) and various analogues, the sialic acids, are found as terminal units of many oligosaccharide sequences of glycoproteins and glycolipids. They play an important role in a series of biochemical and biological processes[2)]. Most of the sialic acids exhibit the same structural skeleton as Neu5Ac. Nevertheless a few species are also found in natural matrices with important structural differences[3,4)]. For example, sialic acid analogue **1**, was isolated in 1970[5)] from sea urchin eggs. As we are interested in new structural variants to investigate the structure-activity-relationships with the enzymes of the sialic acid metabolism[6)] as well as the haemagglutinins of Influenca Viruses, we exchanged the hydrophilic equatorial 4-OH group of Neu5Ac by the hydrophobic methyl group. This compound is structurally related to the sialic acid **1**[5)]. We wish to report now the first synthesis of this branched sialic acid as well as its epimeric congener via a suitable 4-C-methylene derivative.

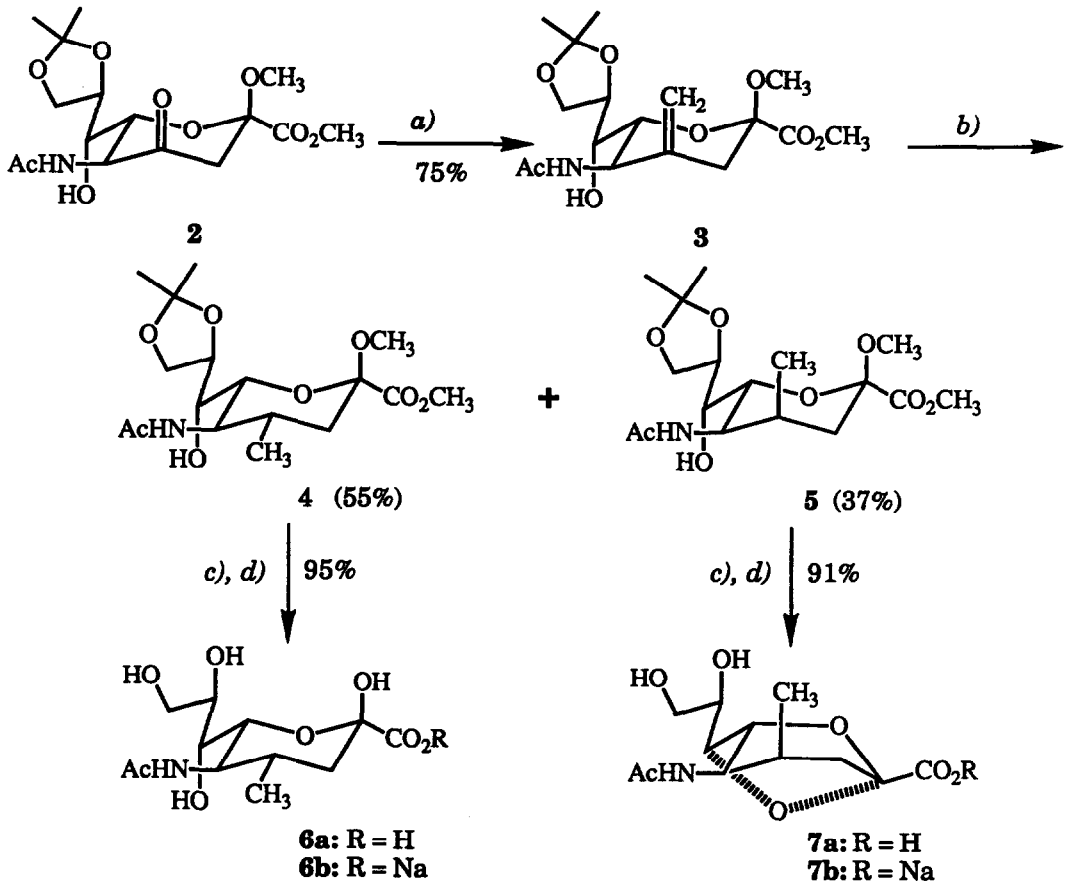


1

R = $CH_3CO_2CH_2CO$
*) stereochemistry not assigned

Our synthetic effort started with the 4-oxo derivative **2**[7)], for which we developed recently an efficient synthesis[8)]. When we tried to prepare a 4-C-methylene derivative by means of Wittig reaction or Peterson olefination we were not

successfull, probably because of the enolization of the ketone **2**. Therefore we applied the triple $Cp_2ZrCl_2/Zn/CH_2I_2$, which is described to form an intermediate carbene complex that reacts easily with enolizable ketones[9,10].



a) Cp_2ZrCl_2, Zn, CH_2I_2; b)H_2-Pd/C; c) 1 M NaOH; d) 0.025 M HCl, Amberlyst 15 H^+.

Thus, stirring 1.3 g Zn, 750 mg zirconocene dichloride and 563 mg **2** (1.5 mmol) in 5 ml anhydrous THF an exothermic reaction took place, when 413 µl of CH_2I_2 were added. After 8 minutes the reaction was quenched by the addition of 15 ml of saturated NH_4Cl solution. Subsequent extraction with ethyl acetate and flash chromatography yielded 395 mg (1.05 mmol) of methyl (methyl-5-acetamido-4-C-methylene-8,9-O-(methyl-ethylidene)-3,5-dideoxy-β-D-*manno*-2-nonulopyranosidon) at **3**[11]. This compound was transformed into a mixture of the two diastereoisomers

4[12] and **5**[13] [3:2] by hydrogenation (H_2 [50 psi], Pd/C, iso-propanol-acetone [1:1]). These two methyl-branched compounds were easily separated by flash chromatography (ethyl acetate).

The unambiguous assignment of the configuration of **4** (D-*glycero*-D-*galacto*) and **5** (D-*glycero*-D-*talo*) was achieved as follows: 1) All coupling constants gave clear evidence that the pyranose exists in the $^2C_5$-conformation. Therefore the coupling constants $J(3_{ax}, 4) = 12.1$ and $J(4, 5) = 10.5$ Hz indicated an axial position of the 4-H in the case of compound **4**. The opposite is true for compound **5** that showed a coupling constant $J(4, 5) = 4.2$ Hz corresponding to equatorial 4-H. 2) ^{13}C-nmr data were in accordance with this assumption for we could observe a high-field shift[14] of 3.35 ppm of the methylcarbon (14.86 ppm) in the axial position to the corresponding equatorial positioned methyl group (18.21 ppm) of compound **4**.

After removal of the protective groups[15] from derivative **5** we obtained the 5-acetamido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid **6a**, which was transformed into its sodium salt **6b**[15] by passing over a column of Dowex 50 Na^+. When we applied the same procedure on **5** we isolated **7a** as the only product which was also transformed into its sodium salt **7b**[17]. This 2,7-anhydro-structure could be assigned by two facts: 1) in the ^{1}H-nmr spectrum the 6-H was found at 4.50 ppm which means a downfield-shift and a small coupling constant $J(5, 6) = 1.0$, which are typical for 2,7-anhydro-sialic acid[18], 2) as a 2,7-anhydro compound the 2-C led to a signal at 107.7 ppm in the ^{13}C-nmr[19].

Acknowledgements

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11. **3**: ^{1}H NMR(250 MHz, $CDCl_3$/TMS): δ = 1.28, 1.35 (2 s, 2 x 3 H, $C(CH_3)_2$), 2.08 (s, 3 H, CH_3CO), 2.56 (ddd, 1 H, 3-H_a), 2.76 (d, 1 H, 3-H_b), 3.25 (s, 3 H, OCH_3), 3.47 (dd, 1 H, 7-H), 3.53 (dd, 1 H, 6-H), 3.78 (s, 3 H, $COOCH_3$), 4.00 (dd, 1 H, 9-H_a), 4.12 (dd, 1 H, 9-H_b), 4.29 (ddd, 1 H, 8-H), 4.67 (dddd, 1 H, 8-H), 4.96 (dd, 1 H, 10-H_a), 5.00 (dd, 1 H, 10-H_b), 5.84 (d, 1 H, N-H); $J(3_a, 3_b)$ = - 14.1 Hz, $J(3_a, 10_a)$ = 1.9, $J(3_a, 10_b)$ = 1.9, J(5, NH) = 9.3, J(5, 6) = 10.6, $J(5, 10_a)$ = 1.9, $J(5, 10_b)$ = 1.9, J(6, 7) = 1.3, J(7, 8) = 8.2, J(8, 9_a) = 5.4, J(8, 9_b) = 6.1, $J(9_a, 9_b)$ = - 8.1.

12. **4**: ^{1}H NMR(250 MHz, $CDCl_3$/TMS): δ = 1.21 (d, 3 H, CH_3), 1.27, 1.35 (2 s, 2 x 3 H, $C(CH_3)_2$), 1.98 (ABM, 2 H, 3-H's), 2.04 (s, 3 H, CH_3CO),2.21 (dddd, 1 H, 4-H), 3.24 (s, 3 H, OCH_3), 3.42 (dd, 1 H, 7-H), 3.70 (dd, 1 H, 6-H),3.75 (s, 3 H, $COOCH_3$), 3.96 (dd, 1 H, 9-H_a), 4.12 (dd, 1 H, 9-H_b), 4.21 (ddd, 1 H, 5-H), 4.30 (ddd, 1 H, 8-H), 5.54 (d, 1 H, N-H); $J(3_{ax}, 4)$ = 12.1, J(4, 5) = 10.5, J(4, 10) = 7.2, J(5, NH) = 9.3, J(5, 6) = 10.5, J(6, 7) = 1.2, J(7, 8) = 8.2, J(8, 9_a) = 6.0, J(8, 9_b) = 6.2, $J(9_a, 9_b)$ = - 8.6, $J(3_{eq}, 4)$ not determined.

13. **5**: ^{1}H NMR(250 MHz, $CDCl_3$/TMS): δ = 0.97 (d, 3 H, CH_3), 1.28, 1.35 (2 s, 2 x 3 H, $C(CH_3)_2$), 1.53 (dd. 1 H, 3-H_{ax}), 1.96-2.18 (m, 2 H, 3-H_{equ}, 4-H), 2.04 (s, 3 H, CH_3CO), 3.27 (s, 3 H, OCH_3), 3.45 (dd, 1 H, 7-H), 3.50 (dd, 1 H, 6-H), 3.65 (ddd, 1 H, 5-H), 3.76 (s, 3 H, $COOCH_3$), 3.99 (dd, 1 H, 9-H_a), 4.12 (dd, 1 H, 9-H_b), 4.30 (ddd, 1 H, 8-H), 5.35 (d, 1 H, N-H); $J(3_{ax}, 3_{eq})$ = - 13.6 Hz, $J(3_{ax}, 4)$ = 12.1, J(4, 5) = 10.5, J(4, 10) = 6.4, J(5, NH) = 8.7, J(5, 6) = 10.2, J(6, 7) = 1.4, J(7, 8) = 8.0, J(8, 9_a) = 5.7, J(8, 9_b) = 6.2, $J(9_a, 9_b)$ = - 8.6.

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15. Typical procedure: 30 mg **5** were dissolved in a mixture of 2 ml 1M NaOH and 1 ml of methanol and stirred 120 min at 40°C. This solution was neutralized with Amberlyst $15H^+$, filtered and lyophilized. The residue was dissolved in 15 ml of 0.025 M HCl and 2 g of Amberlyst were added and heated for 2 h at 80°C.

16. **6a**: ^{1}H NMR(250 MHz, D_2O /DSS): δ = 0.95 (d, 3 H, CH_3), 1.65 (dd, 1 H, 3-H_{ax}), 1.99 (dd, 1 H, 3-H_{equ}), 2.03 (s, 3 H, CH_3CO), 2.06 (ddd, 1 H, 4-H), 3.53 (dd, 1 H, 7-H), 3.59 (dd, 1 H, 9-H_a), 3.67 (dd, 1 H, 5-H), 3.73 (ddd, 1 H, 8-H9, 3.81 (dd, 1 H, 9-H), 3.98 (dd, 1 H, 6-H); $J(3_{ax}, 3_{eq})$ = - 13.3 Hz, $J(3_{ax}, 4)$ = 12.0, $J(3_{eq}, 4)$ = 4.14, J(4, 5) = 11.2, J(5, 6) = 9.0, J(6, 7) = 1.1, J(7, 8) = 9.1, J(8, 9_a) = 5.3, J(8, 9_b) = 2.6, $J(9_a, 9_b)$ = - 11.5.

17. **7a**: ^{1}H NMR(250 MHz, D_2O /DSS): δ = 0.87 (d, 3 H, CH_3), 1.56 (dd, 1 H, 3-H_a), 1.91 (dd, 1 H, 3-H_b), 2.08 (s, 3 H, CH_3CO), 2.39 (dddd, 1 H, 4-H), 3.54 - 3.63 (m, 2 H, 8-H, 9-H_a), 3.75 (dd, 1 H, 9-H_b), 3.96 (dd, 1 H, 5-H), 4.50 (dd, 1 H, 6-H); $J(3_{ax}, 3_{eq})$ = - 14.2 Hz, $J(3_{ax}, 4)$ = 12.7, $J(3_{eq}, 4)$ = 5.1, J(4, 5) = 4.0, J(5, 6) = 1.0, J(6, 7)= 1.2, J(7, 8) = 6.8, J(8, 9_b) = 6.0, $J(9_a, 9_b)$ = - 8.5, J(8, 9_a) not determined.

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