Review

Development of miracle medicines from sialic acids

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(Communicated by Satoshi ŌMURA, M.J.A.)

Abstract: Sialic acids are electronegatively charged C9-sugars and are considered to play important roles in higher animals and some microorganisms. Denoting their significance, understanding and exploiting the complexity of the sialic acids has been referred to as the "the third language of life". In essence, "sialic acid derivatives possess a harmonious shape and good balance between two opposing hydrophilic and hydrophobic parts, meaning that they should display various kinds of potentially unique and possibly conflicting physiological activities (glycolipoids)". Consequently, there are good omens that unprecedented 'miracle' medicines could be developed from sialic acid derivatives. In this review, the first problem, the preparation of sialic acids, is covered, the synthesis of sialic acid derivatives and confirmation of their structures obviously being of critical significance. In addition we needed to confirm their precise stereochemistry and a hydrolysis method has been developed for confirmation of the anomeric position. Several of the compounds have already demonstrated interesting bioactivity.

Keywords: biological activities, DSC, glycosylation, KDN, neuraminic acids, stereochemistry

1. Introduction

This review summarizes typical sialic acids (**1**–**5**) not only for preparations of derivatives, stereochemical determinations, but also physiological activities. Working strategy of this research work is "sialic acids derivatives having good shape of molecule (GLYCOLIPOID[1)]) would elicit physiological activity".[2),3)]

Sialic acids are known important molecules for the human life activities, and also for higher animals, and some microorganisms.[4),5)] Professor Tamio Yamakawa is a pioneer of sialic acids research in Japan.[6)]

2. Preparation of sialic acids

2-1. *N*-Acetyl-D-neuraminic acid (1: Neu5Ac). Most important sialic acid, "*N*-acetyl-D-neuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonic acid)" (**1**) was obtained from edible bird's (*Collocalia* sp.) nest in 5–10% yields by hydrolysis with dil. sulfuric acid.[7),8)] Edible bird's nest was obtained from Chinese food grocery. Structure of the nest mucin was described by Wieruszeski[9)] and Strecker *et al.*,[10)] and for *N*-glycans by Yagi *et al.*[11)]

Configuration of both crystals (fine needles and prisms) of Neu5Ac were confirmed as the *β*-form, by means of IR, CD, and CP-MASS NMR spectra.[12),13)] On the other hand, Neu5Ac in an aqueous solution exists in equilibrium of 5–8% of *α*-anomer and 92–95% of *β*-anomer. Further equilibrium studies of Neu5Ac are summarized in Fig. 2.

Reaction of Neu5Ac (**1**) with alkyl halide gave the corresponding *N*-acetyl-2-*O*-alkyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone [A, C]. On the other hand, acylation of Neu5Ac (**1**) with usual procedures gave 1,7-lactones[14)] [B], and also 2,7-anhydroneuraminic acid (**5**)[15)] [D]. These phenomena are strongly suggested the equilibrium of Neu5Ac (**1**) summarized in Fig. 2.

2-2. *N*-Glycolyl-D-neuraminic acid (2: Neu5Gc). Neu5Gc (**2**) is a sialic acid of some mammals such as pig, equine, rat and some kinds of dog. Also Neu5Gc is important about aging and some diseases such as cancer.[5),16),17)]

Neu5Gc was prepared from Neu5Ac (**1**) as shown Fig. 3 in 20% of overall yields.[18)] A convenient

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doi: 10.2183/pjab.87.328

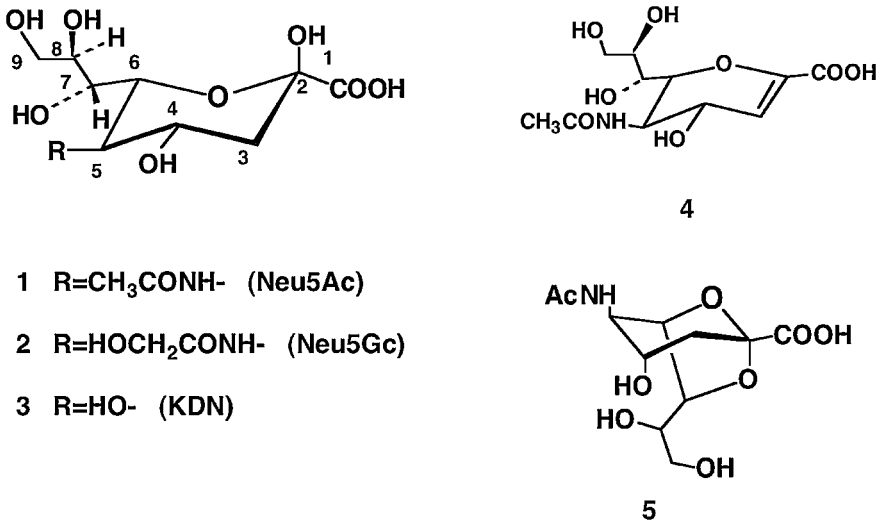


Fig. 1. Typical sialic acids.

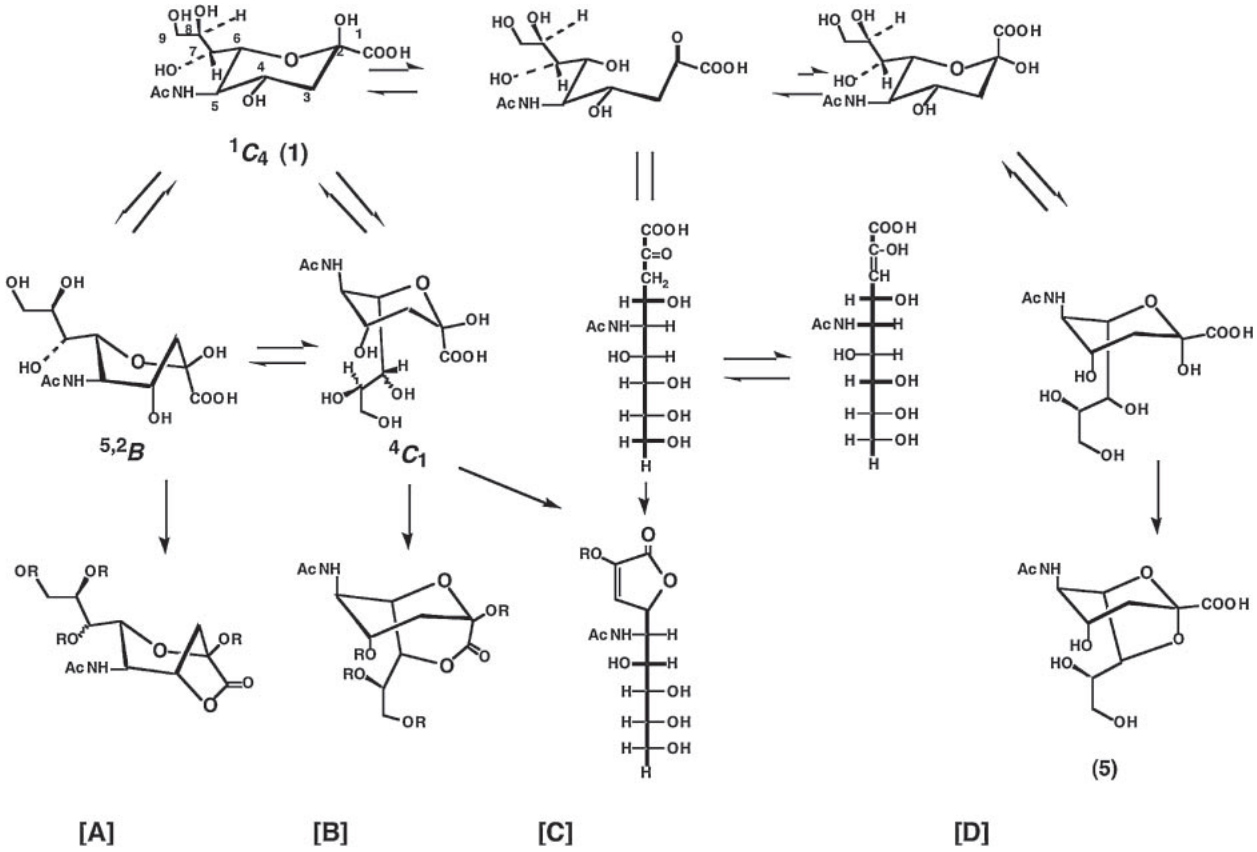


Fig. 2. Equilibrium of Neu5Ac (**1**).

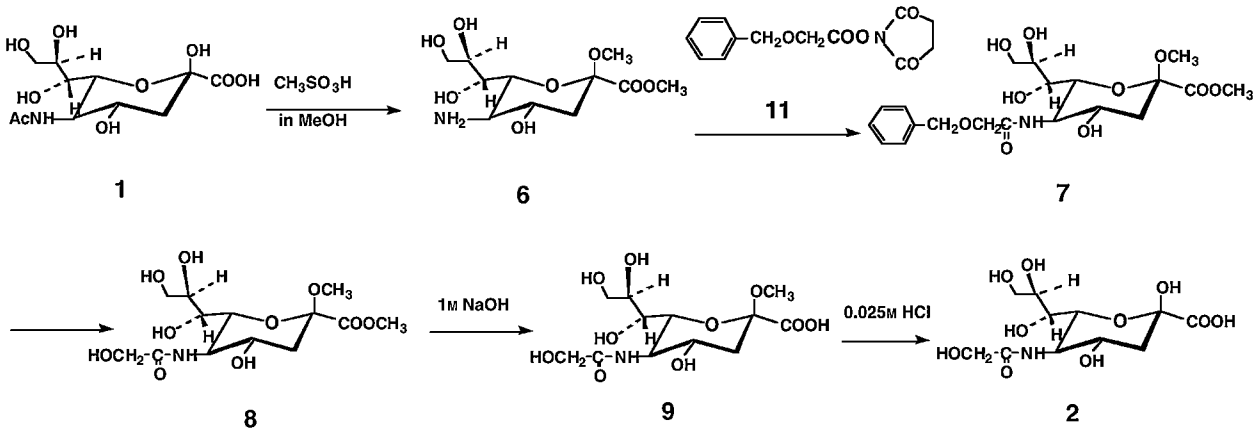


Fig. 3. Synthesis of Neu5Gc (**2**).

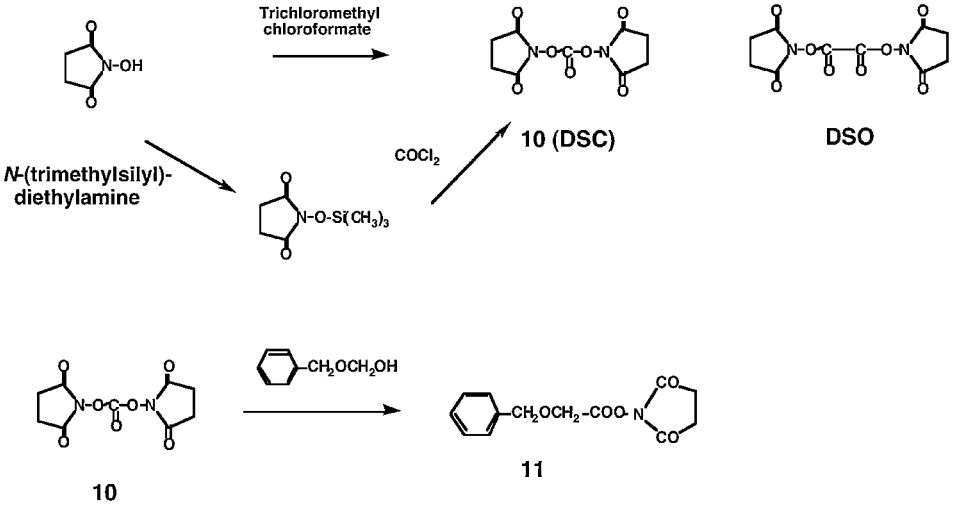


Fig. 4. Synthesis of DSC (**10**).

active ester synthetic reagent *N*,*N*′-disuccinimidyl carbonate (DSC)[19)–21)] was used for active ester synthesis.

N,*N*′-Disuccinimidyl carbonate (**10**: DSC) is prepared as the convenient reagent for active ester and for peptide synthesis, from *N*-hydroxysuccinimide and trichloromethyl chloroformate or *N*-(trimethylsilyl)diethylamine with phosgene.[19)–21)] *N*,*N*′-Disuccinimidyl oxalate (DSO) is also useful for the same purpose.[22)] DSC and DSO are used in the world for peptide and lactam syntheses.[22)–24)]

2-3. 3-Deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (3: KDN). KDN (**3**) was found from unfertilized rainbow trout eggs by Inoue *et al.*[25)] KDN was also obtained from fertilized eggs of chum salmon.[26)] KDN (**3**) was synthesized starting from Neu5Ac by thermal rearrangement of *N*-acetyl-*N*-nitrosoneuraminic acid derivative followed by deprotection.[27)] Structure of methyl glycoside of KDN was confirmed by X-ray analysis.[27)]

Condensation of oxalacetic acid with D-mannose, followed by decarboxylation with nickel chloride as a catalyst gave KDN (**3**) in 70% of yield.[28)] Similar procedure was adopted to D-arabinose, 3-deoxy-D-*manno*-2-octulosonic acid (**14**: KDO) was obtained in 66% of yield.[28)]

KDN has hydroxyl group instead of acetamido group at 5-position of Neu5Ac. As shown in Fig. 7, methyl 3-deoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**15**) was treated with Dowex-50(H^+), in methanol followed by acetic anhydride treatment to yield four compounds. Pyranose-type (**16a,b**) and

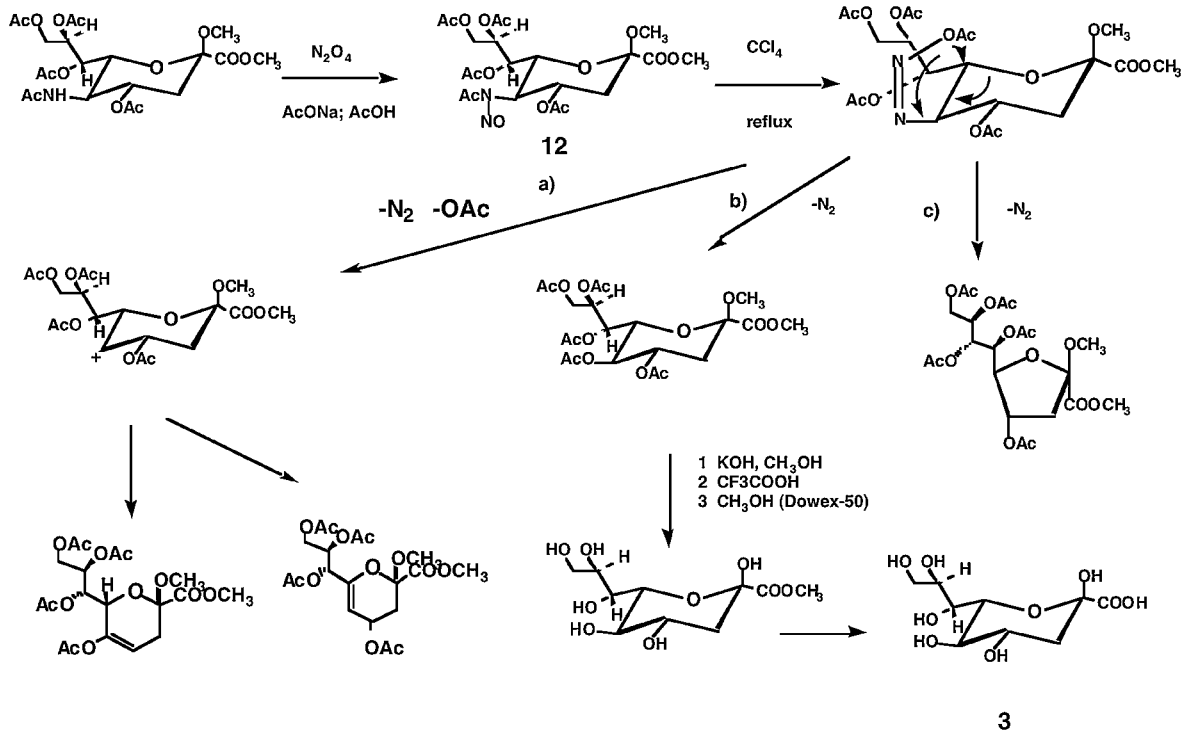


Fig. 5. Synthesis of KDN from Neu5Ac by thermal rearrangement.

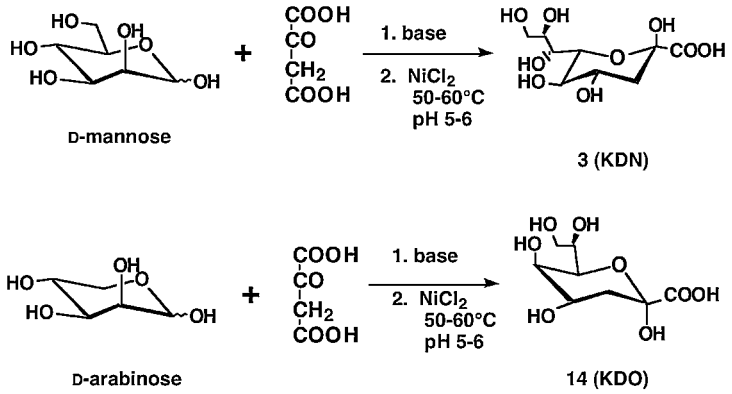


Fig. 6. Synthesis of KDN and KDO.

furanose-type (**17a,b**) compounds were obtained. These structures were confirmed by NMR and X-ray crystallography.[29)]

These experiments strongly suggest that equilibrium of KDN is summarized as shown in Fig. 8.

2-4. 5-Acetamido-2,6-anhydro-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (4; Neu2en5Ac). Neu2en5Ac (**4**) is widely distributed in nature, and have some biological activities.[5)] Methyl 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**18**) was treated with acetic anhydride-sulfuric acid at room temperature to yield Neu2en4,5,7,8,9Ac$_5$ (**19**), and then hydrolyzed to Neu2en5Ac (**4**) as prisms. Structure of Neu2en5Ac was confirmed by X-ray analysis.[30)] The same reaction proceeds at 80 °C, epi-derivative (**20**) was mainly obtained accompanying small amount of by-products (**21**, **22**).

Hydrogenation of methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2,3-dehydro-2-deoxyneuraminate (**19**) with

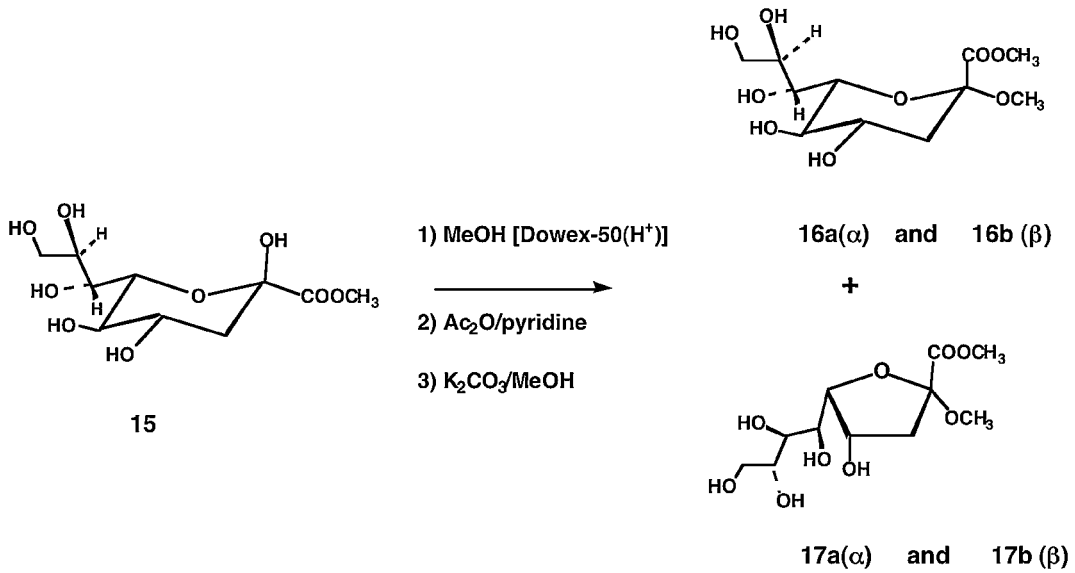


Fig. 7. Methylation of methyl 3-deoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**15**).

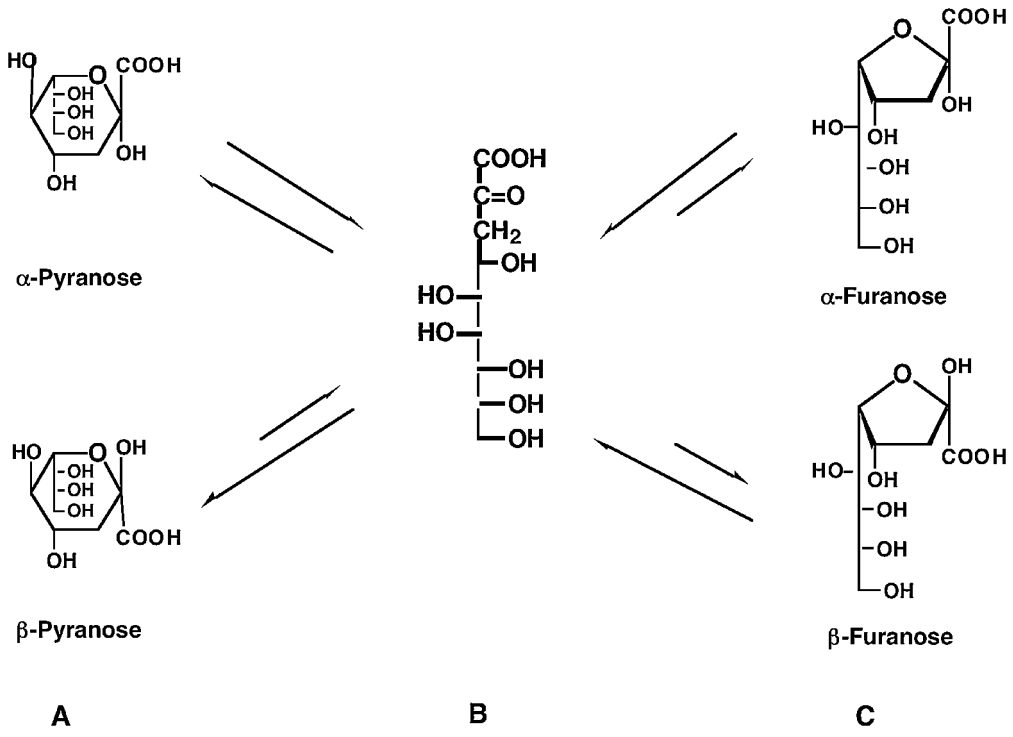
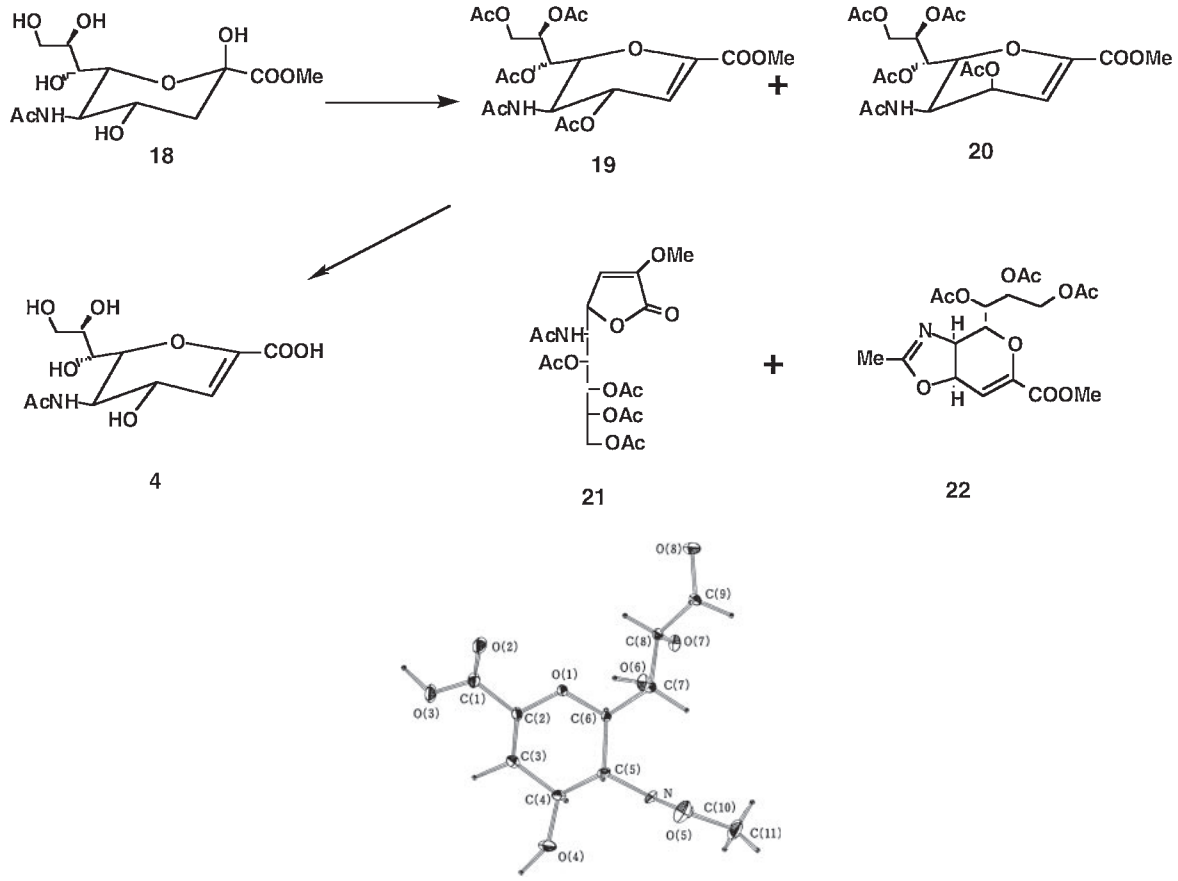


Fig. 8. Equilibrium of KDN (**3**).

platinum oxide under the hydrogen atmosphere yielded 4-deoxy derivative (**23**), and the same compound also obtained from 4-epi-derivative (**20**). Further hydrogenation of these compounds (**19**, **20**, **23**) gave methyl 7,8,9-triacetyl-*N*-acetyl-2,4-dideoxyneuraminate (**25**), further treatment of this compound with 1 mol/L sodium hydroxide afforded *N*-acetyl-2,4-dideoxyneuraminic acid (**27**).[30]

Hydrogenation of **19** and **20** with Pd-C under the hydrogen atmosphere yielded corresponding



A perspective view of Neu2en5Ac (**4**)

Fig. 9. Synthesis of Neu2en5Ac.

saturated compounds **24** and **26**, respectively, as shown in Fig. 10.[31)]

2-5. 2,7-Anhydro-neuraminic acid.

2-5-a. 2,7-Anhydro-N-acetylneuraminic acid (5). 2,7-Anhydro-*N*-acetylneuraminic acid (**5**) was isolated by Suzuki *et al.*[32)] from wet type cerumen. Li *et al.*[33)] reported that leeches contain novel sialidases releasing 2,7-anhydroNeu5Ac quantitatively from α-sialosyl-glycoconjugate.

Preparation of **5** from Neu5Ac (**1**) *via* methyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-2-nonulopyranosonate[34),35)] using 1,1-bis[6-(trifluoromethyl)benzotriazolyl] carbonate (BTBC)[36),37)] as summarized in Fig. 11.

On the other hands, when *S*-methyl glycoside (**34**)[38)] of Neu5Ac was used, 2,7-anhydro derivative (**5**) was obtained in 50% of overall yield.[39)]

2-5-b. 2,7-Anhydro-N-glycolylneuraminic acid (40). 2,7-Anhydro-*N*-glycolylneuraminic acid (**40**; 2,7-anhydro-*N*-glycolyl-3,5-dideoxy-α-D-*glycero*-β-D-*galacto*-2-nonulopyranosonic acid) was prepared starting from methyl 5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**34**),[38)] through hydrolysis, benzylation, and benzoylation reaction to yield benzyl [methyl 5-*N*-(*O*-benzylglycolyl)-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate (**38**) as an intermediate. Intramolecular glycosylation of **38** was performed with dimethyl(methylthio)sulfonium triflate (DMTST) to yield 4,9-di-*O*-benzoyl derivative (**39**). After removal of benzyl and benzoyl group, 2,7-anhydro-*N*-glycolylneuraminic acid (**40**) was obtained as shown in Fig. 12.[39)]

3. Preparation of sialic acids derivatives

3-1. Ester and lactone formation.

3-1-a. Esterification (1,4-lactone). Neu5Ac (**1**) was treated in methanol under reflux with Dowex-50 (H^+) to yield methyl (methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranosid)onate

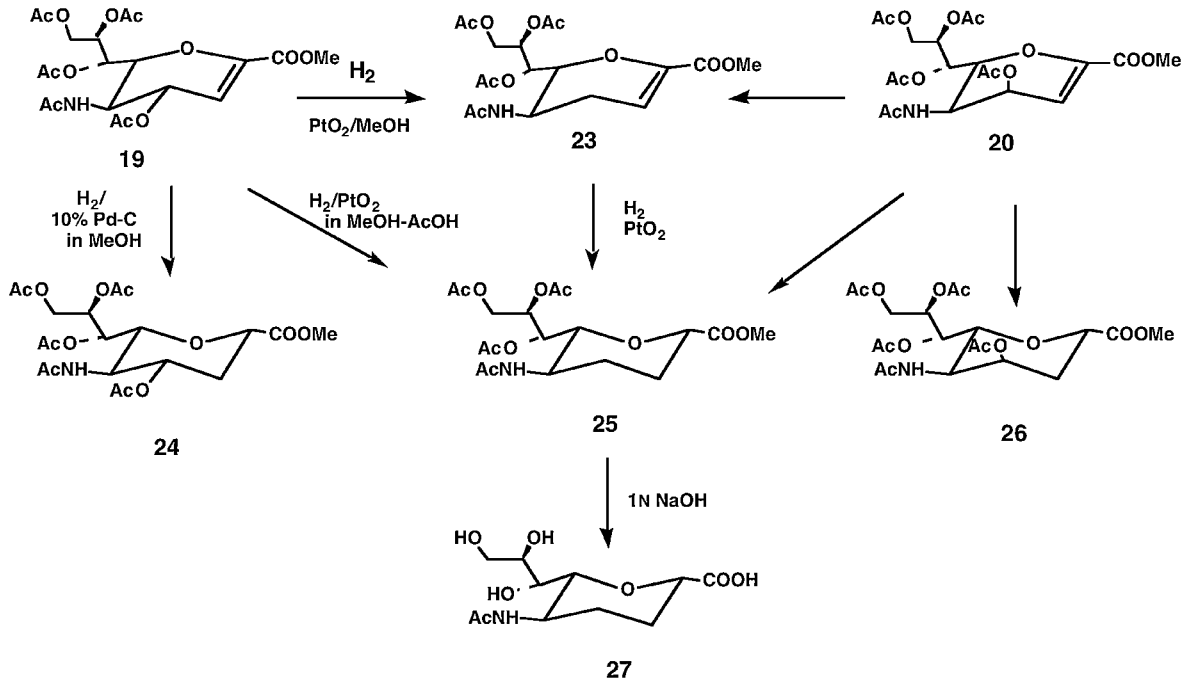


Fig. 10. Reaction of 2,3-dehydro-2-deoxyneuraminic acid (**4**).

(**42**).[8] On the other hand, the reaction was performed under room temperature to yield methyl *N*-acetyl-*β*-D-neuraminate (**41**) in 86% of yield.[8]

When diazomethane was used for formation of methyl ester, compounds **43a** and 1,4-lactone (**44a**) were formed *via* intramolecular cyclizaion of A→B→C. On the other hand, benzyl 5-acetamido-3,5-dideoxy-*β*-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**43b**) was obtained from cesium salt of Neu5Ac and benzyl bromide in good yield. Further treatment of **43b** with cesium carbonate and benzyl bromide, or Neu5Ac (**1**) being treated with excess amount of cesium carbonate and benzyl bromide, gave 5-acetamido-2-*O*-benzyl-3,5-dideoxy-*β*-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**44b**).[15]

Methylation of Neu5Ac (**1**) with methyl iodide yielded 5-acetamido-2-*O*-methyl-3,5-dideoxy-*β*-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**44a**) in fairly good yield. Further, acetylation of **44a** afforded 5-acetamido-6,7,8,9-tetra-*O*-acetyl-2-*O*-methyl-3,5-dideoxy-*β*-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**45**).[14]

Structure of 1,4-lactone was confirmed by means of X-ray analysis of **45** as shown in Fig. 14.

3-1-b. Acylation (1,4-lactone; 1,7-lactone). Acetylation of Neu5Ac (**1**) with acetic anhydride at room temperature, there was obtained 2,4,7,8,9-*penta*-*O*-acetyl-*N*-acetylneuraminic acid (**48**) in 90% yield. Purification of the reaction residue, there was obtained a small amount (6%) of 5-acetamido-2,4,8,9-tetra-*O*-acetyl-3,5-dideoxy-*β*-D-*glycero*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**49**).[40]

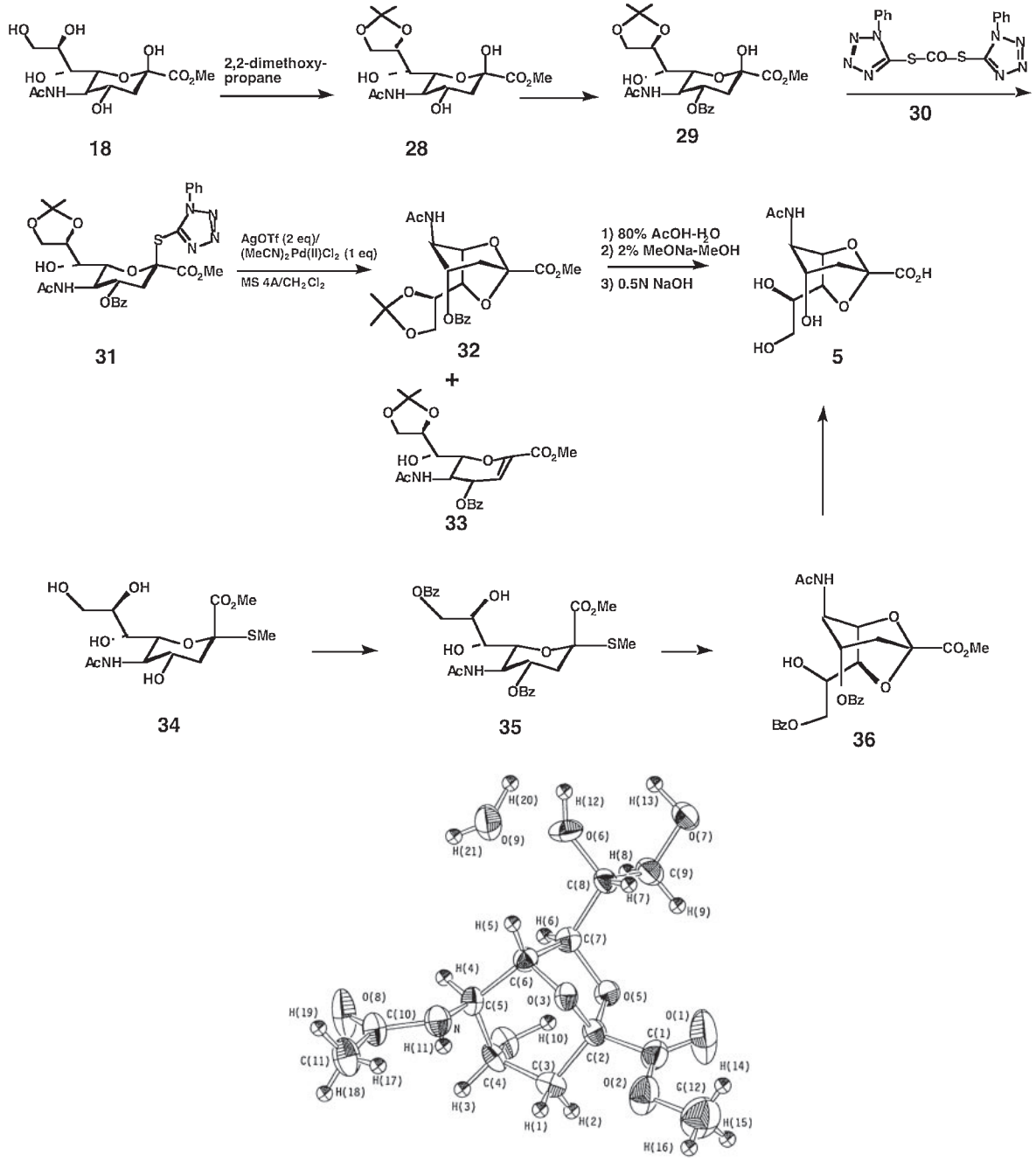
Structure of 1,7-lactone (**49**) was confirmed by means of X-ray analysis as shown in Fig. 16.[40]

Benzoylation of Neu5Ac with benzoyl chloride gave per-*O*-benzoylated 1,7-lactone derivative (**50**) together with small amount of per-*O*-benzoylated 1,4-lactone (**51**) and 2,8,9-tri-*O*-benzoylated 1,7-lactone. The 4 and 7 positions are low reactivity owing to the steric hindrance.

Furthermore, benzoylation with benzoic anhydride gave 2-*O*-benzoylated 1,7-lactone in about 50% yield, while the use of excess amount of reagent, 2,9-di-*O*-benzoylated 1,7-lactone and 2-*O*-benzoylated 1,7-lactone were formed as shown in Fig. 17.

When acylation was performed with pivaloyl chloride, main product is 5-acetamido-2,4,8,9-tetra-*O*-pivaroyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-nonulopyranosono-1,7-lactone (**52**) and small amount of 2,4,9-tri-*O*-substituted and 2,8,9-tri-*O*-substituted compounds were obtained.

Ethoxycarbonylation of Neu5Ac with ethyl chloroformate gave 5-acetamido-2,8,9-tri-*O*-ethoxycarbonyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**53**) and 5-acetamido-2,7,8,9-tetra-*O*-ethoxycarbonyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,4-lactone (**54**).



A perspective view of methyl *N*-acetyl-2,7-anhydroneuraminate (5-Me)

Fig. 11. Synthesis of 2,7-anhydro-*N*-acetylneuraminic acid (**5**).

Treatment of **54** with methanol converted to methyl 5-acetamido-2,7,8,9-tetra-*O*-ethoxycarbonyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**55**).[15)]

Structures of these products were confirmed by means of NMR spectra.

3-1-c. Acylation of 4-position. Treatment of Neu5Ac1Bn (**43b**) with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid gave benzyl 5-acetamido-8,9-*O*-isopropylidene-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate in good yield. Further acetylation of this compound gave

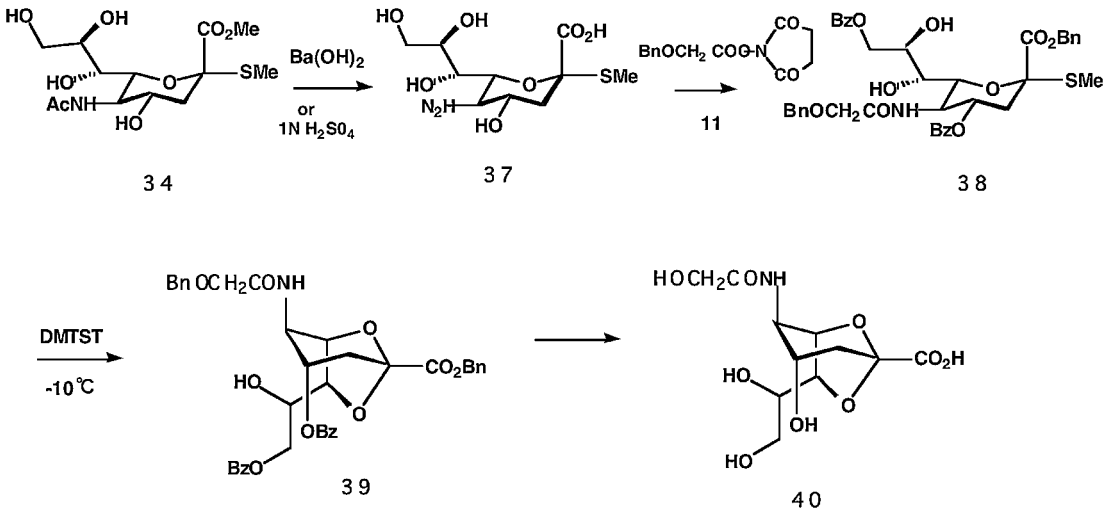


Fig. 12. Synthesis of 2,7-anhydro-*N*-glycolylneuraminic acid (**40**).

benzyl 5-acetamido-4-*O*-acetyl-8,9-*O*-isopropylidene-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**56**). Removal of the *O*-isopropylidene group with acetic acid treatment and then hydrogenolyzed to yield 5-acetamido-4-*O*-acetyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonic acid (**57**).[41]

3-1-d. Acylation of 7-position. Treatment of methyl *N*-acetylneuraminate (**41**) with 2,2-dimethoxypropane and then with *tert*-butyldimethylsilyl chloride gave methyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-*tert*-butyldimethylsilyl-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**58**).

Further acetylation of **58** afforded only 2-acetylated compound (**59**). Thus, the hydroxyl group of 7-position is less reactive than that of 2-position.

Treatment of *α*-methyl glycoside of methyl *N*-acetylneuraminate (**60**) with 2,2-dimethoxypropane gave 8,9-*O*-isopropylidene derivative (**61**), further treatment with *tert*-butyldimethylsilyl chloride obtained 4-*O*-*tert*-butyldimethylsilyl derivative (**62**). Acetylation of **62**, and then was deprotected with acetic acid to give methyl (methyl 5-acetamido-7-*O*-acetyl-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosid)onate (**64**).[42]

3-1-e. Acylation of 9-position. Various 9-*O*-acyl derivatives of Neu5Ac were synthesized by use of ortho esters such as trimethyl orthoformate, trimethyl orthoacetate, trimethyl orthobutyrate, trimethyl orthovalerate, and trimethyl orthobenzoate to give the corresponding 9-*O*-acylated derivatives in fairly good yields. Structures of these compounds (**65 a**–**e**) were confirmed by NMR spectra. Regioselective acylation clearly suggested that the formation of the internal ortho esters as shown in Fig. 20.[41]

3-2. Glycosylation of Neu5Ac.

3-2-a. Glycosyl donor of Neu5Ac. Neu5Ac (**1**) was refluxed in methanol under the presence of Dowex-50 (H^+), *β*-methyl glycoside (**42**) was obtained. On the other hand, under the room temperature condition, methyl *N*-acetyl-*β*-D-neuraminate (**41**) was obtained in 86% of yield.[8] Further, direct treatment with acetyl chloride, methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2-chloro-2-deoxy-*β*-D-neuraminate (**66**) was obtained in 95% of yield as crystals. This compound is the most important intermediate as glycosyl donor. Methanol treatment of the chloride (**66**) gave *α*-glycoside (**67**), further deacetylation with potasium methoxide to yield methyl (methyl 5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-nonulopyranosid)onate (**68**).[8]

Reduction of **68** with sodium borohydride yielded methyl 5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-nonulopyranoside (**69**). On the other hand, methyl 5-acetamido-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-nonulopyranoside (**70**) was prepared from the *β*-anomer (**42**).[8]

3-2-b. S-Glycosyl donor of Neu5Ac. Neu5Ac *S*-glycosyl donor was prepared by use of *S*,*S*′-bis(1-phenyl-1*H*-tetrazol-5-yl) dithiocarbonate (**30**). The reagent is prepared conveniently from 1-phenyl-5-thioxo-4,5-dihydro-1*H*-tetrazole and trichloromethyl chloroformate in 77% of yield.[37] Structure of this reagent was confirmed by means of X-ray analysis (Fig. 23).

Reaction of the reagent (**30**) with allylic alcohols gave 1-phenyltetrazole-5-thio allylic sulfides,

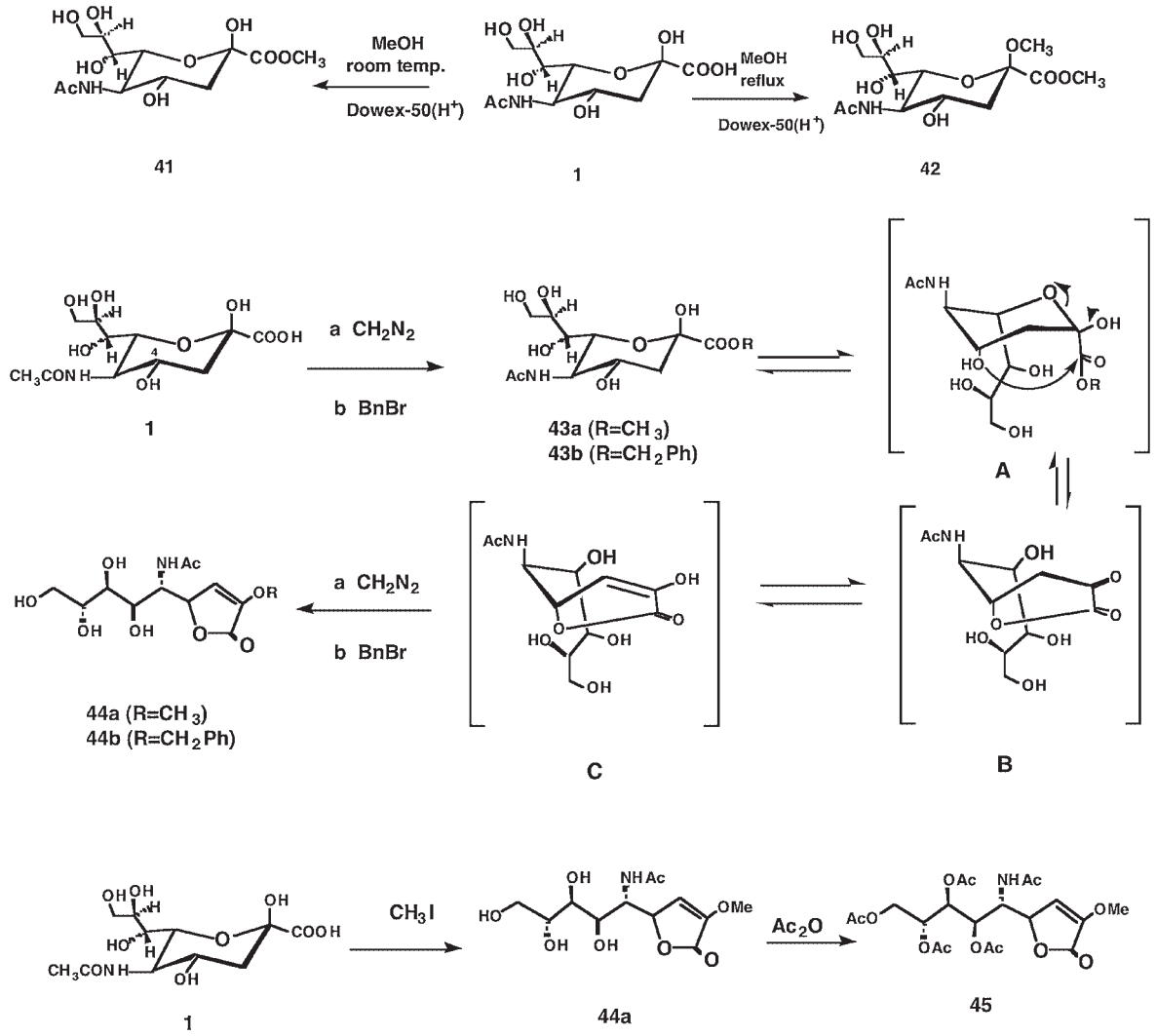


Fig. 13. Esterification of Neu5Ac (**1**).

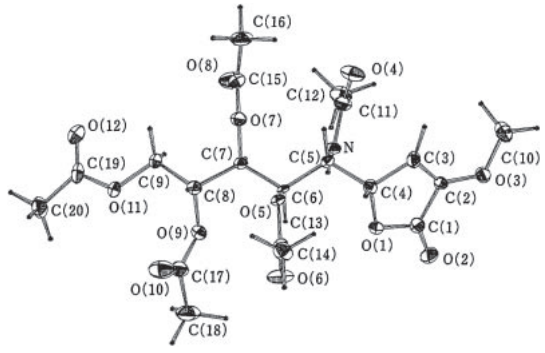


Fig. 14. A perspective view of 5-acetamido-6,7,8,9-tetra-*O*-acetyl-2-*O*-methyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**45**).

and further treatment with Grignard reagents yielded carbon–carbon bond formation product [A].[43),44)]

Reaction of the reagent (**30**) with amines gave isothiocyanates [B], and with carboxylic acids yielded amides [C], esters [D], carbonyl compounds [E] and many kinds of heterocycles [F].[44),45)]

Reaction of BDTC (**30**) with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose gave *S*-1-(1′-phenyl-1*H*-tetrazolyl) 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranose (**71**) by an one-step reaction. Glycosylation of alcohols (methanol, cyclohexanol, cholesterol, and sugars) with **71** gave glycosides (**72**) in good yields (Table 1).[45),46)]

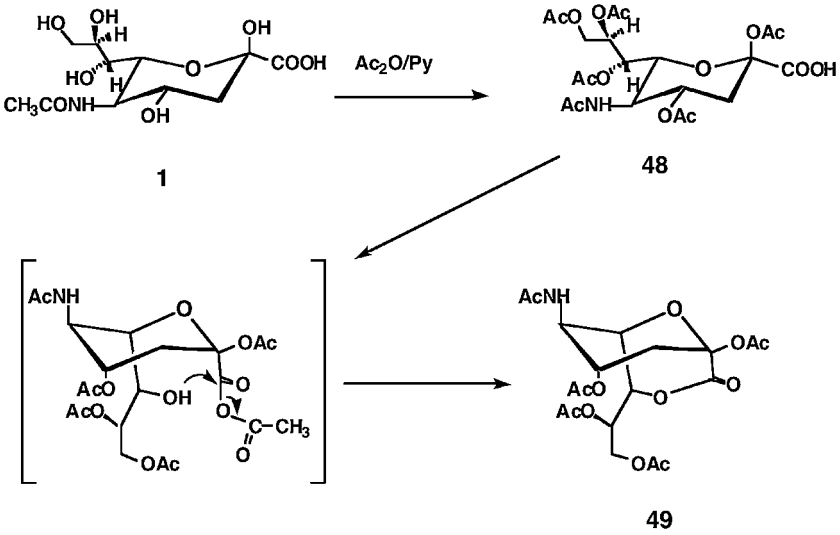


Fig. 15. Acetylation of Neu5Ac (**1**).

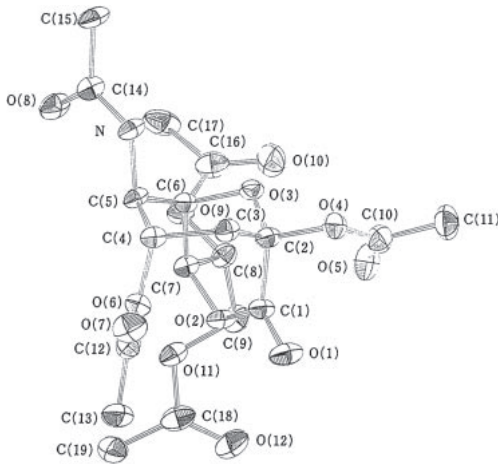


Fig. 16. A perspective view of 5-acetamido-2,4,8,9-tetra-*O*-acetyl-3,5-dideoxy-*β*-D-*glycero*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**49**).

Sialic acid *S*-glycosyl donor (**73**) was prepared efficiently in one step reaction with BDTC (**30**) and methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate to yield stable *S*-glycosyl donor, methyl [1-phenyl-1*H*-tetrazol-5-yl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-*α*- and -*β*-D-*galacto*-2-nonulopyranosid]onate (**73**).[34),46)]

Reaction time of the *S*-glycosyl donor (**73**) with methanol in nitromethane under the presence of Hg-triflate is shorter than dichloromethane solvent. It may be considered that the reaction proceeds owing to the solvent effect, and using silver or mercury triflate occurs *via* an S_N2-like mechanism.[47)]

Further glycosylation of **73** with alcohols gave glycoside, such as methyl, sialosyl-(2→6′)-lactosyl, and cholesteryl derivatives (**74**).[48)]

3-2-c. Disaccharide nucleosides. Glycosylation of Neu5Ac (**1**) by Koenigs–Knorr reaction using key intermediate (**66**) was performed. When an insoluble promoter was used *α*-glycoside was formed, instead, when soluble promoter was used, gave equal amounts of *α*- and *β*-glycosides.[8)]

Koenigs–Knorr reaction of 2′,3′-*O*-isopropylideneuridine with the chloride (**66**) in the presence of mercuric cyanide as a catalyst gave *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→5′)-2′,3′-*O*-isopropylideneuridine (**75a**: R = H) and its 5-fluorouridine derivatives (**75b**: R = F) and their *β*-anomers (**76a**: R = H and **76b**: R = F). Both compounds were treated with 1 mol/L sodium hydroxide to yield corresponding disaccharide nucleosides (**77a**: R = H, **77b**: R = F and **78a**: R = H, **78b**: R = F).[8)]

When silver perchlorate and silver carbonate were used as the catalyst, *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosyl)onate]-(2→N^3)-2′,3′-*O*-isopropylideneuridine (**79**) was obtained in stead of the *β*-anomer. In each case, Neu2en4,5,7,8,9$Ac_5$1Me (**19**) was formed.

Koenigs–Knorr reaction of 2′,3′-di-*O*-acetylinosine with the chloride (**66**) as a glycosyl donor gave

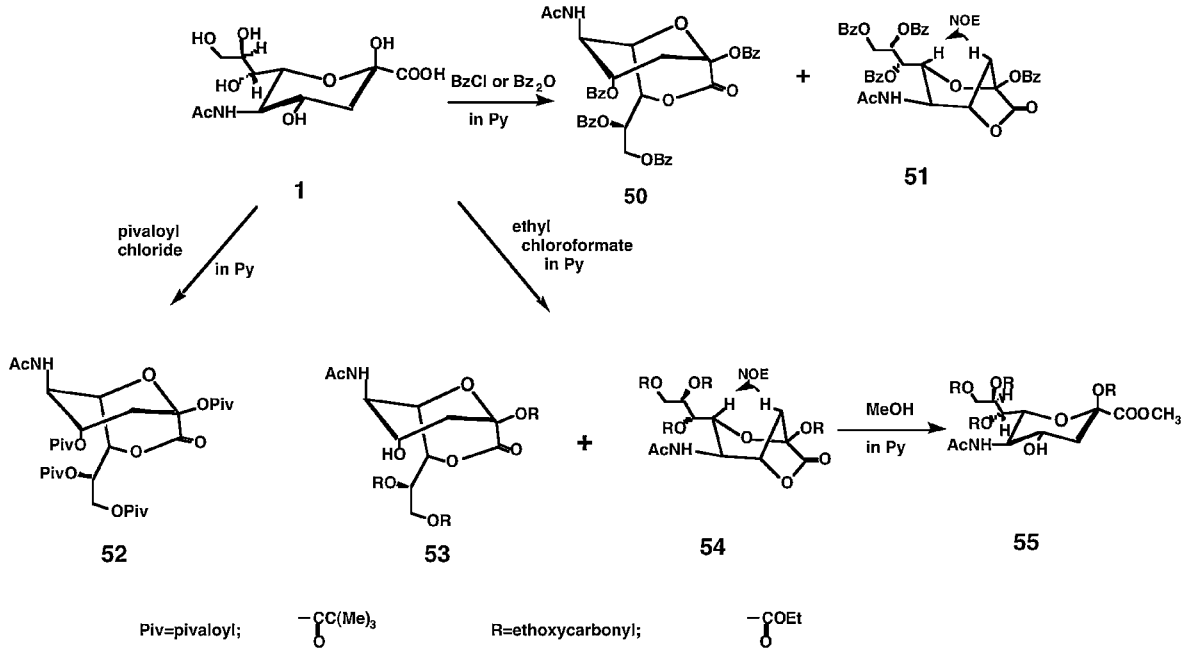


Fig. 17. Acylation of Neu5Ac (**1**).

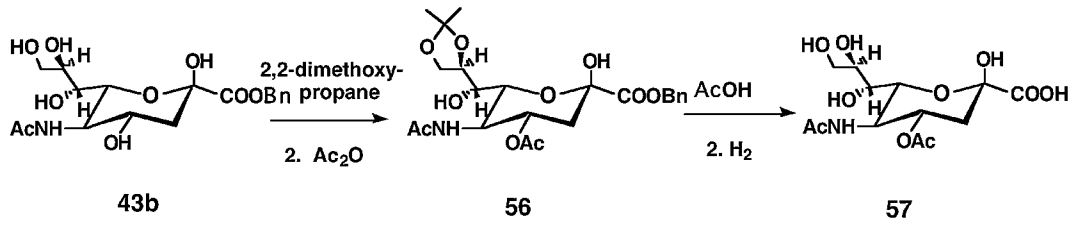


Fig. 18. Acetylation of 4-position.

methyl [*N*-acetyl-4″,7″,8″,9″-tetra-*O*-acetyl(2′,3′-di-*O*-acetylinosin-5′-yl)-α- and -β-D-neuraminosid]onate (**80a**: α-anomer; **80b**: β-anomer). Similar reaction was adopted to 2′,3′-di-*O*-acetyl-*N*-benzoylcytidin as glycosyl acceptor, also gave methyl [*N*-acetyl-4″,7″,8″,9″-tetra-*O*-acetyl(2′,3′-di-*O*-acetyl-*N*-benzoylcytidin-5′-yl)-α- and -β-D-neuraminosid]onate (**82a**: α-anomer; **82b**: β-anomer) were obtained. In each case, methyl *N*-acetyl-4,7,8,9-tetra-*O*-acetyl-2,3-dehydro-2-deoxyneuraminate (**19**) was formed.[48]

Further saponification of these compounds (**80a**,**b** and **82a**,**b**) gave *N*-acetyl(inosin-5′-yl)-α- and -β-D-neuraminosidoic acids (**81a**,**b**), *N*-acetyl-(cytidin-5′-yl)-α- and -β-D-neuraminosidoic acids (**83a**,**b**).

3-2-d. N-Glycoside nucleosides. *N*-Glycosyl derivatives of Neu5Ac (**1**) were prepared from methyl 2,4,7,8,9-penta-*O*-acetyl-*N*-acetyl-β-D-neuraminate (**84**) with trimethylsilylpyrimidine or 5-fluorotrimethylsilylpyrimidine. There was obtained a 1:1 ratio of anomeric mixture (**85a**,**b** or **86a**,**b**). On the other hand, the chloride (**66**) was used as a starting material, only the β-anomers (**85b**; **86b**) were formed. In this case, methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2,3-dehydro-2-deoxyneuraminate (**19**) was separated.[49]

3-2-e. Mucin analogs. Mucin is one of the important substance in sialoglycoproteins. *N*-Acetylglucosamine treated with acetyl chloride yielded chloride (**87**), followed by treatment with Cbz-serine to yield **88**. Further treatment of this compound (**88**) with triethylamine, trityl chloride, acetic anhydride, and hydrobromide, successively gave **89**, followed by coupling with the chloride (**66**) to yield **90**, and then deacetylation afforded Neu5Acα(2→6)GluNAcβ1→Ser (**91**).[7]

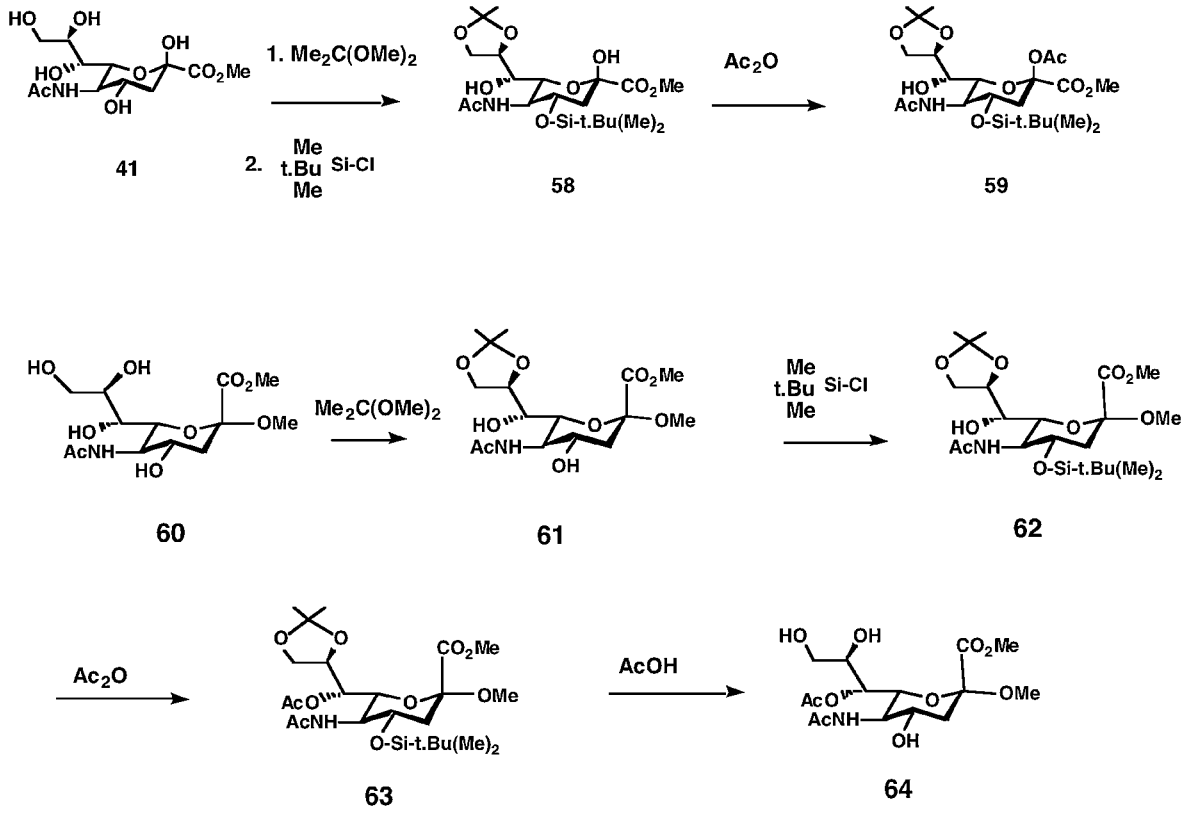


Fig. 19. Acetylation of 7-position.

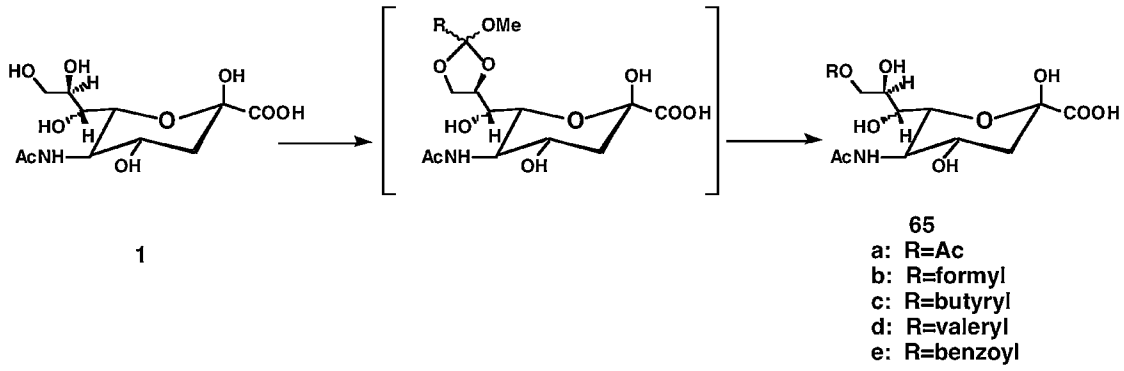


Fig. 20. Acylation of 9-position.

On the other hand, Neu5Acα(2→6)GluNAcα(1→)Ser (**95**) was synthesized from bromo-derivative (**92**) as shown in Fig. 27.[7)]

3-2-f. Sialyllactose. Sialyl oligosaccharides from human, bovine, and rat milk include α(2→3)- and α(2→6)-linked sialyllactose.[5)] α(2→6)Sialyllactose was synthesized from 1,6-anhydro-2,2′,3,3′,4′,6′-hexa-*O*-acetyl-β-D-lactose (**96**) by removing the acetyl group, followed by tritylation, and then benzoylation to give *O*-(2,3,4-tri-*O*-benzyl-β-D-galactopranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (**97**) and reaction with the chloride (**66**) under the Koenigs–Knorr reaction conditions.[50)]

There was obtained the anomeric mixture of the product (**100**). With further treatment of

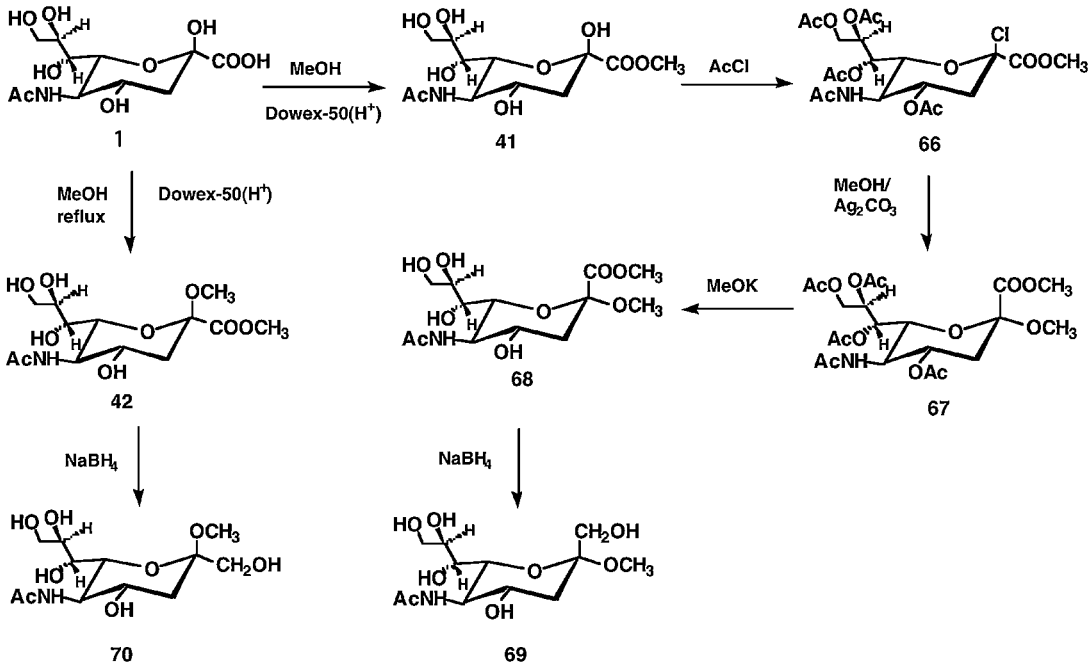


Fig. 21. Synthesis of glycosyl donor (**66**).

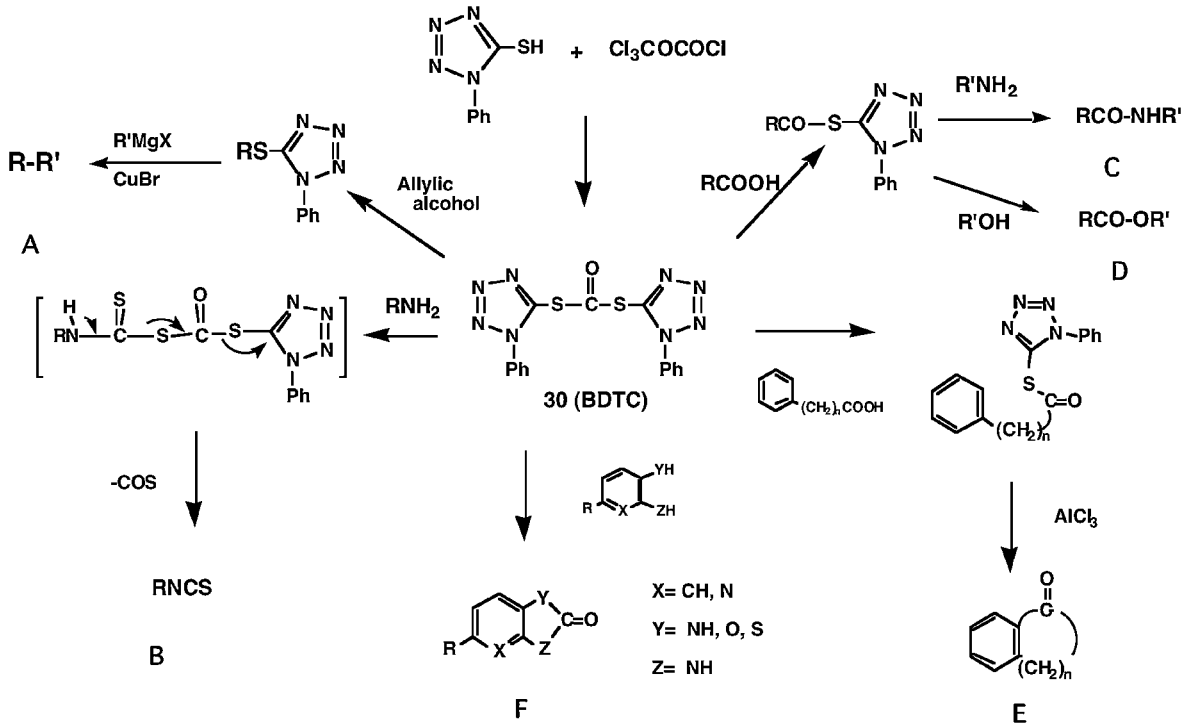


Fig. 22. Synthesis of *S*,*S*′-bis(1-phenyl-1*H*-tetrazol-5-yl)dithiocarbonate (**30**) and its reactions.

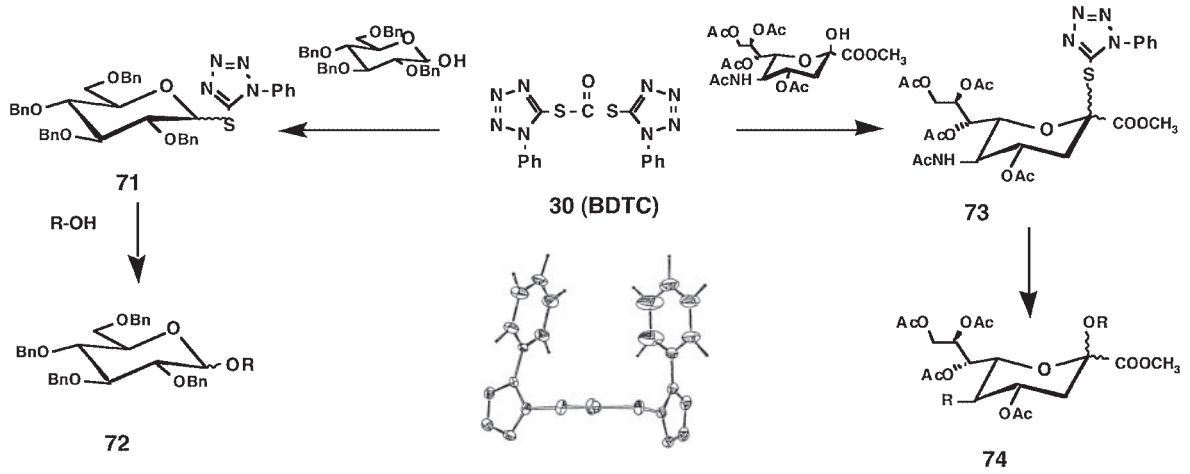


Fig. 23. Synthesis of *S*-glycosyl donor.

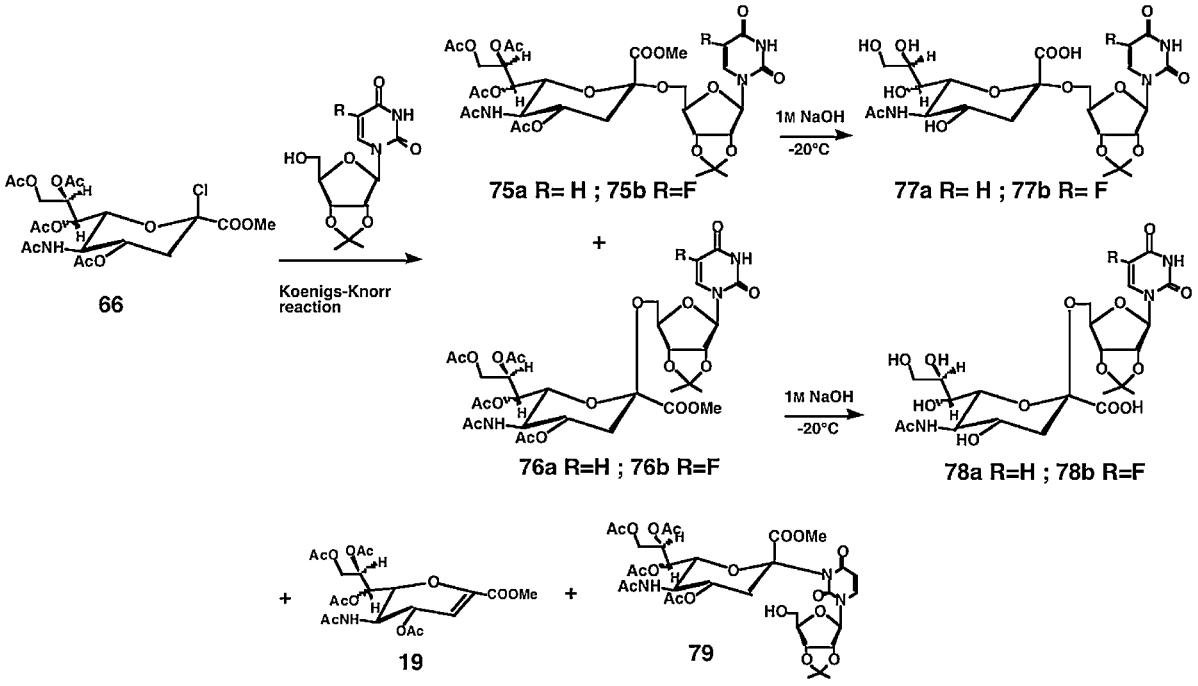


Fig. 24. Disaccharide nucleoside of Neu5Ac (No. 1).

Table 1.

Compound	methanol	cyclohexanol	cholesterol	sugar*	sugar**
Yield (%)	87	95	95	48	71

*1-methyl-2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α- and -β-D-glucopyranosyl)-α-D-glucopyranose.

**-*O*-(2,3,4,6-tetra-*O*-benzyl-α- and -β-D-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-α-D-glucopyranose.

deprotection and separation, there was obtained α- and β-anomeric Neu5Ac(2→6) lactose (**101a**,**b**).

O-(5-Acetamido-9-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonicacid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**102**) and 9-*O*-butyroyl derivative (**103**) were prepared from the sialyllactose (**101a**,**b**).

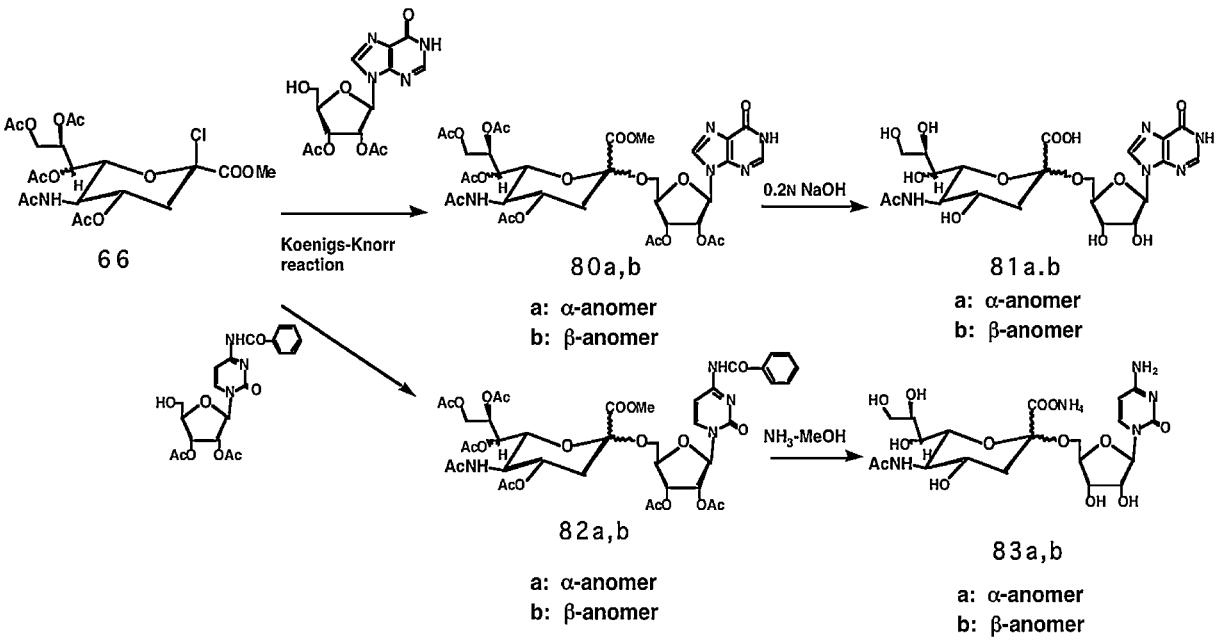


Fig. 25. Disaccharide nucleoside of Neu5Ac (No. 2).

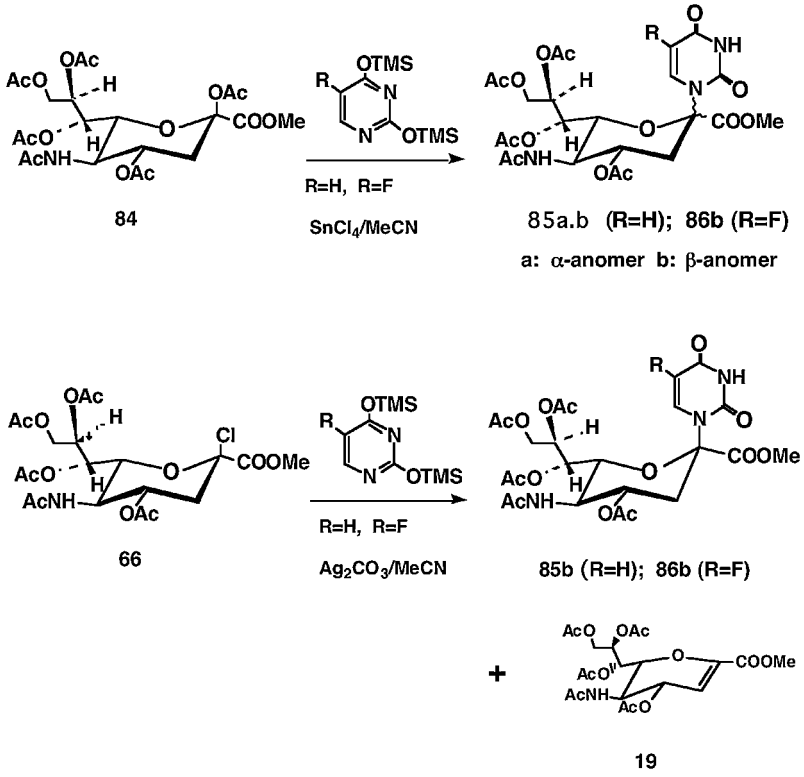


Fig. 26. *N*-Glycoside nucleoside.

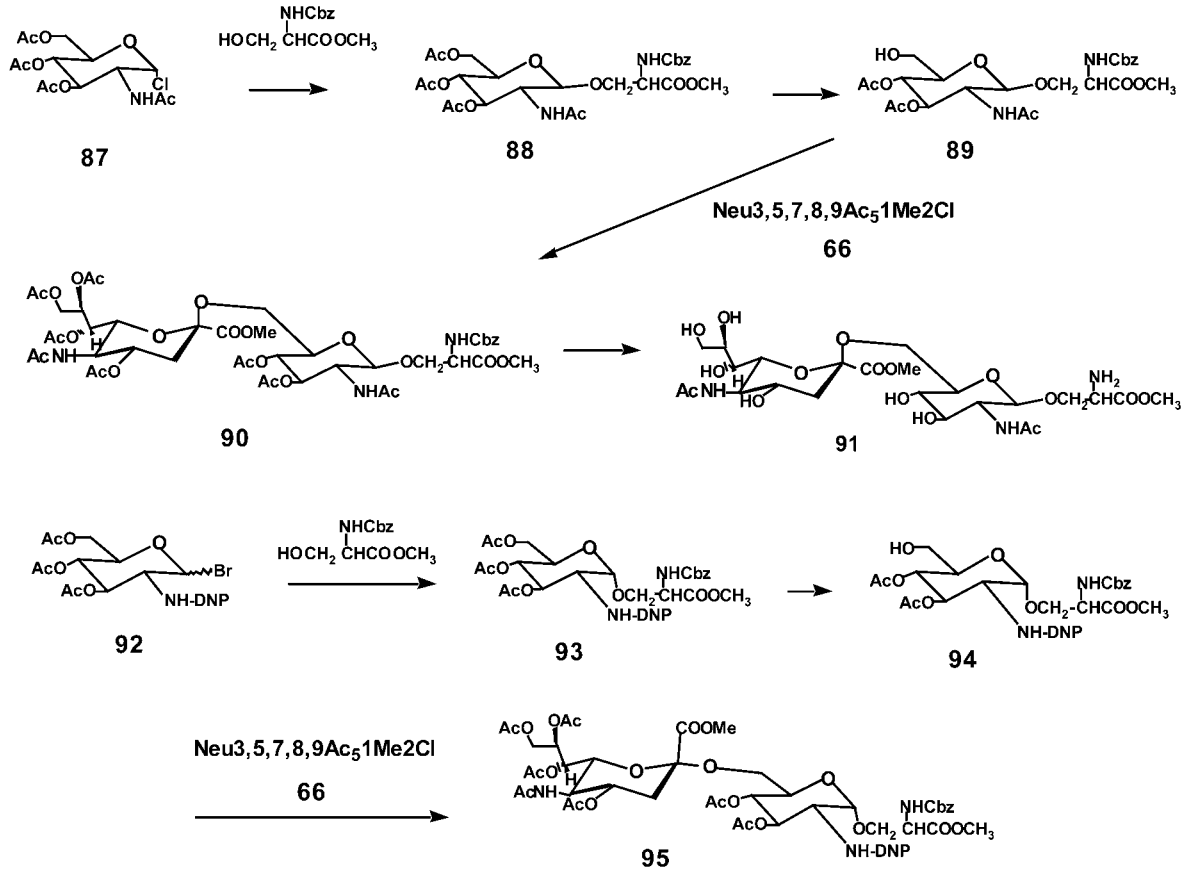


Fig. 27. Synthesis of mucin analogs.

When *S*-glycosyl donor (**73**) was used instead of the chloride (**66**), α- and β-anomeric mixture of **98** was obtained in 34–54% yields.

3-2-g. Sialylcholesterol. Cholesterol is one of the most important molecule in the animal cell membranes, and sialylated cholesterol could not be found in the animal cells. Of our interest to prepare glycolipoids, sialylcholesterol and GM3 analog are synthesized.

Koenigs–Knorr-like reaction of the chloride (**66**) and cholesterol under various conditions gave α- and β-anomers of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-*2*-(5-cholesten-3β-yloxy)-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**104a**,**b**).

As shown in Table 2, silver trifluoromethanesulfonate was used as a promoter, the yield is 60% after chromatographic purification. When silver carbonate and iodine were used as promoters, α-anomer was obtained in the ratio of 11.5:1. Mercury salts were not so good promoter, because the yield and stereospecificity of the product are low together with a lot of by-product (**19**).[51]

When *S*-glycosyl donor (**73**) was used instead of the chloride (**66**), and β-anomer rich of **104** was obtained in 64–70% yields.[34]

Saponification of these acetates (**104**) with 2 M sodium hydroxide afforded the α- and β-anomers of *N*-acetyl-*2*-(5-cholesten-3β-yloxy)-D-neuraminic acid in fair yields, their sodium salts (**105a**,**b**) were prepared with an equimolar amount of sodium hydroxide.[49]

Koenigs–Knorr-like reaction of hepta-*O*-acetyl-D-lactosyl halides (**106**, **107**) and cholesterol gave α- and β-anomers of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl(1→4)-3,6-di-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**108a**,**b**) and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**109**).

This compound (**109**) was successively, 1) deacetylated, 2) the hydroxyl groups of 4- and 6-positions of galactose moiety were protected, 3) acetylated, and then 4) debenzylidenation to yield 2,3-di-*O*-acetyl-β-D-galactopyranosyl(1→4)-2,3,6-tri-

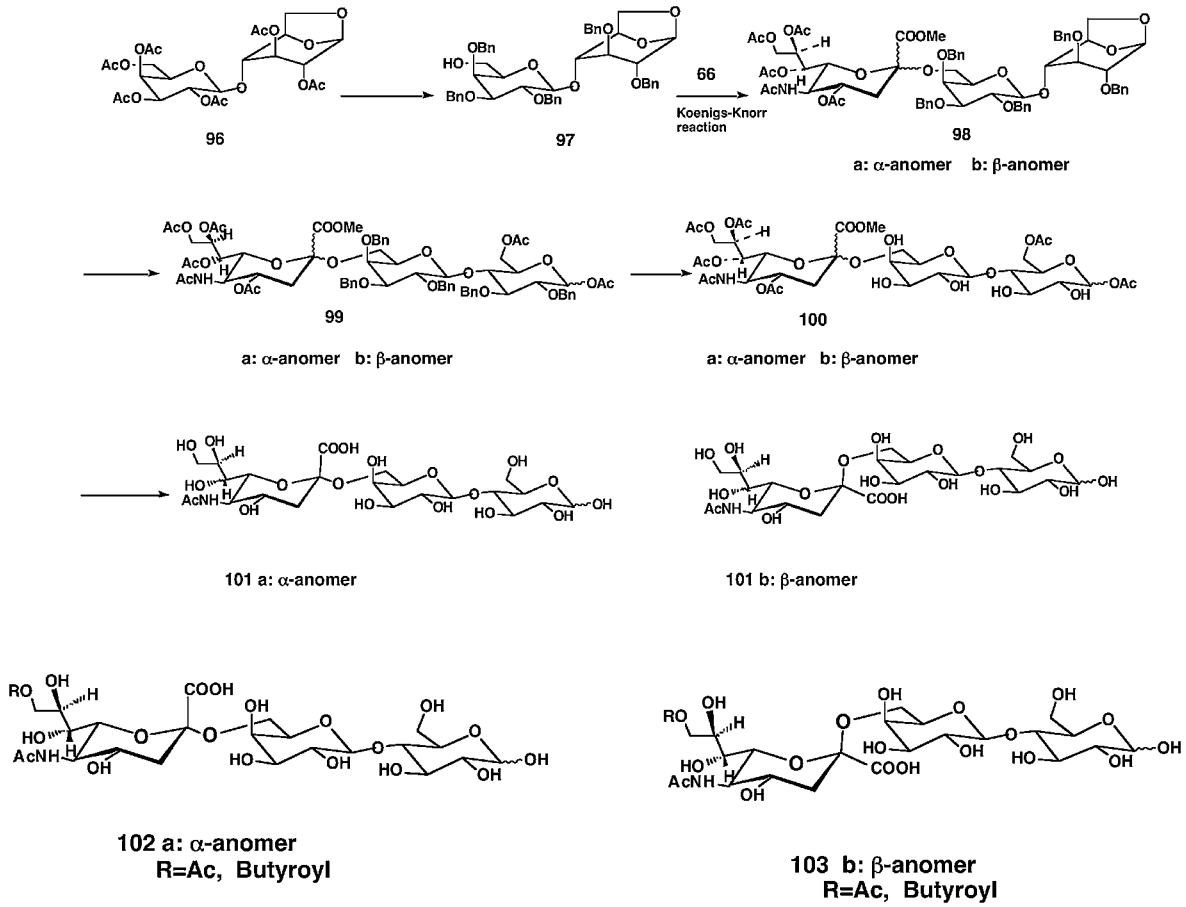


Fig. 28. Synthesis of Neu5Ac(2→6)lactose and 9-*O*-acyl derivatives.

Table 2.

Glycosyl donor	Promoter	Solvent	Yield (%)	Ratio of products α-104:β-104	By-product 19 (%)
a	Ag_2CO_3/I_2	benzene	22	11.5:1	21
a	$AgOSO_2CF_3$	CH_2Cl_2	60	1:1	10
b	$TMSOSO_2CF_3$	CH_2Cl_2	5	0:1	42
c	$AgOSO_2CF_3$/ $SnCl_2$	benzene	42	1:1.3	33
d	$BF_3.Et_2O$	CH_2Cl_2	56	0:1	0

O-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**110**) in 56% yield. Then the compound **110** and the chloride (**66**) were subjected to Koenigs–Knorr-like reaction, when silver trifluoromethanesulfonate was used as promotor, α- and β-anomers of 6-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyransyl)onate]-(2→6)-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-(5-cholesten-3β-yloxy)-β-D-glucopyranose (**111a**,**b**) were obtained.[52)]

3-2-h. Partially acetylated of 4-methylcoumarin derivatives. In Chapter 3, already summarized on the synthesis of partially *O*-acetylated Neu5Ac. Synthesis of various partially acetylated 4-methylcoummarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acids is described as new fluorogenic substrate for neuraminidase.[53)]

Benzyl esterification of 4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid was carried out with benzyl bromide to yield benzyl (4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**112**) in 90% yield. Further treatment of **112** with trimethyl orthoacetate to give 9-*O*-acetylated (**113**), followed by hydrogenolysis to obtain 4-methylcoumarin-7-yl 5-acetamido-9-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**114**).

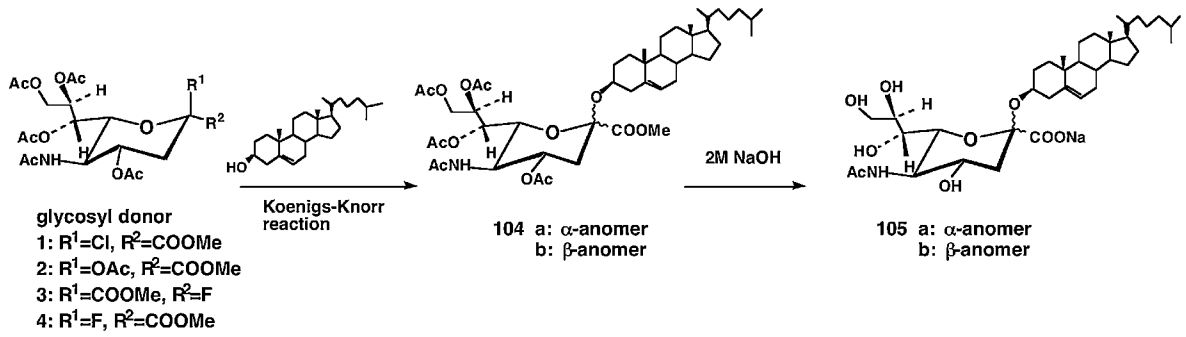


Fig. 29. Synthesis of sialylcholesterol.

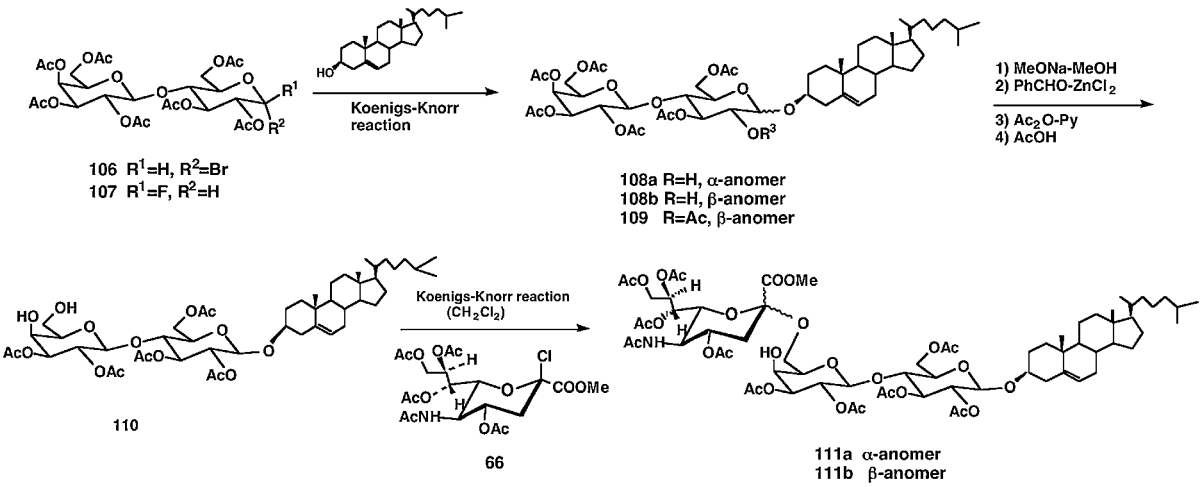


Fig. 30. Synthesis of GM3 analog.

Reaction of **112** with 2,2-dimethoxypropane gave benzyl (4-methylcoumarin-7-yl 5-acetamido-8,9-*O*-isopropylidene-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**115**). Acetylation at 4-hydroxyl group with acetic anhydride at 20 °C yielded **116**, further removal of the isopropylidene and benzyl groups afforded 4-methylcoumarin-7-yl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**117**).

After protection of the 4-hydroxyl group with *tert*-butyldimethylchlorosilane, treatment with acetic anhydride gave benzyl (4-methylcoumarin-7-yl 5-acetamido-7-*O*-acetyl-4-*tert*-butyldimethylsilyl-8,9-*O*-isopropylidene-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**118**). Removal of the protecting groups of **118** gave benzyl (4-methylcoumarin-7-yl 5-acetamido-7-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**119**) with small amount of by-product (**120**). Removal of the benzyl group by catalytic hydrogenation gave 4-methylcoumarin-7-yl 5-acetamido-7-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**121**).

Di-*O*-acetyl derivative was synthesized from benzyl (4-methylcoumarin-7-yl 5-acetamido-4,9-di-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**122**).[52] Acetylation with acetic anhydride gave benzyl (4-methylcoumarin-7-yl 5-acetamido-7,8-di-*O*-acetyl-4,9-bis-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**123**) in 78% of yield. Further removal of the *O*-*tert*-butyldimethylsilyl groups with acetic acid, and then hydrogenation gave 4-methylcoumarin-7-yl 5-acetamido-7,8-di-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**124**).[54]

3-2-i. Glycosylation of mitomycin. Mitomycins are known as excellent antitumor antibiotics, and are look forward having enhanced antitumor activity with decreased toxicity than natural mitomycins. A part of this program on the synthesis of 7-*O*-glycosyl-9a-methoxymitosanes, 7-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-

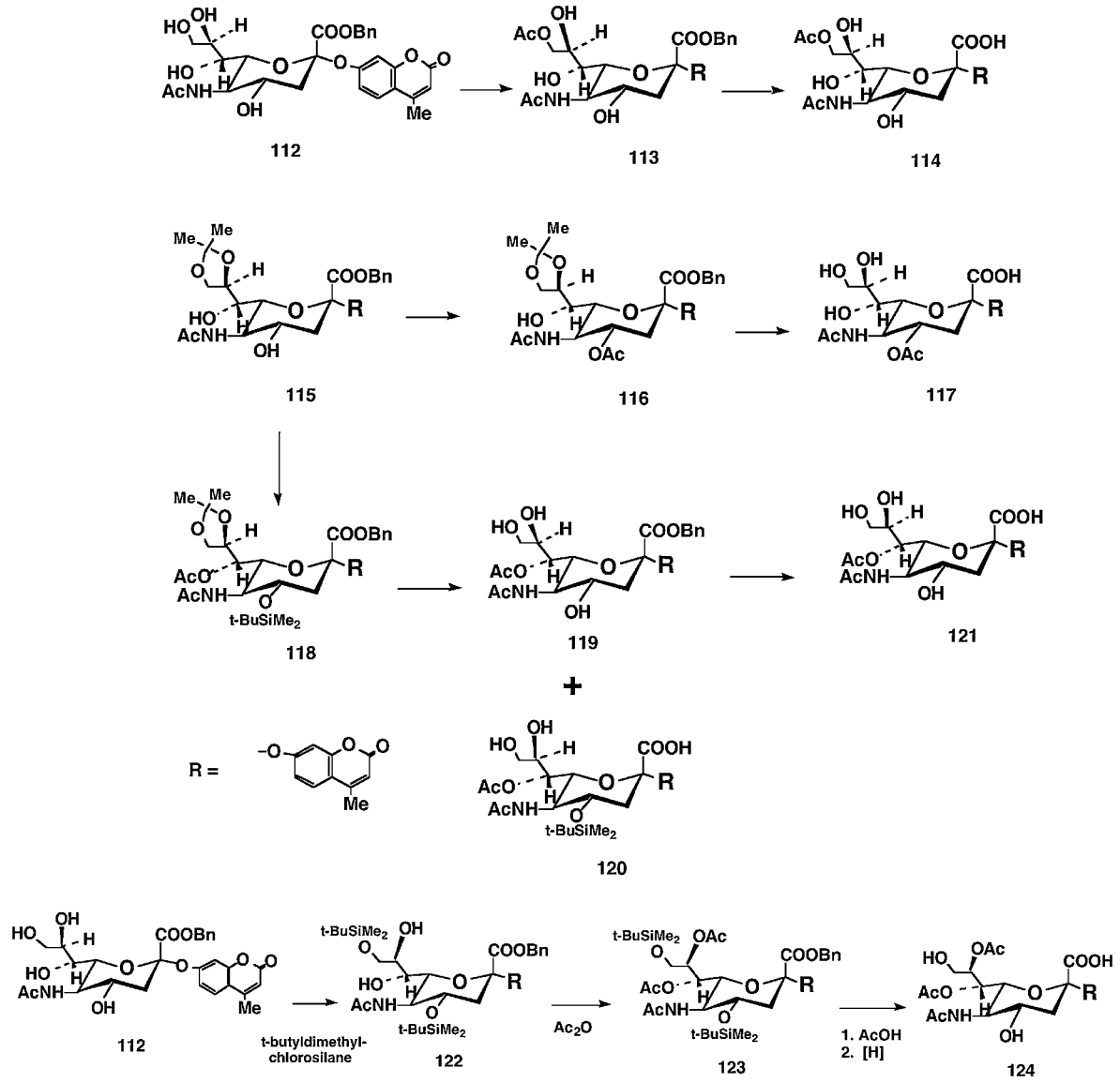


Fig. 31. Synthesis of partially acetylated 4-methylcoumarin derivatives.

β-D-glucopyranosyl)-9a-methoxymitosane (**126a**), 7-*O*-(2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-9a-methoxymitosane (**126b**), 7-*O*-(2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-galactopyranosyl)-9a-methoxymitosane (**126c**), and 7-*O*-(hepta-*O*-acetyl-β-D-lactosyl)-9a-methoxymitosane (**126d**) were prepared.[55]

Treatment of mitomycin A (**125**) and 4-aminophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside gave 7-*N*-{4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)phenyl}-9a-methoxymitosane (**127**) in 69% of yield. Deacetylation was performed with sodium methoxide in methanol.

Intermediate, 4-aminophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidoic acid (**128**) was prepared starting from Neu4,5,7,8,9Ac$_5$1Bn *via* 2-chloride. Glycosylation of the chloride with 4-nitrophenol afforded benzyl (4-nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate, and the nitro group and the benzyl group were hydrogenated to yield the intermediate (**128**).

Reaction of mitomycin A (**125**) with **128** afforded 7-*N*-{4-*O*-(sodium 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-

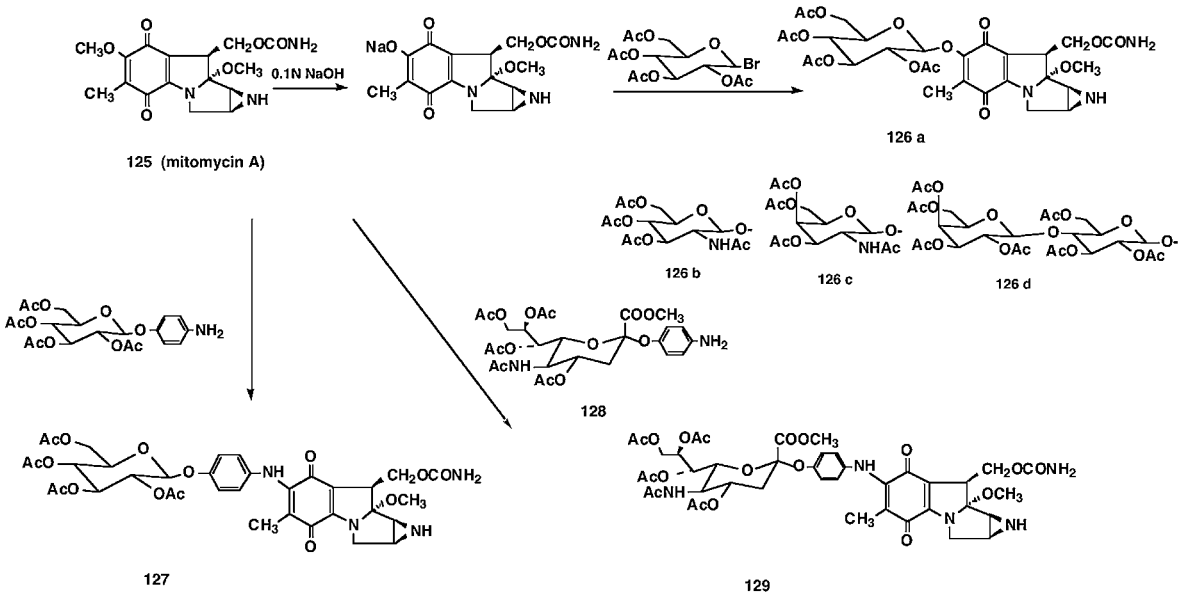
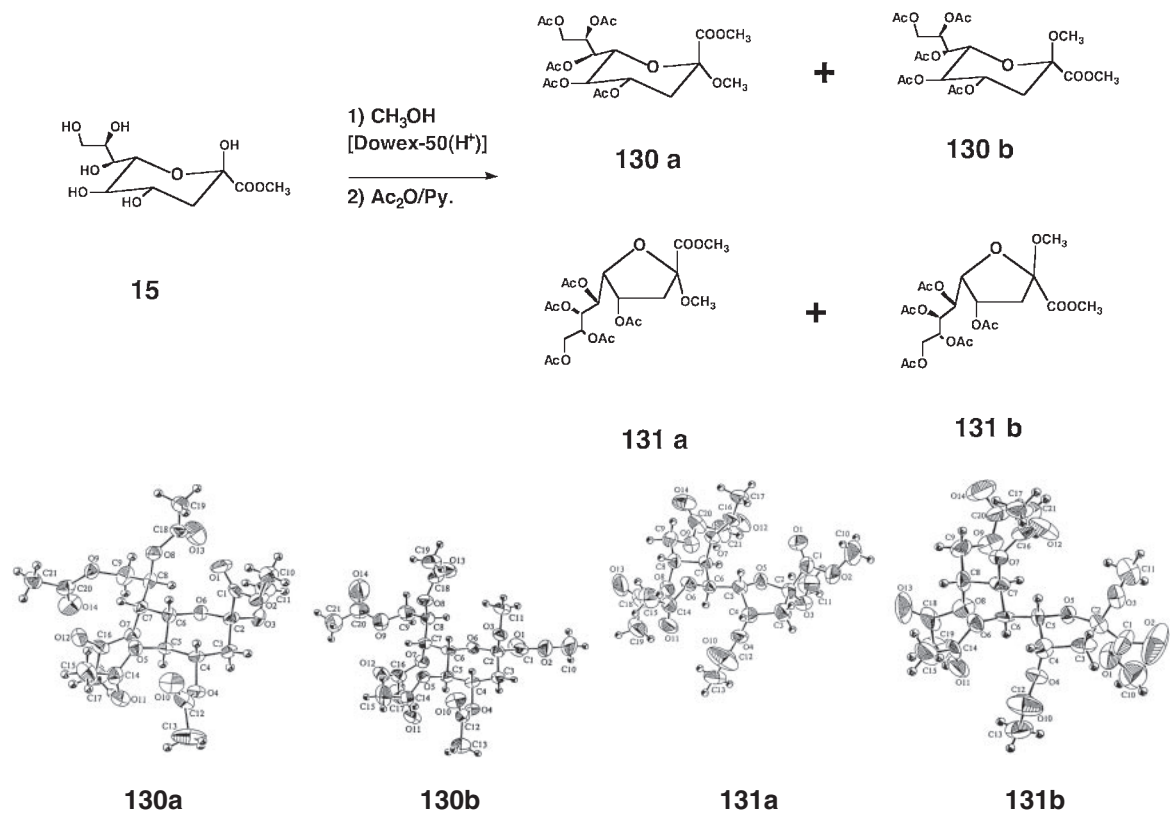


Fig. 32. Glycosylation of mitomycins.



Perspective view of **130a**,**b** and **131a**,**b**

Fig. 33. Fischer's methyl glycosylation of KDN.

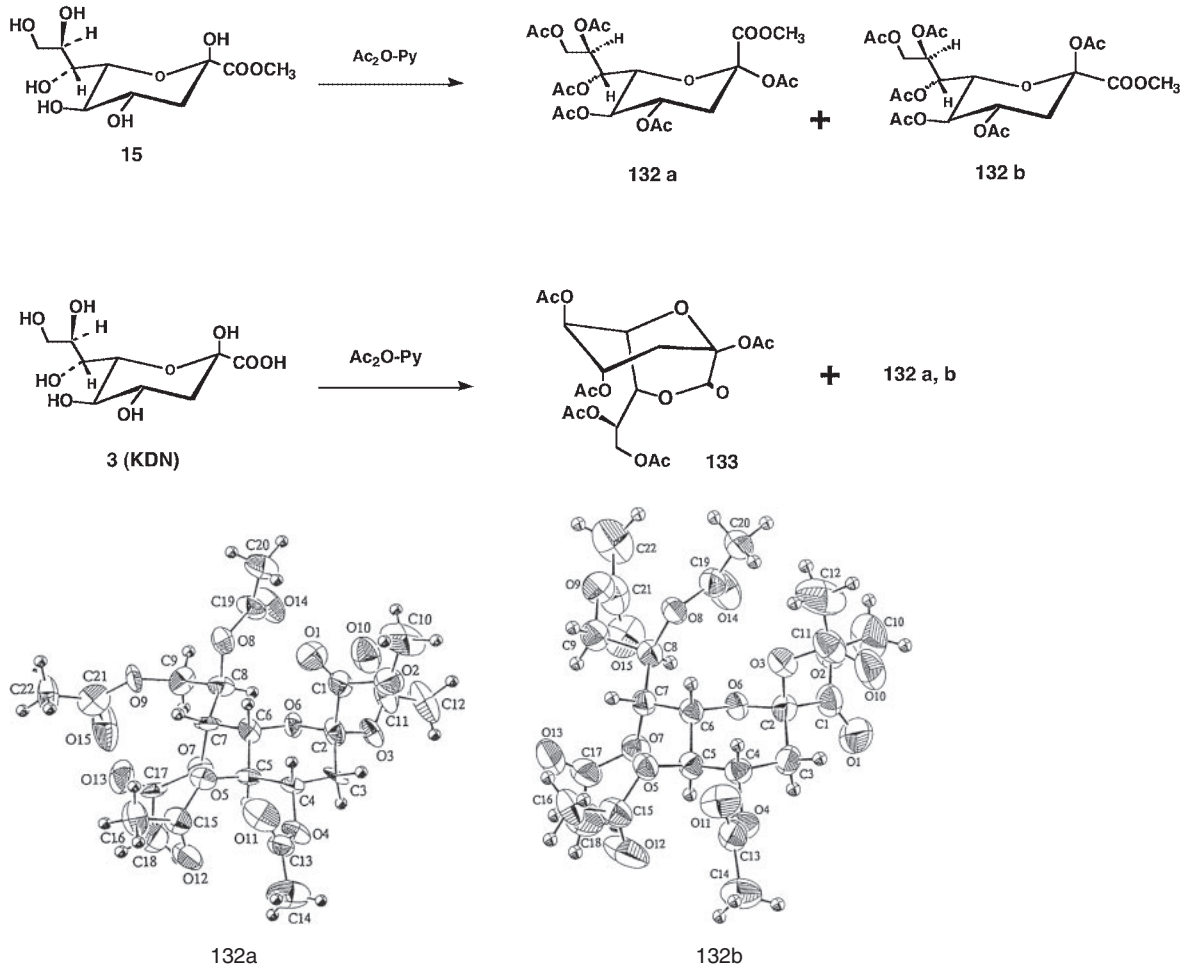


Fig. 34. Acetylation of KDN.

nonulopyranosylonate)phenyl}-9a-methoxymitosane (**129**) after treatment with $NaHCO_3$.[56)]

4. Preparation of KDN derivatives

4-1. Glycosylation of KDN.

4-1-a. Fischer's methyl glycosylation of KDN. KDN is different at the 5-hydroxyl function instead of amino group of Neu5Ac. Methyl ester (**15**) of KDN was treated with methanol under the presence of Dowex-50(H^+), followed by acetylation with acetic anhydride to give four compounds; methyl 2,4,5,7,8,9-hexa-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulo-pyranosonates (**130a**,**b**) and -furanosonates (**131a**,**b**) as shown in Fig. 33.

Ratio of the products depends upon the glycosylation conditions, as shown in Table 3. When the glycosylation was run at 20 °C, furanosides were mainly obtained. On the other hand, the reaction proceeded at 70 °C 15 hr, β-pyranoside mainly obtained.[28)] These results indicated that furanoside formed by a kinetic control and pyranoside formed by a thermodynamic control.

Table 3.

Reaction temperature	Time (hr)	Yield (%)			
		130a	**130b**	**131a**	**131b**
20 °C	240	0	5	32	48
70 °C	1	1	16	21	39
	3	2	27	15	33
	5	5	67	2	4
	15	4	74	0	0

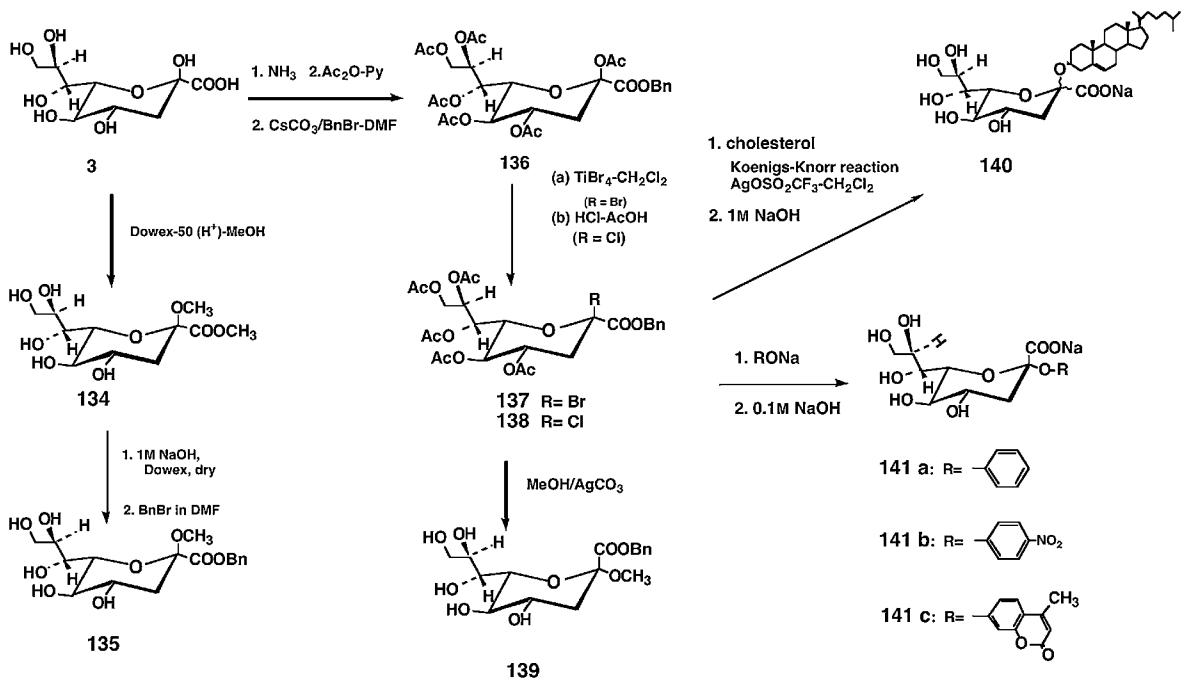


Fig. 35. Glycosylation of KDN.

Structures of these compounds were confirmed by X-ray analysis.[29]

These methyl glycosides (**130ab**, **131ab**) were deacetylated with potassium carbonate in methanol to give methyl (methyl 3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosid)onates, respectively.[29]

4-1-b. Acylation of KDN. Methyl ester (**15**) of KDN was treated with acetic anhydride to afford methyl 2,4,5,7,8,9-hexa-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosonates (**132a**,**b**). On the other hand, acetylation of KDN (**3**) directly, gave 2,4,5,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosono-1,7-lactone (**133**) accompanied by small amount of **132a**,**b**.

Structures of **132a** and **132b** were confirmed by X-ray analysis.[29] Structure of 1,7-lactone (**133**) was elucidated by NMR spectra comparison with the corresponding Neu5Ac derivative (**49**).

4-1-c. Glycosylation of KDN. A solution of KDN (**3**) in dry methanol was stirred with Dowex-50(H^+), there was obtained methyl glycoside (**134**). Further treatment with alkaline and benzyl bromide, yielded benzyl (methyl 3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (**135**). On the other hand, glycosyl donor, benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl bromid)onate (**137**) was prepared from **136** with titanium tetrabromide (path a). The chloridonate (**138**) was prepared from **136** with HCl gas in acetic acid solution (path b).[57)–59)] Further treatment of the bromide (**137**) with methanol, and then sodium hydroxide gave benzyl (methyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**139**).[57]

Table 4.

Substituent (R)	Yields (%)		
	phenyl	*p*-nitirophenyl	4-methylumbelliferonyl
1. RONa	31	77	66
2. 0.1 M NaOH	81	87	80

When cholesterol as a glycosyl acceptor was reacted with the bromide (**137**), benzyl 4,5,7,8,9-penta-*O*-acetyl-2-(5-cholesten-3β-yloxy)-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosonates (**140**) accompanied with large amount of 2,3-dehydro derivative (benzyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-2,3-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate) were obtained.[57]

Condensation of the chloride (**138**) with sodium salts of phenol, *p*-nitrophenol, and 4-methylumbelliferone gave the corresponding α-glycosides, benzyl (substituted 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)-onate. These compounds were deprotected with

sodium hydroxide to give sodium (phenyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**141a**), sodium (*p*-nitrophenyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**141b**), and sodium (4-methylumbelliferonyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**141c**) in good yields as shown in Fig. 35 and Table 4.[58)–61)]

4-1-d. N-Glycosylation of KDN. Glycosylation of benzyl and methyl 2,4,5,7,8,9-hexa-*O*-acetyl-3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonates (**136**) with trimethylsilyl derivatives of pyrimidine, 5-fluoropyrimidine and 5-methylpyrimidine under Vorbrüggen reaction conditions gave anomeric mixuture of benzyl and methyl 2,3-dideoxy-2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-D-*glycero*-D-*galacto*-2-nonulopyranosonates (**142**) in poor yields.

On the other hand, methyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-2,3-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**138**; R = Me) and sodium hydride was used, only the α-isomers (**143**) were formed in rather good yield. Structure of **143** (R = Me, R′ = H) was confirmed by X-ray diffraction analysis.[60)]

The 2-chloro derivative (**138**; R = Me) was reacted with azidotrimethylsilane to yield methyl 4,5,7,8,9-penta-*O*-acetyl-2-azido-2,3-dideoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosonates (**144**; R = Me). Treatment of **144** with 0.01 M sodium hydroxide gave methyl 2-azido-2,3-dideoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosonates (**145**; R = Me) as shown in Fig. 36.

4-1-e. Photocycloaddition of 2,3-dimethyl-2-butene. Photocycloaddition reaction of 2,3-dimethyl-2-butene with **143** gave methyl 4,5,7,8,9-penta-*O*-acetyl-2,3-dideoxy-2-[(1*R*,6*S*)- (**146**) and (1*S*,6*R*)-7,7,8,8-tetramethyl-*cis*-2,4-diazabicyclo[4.2.0]octane-3,5-dioxo-2-yl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**147**).[62)]

Photocycloaddition of 2,3-dimethyl-2-butene to 2′-deoxyribonucleoside,[63)] cytosine and 2′-deoxycytidines,[64)] deoxyuridines,[65)] benzoylated 2′-deoxyribonucleoside,[66)] and kinetics and mechanism of photocycloaddition of deoxyuridines to 2,3-dimethyl-2-butene were reported.[67)]

4-1-f. Glycosylation of KDN with S-glycosyl donor. Reaction of methyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonates (**148**) prepared from the chloride (**138**) with BDTC (**30**) afforded methyl (1-phenyl-1*H*-tetrazol-5-yl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-2-thio-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (**149**) and methyl (1-phenyl-5-thioxo-1*H*,4*H*-tetrazol-4-yl 4,5,7,8,9-penta-*O*-acetyl-2,3-dideoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosid)onate (**150**). Structures of these compounds were confirmed by means of UV, CD and NMR spectra, and X-ray analysis of methyl (1-phenyl-1*H*-tetrazol-5-yl 3-deoxy-2-thio-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (**154**).

These glycosides (**149**, **150**) were applied to *O*-glycosylation with 2-propanol to give methyl (isopropyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosid)onate (**151**). Reaction of *S*-glycoside (**149**) with 2-trimethylsilyloxypropene gave methyl [4,5,7,8,9-penta-*O*-acetyl-2-*C*-(2-oxopropyl)-2,3-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranos]onate (**152**).

Similar reaction with 1-phenyl-1-(trimethylsilyloxy)ethylene gave methyl [4,5,7,8,9-penta-*O*-acetyl-2-*C*-(2-oxo-2-phenylethyl)-2,3-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranos]onate (**153**).[68)]

5. Confirmation of stereochemistry

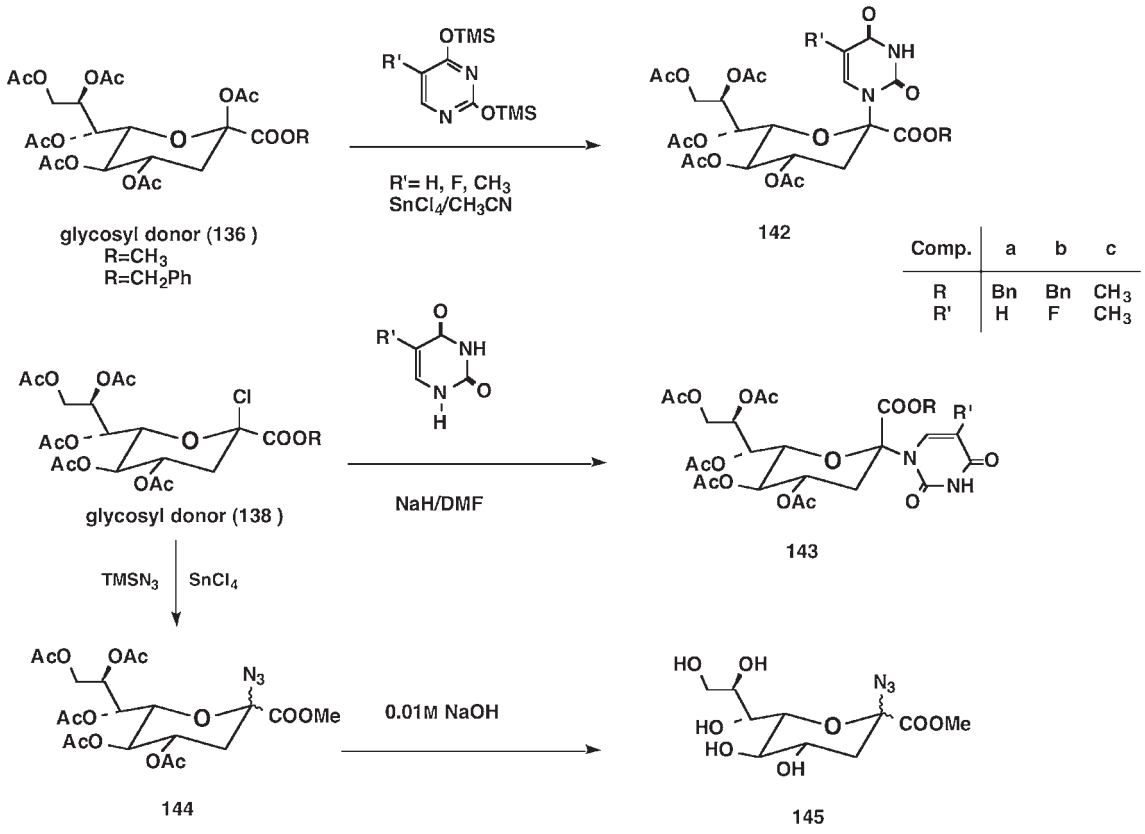
Structure and stereochemistry of sialic acids and their derivatives were confirmed by means of NMR and CD spectra. Furthermore, hydrolysis method was developed.

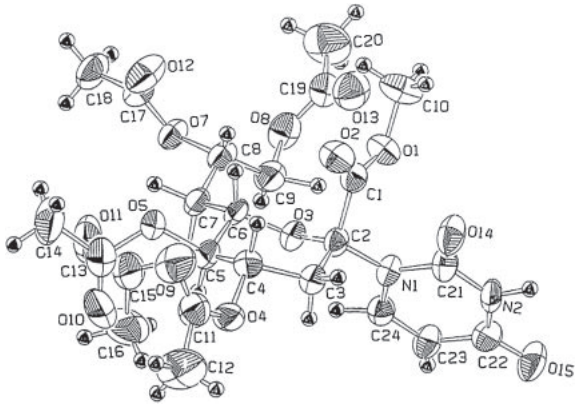
5-1. NMR spectra. In the NMR spectra, the chemical shifts at 3-Heq double-doublet resonance of Neu5Ac and its derivatives indicated 2.6–2.8 ppm for α-anomers. For β-anomers the range is 2.1–2.5 ppm. The coupling constant $J_{7,8}$ value is 7–9 Hz for the α-anomers, and 2–3 Hz for the β-anomers.[34),41)]

As summarized in Table 5, the values of chemical shifts of *N*-nucleoside at 3-Heq (δ: α 3.05 and 2.93 ppm; β 3.09 and 2.89 ppm) and $J_{7,8}$ values of KDN derivatives (α 8.7 Hz; β 9.0 Hz) are quite different from the usual data. This problem could be explained by the anisotropic effect of the aromatic moiety at the 2′-position. The stereochemistry of sialic acids derivatives at the anomeric position could not be assessed from the NMR data.

5-2. CD spectra. CD spectra of sialic acids derivatives are valuable for the stereochemical confirmation. The peak around 220–230 nm was assigned to the n–π* Cotton effect of the carboxyl group. The negative Cotton effect was assigned to the α-configuration, and the positive Cotton effect was assigned to the β-configuration. As shown in Fig. 39, negative Cotton effect around 220–230 nm, supporting the α-configuration. On the other hand, the β-anomer shows a positive Cotton effect.[12),13)]

As shown in Fig. 39, β-methyl neuraminate shows positive Cotton effect around 217 nm, and the negative one for the α-anomer at around 223 nm. Neu5Ac crystals show β-form both in water and KBr.





Perspective view of **143** (R=Me. R'=H)

Fig. 36. *N*-Glycosylation of KDN.

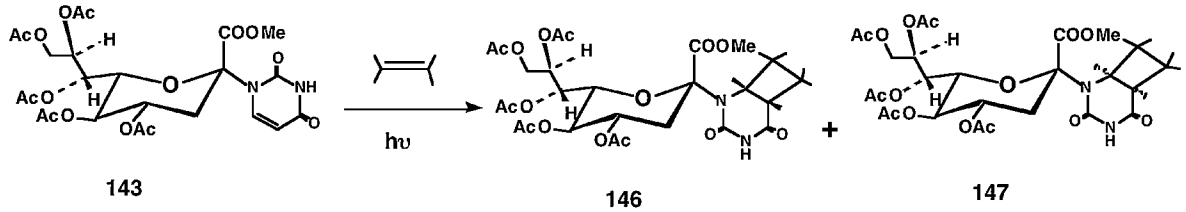


Fig. 37. Photocycloaddition of 2,3-dimethyl-2-butene.

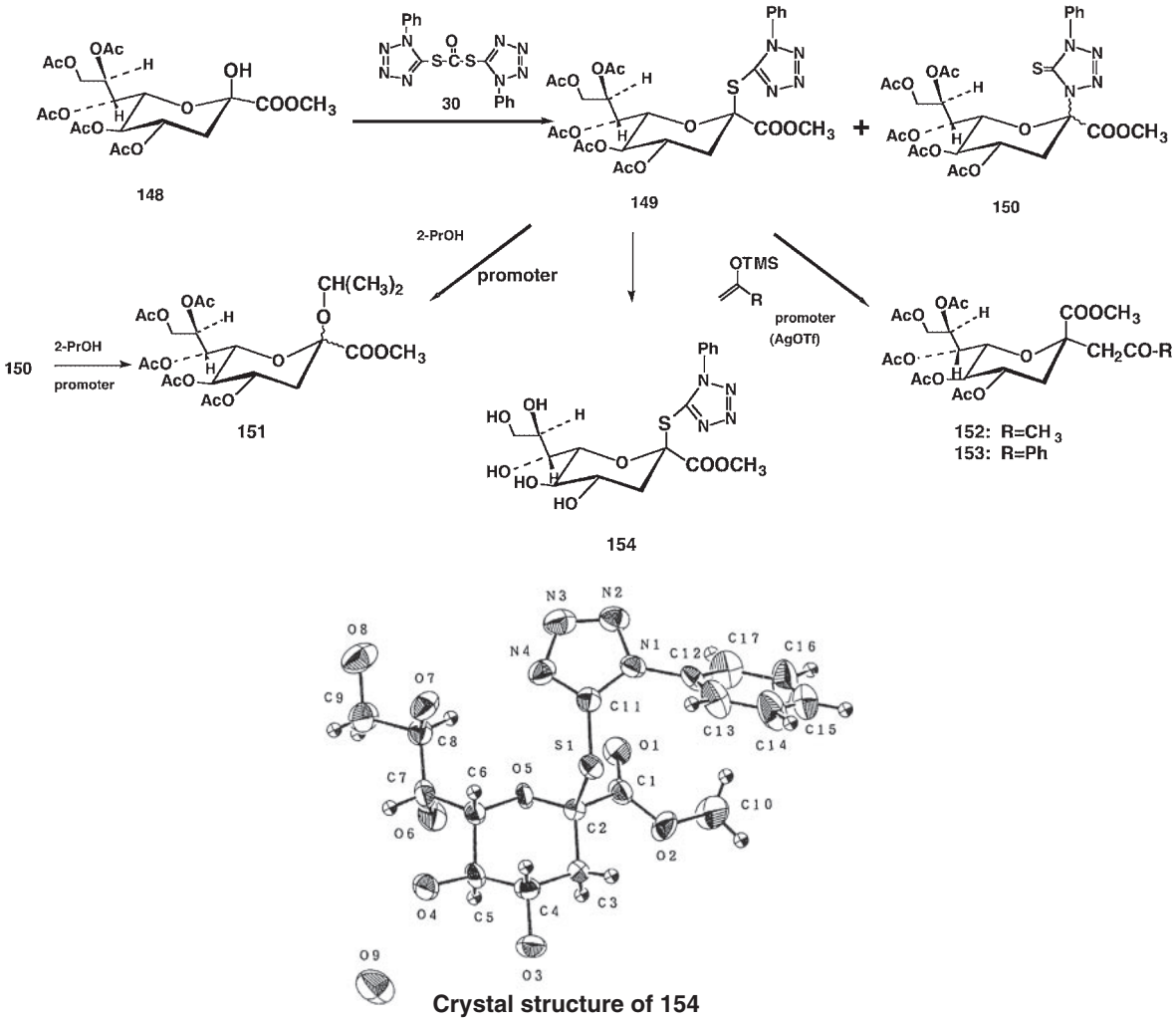


Fig. 38. Glycosylation of KDN with BDTC.

This conclusion was supported in KDN derivatives as shown in Fig. 40.[29),51),52)]

As shown in Figs. 39–41, the peak around 220–230 nm in several derivatives of Neu5Ac and KDN was assigned to the n–π^* Cotton effect of the carboxyl group and the positive Cotton effect is β- and negative one is α-configuration. Although this empirical rule does not apply to sialosyl-cytidine and -uracil derivatives as shown in Fig. 41.[48),49),57)] Then, hydrolysis method was exmined.

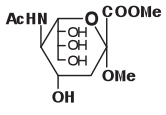
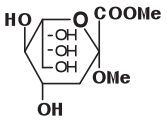
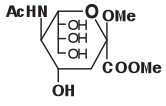
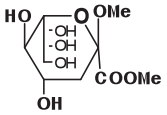
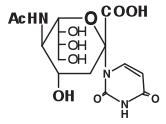
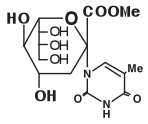
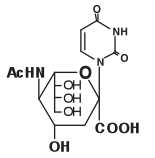
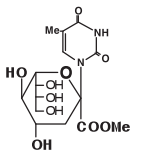
5-3. Hydrolysis method. Hydrolysis of α- and β-methyl neuraminate was performed in water at 80 °C, α-anomer was hydrolyzed completely in 1 hr, while β-anomer was stable even after 5 hr as shown in Fig. 42(a). Further examination was performed on Neu5Ac2Lac (Fig. 42(b)), α- and β-anomers were stable in 0.1 M sulfuric acid at 20 °C, while, at 80 °C, the α-anomer was hydrolyzed completely in 1 hr in water.[50)]

This conclusion was supported in disaccharide nucleoside, *N*-glycoside,[49)] and KDN glycosides[57)] as shown Figs. 43, 44. These results indicate that the measurement of the rate of hydrolysis may be useful for the confirmation of stereochemistry in sialic acids chemistry.[7)] This is supported by Thiem *et al.*[69)]

6. Biological activities of glycolipoid

6-1. Disaccharide nucleoside analogs. *O*-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5,-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosyl)onate]-(2→5′)-5-fluoro-2′,3′-*O*-isopropylideneuridine (**75b**) and inosine derivative (**80**) are capable of enhancing the induction of suppressor T cells by concanavalin A, and can also induce suppressor T cells by

Table 5.

Compound	Ref.	H-3eq (δ; ppm)	Compound	Ref.	H-3eq (δ; ppm)
	8	2.69		29	2.55
	8	2.30		29	2.26
	49	3.05		61	2.93
	49	3.09		61	2.89

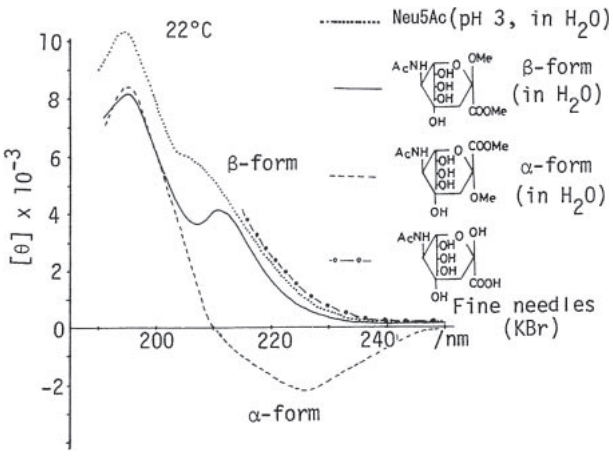


Fig. 39. CD spectra of sialic acids Neu5Ac and its α- and β-methyl glycosides.

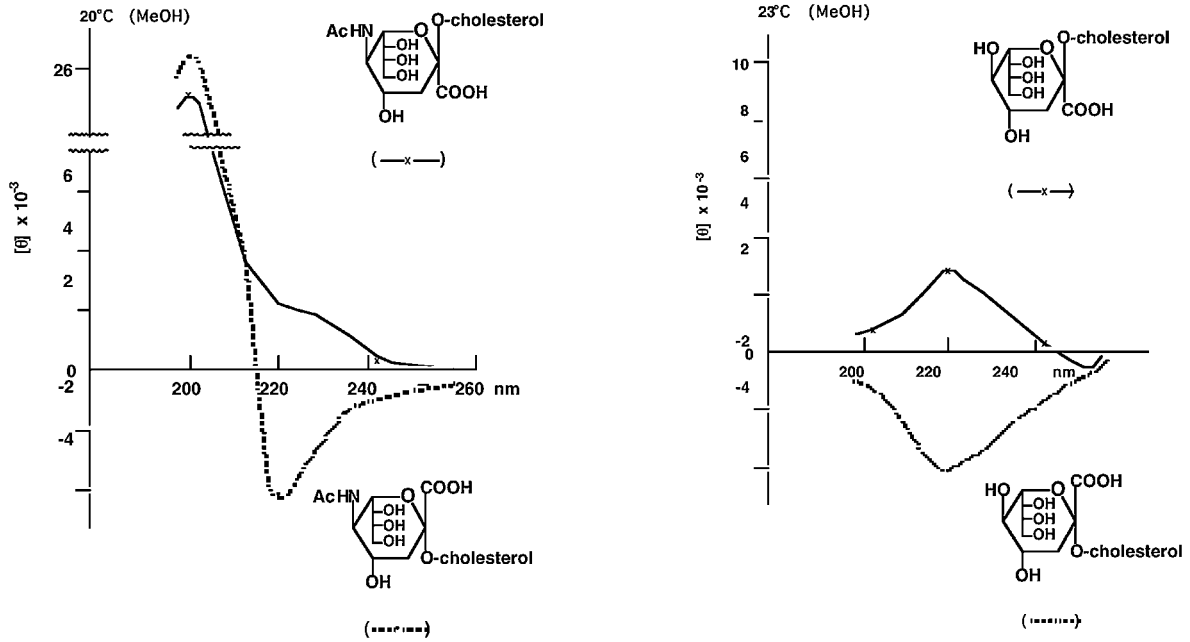


Fig. 40. CD spectra of sialic acids. Cholesterol derivatives of Neu5Ac and KDN.

themselves. They reduced incorporation of sialic acid into glycoconjugates on the murine lymphocyte surface.[70),71)]

Metastatic processes on cancer are very complicated, because they involve various factors and important problems. Sialyltransferase inhibitor, 5-fluorouridine derivative was effective in the experimental lung metastasis of colon adenocarcinoma of NL-17 (high metastatic potential) or NL-44 (low metastatic potential) cells.[72),73)]

β-Anomer (**76b**) of the 5-fluorouridine derivative, and a mixture of α- and β-anomer also inhibited

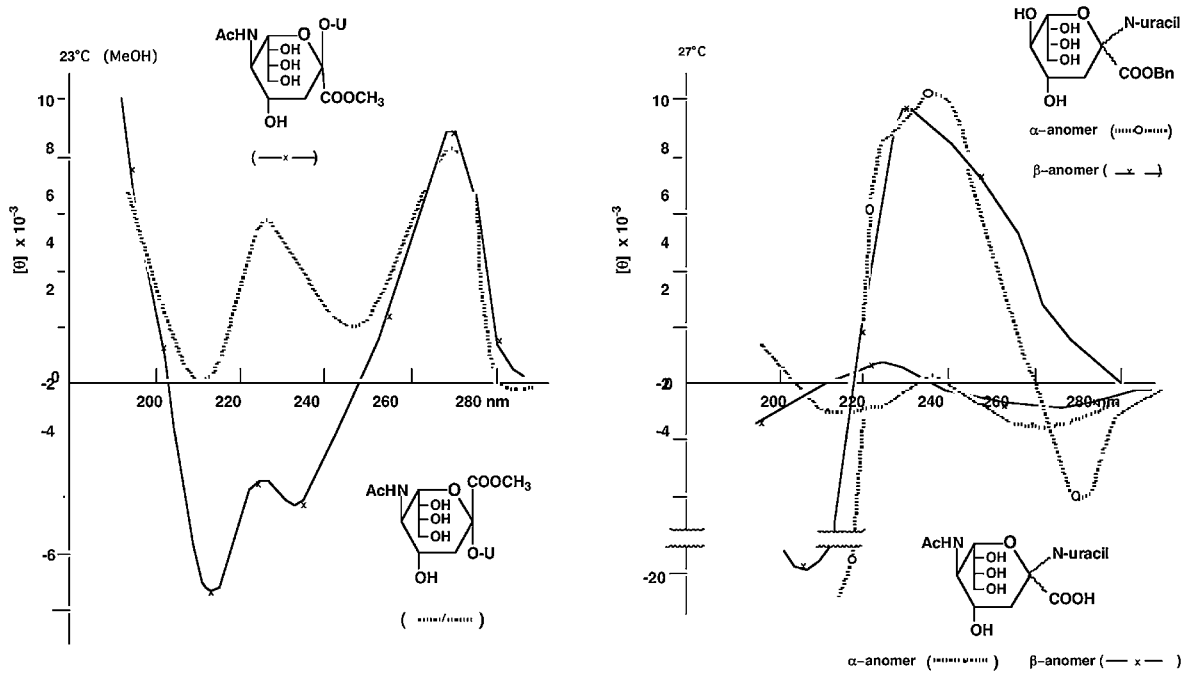


Fig. 41. CD spectra of sialic acids. Uridine and uracil derivatives of Neu5Ac and KDN.

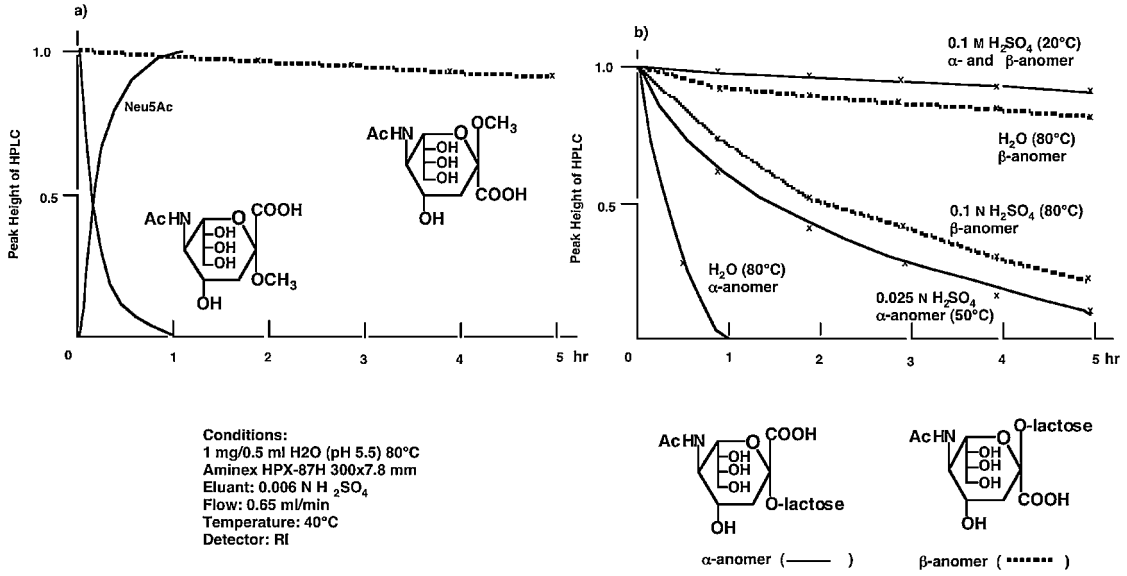


Fig. 42. (a, b) Hydrolysis method of Neu5Ac2Me (a) and Neu5Ac2Lac (b).

the metastatic ability of NL cells.[72] On further experiment of compounds **75** and **76**, they inhibited the metastasis to liver.[73]

6-2. Sialosylcholesterol. Sialosylcholesterol (**105a**,**b**) showed potent activity for the propagation of neurites (neuro 2a) and induced the morphological conversion of normal rat glioblasts from a flat epithelioid morphology to an astrocytic process-bearing morphology by glia maturation factor (GMF).[74)–76] The activity of α-sialosylcholesterol (**105a**) is 420 times as high as that of GM1 and 270 times that of GQ1b, and shows a strong activity for the propagation of neurites.[74)–76]

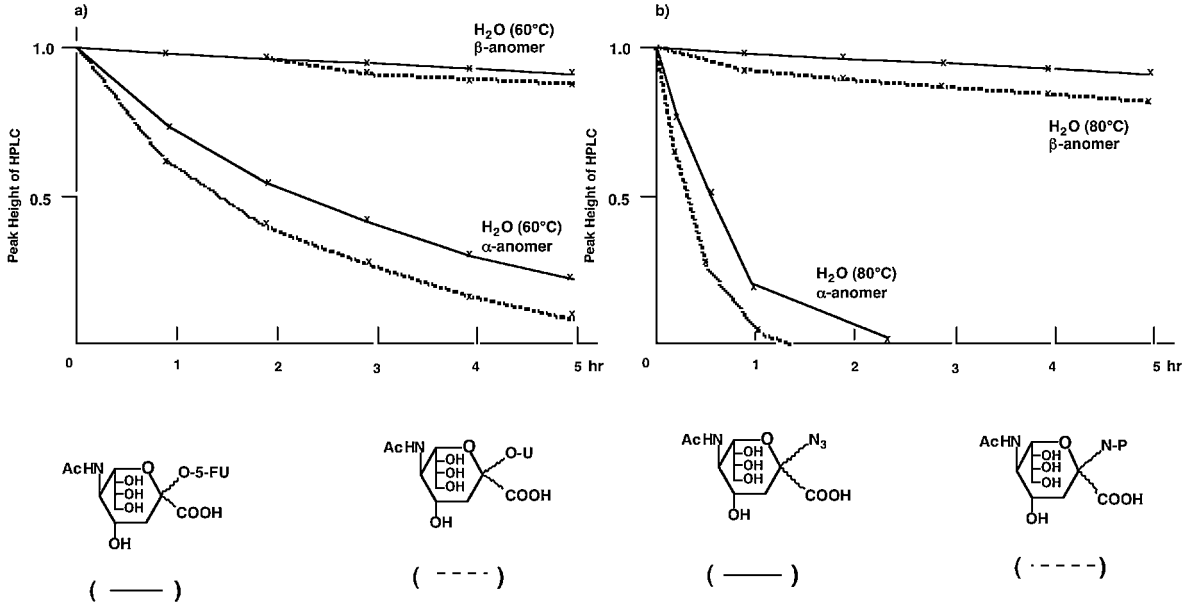


Fig. 43. (a, b) Hydrolysis method of Neu5Ac derivatives.

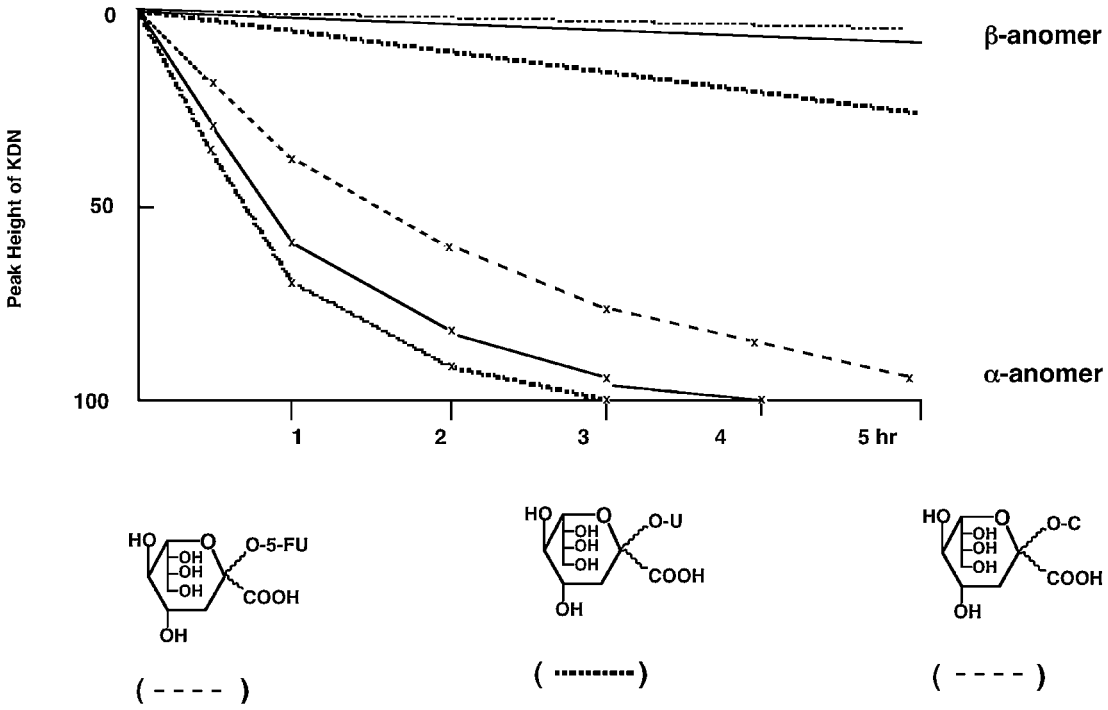
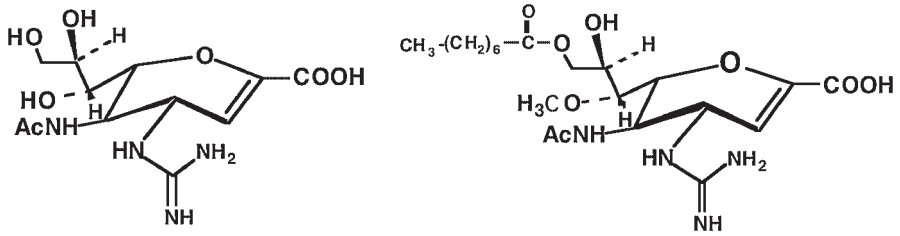


Fig. 44. Hydrolysis method of KDN derivatives.

β-KDN-cholesterol has a similar activity for the propagation of the neurite.[74)–76)] As shown in Table 6, the differentiation-inducing activity of α-Neu5Ac-cholesterol to HL-50 cells is greater than that of sialoglyceride, β-anomer, and KDN-cholesterol.[77),78)]

Sialosylcholesterol (**105a**,**b**) and GM1 are incorporated to mouse Neuro 2a in 24 hr. Cell fractionation experiments of ^{14}C-**105a**,**b** showed $\sim$40% of the incorporated ^{14}C-sialosylcholesterol was localized in the nucleus, 25% in the plasma



145 (zanamivir) 146 (inavir)

Fig. 45. Antiviral agent.

Table 6. Neutrogenic effects of sialosylcholesterol neurite extention neuro 2a cells

Compound	Dose (M)	Length
control		6.33 ± 0.58
α-Neu-cholesterol	10^{-7}	12.33 ± 2.08
	10^{-6}	15.00 ± 1.00
β-Lac-cholesterol	10^{-7}	9.33 ± 0.58
	10^{-6}	9.33 ± 0.58
	10^{-5}	9.33 ± 2.31
α-KDN-cholesterol	10^{-7}	9.33 ± 0.58
	10^{-6}	#
	10^{-5}	#
β-KDN-cholesterol	10^{-7}	10.67 ± 1.15
	10^{-6}	11.67 ± 1.53
	10^{-5}	13.00 ± 1.53

Table 7. Intracellular distribution of incorporated sialosylcholesterol and GM1

Fraction	α-Sialosyl-cholesterol	β-Sialosyl-cholesterol	GM1
Plasma membrane	25.1 ± 1.47	25.4 ± 1.62	21.7 ± 1.12
Granule	14.3 ± 10.78	11.1 ± 0.66	25.4 ± 1.31
Nucleus	42.6 ± 2.49	41.2 ± 2.23	25.5 ± 1.31

membrane fractions, and 11–14% in the granule fraction (Table 7).[79),80)]

In conclusion, sialyl derivatives of cholesterol have strong biological activities. Addition of α-sialylcholesterol stimulated mouse brain and release acetylcholine from synaptosomes. The β-anomer also increased the neurotransmitter release, but the effect was weak.[81),82)]

6-3. Sialidase inhibitors. Partially *O*-acetylated (4, 7, and 9-position) 4-methylumbelliferyl-α-*N*-acetylneuraminic acids (*cf.* 3-2-h) were tested as substrates of sialidases of *Vibrio cholerae* and of *Clostridium perfringens*. The relative substrate specificity of the *Vibrio cholerae* sialidase is Neu5Ac-MU > Neu5,7Ac_2-MU ≈ Neu5,9Ac_2-MU.[83),84)] Activity of sialidases inhibitor is weak.

Zanamivir (**145**; *N*-acetyl-2,3-didehydro-4-deoxy-4-guanidinoneuraminic acid: 5- acetamido-2,3-didehydro-3,4,5-trideoxy-4-guanidino-α-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid: 4-guanidino-Neu5Ac2en) is a potent neuraminidase inhibitor for antiviral against influenza viruses.[85),86)]

Modified antiviral agent inavir (**146**; laninamivir octanoate) is also used as a long-acting and a single inhalation neuraminidase inhibitor.[87)]

6-4. Edible bird's nest. Edible bird's nest is the nest made by saliva of *Collocalia* sp. and used as the drug for keeping health and for enhancing immunocompetence since it was used in ancient China.[4)] Recently, edible bird's nest stimulates the growth factor for epidermal tissue resulting the repairing of cells.[88),89)]

Extract of edible bird's nest strongly inhibits infection with influenza viruses and inhibits hemagglutination of influenza viruses to erythrocytes. Edible bird's nest is the safe and valid natural source for the prevention of influenza viruses.[90)]

6-5. *N*-acetyl-D-neuraminic acid. *N*-Acetyl-D-neuraminic acid showed mucospissic and mucociliary clearance effects, and is expected as a pollinosis agent.[91),92)]

Acknowledgements

This review summarizes of our studies about sialic acids performed in School of Pharmaceutical Sciences, Kitasato University. I am grateful to Professor Yamakawa for his kind guidance through the research of sialic acids, and for his encouragement in the preparation of this review.

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(Received Mar. 22, 2011; accepted Apr. 26, 2011)

Profile

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