Tetrahedron Letters,Vol.29,No.35,pp 4449-4452,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

THERMAL REARRANGEMENT OF N-ACETYL-N-NITROSONEURAMINIC ACID DERIVATIVE: SYNTHESIS OF 3-DEOXY-D-NONULOSONIC ACID (KDN)[1-3]

Ryuichi Shirai, Mitsunobu Nakamura, Sumiko Hara,
Hiroaki Takayanagi, and Haruo Ogura*

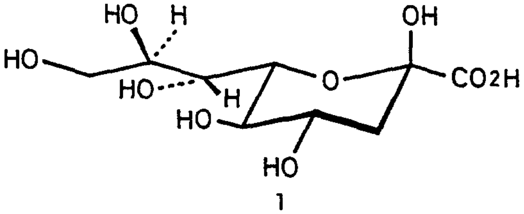
School of Pharmaceutical Sciences, Kitasato University,
Shirokane, Minato-ku, Tokyo 108, Japan

ABSTRACT: Thermal rearrangement of N-acetyl-N-nitrosoneuraminic acid derivative followed by deprotection gave 3-deoxy-D-nonulosonic acid (KDN).

Recently, an unknown acidic sugar was isolated by Inoue *et al*. from polysialoglycoprotein (PSGP) of rainbow trout eggs.[4] The structure of this monosaccharide was determined to be 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) (1). The KDN residues were located at the non-reducing termini in PSGP. Terminal capping of oligo(poly)sialyl chains by the KDN residues protects these chains from exosialidases, and thereby helps them to perform some required, but as yet unidentified function during egg activation or early development.

Inoue *et al*. synthesized protected KDN (8) by converting an amino group of N-deacylated sialic acid derivative to a hydroxy group in low yield *via* a diazonium intermediate.[4] The first practical synthesis of KDN (1) was achieved recently by Augé *et al*. by condensation of D-mannose with pyruvate, catalyzed by immobilized acylneuraminate pyruvate lyase.[5]

In this paper, we wish to report a convenient chemical method to convert protected N-acetylneuraminic acid to protected KDN (4) *via* N-nitrosoation of acetamide moiety to give N-acetyl-N-nitrosoneuraminic acid derivative (3) followed by thermal rearrangement (Scheme 1).



1

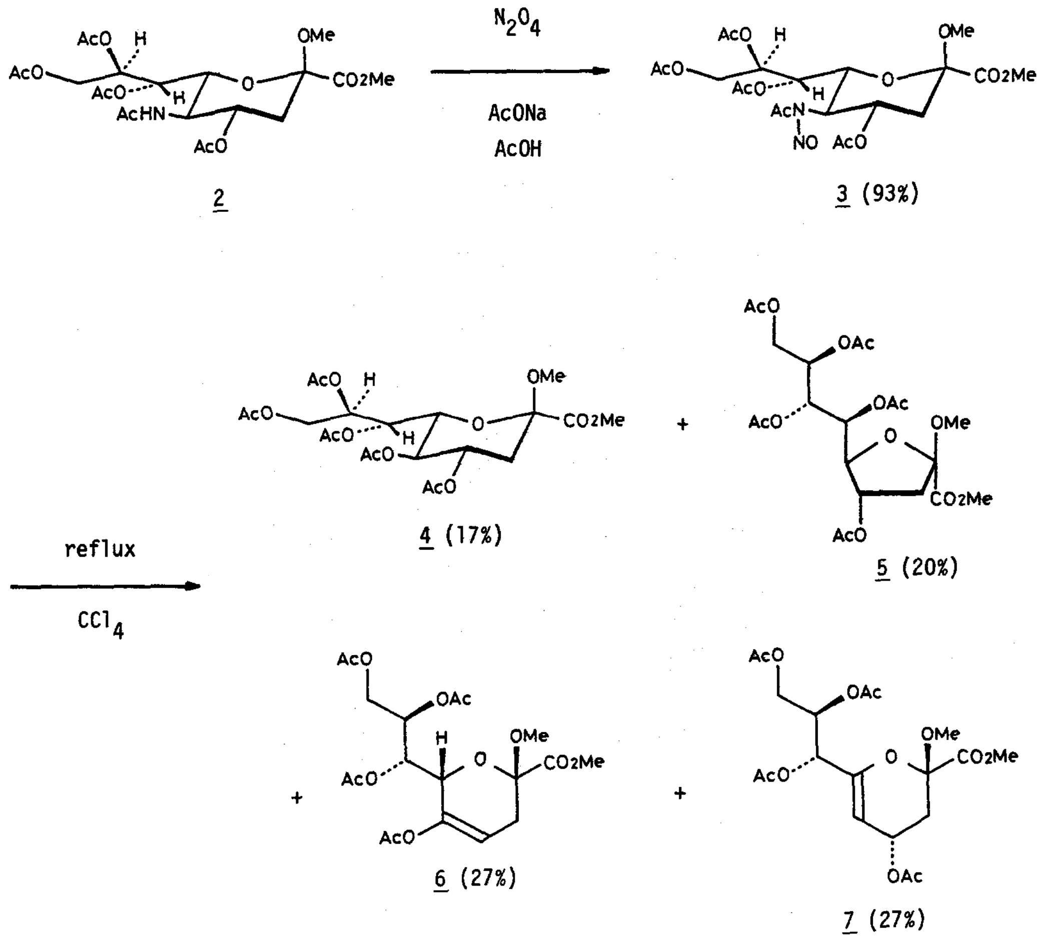
Methyl N-acetyl-4, 7, 8, 9-tetra-O-acetylneuraminate β-methyl glycoside (2) was nitrosoated by White's method using dinitrogen tetraoxide.[6] To the frozen mixture of 2, anhydrous sodium acetate and acetic acid, was added dinitrogen tetraoxide in acetic acid. Stirring was continued for several hours at room temperature and produced yellow N-nitrosoamide (3) in a quantitative yield.

Thermal rearrangement was performed by refluxing the reaction mixture in CCl_4 for about 2 hours untill the yellow color disappeared. Reaction products were the desired 4 (17%), abnormally rearranged 5 (20%), and the inseparable mixture of eliminated products 6 and 7 (total 54%, 1 : 1). These structures were determined by 300 MHz ^{1}H-NMR spectra.

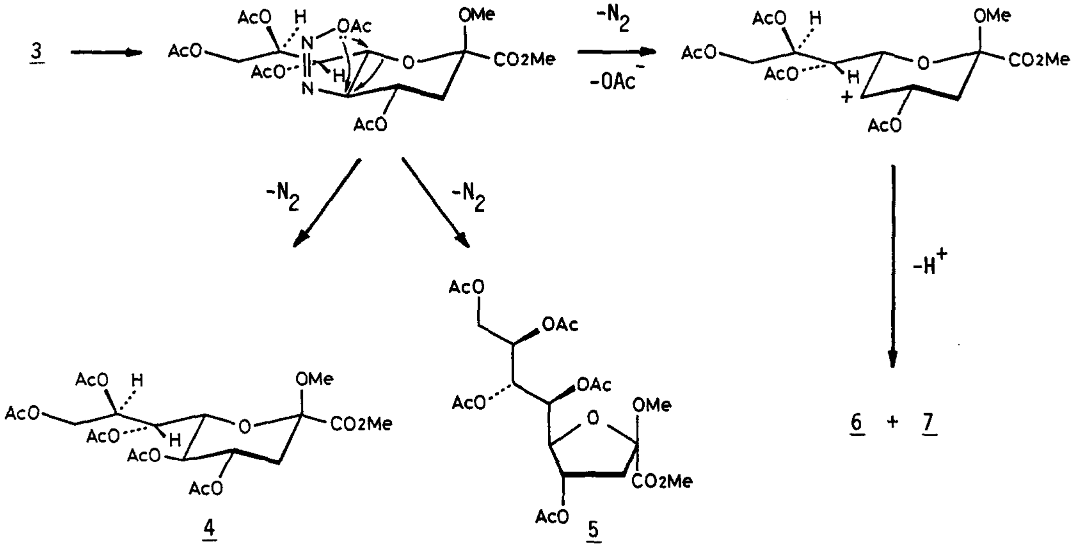
White reported that this type of thermal rearrangement in non-polar solvent retained its stereochemistry at the chiral center.6) In our case, however, abnormally rearranged isomer 5 was formed. Possible pathways of this reaction are depicted in Scheme 2.

The desired product 4 was deprotected to afford KDN (1) as shown in Scheme 3. Thus, KDN (1) was synthesized from *N*-acetylneuraminic acid. ^{1}H- and ^{13}C-NMR spectra of KDN ammonium salt (10) are in good agreement with those reported earlier.5) The structure of deacetylated compound (8), which was firstly synthesized by Inoue *et al*. from *N*-glycolylneuraminic acid and from PSGP of rainbow trout eggs,4) was also determined by X-ray crystallographic analysis for complete determination of its stereochemistry. The molecular structure of 8 is illustrated in Figure 1.7)

Scheme 1



Scheme 2



Scheme 3

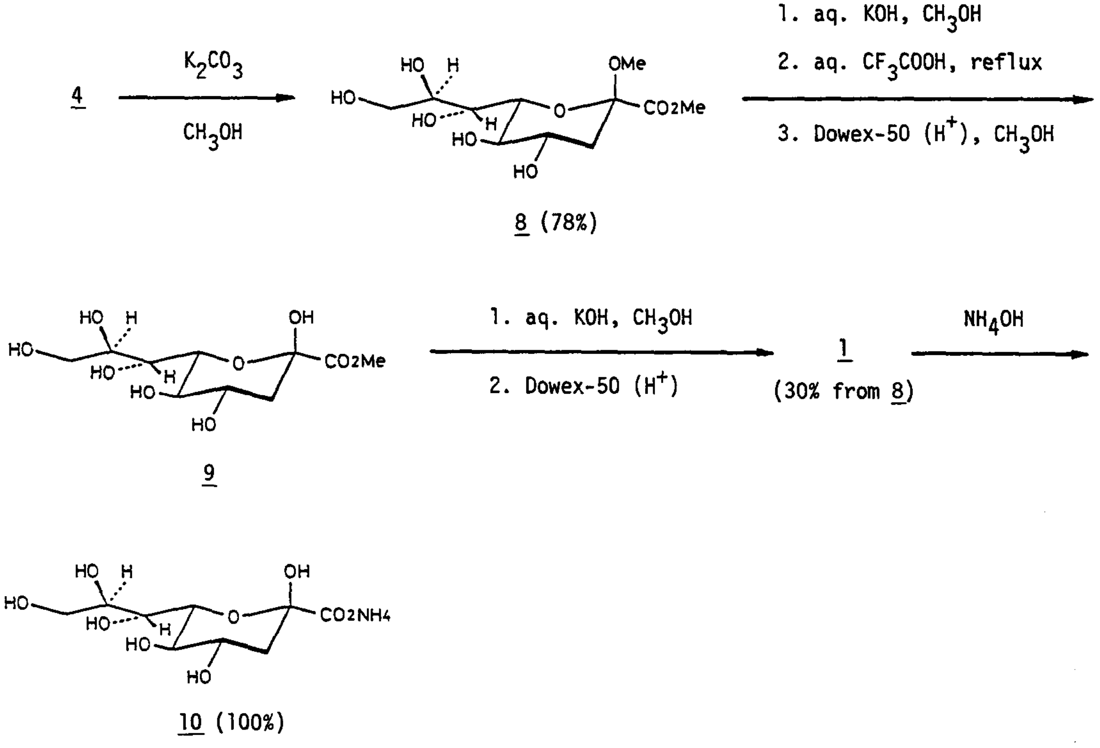
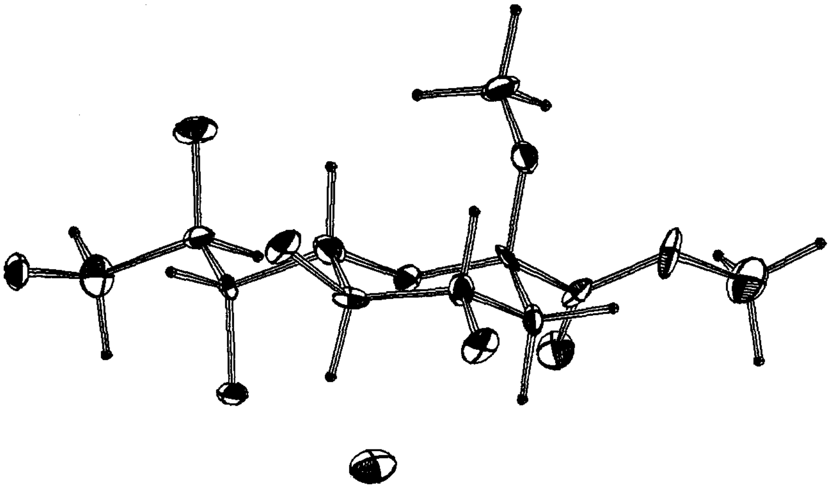


Figure 1 Perspective Drawing of 8



Acknowledgements This work was supported in part by Grants-in-Aid for Scientific Research (No. 61010096, No. 62771852, No. 63470129) from the Ministry of Education, Science and Culture, and by Research Aid of Inoue Foundation for Science. N-Acetylneuraminic acid was supplied by MECT., Ltd..

References and Notes

1) This communication is dedicated to Prof. Bunsuke Umezawa (Faculty of Pharmaceutical Sciences, Science University of Tokyo) deceased on May 24, 1988.

2) Part XII of the series ''Studies on Sialic Acid'' (for part XI, S. Sato, K. Furuhata, M. Itoh, Y. Shitori, and H. Ogura, Chem. Pharm. Bull., 36, 914 (1988).

3) R. Shirai, M. Nakamura, H. Takayanagi, and H. Ogura, Presented at the 16th International Symposium on the Chemistry of Natural Product (IUPAC), May 29 - June 3, Kyoto, Japan

4) D. Nadano, M. Iwasaki, S. Endo, K. Kitajima, S. Inoue, and Y. Inoue, J. Biol. Chem., 261, 11550 (1986).

5) C. Augé and C. Gautheron, J. Chem. Soc., Chem. Commun., 1987, 859.

6) E. H. White, J. Am. Chem. Soc., 77, 6003 (1955).; idem, ibid., 77, 6011 (1955).; idem, ibid., 77, 6014 (1955).

7) The crystal data were : 8, $C_{11}H_{20}O_9$, Mol. Wt. = 296.3. Orthorhombic, space group $P2_12_12_1$, Z = 4, Lattice constants, a = 8.568 (1), b = 25.782 (4), c = 6.732 (1) Å, V = 1487 $Å^3$, D_{calc} = 1.404 gcm^{-3}, μ for $CuK\alpha$ = 1.17 cm^{-1}. A total of 1618 reflections were measured as being above the 3σ (I) level in the 2θ range of 0 - 140°. The crystal structure was determined by the direct method and atomic parameters were refined by least squares calculations with block-diagonal matrix approximations. The final R value was 0.092 for 924 reflections including 15 hydrogen atoms, for which isotropic thermal parameters were assigned. The final atomic parameters will be deposited with the Cambridge Crystallographic Data Center.

(Received in Japan 18 March 1988; accepted 28 June 1988)