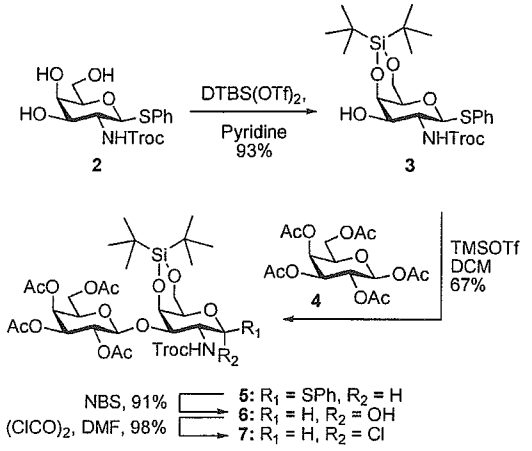
our DTBS-directing α-selective galactosylation[6] for 4-MU T-antigen synthesis.

Taking the advantage of the compatibility of our α-selective galactosylation with acyl functionality on C-2 amino groups, we designed the *N*-2,2,2-trichloroethoxycarbonyl (Troc)-protected disaccharide **7** as a DTBS glycosyl donor. Treatment of the readily accessible 2-*N*-Troc galactothioglycoside **2**[7] with DTBS(OTf)$_2$ in pyridine[8] gave 4,6-silylated **3** in 93% yield, which was then orthogonally glycosylated with the 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose **4** catalyzed by trimethylsilyl trifluoromethanesulfonate[9] to afford disaccharide **5** in 67% yield. The hemiacetalization of **5** with NBS in aqueous acetone[10] produced **6**. Finally, the hemiacetal **6** was converted into the corresponding chloride **7** by the action of Vilsmeier's reagent[11] (Scheme 1).

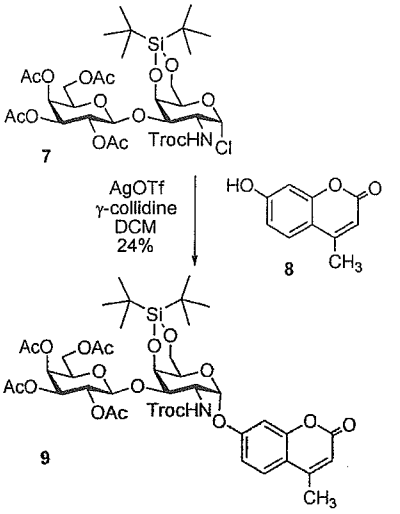
Scheme 1. Preparation of Gal β(1→3) GalN Disaccharide



With the glycosyl chloride **7** in hand, we then subjected it to a DTBS-directing α-glycosidation with 4-methylumbelliferone. Initially, we attempted reaction of the α-chloride **7** with 4-methylumbelliferone **8** in the presence of the silver triflate−γ-collidine complex.[5] This reaction provided the α-glycoside **9** exclusively in 24% yield together with the hemiacetal **6** as the main byproduct (Scheme 2). However, the yield could not be elevated any further.

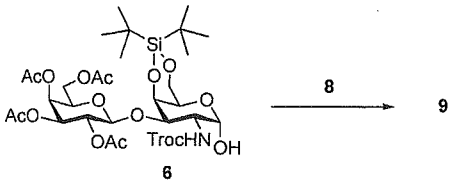
Accordingly, we next investigated the utility of the Mitsunobu reaction in this capacity.[12] Thus, the hemiacetal

Scheme 2. Condensation of **7** and 4-Methylumbelliferone **8**



6 was reacted with **8** in the presence of various combinations of trialkyl phosphines (TPP,[12] TBP,[13,15] DPPE[14]) and azocompounds (DEAD,[12] ADDP,[13] TMAD,[15] DIAD[12,14]) as summarized in Table 1. Surprisingly, the anomeric configuration of **6** was mostly retained; the α-glycoside **9** predominating in all these reactions. Interestingly, the yield of **9** increased when the reaction was performed at higher

Table 1. 4-Methylumbelliferylation by Mitsunobu Reaction



entry	phosphine/azo compd[b]	MU-OH (equiv)	solvent	*T* (°C)	% yield[c] (α/β)
1[d]	TPP/DEAD	3.0	THF	80	47:5
2[e]	TBP/ADDP	3.0	THF	80	20:−
3[f]	TBP/TMAD	3.0	THF	80	8:−
4[g]	DPPE/DIAD	3.0	THF	80	no reaction
5	TPP/DEAD	3.0	toluene	130	74:8
6	TBP/ADDP	3.0	toluene	130	62:15
7	TPP/DEAD	8.0	toluene	130	80:9

[a] Every reaction was conducted under reflux condition. [b] TPP, triphenylphosphine, TBP, tributylphosphine, DPPE, 1,2-bis(diphenylphosphino)-ethane, DEAD, diethyl azodicarboxylate, ADDP, 1,1′-(azodicarbonyl)dipiperidine, TMAD, 1,1′-azobis(*N*,*N*′-dimethylformamide), DIAD, diisopropyl azodicarboxylate. [c] Isolated yield. [d] See ref 12. [e] See ref 13. [f] See ref 15. [g] See ref 14.

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(7) Compound **2** was derived from galactosamine hydrochloride through four-step manipulation (65% overall) according to the method of 2-*N*-Troc glucothioglycoside: Yan, F.; Mehta, S.; Eichler, E.; Wakarchuk, W. W.; Gilbert, M.; Schur, M. J.; Whitfield, D. M. *J. Org. Chem.* **2003**, *68*, 2426−2431.
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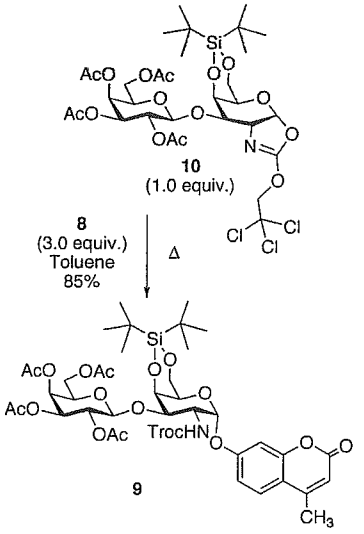
temperature (entries 5 and 6). Additionally, the use of excess 4-MU-OH (8.0 equiv) led to the optimum product yield (80%) (entry 7).

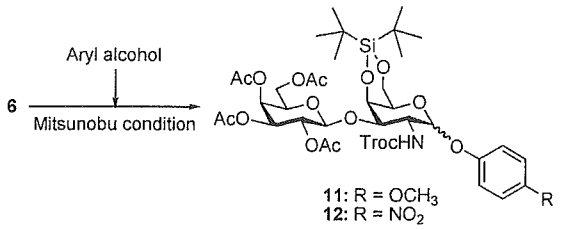
In contrast, in entries 1 and 2, the trichloroethoxyoxazole **10** was observed as a major byproduct. Furthermore, we observed the transitory formation of **10** during all our reactions according to TLC monitoring. This result implied that the oxazole **10** is a reaction intermediate; in fact, the isolated oxazole **10** reacted with 4-MU-OH in toluene under reflux to afford α-glycoside **9** in 85% yield (Scheme 3).

Scheme 3. Coupling of **10** and **8** in the Absence of Activators



Significantly, we have shown that this reaction can produce other α-aryl glycosides **11** and **12** in moderate yields (Table 2). Taking into consideration these results, we hypothesize that the coupling reactions proceed as follows: (i) initial formation of the oxazole intermediate results from triphenylphosphine oxide elimination, (ii) oxocarbenium ion formation then occurs, (iii) "through-space" electron-donation[16] from axially oriented C4 or C6 hydroxyl places the ^{t}Bu moiety closer to the anomeric carbon, and (iv) attack of 4-MU-OH is then restricted to the α-face of anomeric center due to steric hampering by the DTBS group (Scheme 4).

Table 2. Various Arylations by Mitsunobu Reaction[a]



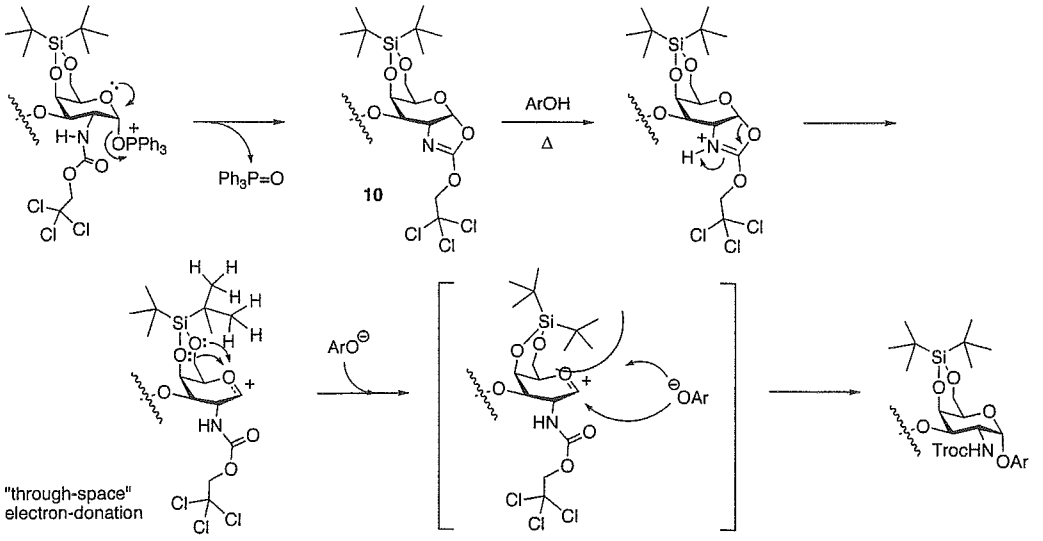
entry	aryl alcohol	product	% yield (α/β)
1	MPOH	**11**	56:–
2	PNPOH	**12**	41:17

[a] All reaction conditions are the same as entry 5 in Table 1.

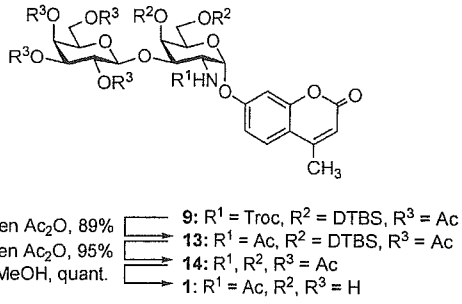
Finally, as depicted in Scheme 5, 4-MU glycoside **9** was transformed into **1**. Thus, deprotection of Troc group by the action of Zn and subsequent acetylation provided acetamide **13** in 89% yield. Removal of 4,6-*O*-DTBS group by TBAHF[17] and sequential acetylation yielded fully acetylated 4-MU T-antigen **14** in 95% yield, which was subjected to de-*O*-acetylation[18] to afford free 4-methylumbelliferyl T-antigen **1**.

In conclusion, we have found a new method for forming α-4-MU galactosaminide based upon the DTBS effect and the Mitsunobu reaction. The synthesized 4-MU T-antigen will serve as a powerful probe for enzymatic studies, e.g., seeking the unknown *endo*-α-*N*-acetylgalactosaminidase.

Scheme 4. Expected Reaction Mechanism on Aryl Glycosylation



Scheme 5. Final Deprotections



Now we are also undertaking the synthesis of other tumor-associated glycan antigen probes having 4-MU.

Acknowledgment. This work was partly supported by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan (Grant-in-Aid for Scientific Research to M. Kiso, No. 17101007) and CREST of JST (Japan Science and Technology Corporation.). We thank Ms. Kiyoko Ito for technical assistance.

Supporting Information Available: Full experimental details and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL051592Z

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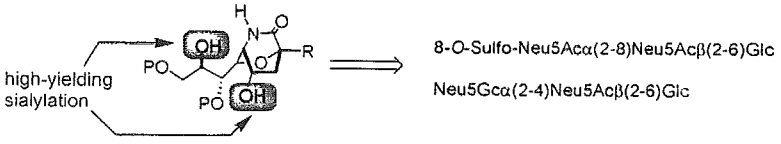
Sialylation

1,5-Lactamized Sialyl Acceptors for Various Disialoside Syntheses: Novel Method for the Synthesis of Glycan Portions of Hp-s6 and HLG-2 Gangliosides



A dramatic enhancement of the reactivity of the C4- and C8-hydroxy groups of sialic acid has been demonstrated by 1,5-lactam bridging. Sialyl-α(2→4)sialoside and sialyl-α(2→8)sialoside were made available in high yields through direct sialylation (see scheme). Furthermore, the glycan parts of the new gangliosides Hp-s6 and HLG-2 were synthesized for the first time.

H. Ando,* Y. Koike, S. Koizumi, H. Ishida, M. Kiso* _____ 6759 – 6763

Keywords: gangliosides · glycosylation · lactams · sialic acids

2005 – 44/41

WILEY-VCH

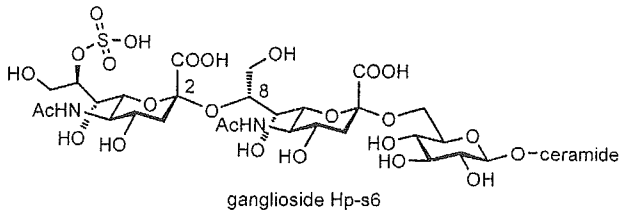
Sialylation

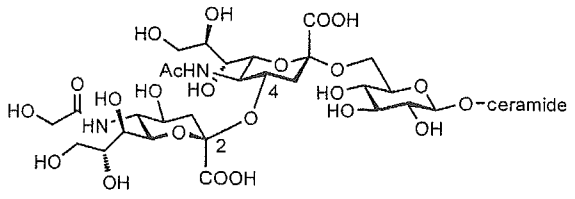
DOI: 10.1002/anie.200501608

1,5-Lactamized Sialyl Acceptors for Various Disialoside Syntheses: Novel Method for the Synthesis of Glycan Portions of Hp-s6 and HLG-2 Gangliosides**

Hiromune Ando, Yusuke Koike, Sachiko Koizumi, Hideharu Ishida, and Makoto Kiso**

The ongoing studies on oligosaccharide synthesis have resulted in the development of precise synthetic methods by which a large portion of the complex natural oligosaccharides can be duplicated.[1] Although the synthesis of the sequence Neu5Acα(2→8)Neu5Ac (α(2→8)disialic acid; Neu5Ac = *N*-acetylneuraminic acid) has been a major difficulty, the emergence of several exquisite methods[2] that employ indirect coupling by using a C3-functionalized *N*-acetyl sialyl donor and direct coupling by using an *N*-trifluoroacetyl (TFAc)-protected sialic acid donor with the help of the nitrile solvent effect have paved the way for the successful synthesis of α(2→8)disialic acid containing oligosaccharides, such as those with GD3[2c] and GQ1b[3] glycan portions. However, it is obvious that the synthesis of new congeners of disialic acid, such as 8-*O*-sulfo-Neu5Acα(2→8)Neu5Ac in ganglioside Hp-s6[4] and Neu5Gcα(2→4)Neu5Ac in ganglioside HLG-2[5] (Scheme 1), is still difficult because of the diverse modifications possible at the functionality level. On the basis of the predicted biological functions of the disialic acid congener containing oligosaccharides relevant to functions such as neural network formation and fertilization, the establishment of an expedient synthetic method that includes the entire disialic acid family seems essential not only for the progress of glycochemistry but also for studying in detail the molecular





Scheme 1. Structures of novel disialyl gangliosides Hp-s6 and HLG-2.

basis underlying the biological functions of these compounds. In this study, we report a novel synthetic method for the synthesis of disialic acid congener containing glycans that uses highly reactive lactamized sialyl acceptors and an *N*-2,2,2-trichloroethoxycarbonyl (Troc)-protected sialyl donor.

Recently, we reported an *N*-Troc-protected sialyl donor (*N*-Troc donor **1**) that shows elevated reactivity and a high degree of accessibility for various sialic acid congeners such as *N*-glycolylneuraminic acid (Neu5Gc), 8-*O*-sulfo-Neu5Ac, and 1,5-lactam-Neu.[6] Initially, we anticipated that use of the *N*-Troc donor would enable the design of HLG-2 and Hp-s6 glycan sequences in an expedient manner. However, as depicted in Scheme 2, the results of the condensations with 4-OH and 8-OH sialyl acceptors, **2** and **3**, respectively, did not meet expectations with regard to yields and stereoselectivity. Even in the case of **2**, which showed the relatively higher reactivity, α-disialyl glycoside was obtained in less than 5%. We hypothesized that the poor results were mainly due to unfavorable hydrogen bonding with the amide moiety at C5, as proposed previously by Tsvetkov and Schmidt.[7] This hypothesis was the basis of the idea that the conformational transformation from the $^2C_5$ chair form to the fixed boat form with the 1,5-lactam bridge would result in increased reactivity of both the C4- and C8-hydroxy groups.[8]

To form the 1,5-lactam bridge in the sialoside, the previously reported *N*-TFAc-sialic acid derivative **6**[9] was used as the key precursor (Scheme 3). After the coupling reaction of **6** and tribenzylated glucosyl acceptor **7**, the resulting sialyl-α(2→6)Glc disaccharide, **8**, was subjected to 1,5-lactamization. First, we attempted a carbodiimide-mediated intramolecular amide formation after the complete deacylation and saponification of **8**, but this reaction yielded a complex mixture. The optimum yield was obtained when **8** was treated with methanolic sodium methoxide in the presence of Drierite under reflux to provide the 1,5-lactam-sialyl glucoside **9** in 85% yield; through regioselective benzoylation of the C9-hydroxy group of **9** with benzoyl chloride and pyridine, under kinetic control, triol acceptor **10** was produced. For the synthesis of the 8-hydroxy-1,5-lacta-

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[**] Synthetic Studies on Sialoglycoconjugates, Part 139. This work was financially supported by CREST of JST (M.K.), MEXT of Japan (Grant-in-Aid for Scientific Research; no. 16780083 to H.A., no. 16580086 to H.I., and no. 17101007 to M.K.), and the Mitsubishi Chemical Corporation Fund (H.A.). We thank Ms. Kiyoko Ito for technical assistance. For Part 138, see: M. Yamaguchi, H. Ishida, A. Kanamori, R. Kannagi, M. Kiso, *Glycoconjugate J.* **2005**, *22*, 83–96.

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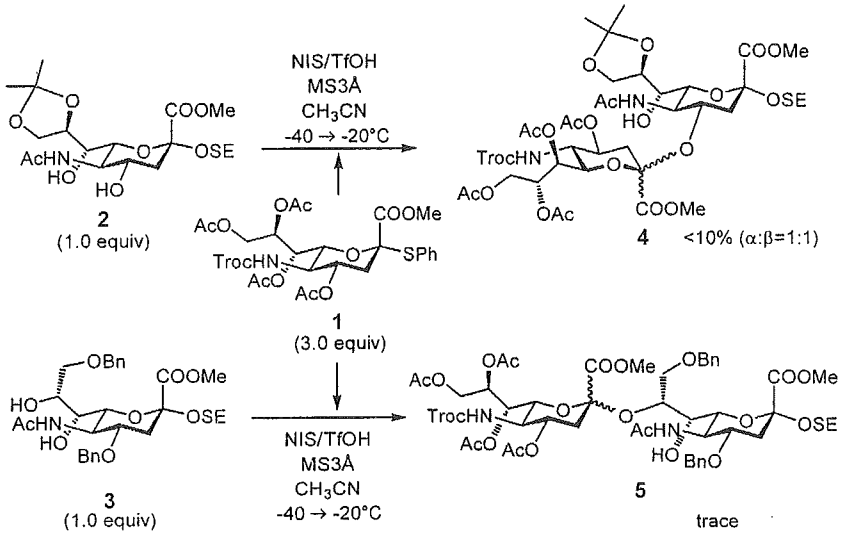
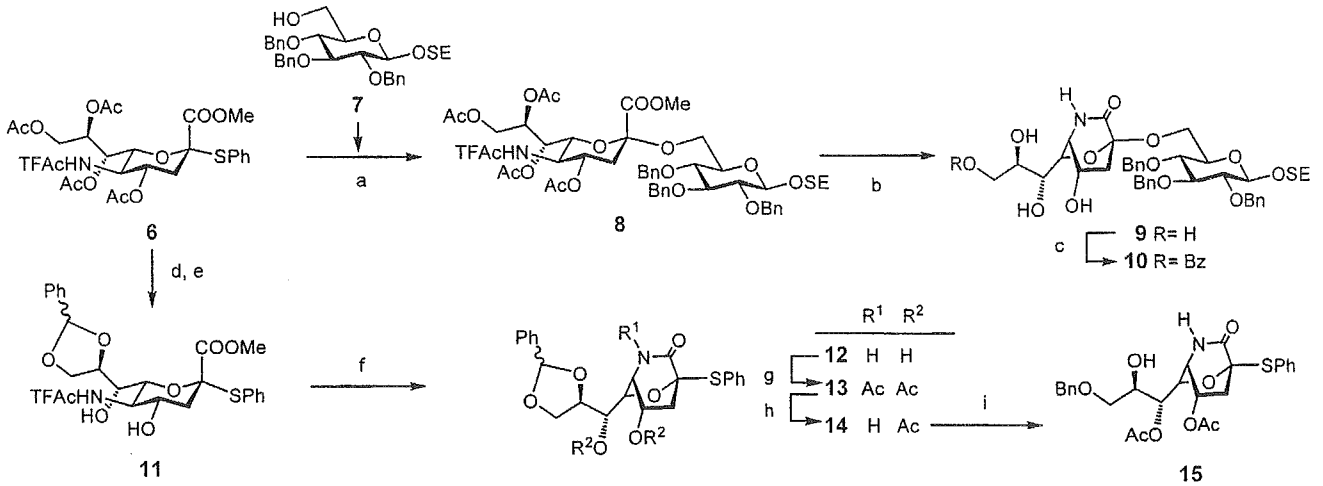


Scheme 2. Unsuccessful sialylation to 4-OH and 8-OH sialyl acceptors. Bn = benzyl, MS = molecular sieves, NIS = *N*-iodosuccinimide, SE = 2-(trimethylsilyl)ethyl, TfOH = trifluoromethanesulfonic acid.

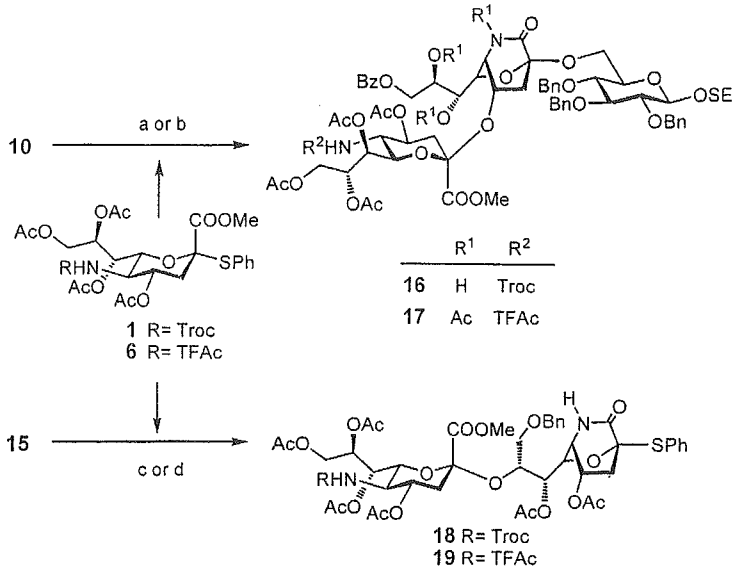
mized sialyl acceptor, 8,9-*O*-benzylidenation was required prior to the lactamization because 8,9-*O*-acetalization of the lactamized derivative was unsuccessful. Thus, compound **6** was de-*O*-acetylated and this was followed by the conventional 8,9-*O*-bezylidenation with benzaldehyde dimethyl acetal and camphorsulfonic acid to produce **11**, which was then subjected to the one-pot 1,5-lactam formation mentioned earlier to yield bicyclo-sialoside **12** in 89% yield. Next, **12** was completely acetylated with Ac_2O in the presence of pyridine and the product was successively de-*N*-acetylated with hydrazinium acetate in a chemoselective manner to produce compound **14**. Finally, reductive ring opening of the benzylidene acetal moiety, influenced by $BH_3{\cdot}NMe_3$ and $AlCl_3$ in THF,[10] produced the 8-OH lactam acceptor **15**.

Next, we carried out the glycosylation of the lactam acceptors **10** and **15** with *N*-Troc- and *N*-TFAc-sialyl donors to evaluate their properties as glycosyl acceptors (Scheme 4). First, the triol acceptor **10** was treated with *N*-Troc donor **1** in the presence of NIS, TfOH, and a molecular sieve in EtCN[11] at −40°C to provide the Neuα(2→4)Neuα-(2→6)Glc sequence **17**, along with the corresponding β isomer. The anomeric configuration of the new ketosidic linkage was determined on the basis of previous reports[12] by measuring the long-range $^3J_{C1,H3ax}$ coupling constants. For compound **17** this coupling constant was 5.4 Hz, whereas for the β isomer it was less than 1.0 Hz, a fact indicating that the amomeric configuration of **17** was α. Similarly, the coupling reaction with the *N*-TFAc donor **6** and the complete acetylation that followed yielded the corresponding Neuα(2→4)Neuα(2→6)Glc sequence **17** in 41% yield, along with the β isomer (10%) and the Neu(2→8){[Neu(2→4)]Neuα}(2→6)Glc sequences as an anomeric mixture (8%).

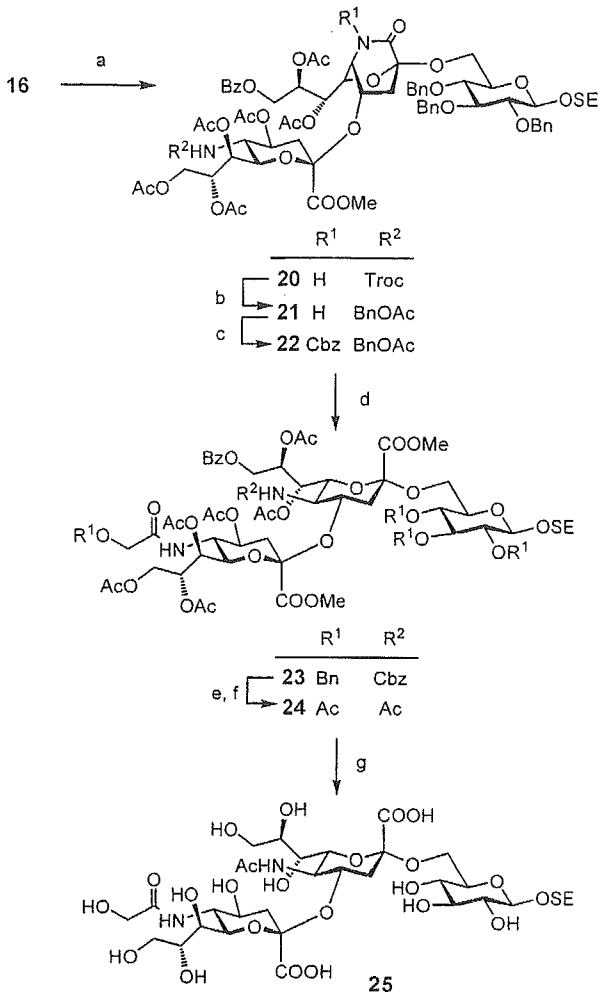
Next, we attempted to fashion the purest form of Neuα(2→8)Neu sequence (Scheme 4). As initially expected, the glycosylation reactions of the lactam acceptor **15** with *N*-Troc and *N*-TFAc donors (**1** and **6**) yielded the corresponding Neuα(2→8)Neu sequences. Thus, *N*-Troc donor **1** and *N*-TFAc donor **6** were incorporated, in the presence of NIS, TfOH, and a molecular sieve in EtCN, at −80°C to yield α(2→8)disialosides **18** and **19** in 49 and 71% yield, respectively; no corresponding β form was generated in either event. To the best of our knowledge, the yield of addition to the C8-hydroxy group of sialic acid (71%) during the sialylation process was the highest value obtained by direct coupling methods[2] In keeping with the results of the previous experiments, the anomeric configuration of the new linkages was determined to be α from $^3J_{C1,H3ax}$ coupling constants that



Scheme 3. a) **7**, NIS, TfOH, CH_3CN/CH_2Cl_2, MS (3Å), −30°C, 5 min, 74%; b) NaOMe, MeOH, Drierite, reflux, 44 h, 85%; c) BzCl, py/CH_2Cl_2, −40°C, 90 min, 79%; d) NaOMe, MeOH, room temperature, 29 h; e) $PhC(OMe)_2$, CSA, DMF, 40°C, 2 h, 88% (2 steps); f) NaOMe, MeOH, Drierite, reflux, 5 d, 89%; g) Ac_2O, py, DMAP, room temperature, 3 h; h) $NH_2NH_2{\cdot}AcOH$, THF, room temperature, 80 min, 94% (2 steps); i) $BH_3{\cdot}NMe_3$, $AlCl_3$, THF, MS (4Å), 0°C→RT, 6 h, 74%. Bz = benzoyl, CSA = (±)-10-camphorsulfonic acid, DMF = *N*,*N*-dimethylformamide, DMAP = 4-dimethylaminopyridine, py = pyridine, THF = tetrahydrofuran.



Scheme 4. a) **1** (2.0 equiv), NIS (3.0 equiv), TfOH (0.3 equiv), EtCN, MS (3 Å), −40 °C, 6 h, 84% (α/β 66:18); b) 1. **6** (2.0 equiv), NIS, TfOH, EtCN, MS (3 Å), −40 °C, 6 h; 2. Ac_2O, py, DMAP, 40 °C, 17 h, 51%; c) **1** (3.0 equiv), NIS, TfOH, EtCN, MS (3 Å), −80 °C, 5 h, 49% (α only); d) **6** (3.0 equiv), NIS, TfOH, EtCN, MS (3 Å), −80 °C, 3 h, 71% (α only).



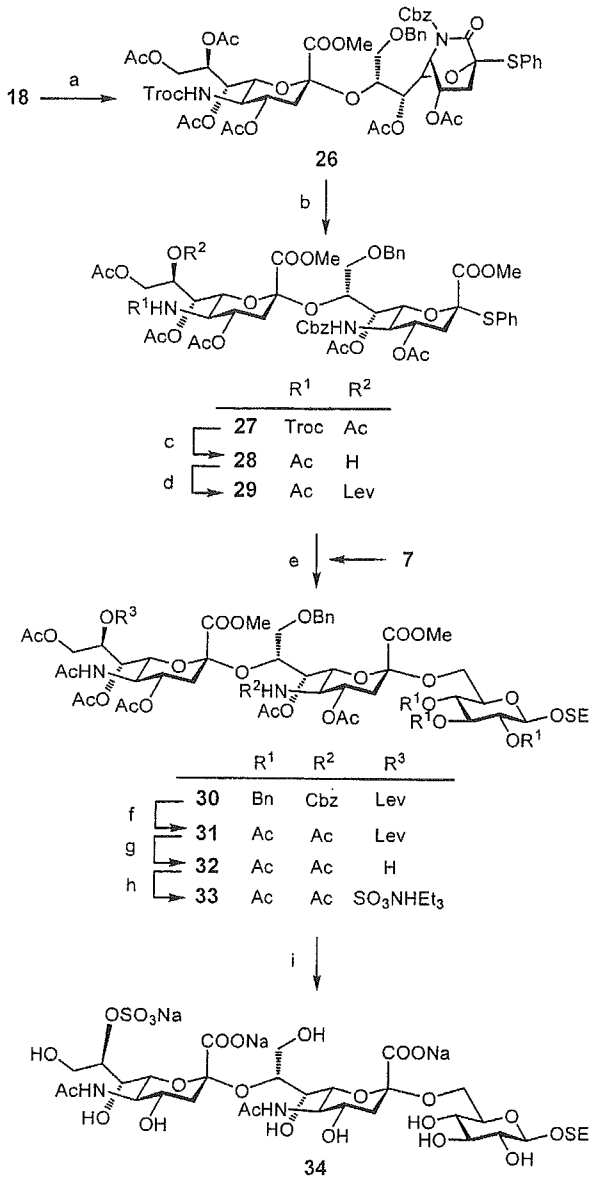
Scheme 5. a) Ac_2O, py, room temperature, 10 h, 89%; b) 1. Zn, AcOH, room temperature, 2 h; 2. BnOAcCl, THF, room temperature, 1 h, 74% (2 steps); c) Cbz_2O, DMAP, py, 40 °C, 26 h, 95%; d) 1. Et_3N, H_2O/CH_3CN, room temperature, 2 d; 2. MeI, K_2CO_3, DMF, room temperature, 30 min, 74% (2 steps); e) 1. H_2, 10% $Pd(OH)_2$/C, NH_3, EtOH, room temperature, 2 h; 2. AcCl, room temperature, 1 h, 68% (2 steps); f) 1. H_2, 10% $Pd(OH)_2$/C, EtOH; 2. Ac_2O, py, room temperature, 54% (2 steps). Cbz = benzyloxycarbonyl.

ranged from 6.7 to 6.9 Hz. Furthermore, the phenylsulfenyl group at the bridgehead anomeric center of acceptor **15** remained unaffected during the coupling reactions. This result confirmed our initial hypothesis, based on Bredt's rule, suggesting the basis of a novel method for the complete deactivation of a sialyl donor.

On the basis of the results obtained with regard to the performance of 1,5-lactamized sialic acid acceptors **10** and **15** in the sialylation reactions, we focused on the synthesis of the glycan portions of HLG-2 and Hp-s6 gangliosides of **16** and **18**, respectively, in order to demonstrate the practical efficacy of the synergic strategy for synthesizing variant disialosides from the 1,5-lactam-sialyl acceptor and *N*-Troc-sialyl donor.

In the initial stages of the synthesis of the HLG-2 glycan portion (Scheme 5), the trisaccharide **16** was *O*-acetylated to provide **20**, to which the *N*-glycolyl moiety was introduced by our reported method,[6] thereby providing **21** in a relatively high yield (66% from **16**). Next, we attempted to recover the $^2C_5$ conformation of the inner sialic acid unit. The following reaction sequences supplied HLG-2 glycan frame **23** in a high yield: *N*-benzyloxycarbonylation, basic hydrolysis, and ensuing methylation of the carboxy group. Debenzylation and acetylation to replaced the Cbz group of **23** by the acetyl group and full deprotection of the product **24** yielded the HLG-2 glycan structure **25**.

In the case of the Hp-s6 glycan frame, the "locked-up" phenylsulfenyl group at the bridgehead carbon atom of **18** was converted into an active state in the initial stages (Scheme 6). To be precise, the reaction sequences mentioned earlier yielded $^2C_5$ conformer **27** in 62% overall yield. For the purpose of 8-*O*-sulfonylation in the final stages of the synthesis, **27** was further transformed into the 8-hydroxy derivative **28** by our regioselective acetyl-transfer method,[6] and the C8 hydroxy group was capped with a levulinoyl group to produce high yields (89%) of the suitably protected disialic acid donor **29** in two steps. Compound **29** was then treated with glucosyl acceptor **7**, influenced by the NIS/TfOH activator system in EtCN at −80→−60 °C, to provide Neuα-(2→8)Neuα(2→6)Glc sequence **30** in 66% yield, predominantly in the α configuration. Next, replacement of the Cbz and benzyl groups of trisaccharide **30** by the acetyl group, followed by chemoselective deblocking of the levulinoyl group with hydrazinium acetate[13] and sulfonylation with SO_3·pyridine resulted in the formation of a completely protected Hp-s6 glycan frame, **33**.[14] The ^{1}H NMR signal for the C8 proton of the outer sialic acid appeared in compound **33** at lower magnetic field ($\delta = 4.92$ ppm) than in compound **32** ($\delta = 4.22$ ppm), and the heteronuclear multiple-bond coherence (HMBC) spectrum of compound **33** contained cross-coupling signals between carbonyl carbon atoms of acetyl groups at C7 and C9, and H7 ($\delta = 5.40$ ppm) and H9



Scheme 6. a) CbzOSu, DMAP, py, room temperature, 42 h, 79%; b) 1. Et_3N, H_2O/CH_3CN, 40°C, 45 h; 2. MeI, K_2CO_3, DMF, room temperature, 3 h, 79% (2 steps); c) Zn, AcOH, THF, room temperature, 28 h, 94%; d) LevOH, DCC, DMAP, CH_2Cl_2, room temperature, 2 h, 95%; e) **7**, NIS, TfOH, EtCN, MS (3 Å), −80→−60°C, 4 d, 66%; f) 1. H_2, 10% $Pd(OH)_2/C$, NH_3, EtOH, room temperature, 1 h; 2. Ac_2O, py, room temperature, 30 min; 3. H_2, 10% $Pd(OH)_2/C$, EtOH, 40°C, 3 h; 4. Ac_2O, py, room temperature, 12 h, 86% (4 steps); g) NH_2NH_2·AcOH, EtOH, room temperature, 6 h, 90%; h) SO_3·py, py, room temperature, 7 h, 65%. Lev = levulinoyl = 4-oxopentanoyl, Su = succinimidyl, DCC = *N*,*N*′-dicyclohexyl carbodiimide.

($\delta = 4.19$ ppm). Thereby, the installation of the sulfonyl group on the C8-hydroxy group was determined.

In conclusion, we have discovered that 1,5-lactam bridging in sialic acid endows high reactivity to the C4- and C8-hydroxy groups, thereby leading to the supply of α(2→4)- and α(2→8)disialic acid sequences in high yields. Furthermore, the practical efficacy of the synergic synthetic approach toward diverse disialic acid containing oligosaccharides, based on the *N*-Troc donor and the lactamized acceptors as the main units, has been demonstrated by the novel method for the synthesis of the HLG-2 and Hp-s6 glycan chains. On the basis of these results, we are now investigating the synthesis of α(2→8)-linked oligosialic acids.

Received: May 11, 2005
Revised: July 13, 2005
Published online: September 27, 2005

Keywords: gangliosides · glycosylation · lactams · sialic acids

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小脳グリア細胞の分化機構—糖鎖生物学の視点から

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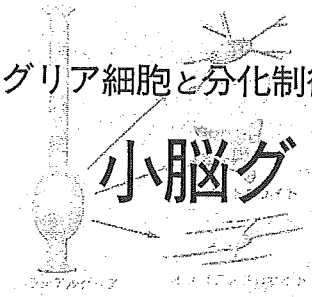
CLINICAL NEUROSCIENCE 別冊

Vol. 23 No. 2　2005年2月1日発行

中外医学社



グリア細胞と分化制御

小脳グリア細胞の分化機構 — 糖鎖生物学の視点から

加藤啓子　平林義雄

▣ はじめに

脳の発生過程において，神経前駆体細胞が定められた位置に移動しシナプスを成熟させていく際に，ニューロン-グリア間の相互作用が極めて重要な鍵を担っている．特に，小脳の層構造は組織学的解析，およびその構造機能相関の解明が進んでいることから，小脳をモデルにしたニューロン-グリア相関に関する知見が多く集積されてきている．本稿では小脳皮質の代表的アストロサイトであるBergmannグリアを中心に，ポストゲノムとして最近注目を集めている糖鎖の関与について，マウスの例を中心に紹介した．

マウス小脳発生の形態学的基礎[1,2]

小脳発生は胎生期に始まるが，その大部分は出生後に決定される(図1)．マウス胎生9〜11日頃の第四脳室レベルに位置する脳室帯ventricular zoneで神経前駆細胞とグリア細胞が発生する(図1A)．その後，神経前駆細胞が外側基底膜(軟膜)を目指して遊走する一方で，グリア細胞は放射状グリアradial Bergmann gliaとして細胞体を脳室帯に残したまま放射状突起を軟膜まで伸展する．胎生14日になると，小脳板cerebellar plateから小脳原基cerebellar primordiumが発生し，細胞レベルでは，軟膜を目指して遊走していた神経上皮細胞が辺縁帯marginal zoneの外側に到達し，外顆粒層external granular layer(EGL)を形成する(図1B)．胎生17日には，この外顆粒層が神経上皮細胞と脈絡叢choroid plexusと接する胚三角錐germinal trigoneが出現するようになる(図2)．外顆粒層が出現すると，すぐに脳室帯に位置していたPurkinje細胞が放射状Bergmannグリアの突起に沿って中間帯intermediate zoneに遊走する．放射状グリアはPurkinje細胞の遊走を誘導した後，放射状突起を短縮し，Purkinje細胞層に移動する(図1C)．一方，この頃オリゴデンドロサイトも脳室帯に出現する．

生後すぐに，外顆粒層に位置する3種の介在ニューロン前駆細胞(顆粒細胞，バスケット細胞，星状細胞)が分裂を開始し，生後1日目以降には，分裂を停止した顆粒細胞が順次，外顆粒層の内側へと遊走を開始する(inner EGL，将来の分子層)(図1C)．生後3週までには，これら3種の介在ニューロンが将来の分子層に位置するようになる．一方で顆粒細胞は，生後8〜10日頃にBergmannグリアに沿って分子層からPurkinje細胞層の直下にまで遊走し，生後21日には内顆粒層inner granular layerを形成する(図1D)．その後，脊髄から小脳に投射される苔状線維とシナプスを形成するようになる．生後21日には，外顆粒層は消失し，顆粒細胞へのBergmannグリアの関与は終了する．

一方，Purkinje細胞は生後4〜5日で小脳原基の発達に伴い，辺縁帯の直下の皮質板cortical plateに到達する(図1C)．すると下オリーブ核から登上線維が小脳に進入し，Purkinje細胞を神経支配する．このころになると(生後5〜15日)，Bergmannグリアが Purkinje細胞およびその樹状突起と直接に接し，一時期Purkinje細胞の樹状突起が登上線維で埋め尽くされる．この時Bergmannグリアは，樹状突起の伸展と発芽sproutingおよび，シナプスの構造的成熟に何らかの作用を示していると考えられている．生後20日になると，Purkinje細胞は平行線維からのシナプス入力を受けるようになり，生後21日目には，Purkinje細胞は1本の登上線維からの入力のみを受けるようになる．この後Bergmannグリアは，Purkinje細胞の細胞体および樹状突起を継続的に支持する．

最近まで，小脳での神経細胞の遊走に関わるグリア細胞は，Bergmannグリアのみが興味の対象となっていた．しかしながら，オリゴデンドロサイトを生後1〜20日間排除した際，Purkinje細胞と顆粒細胞の遊走異常および，バスケット細胞や星状細胞の遊走異常も観察されており，このことは，Bergmannグリアに加えてオリゴデンドロサイトも神経細胞の移動に積極的に関与していることを示している[3]．

成熟後の小脳入力は，脊髄から投射された苔状線維の情報が，顆粒細胞に伝えられ，顆粒細胞の軸索である平行線維によりPurkinje細胞の樹状突起に伝えられる．登上線維の情報は，上述のように直接Purkinje細胞の樹状突起に伝

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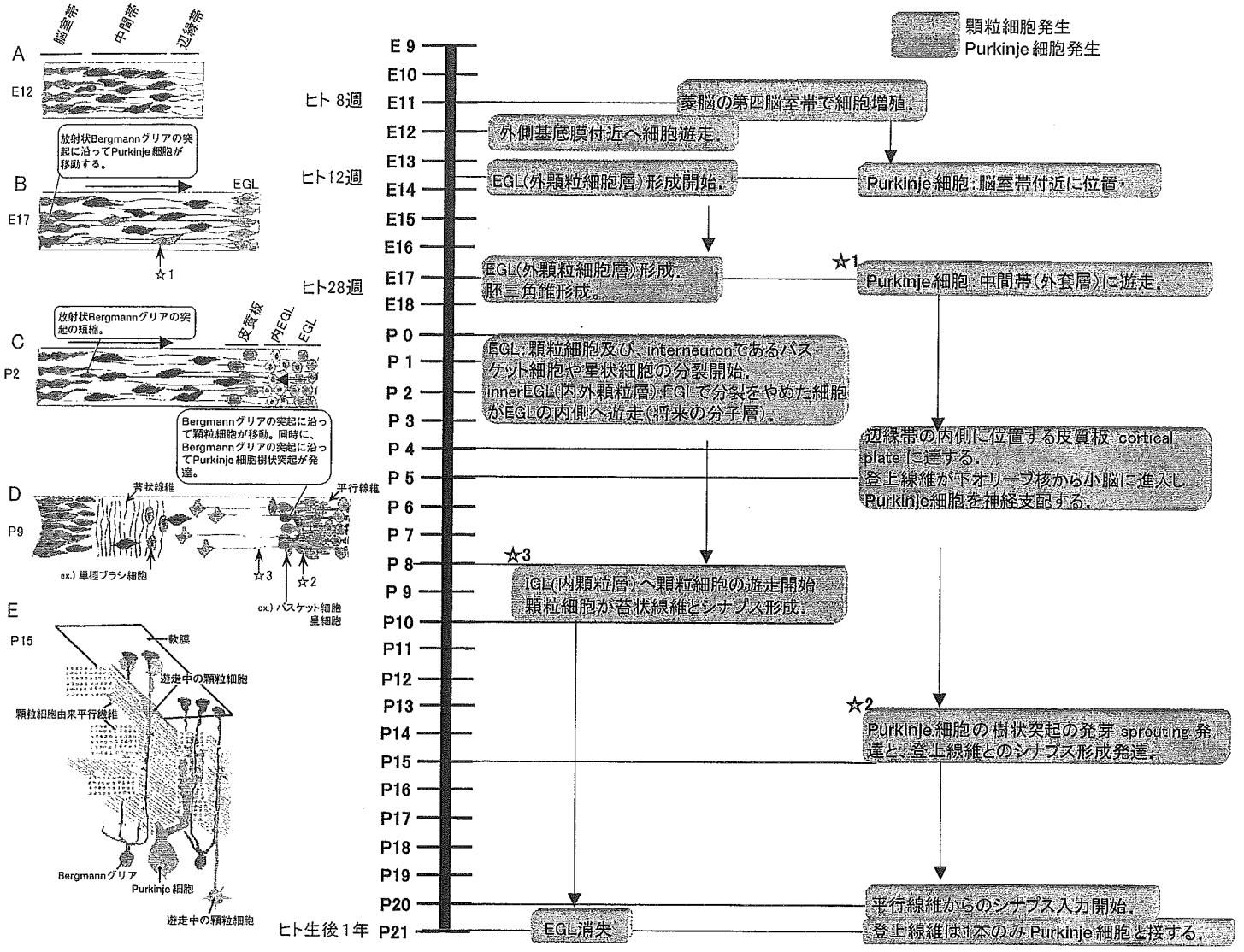


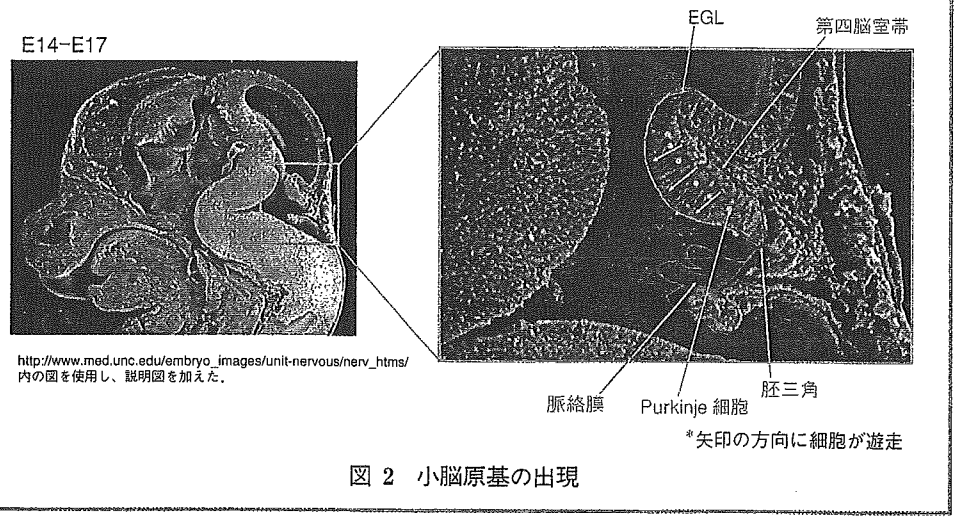
図 1　マウス小脳発生

☆：Bergmann グリアの関与を示す．E：Bergmann グリアに沿って顆粒細胞が移動しているのと同時に，Purkinje 細胞の樹状突起が Bergmann グリアに沿いながら発達している．

えられる．Golgi 細胞は顆粒細胞に，星状細胞は Purkinje 細胞に対して抑制性に作用する．そして小脳からの出力は唯一 Purkinje 細胞の軸索のみである．

小脳グリアの分化と糖鎖修飾

Bergmann グリアは，Purkinje 細胞と顆粒細胞の発生に不可欠な働きを示し，発生初期にみられる Purkinje 細胞の遊走(図 1 ☆ 1)と，後期にみられる Purkinje 細胞の樹状突



http://www.med.unc.edu/embryo_images/unit-nervous/nerv_htms/ 内の図を使用し、説明図を加えた．

図 2　小脳原基の出現

小脳グリアに発現する糖鎖構造とニューロンとの相関性

糖鎖構造	糖鎖修飾分子	発現細胞	ニューロンとの相関性	参考文献
Siaα 2-3 Galβ 1-3 GlcNAcβ 1-2 Man 1 α-Ser/Thr	αジストログリカン	Bergmann グリア	軟膜のラミニンと結合し，ニューロンの遊走	7, 18
9-*O*-acetyl GD 3	ガングリオシド	Bergmann グリア	顆粒細胞の外顆粒層から内顆粒層への遊走	8
コンドロイチン硫酸	PTPζ	Bergmann グリアと神経細胞	Bergmann グリアが発現するプレイオトロピンと結合し，Purkinje 細胞の樹状突起の発達促進	9
Siaα 2-3 Galβ 1-3 GlcNAcβ 1-3 Fuc- Ser/Thr	Notch	Bergmann グリア	Fringe(fucose 転移酵素)の発現がニューロンである	13, 14, 15
CD 15(Le^x)	糖タンパク質あるいは糖脂質	Bergmann グリア	？	19

起の成熟(☆2)，さらには顆粒細胞の外顆粒層から内顆粒層への移動(☆3)に関与している．特に，生後10日前後に見られるPurkinje細胞の樹状突起の発芽時期に一致して，Bergmannグリアはその細胞質突起を放射状から網状に変化させ，樹状突起と密接に接触する知見が報告されている[4]．しかしながら，Bergmannグリアを中心としたニューロン-グリア相関の分子メカニズムはほとんど不明であった．しかし，最近になってようやくその分子メカニズムに迫る知見が報告され，その中でも特に糖鎖の関与を示した知見は注目に値する．

まず，顆粒細胞の発達に関わる糖鎖の役割を示唆する例を紹介する．顆粒細胞の外顆粒層から内顆粒層への移動には，Bergmannグリアが発現する**O-グリカン化αジストログリカン**が関与する[5,6]．αジストログリカンはO-linked mannose β1,2-N-acetylglucosaminyltransferase(POMGnT 1)によりO-グリカン糖鎖修飾を受け，この酵素が欠損すると中枢神経症状を合併する遺伝性の筋ジストロフィーを発症することが知られている[7]．胎生14日までにBergmannグリアは放射状突起を軟膜にのばし，Bergmannグリア側のO-グリカンと軟膜側のラミニンが結合することにより，顆粒細胞の遊走を可能にする．αジストログリカン上のO-グリカンが欠損すると，顆粒細胞の遊走が果たせず，小脳発生が終了した後も顆粒細胞を含んだ外顆粒層が軟膜側に残ってしまう．

この顆粒細胞の移動には，**9-O-アセチルGD 3ガングリオシド**も関与する[8]．スフィンゴ糖脂質の中でシアル酸を含むガングリオシドは脳に特徴的に多く含まれ，その組成が哺乳動物間で保存されている．生後9日頃のBergmannグリアが9-O-アセチルGD 3ガングリオシドを発現しており，この時期に9-O-アセチルGD 3ガングリオシドに対する特異抗体を脳室内に投与すると，抗体はBergmannグリアにのみ結合し，顆粒細胞の外顆粒層から内顆粒層への移動を阻害する．これは，9-O-アセチルGD 3ガングリオシドを介したニューロン-グリア間の分子相互作用の存在を暗示している．しかし，GD 3ガングリオシド合成酵素のノックアウトマウスは，見かけ上脳の基本的形態には異常がないので，他の陰性荷電を持った糖鎖により機能補完されていると考えられている．

小脳発生後期に見られるPurkinje細胞の樹状突起の発達とシナプスの成熟に，Bergmannグリアとの物理的接触が必須であるといわれている．このニューロン-Bergmannグリア間の相互作用に，ある種のプロテオグリカンが機能していることがわかってきた．**プロテオグリカン**とは，コンドロイチン硫酸，デルマタン硫酸，ヘパラン硫酸，ヘパリン，ケラタン硫酸等のグリコサミノグリカン(GAG鎖)と呼ばれる硫酸化多糖がタンパクに共有結合してできる糖タンパク質のことである．GAG鎖の基本構造は，ウロン酸(あるいはガラクトース)とアミノ糖であるヘキソサミン(グルコサミン・ガラクトサミン)からなる二糖構造が繰り返し直列に結合している糖鎖であり，プロテオグリカン上の糖鎖は，2種以上のGAG鎖が含まれる場合や，通常の糖タンパク質に見られるようなN-結合型オリゴ糖やO-結合型オリゴ糖も含む．脳では特にコンドロイチン硫酸やヘパラン硫酸からなるプロテオグリカンが多く存在する．

コンドロイチン硫酸プロテオグリカンであるPTPζ/phosphacanは，Purkinje細胞とBergmannグリア双方が発現する．その一方で，ヘパリン結合成長因子・プレイオトロピンがそのリガンドとしてBergmannグリアより分泌され，Purkinje細胞とBergmannグリア膜上のPTPζに結合する．この結合にPTPζ上のコンドロイチン硫酸が必須であり，コンドロイチン硫酸がなくなるとBergmannグリアに特異的に発現するGLAST(グルタミン酸トランスポーターの一種)の発現量が減少し，Purkinje細胞の樹上突起は未発達に止まる．実際に，遊離コンドロイチン硫酸あるいは，コンドロイチナーゼABCを添加した場合，近位樹状突起の走行異常が観察されている[9]．コンドロイチン硫酸を介した細胞内シグナルがどのように伝えられるのかは多くの点で疑問を残しているが，最近になって，そのシグナル系を示唆する傍証がいくつか報告されてきている．

甲状腺機能低下症が小脳外顆粒層における細胞分裂の低下を引きおこし，その結果，外顆粒層から内顆粒層への顆粒細胞の移動の低下や，Purkinje細胞の発達不全を引きお

こす[10]．この分子メカニズムもニューロン-グリア相関が関与していることがわかってきた[11]．小脳アストロサイトが，甲状腺ホルモン(Thyroid hormone T 3)の刺激を受け，EGFを分泌し小脳顆粒細胞の分裂を誘導する．その一方で，アストロサイト自身のEGF受容体がEGFを受け取り，ERK 1/2が活性化される．その結果フィブロネクチンやラミニンの発現が上昇し，神経突起伸長を誘導する．ERK 1/2の活性化はコンドロイチン硫酸の発現により制御されていることが，メラノーマ細胞を用いた解析から明らかになっており[12]，このことは，T 3により誘導される小脳顆粒細胞の発達にもコンドロイチン硫酸の関与が考えられる．

以上，図1☆に記したニューロンの発生に関わるBergmannグリアの関与について，糖鎖を介した分子シグナルを中心に記した．一方，上述以外にもニューロン-グリア相関に関わる糖鎖合成の役割を示唆する知見がある．例えば，体節決定に関わる**Notchとそのリガンドである Jagged**は共にBergmannグリアに発現している[13]．その一方で，以前よりNotch-シグナルのスイッチとして知られていた**Fringe**は長い間その生化学的機能がわからなかったが，最近になって，Notchに*O*-グリカンを付加する酵素(fucose-specific β1,3-*N*-acetylglucosaminyltransferase)であることが明らかとなり[14]，小脳神経細胞に強く発現することもわかってきた[15]．これらの知見は，Fringeによる Notch シグナルの開始スイッチも，ニューロン-Bergmann グリア相関に関与している可能性が高いことを示している．またヘパラン硫酸プロテオグリカン上のヘパラン硫酸も小脳発生に関わることがわかってきた．ヘパラン硫酸糖鎖合成ポリメラーゼ(グルクロン酸/N-アセチルグルコサミン転移酵素，EXT 1)のノックアウトマウスは，驚いたことに，胎生18.5日齢で小脳が欠失する[16]．現在までの知見では，ヘパラン硫酸を介したシグナル系がシナプス形成時に機能することが示されているが[17]，グリアからの制御の有無は不明であり，今後の解析が待たれる．

■むすび

以上述べてきたように，小脳発生におけるグリアの機能に，糖鎖を介したシグナル系が深く関わっていることが判明しつつある．その一方で，Bergmannグリアは，小脳グリアの中でもっともよく理解されているグリアであるが，その他のグリアは，その存在は知られているものの，機能はほとんど分かっていないのが現状である．また糖鎖に関しては，その構造および機能解析に関する本格的研究がスタートしたばかりであるが，今回紹介した例でわかるように，糖鎖合成の初期過程が機能的に特に重要であることが示されている．今後，小脳グリアとニューロン間相互作用に関わる糖鎖を介したシグナル系の解析が，更に進展することを期待したい．

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