Chemical Synthesis of Core Fucose Containing Complex-Type *N*-Glycan

コアフコース含有複合型 *N*-結合型糖鎖の化学合成

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Table of Contents

Abbreviations

Ac	Acetyl
ADCC	Antibody-dependent cellular cytotoxicity
Asn	Asparagine
AzClBn	4-Azido-3-chlorobenzyl
Bn	Benzyl
Bu	Butyl
Bz	Benzoyl
cod	1,5-Cyclooctadiene
CPME	Cyclopentylmethylether
DDQ	2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
EGFR	Epidermal growth factor receptor
ESI	Electrospray ionization
Et	Ethyl
FGFR	Fibroblast growth factor receptor
Fmoc	9-Fluorenylmethyloxycarbonyl
ER	Endoplasmic reticulum
Fuc	Fucose
FUT8	Fucosyl transferase 8
Gal	Galactose
GlcNAc	*N*-acetylglucosamine
HPLC	High performance liquid chromatography

HBTU	*O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
HR	High resolution
IgG	Immunoglobulin G
MALDI	Matrix assisted laser desorption/ionization
Man	Mannose
Me	Methyl
MS	Mass spectrum
MS4A	Molecular sieves 4A
Neu5Ac	*N*-acetylneuraminic acid
NMR	Nuclear magnetic resonance
PET	Positron emission tomography
Ph	Phenyl
Phth	Phthaloyl
PMP	*p*-Methoxyphenyl
p-TsOH	*p*-Toluenesulfonic acid
Ser	Serine
rt	room temperature
TBS	*tert*-Butyldimethylsilyl
*t*Bu	*tert*-Butyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin-layer chromatography

TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl trifluoromethylsulfate
TOF	Time of flight
Troc	2,2,2-Trichloroethoxycarbonyl
UV	Ultraviolet
Z	Benzyloxycarbonyl

Chapter 1. Introduction

1-1. Structure and Function of *N*-Glycan

Oligosaccharides are widely expressed in our body and play important roles. Many kinds of oligosaccharides are linked to other biomolecules including proteins. Glycosylation is the most abundant post-translational modification of proteins; approximately 50% of proteins possesses oligosaccharides.[1)] The oligosaccharides on proteins are classified as two categories, i.e., *O*-glycan linked to Ser/Thr side chain and *N*-glycan linked to Asn side chain of (Asn-X-Ser/Thr) sequence. *N*-Glycans are divided into three types: high mannose-type, complex-type, and hybrid-type (Figure 1-1). All these glycans are produced from the common *N*-glycan composed of three glucoses, nine mannoses, and two *N*-acetyl glucosamines, which is transferred to proteins in the endoplasmic reticulum (ER) after the ribosomal protein biosynthesis. The protein-bound *N*-glycan is subjected to oligosaccharide processing and the processed glycans work as tags for the quality control of proteins during the protein folding process in the ER.[2)] Further structural modifications are then effected with various enzymes in Golgi apparatus to generate a variety of *N*-glycans. *N*-Glycans in nature are generally heterogeneous, even for one specific glycosylation site. These structural diversities are called glycoform.

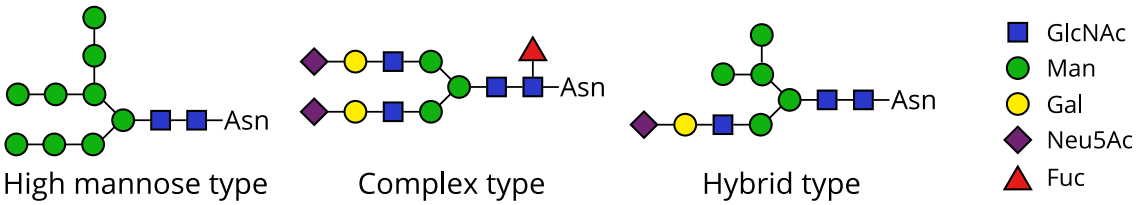


Figure 1-1. Three types of *N*-glycans.

Complex-type *N*-glycans play important roles in various life phenomena and diseases, including regulation of glycoprotein dynamics[3)], cell development,[4)] immunity,[5)] virus infection,[6)] and cancer invasion.[7)] For exmaple, sialic acid controls *in vivo* dynamics of glycoproteins such as erythropoietin.[3a, 3b)]

Structure of *N*-glycans influences the function and dynamics of glycoproteins *in vivo*. For example, our research group conducted *in vivo* PET imaging of *N*-glycan using glycoprotein and dendrimer-type glycoclusters to reveal the remarkable dependence of the *in vivo* dynamics and bio-distributions in the glycan structure, i.e., the presence or absence of sialic acid moieties as well as the linking of sialic acid to galactose 3-OH or 6-OH positions (Figure 1-2).[8)]

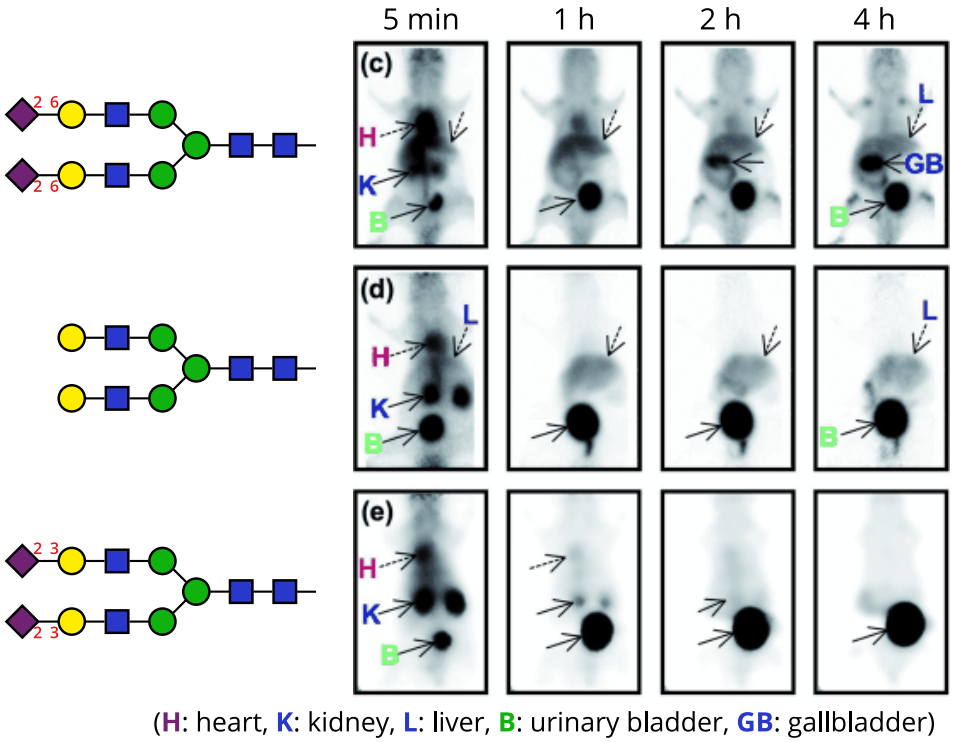


Figure 1-2. *In vivo* imaging of three kinds of *N*-glycan.[8)]

1-2. Core Fucose in *N*-Glycan

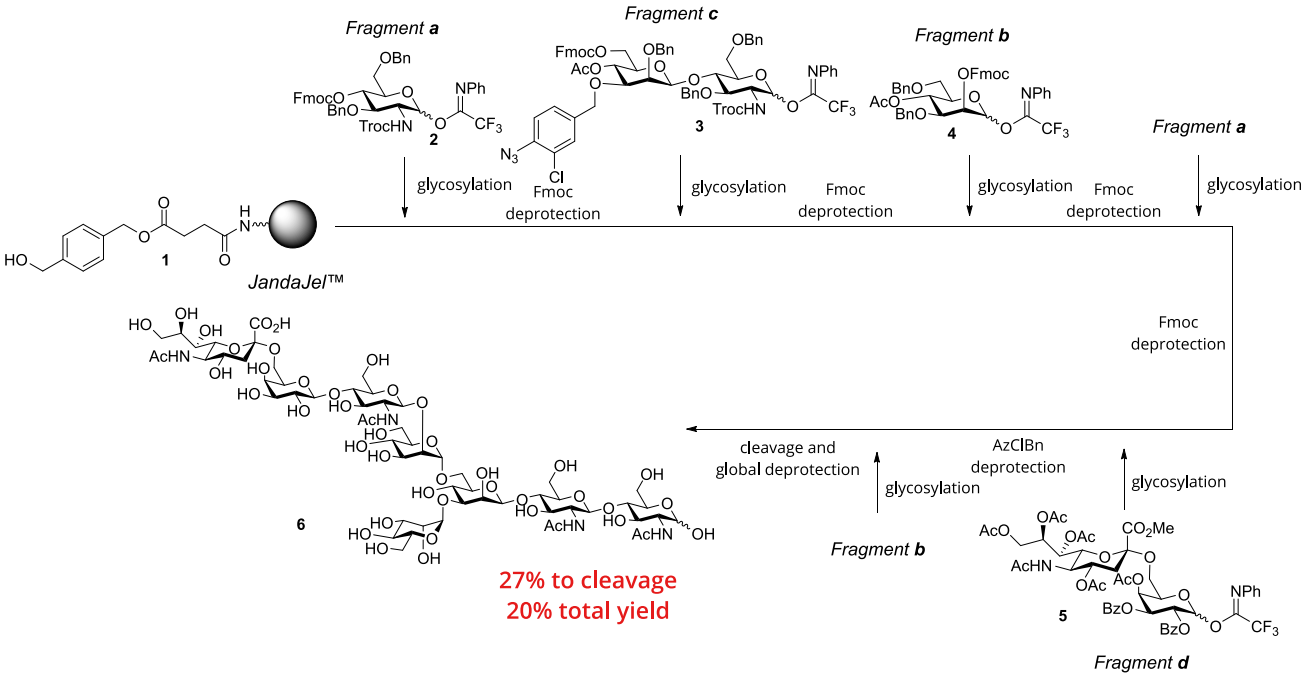
Fucose residue linked to the reducing end GlcNAc with α-linkage is termed core fucose which has α(1-6) structure in mammals whereas α(1-3) in plants.[7c)] Core fucose is one of the major modifications in complex-type *N*-glycans. Mammalian core fucose is transferred to complex-type *N*-glycans by fucosyl transferase 8 (FUT8).[9)] It has been demonstrated that core fucose plays important roles in various physiological and pathological conditions. The Fut8 knockout mice exhibited severe growth retardation, and mortality rate was 70% during the 3 postnatal days.[10)] Core fucosylated immunoglobulin G (IgG)

showed the ADCC activity 100-fold weaker than IgG without core fucose.[11] Implication of core fucose in various cancers has also been demonstrated. For example, α-fetoprotein having core fucose increase significantly in hepatocellular carcinoma to be used as a liver cancer marker.[12] Moreover, core fucose at glycans on many kinds of growth factor receptor including EGFR, FGFR, etc. activates these receptors.[10a, 10b, 13] However, the detail of the functions of core fucose and the action mechanisms are still unclear. Core fucose-binding lectin has not been found in mammals, despite several core fucose-binding lectins are found in plants,[14] fungi,[15] and bacteria.[16]

1-3. Chemical Synthesis of *N*-Glycan for Its Functional Study

Since various natural glycans have high complexity and heterogeneity, it has been not easy to elucidate the biological functions at molecular level based on their structures. Although genetic techniques such as the knockout of glycosyl transferase have revealed various functions of glycans, it is difficult to reveal the structure responsible for each biological function because of the inherent heterogeneity of glycans. Therefore, bio-functional studies by using the homogeneous *N*-glycans are important to address these issues. Although various series of *N*-glycans are now available from natural sources,[17] preparation of diverse *N*-glycan structures such as core fucosylated *N*-glycan is still difficult issue. Therefore, chemical synthesis is required to obtain a pure and homogeneous *N*-glycan containing core fucose.

Syntheses of complex-type *N*-glycans have been reported by many groups. Danishefsky and co-workers have succeeded to synthesize various kinds of *N*-glycans including core fucosylated glycan and triantennary glycan.[18] They also developed some important techniques on *N*-glycan synthesis such as the introduction of asparagine to synthetic glycans.[19] Unverzagt and co-workers also reported the syntheses of *N*-glycans with various structures including core fucose.[20] In addition, our research group reported the solid-phase synthesis of *N*-glycan in 2009 (Scheme 1-1).[21] In that synthesis, *JandaJel*™ resin with good swearing property in various solvents was used and fluorous solvent was used in glycosylations to improve the yield owing to the reagents concentration effect.



Scheme 1-1. Solid-phase synthesis of sialic acid containing *N*-glycan.

In this study, the author synthesized core fucose containing complex-type *N*-glycan **7**, which has the asparagine-linked dodecasaccharide structure (Figure 1-3). The author selected this glycan as a synthetic target in order to investigate the biological function of core fucose, since the asparagine-linked *N*-glycan having the same undecasaccharide structure without core fucose can be obtained from natural source[17a)] and commercially available. In addition, various types of core fucose containing *N*-glycan can be obtained by the treatment of sialidase and galactosidase.

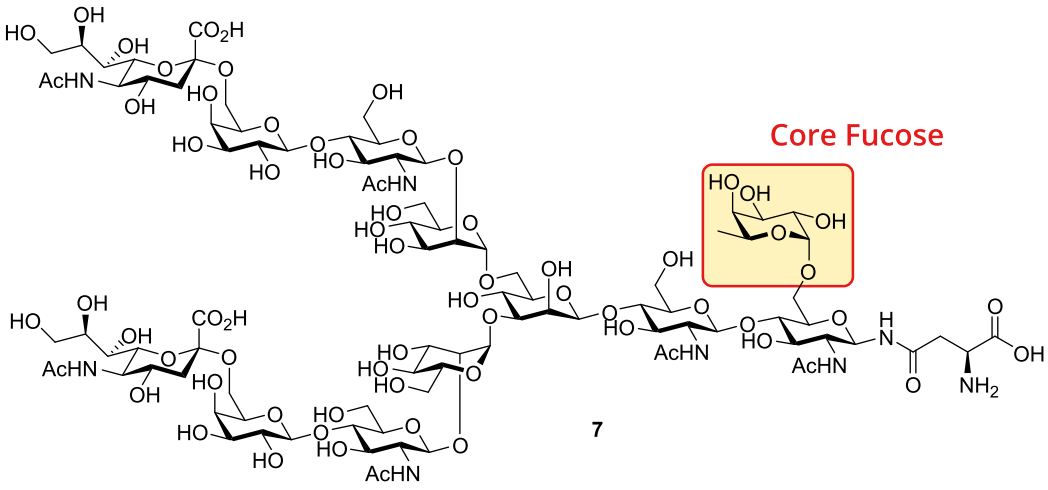
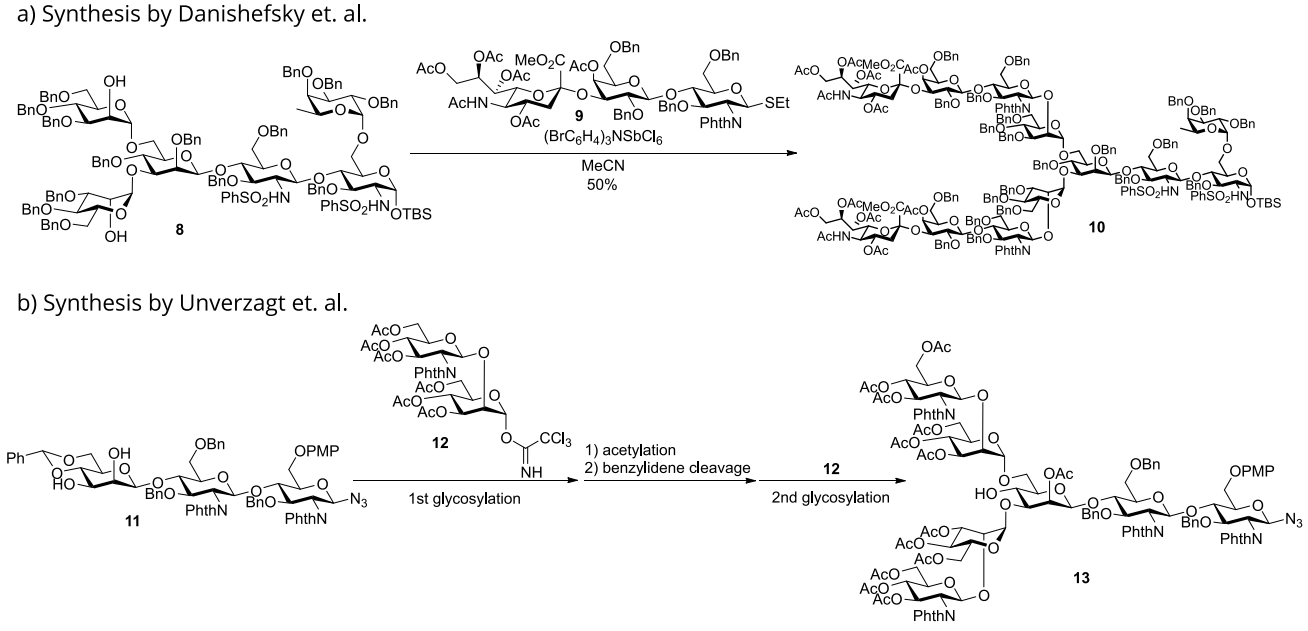


Figure 1-3. Core fucose containing complex-type *N*-glycan **7**.

1-4. Convergent Synthetic Strategy

The author chose liquid-phase synthesis in order to furnish the synthetic glycan for further functional studies of core fucose. In our former solid-phase synthesis of *N*-glycan, we used mono- or disaccharide fragments to link one after another to the resin. On the other hand, more convergent synthetic strategy is desired for the solution-phase synthesis in order to reduce steps of the longest linear sequence. Danishefsky and co-workers used a glycosyl acceptor consisting of hexasaccharide, which has mannoses in both antennae (these mannoses are common in every type of *N*-glycan) for the synthesis of core fucose containing dodecasaccharide (Scheme 1-2a).[18a)] Non reducing-end trisaccharide moiety was successfully introduced to 2-position of mannose in the hexasaccharide. Unverzagt and co-workers adopted another strategy, in which the stem oligosaccharides were connected to mannose residue at the branched position in the trisaccharide (Scheme 1-2b).[20a, 20c)] In this way, the longest linear sequence is shorter than Danishefsky's route. However, stereoselectivity in glycosylations between stem and branch can be a problem because they cannot use neighboring group participation.



Scheme 1-2. Synthetic strategy of biantennary *N*-glycan reported in past.

In this study, the author chose a similar strategy as Unverzagt's one (Figure 1-4). The target dodecasaccharide-Asn backbone was planned to be constructed with core tetrasaccharide-Asn fragment **14** and branch tetrasaccharide donor **15**. Hydroxy groups at 3- and 6-position in branching mannose of **14** were orthogonally protected: 3-OH was protected by 4-azido-3-chlorobenzyl (AzClBn) developed by our group,[22] and 6-OH was protected by 4,6-benzylidene acetal. AzClBn protection can be cleaved by phosphine reduction followed by DDQ oxidation. On the other hand, benzylidene acetal can be cleaved by acid. This strategy gives us a powerful method to approach to a variety of *N*-glycans including asymmetric ones. Key intermediates **14** and **15** would be synthesized by coupling two disaccharides, **16** and **17** for **14**, and **18** and **19** for **15**, respectively.

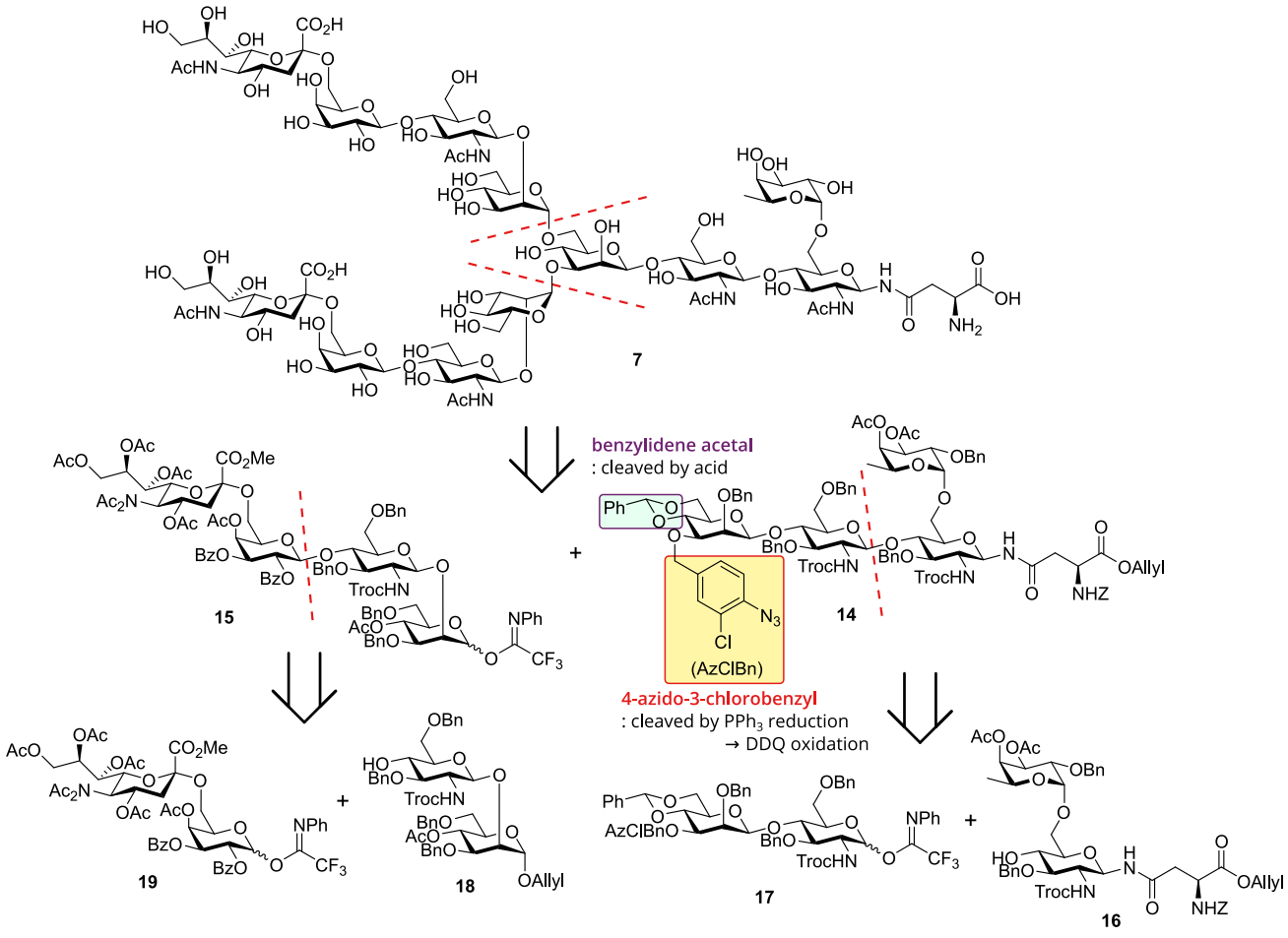


Figure 1-4. Synthetic strategy of *N*-glycan **7**.

1-5. Previous Synthesis of Fragments by Microfluidic Glycosylation

Large scale preparation of small saccharide fragments **16**, **17**, and **19** were previously investigated by using microfluidic methods. Our research group has applied microfluidic method to the syntheses of bioactive natural products including *N*-glycan.[23] For *N*-glycan synthesis, microfluidic reaction was utilized to construct **16**, **17**, and **19** with high yields and stereoselectivity.

Microfluidic synthesis is an innovative technology for organic synthesis using a micromixer, which enables efficient mixing of substrates and reagents in the micro-channel and rapid heat transfer owing to the high surface-to-volume ratio of microreactors.[24] In addition, large-scale synthesis is possible by the continuous flow process (Figure 1-5). New synthetic methodologies have been developed in order to improve the yields and selectivity based on these inherent features of microreactors.

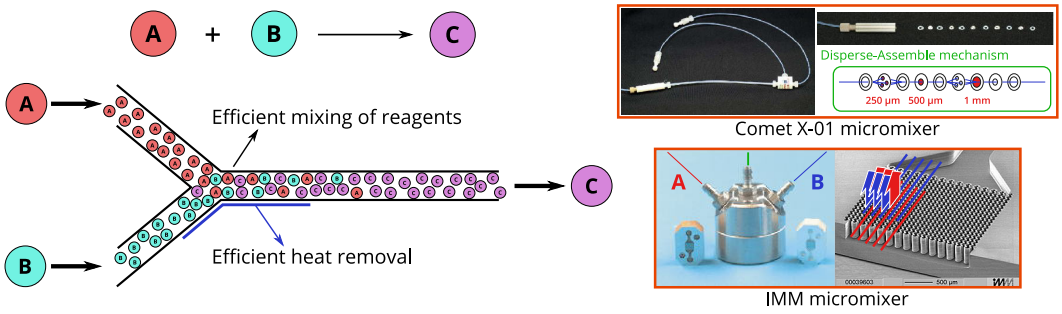


Figure 1-5. Microfluidic Reaction Using Micromixer.

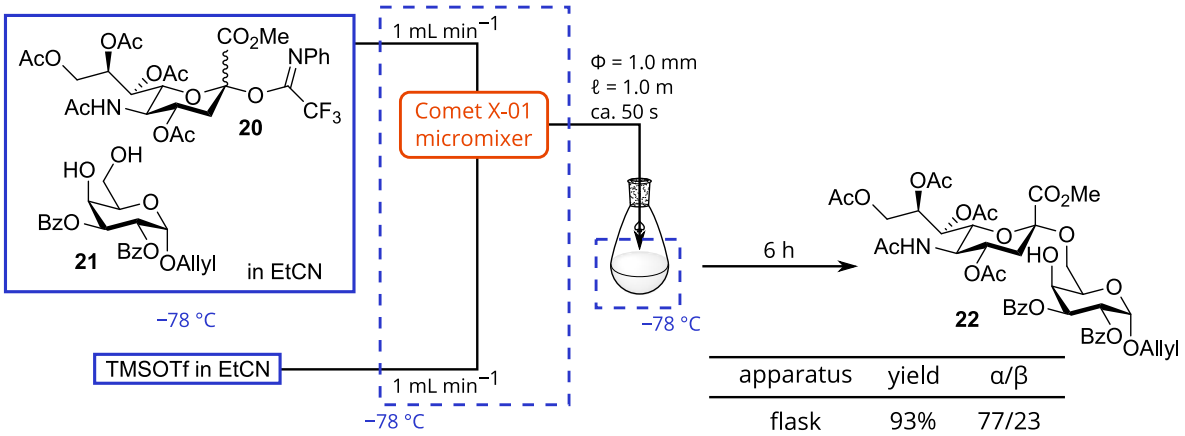
One of the important glycosylations is α-sialylation. It has been thought that α-sialylation using sialyl donor with 5-NHAc group would result in low stereoselectivity and modification of 5-substitution would be essential for highly selective α-sialylation. We re-investigated sialylation using 5-NHAc donor **20** under microfluidic condition with Comet X-01 micromixer,[25] and microfluidic sialylation showed much better selectivity than a reaction in flask (Figure 1-6a).[26]

β-Selective mannosylation was also investigated using micromixer (Figure 1-6b).[23a, 27] It was difficult to scale-up β-mannosylation in batch method probably owing to the local heat generation by mixing Lewis acid. Mixing with Comet X-01 micromixer removed the local heat efficiently to obtain

disaccharide **25** in high yield and with high stereoselectivity even in large scale.

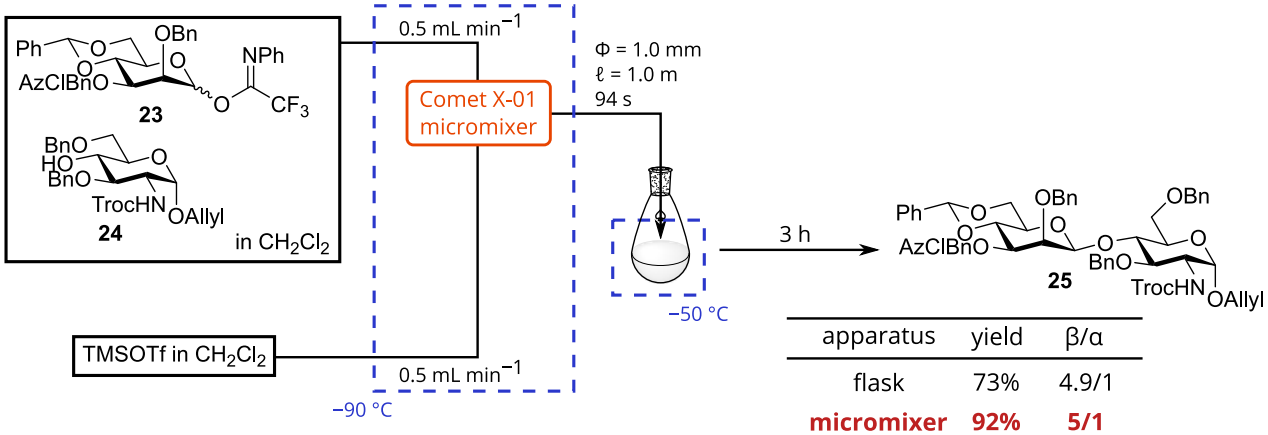
We also employed microfluidic *N*-glycosylation for the synthesis of asparagine-linked disaccharide **28** (Figure 1-6c).[28] *N*-Glycosylation of asparagine was thought to be difficult because of the low nucleophilicity of amide nitrogen. Takahashi and co-workers found that *N*-glycosylation proceeded very well in nitromethane to give corresponding *N*-glycosides in good yields.[29] However, we decided not to use this condition for the large-scale synthesis, since nitromethane is highly flammable and explosive. Our group thus investigated *N*-glycosylation in other solvents under various reaction conditions and established an efficient *N*-glycosylation conditions using glycosyl *N*-phenyltrifluoroacetimidates with asparagine amide under integrated microfluidic/batch conditions. Disaccharide-Asn **28** thus was obtained in 84% yield while a reaction in a flask gave **28** in 47% yield. The author also investigated the both microfluidic and batch conditions and found that careful addition of TMSOTf under batch conditions afforded comparable yields. Therefore, the success for *N*-glycosylation must be due to the temperature control, which inhibits decomposition of the reactants and/or the formation of complicated glycosyl intermediates between the donor, acceptor, and activator.

a) microfluidic α-sialylation



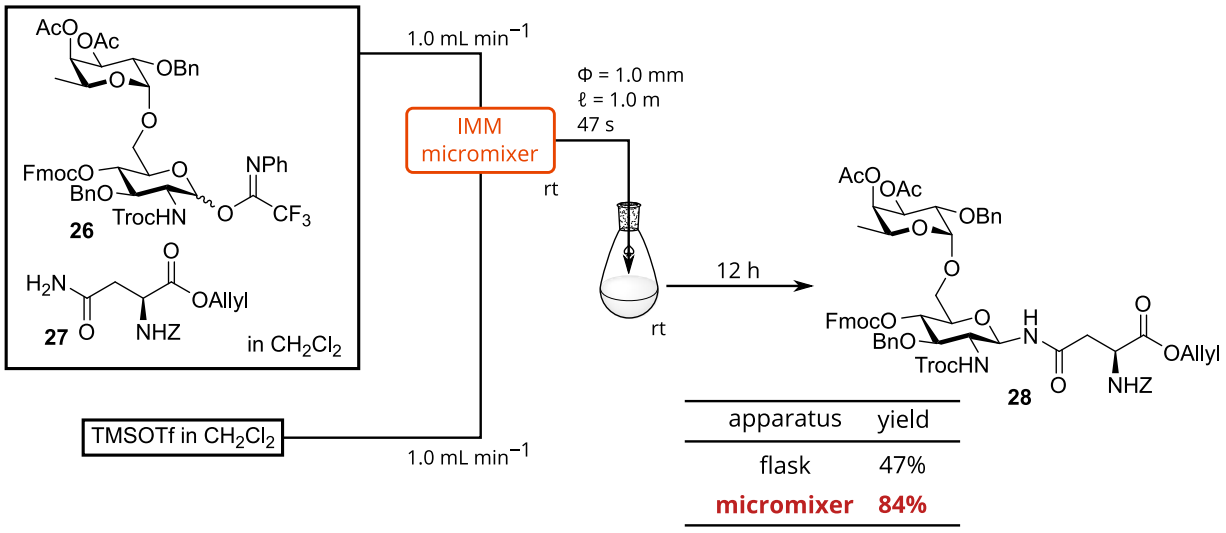
apparatus	yield	α/β
flask	93%	77/23
micromixer	**89%**	**94/6**

b) microfluidic β-mannosylation



apparatus	yield	β/α
flask	73%	4.9/1
micromixer	**92%**	**5/1**

c) microfluidic *N*-glycosylation



apparatus	yield
flask	47%
micromixer	**84%**

Figure 1-6. Glycosylations under microfluidic condition.

Efficient *N*-glycosylation furnishes an asparagine-linked saccharide as a starting material for many glycan synthesis. Success in *N*-glycosylation provided a new synthetic strategy of *N*-glycan. In previous synthetic studies, asparagine was introduced to oligosaccharide after construction of whole oligosaccharide structure. The most widely used methods are amination of non-protected oligosaccharide with NH_4HCO_3 followed by condensation with aspartic acid (Figure 1-7a),[30] and phosphine-mediated coupling of 1-N_3 oligosaccharide with aspartic acid (Figure 1-7b).[31] These reactions are carried out for non-protected, complex oligosaccharide after deprotection. This nature increases the number of reaction steps using large compounds with high polarity. In addition, condensation with aspartic acid can have a selectivity problem with carboxyl groups of sialic acids. Early stage introduction of Asn solves these problems. After the backbone construction, global deprotection gives Asn-linked *N*-glycan directly. This synthetic strategy was adopted for the target *N*-glycan **7**.

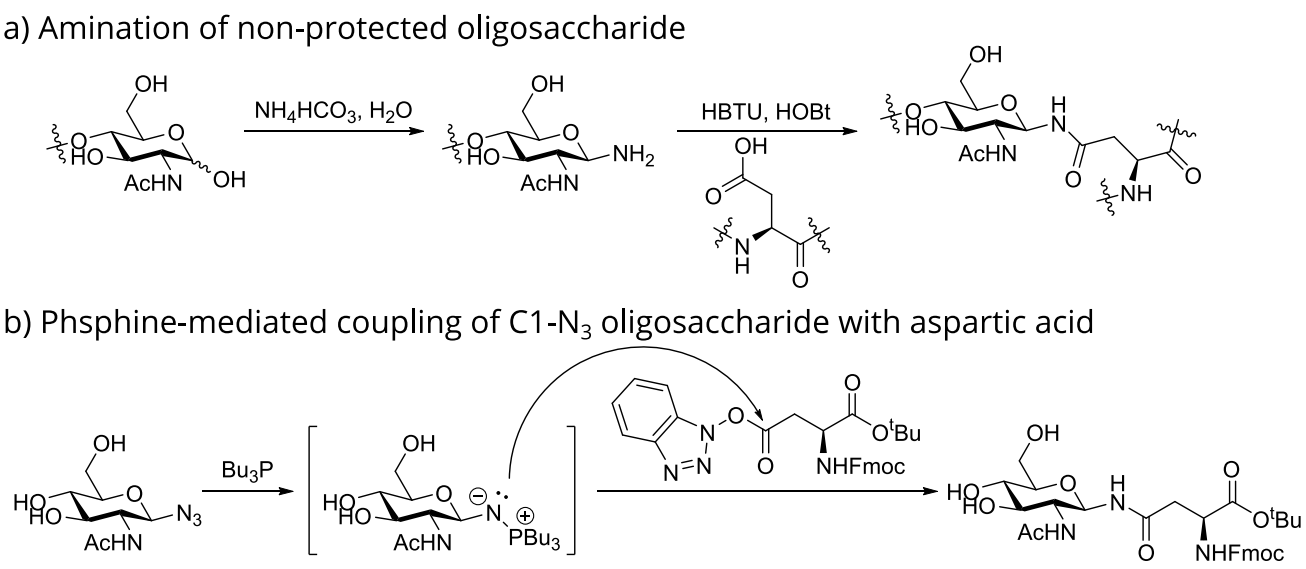


Figure 1-7. Previous methods to introduce asparagine to oligosaccharide.

References

1) Apweiler, R.; Hermjakob, H.; Sharon, N. *Biochim. Biophys. Acta* **1999**, *1473*, 4, DOI: 10.1016/S0304-4165(99)00165-8.

2) Helenius, A.; Aebi, M. *Annu. Rev. Biochem* **2004**, *73*, 1019, DOI: 10.1146/annurev.biochem.73.011303.073752.

3) a) Imai, N.; Higuchi, M.; Kawamura, A.; Tomonoh, K.; Oh-Eda, M.; Fujiwara, M.; Shimonaka, Y.; Ochi, N. *Eur. J. Biochem.* **1990**, *194*, 457, DOI: 10.1111/j.1432-1033.1990.tb15639.x; b) de Frutos, M.; Cifuentes, A.; Diez-Masa, J. C. *Electrophoresis* **2003**, *24*, 678, DOI: 10.1002/elps.200390080; c) Solá, R. J.; Griebenow, K. *J. Pharm. Sci.* **2009**, *98*, 1223, DOI: 10.1002/jps.21504.

4) a) Cipollo, J. F.; Awad, A. M.; Costello, C. E.; Hirschberg, C. B. *J. Biol. Chem.* **2005**, *280*, 26063, DOI: 10.1074/jbc.M503828200; b) Hwang, H.-Y.; Olson, S. K.; Esko, J. D.; Horvitz, H. R. *Nature* **2003**, *423*, 439, DOI: 10.1038/nature01634; c) Mizuguchi, S.; Uyama, T.; Kitagawa, H.; Nomura, K. H.; Dejima, K.; Gengyo-Ando, K.; Mitani, S.; Sugahara, K.; Nomura, K. *Nature* **2003**, *423*, 443, DOI: 10.1038/nature01635.

5) a) Zhou, R. W.; Mkhikian, H.; Grigorian, A.; Hong, A.; Chen, D.; Arakelyan, A.; Demetriou, M. *Nat. Immunol.* **2014**, *15*, 1038, DOI: 10.1038/ni.3007; b) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626, DOI: 10.1126/science.278.5343.1626; c) Kannagi, R.; Goto, Y.; Fukui, F. *Trends Glycosci. Glycotech.* **2004**, *16*, 211, DOI: 10.4052/tigg.16.211; d) Bakker, T. R.; Piperi, C.; Davies, E. A.; van der Merwe, P. A. *Eur. J. Immunol.* **2002**, *32*, 1924, DOI: 10.1002/1521-4141(200207)32:7<1924::AID-IMMU1924>3.0.CO;2-N.

6) a) Kobasa, D.; Takada, A.; Shinya, K.; Hatta, M.; Halfmann, P.; Theriault, S.; Suzuki, H.; Nishimura, H.; Mitamura, K.; Sugaya, N.; Usui, T.; Murata, T.; Maeda, Y.; Watanabe, S.; Suresh, M.; Suzuki, T.; Suzuki, Y.; Feldmann, H.; Kawaoka, Y. *Nature* **2004**, *431*, 703, DOI: 10.1038/nature02951; b) Mahdavi, J.; Sondén, B.; Hurtig, M.; Olfat, F. O.; Forsberg, L.; Roche, N.; Angstrom, J.; Larsson, T.; Teneberg, S.; Karlsson, K.-A.; Altraja, S.; Wadström, T.; Kersulyte, D.; Berg, D. E.; Dubois, A.; Petersson, C.; Magnusson, K.-E.; Norberg, T.; Lindh, F.; Lundskog, B. B.; Arnqvist, A.; Hammarström, L.; Borén, T. *Science* **2002**, *297*, 573, DOI: 10.1126/science.1069076.

7) a) Zhao, Y.-Y.; Takahashi, M.; Gu, J.-G.; Miyoshi, E.; Matsumoto, A.; Kitazume, S.; Taniguchi, N. *Cancer Sci.* **2008**, *99*, 1304, DOI: 10.1111/j.1349-7006.2008.00839.x; b) Hedlund, M.; Ng, E.; Varki, A.; Varki, N. M. *Cancer Res.* **2008**, *68*, 388, DOI: 10.1158/0008-5472.can-07-1340; c) Becker, D. J.; Lowe, J. B. *Glycobiology* **2003**, *13*, 41R, DOI: 10.1093/glycob/cwg054; d) Nakagoe, T.; Sawai, T.; Tsuji, T.; Jibiki, M.-a.; Nanashima, A.; Yamaguchi, H.; Kurosaki, N.; Yasutake, T.; Ayabe, H. *J. Gastroenterol.* **2001**, *36*, 166, DOI: 10.1007/s005350170124.

8) Tanaka, K.; Siwu, E. R. O.; Minami, K.; Hasegawa, K.; Nozaki, S.; Kanayama, Y.; Koyama, K.; Chen, W. C.; Paulson, J. C.; Watanabe, Y.; Fukase, K. *Angew. Chem. Int. Ed.* **2010**, *49*, 8195, DOI: 10.1002/anie.201000892.

9) a) Wilson, J. R.; Williams, D.; Schachter, H. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 909, DOI: 10.1016/S0006-291X(76)80218-5; b) Kötzler, M. P.; Blank, S.; Bantleon, F. I.; Spillner, E.; Meyer, B. *Biochim. Biophys. Acta* **2012**, *1820*, 1915, DOI: 10.1016/j.bbagen.2012.08.018.

10) a) Wang, X.; Inoue, S.; Gu, J.; Miyoshi, E.; Noda, K.; Li, W.; Mizuno-Horikawa, Y.; Nakano, M.; Asahi, M.; Takahashi, M.; Uozumi, N.; Ihara, S.; Lee, S. H.; Ikeda, Y.; Yamaguchi, Y.; Aze, Y.; Tomiyama, Y.; Fujii, J.; Suzuki, K.; Kondo, A.; Shapiro, S. D.; Lopez-Otin, C.; Kuwaki, T.; Okabe, M.; Honke, K.; Taniguchi, N. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 15791, DOI: 10.1073/pnas.0507375102; b) Wang, X.; Gu, J.; Miyoshi, E.; Honke, K.; Taniguchi, N. *Methods Enzymol.* **2006**, *417*, 11, DOI: 10.1016/S0076-6879(06)17002-0; c) Wang, X.; Fukuda, T.; Li, W.; Gao, C.-X.; Kondo, A.; Matsumoto, A.; Miyoshi, E.; Taniguchi, N.; Gu, J. *J. Biochem.* **2009**, *145*, 643, DOI: 10.1093/jb/mvp022.

11) a) Shinkawa, T.; Nakamura, K.; Yamane, N.; Shoji-Hosaka, E.; Kanda, Y.; Sakurada, M.; Uchida, K.; Anazawa, H.; Satoh, M.; Yamasaki, M.; Hanai, N.; Shitara, K. *J. Biol. Chem.* **2003**, *278*, 3466, DOI: 10.1074/jbc.M210665200; b) Niwa, R.; Shoji-Hosaka, E.; Sakurada, M.; Shinkawa, T.; Uchida, K.; Nakamura, K.; Matsushima, K.; Ueda, R.; Hanai, N.; Shitara, K. *Cancer Res.* **2004**, *64*, 2127, DOI: 10.1158/0008-5472.can-03-2068; c) Shields, R. L.; Lai, J.; Keck, R.; O'Connell, L. Y.; Hong, K.; Meng, Y. G.; Weikert, S. H. A.; Presta, L. G. *J. Biol. Chem.* **2002**, *277*, 26733, DOI: 10.1074/jbc.M202069200; d) Satoh, M.; Shitara, K.; Hanai, N. *Trends Glycosci. Glycotech.* **2006**, *18*, 129, DOI: 10.4052/tigg.18.129.

12) a) Noda, K.; Miyoshi, E.; Uozumi, N.; Yanagidani, S.; Ikeda, Y.; Gao, C.-x.; Suzuki, K.; Yoshihara, H.; Yoshikawa, M.; Kawano, K.; Hayashi, N.; Hori, M.; Taniguchi, N. *Hepatology* **1998**, *28*, 944, DOI: 10.1002/hep.510280408; b) Miyoshi, E.; Noda, K.; Ko, J. H.; Ekuni, A.; Kitada, T.; Uozumi, N.; Ikeda, Y.; Matsuura, N.; Sasaki, Y.; Hayashi, N.; Hori, M.; Taniguchi, N. *Cancer Res.* **1999**, *59*, 2237; c) Noda, K.; Miyoshi, E.; Gu, J.; Gao, C.-X.; Nakahara, S.; Kitada, T.; Honke, K.; Suzuki, K.; Yoshihara, H.; Yoshikawa, K.; Kawano, K.; Tonetti, M.; Kasahara, A.; Hori, M.; Hayashi, N.; Taniguchi, N. *Cancer Res.* **2003**, *63*, 6282; d) Miyoshi, E.; Noda, K.; Yamaguchi, Y.; Inoue, S.; Ikeda, Y.; Wang, W.; Ko, J. H.; Uozumi, N.; Li, W.; Taniguchi, N. *Biochim. Biophys. Acta* **1999**, *1473*, 9, DOI: 10.1016/S0304-4165(99)00166-X.

13) a) Liu, Y.-C.; Yen, H.-Y.; Chen, C.-Y.; Chen, C.-H.; Cheng, P.-F.; Juan, Y.-H.; Chen, C.-H.; Khoo, K.-H.; Yu, C.-J.; Yang, P.-C.; Hsu, T.-L.; Wong, C.-H. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 11332, DOI: 10.1073/pnas.1107385108; b) Wang, M.; Wang, J.; Kong, X.; Chen, H.; Wang, Y.; Qin, M.; Lin, Y.; Chen, H.; Xu, J.; Hong, J.; Chen, Y.-X.; Zou, W.; Fang, J.-Y. *Scientific Reports* **2014**, *4*, 6145, DOI: 10.1038/srep06145.

14) a) Howard, I. K.; Sage, H. J.; Stein, M. D.; Young, N. M.; Leon, M. A.; Dyckes, D. F. *J. Biol. Chem.* **1971**, *246*, 1590; b) Debray, H.; Decout, D.; Strecker, G.; Spik, G.; Montreuil, J. *Eur. J. Biochem.* **1981**, *117*, 41, DOI: 10.1111/j.1432-1033.1981.tb06300.x; c) Allen, A. K.; Desai, N. N.; Neuberger, A. *Biochem. J* **1976**, *155*, 127, DOI: 10.1042/bj1550127.

15) a) Kochibe, N.; Furukawa, K. *Biochemistry* **1980**, *19*, 2841, DOI: 10.1021/bi00554a004; b) Kobayashi, Y.; Tateno, H.; Dohra, H.; Moriwaki, K.; Miyoshi, E.; Hirabayashi, J.; Kawagishi, H. *J. Biol. Chem.* **2012**, *287*, 33973, DOI: 10.1074/jbc.M111.327692.

16) Matsumura, K.; Higashida, K.; Ishida, H.; Hata, Y.; Yamamoto, K.; Shigeta, M.; Mizuno-Horikawa, Y.; Wang, X.; Miyoshi, E.; Gu, J.; Taniguchi, N. *J. Biol. Chem.* **2007**, *282*, 15700, DOI: 10.1074/jbc.M701195200.

17) a) Kajihara, Y.; Suzuki, Y.; Yamamoto, N.; Sasaki, K.; Sakakibara, T.; Juneja, L. R. *Chem. Eur. J.* **2004**, *10*, 971, DOI: 10.1002/chem.200305115; b) Makimura, Y.; Kiuchi, T.; Izumi, M.; Dedola, S.; Ito, Y.; Kajihara, Y. *Carbohydr. Res.* **2012**, 41, DOI: 10.1016/j.carres.2012.10.011; c) Sumiyoshi, W.; Nakakita, S.-i.; Miyanishi, N.; Hirabayashi, J. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 543, DOI: 10.1271/bbb.80598.

18) a) Wu, B.; Hua, Z.; Warren, J. D.; Ranganathan, K.; Wan, Q.; Chen, G.; Tan, Z.; Chen, J.; Endo, A.; Danishefsky, S. J. *Tetrahedron Lett.* **2006**, *47*, 5577, DOI: 10.1016/j.tetlet.2006.05.086; b) Wang, P.; Zhu, J.; Yuan, Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2009**, *131*, 16669, DOI: 10.1021/ja907136d; c) Walczak, M. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 16430, DOI: 10.1021/ja307628w; d) Walczak, M. A.; Hayashida, J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2013**, *135*, 4700, DOI: 10.1021/ja401385v.

19) a) Li, X.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2008**, *130*, 5446, DOI: 10.1021/ja800612r; b) Wu, X.; Yuan, Y.; Li, X.; Danishefsky, S. J. *Tetrahedron Lett.* **2009**, *50*, 4666, DOI: 10.1016/j.tetlet.2009.06.005.

20) a) Schuberth, R.; Unverzagt, C. *Tetrahedron Lett.* **2005**, *46*, 4201, DOI: 10.1016/j.tetlet.2005.04.061; b) Eller, S.; Schuberth, R.; Gundel, G.; Seifert, J.; Unverzagt, C. *Angew. Chem. Int. Ed.* **2007**, *46*, 4173, DOI: 10.1002/anie.200604788; c) Ott, D.; Seifert, J.; Prahl, I.; Niemietz, M.; Hoffman, J.; Guder, J.; Mönnich, M.; Unverzagt, C. *Eur. J. Org. Chem.* **2012**, 5054, DOI: 10.1002/ejoc.201200468.

21) Tanaka, K.; Fujii, Y.; Tokimoto, H.; Mori, Y.; Tanaka, S.-i.; Bao, G.-m.; Siwu, E. R. O.; Nakayabu, A.; Fukase, K. *Chem. Asian J.* **2009**, *4*, 574, DOI: 10.1002/asia.200800411.

22) a) Egusa, K.; Fukase, K.; Kusumoto, S. *Synlett* **1997**, 675, DOI: 10.1055/s-1997-3258; b) Egusa, K.; Fukase, K.; Nakai, Y.; Kusumoto, S. *Synlett* **2000**, 27, DOI: 10.1055/s-2000-6468; c) Manabe, Y.; Kitawaki, Y.; Nagasaki, M.; Fukase, K.; Matsubara, H.; Hino, Y.; Fukuyama, T.; Ryu, I. *Chem. Eur. J.* **2014**, *20*, 12750, DOI: 10.1002/chem.201402303.

23) a) Tanaka, K.; Fukase, K. *Beilstein J. Org. Chem.* **2009**, *5*, 40, DOI: 10.3762/bjoc.5.40; b) Tanaka, K.; Fukase, K. *Org. Process Res. Dev.* **2009**, *13*, 983, DOI: 10.1021/op900084f.

24) a) Yoshida, J.-i.; Saito, K.; Nokami, T.; Nagaki, A. *Synlett* **2011**, 1189, DOI: 10.1055/s-0030-1259946; b) Fukuyama, T.; Rahman, M. T.; Sato, M.; Ryu, I. *Synlett* **2008**, 151, DOI: 10.1055/s-2007-1000884.

25) Techno-applications, http://homepage3.nifty.com/techno-applications/

26) Uchinashi, Y.; Nagasaki, M.; Zhou, J.; Tanaka, K.; Fukase, K. *Org. Biomol. Chem.* **2011**, *9*, 7243, DOI: 10.1039/c1ob06164j.

27) Tanaka, K.; Mori, Y.; Fukase, K. *J. Carbohydr. Chem.* **2009**, *28*, 1, DOI: 10.1080/07328300802571129.

28) Tanaka, K.; Miyagawa, T.; Fukase, K. *Synlett* **2009**, 1571, DOI: 10.1055/s-0029-1217343.

29) Tanaka, H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T. *J. Am. Chem. Soc.* **2005**, *127*, 1630, DOI: 10.1021/ja0450298.

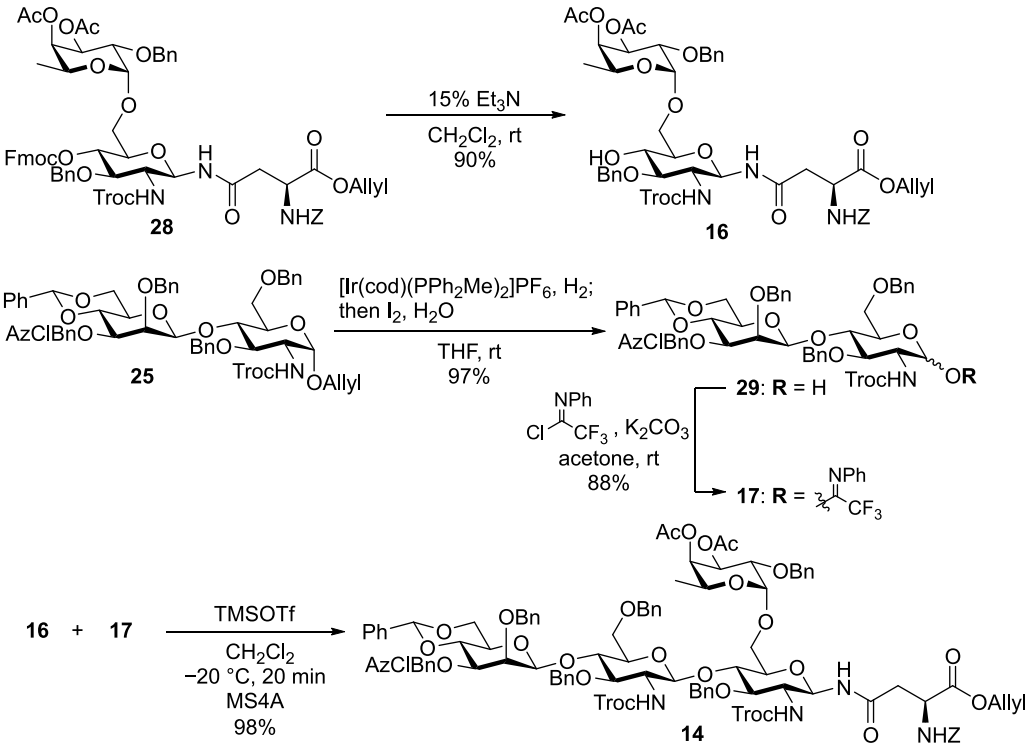
30) Cohen-Anisfeld, S. T.; Lansbury, P. T. *J. Am. Chem. Soc.* **1993**, *115*, 10531, DOI: 10.1021/ja00076a010.

31) a) Inazu, T.; Kobayashi, K. *Synlett* **1993**, 869, DOI: 10.1055/s-1993-22637; b) Doores, K. J.; Mimura, Y.; Dwek, R. A.; Rudd, P. M.; Elliott, T.; Davis, B. G. *Chem. Commun.* **2006**, 1401, DOI: 10.1039/b515472c.

Chapter 2. Preparation of Tetrasaccharide Fragments by Controlling Intermolecular Hydrogen Bonding

2-1. Synthesis of Tetrasaccharide-Asn Fragment

The author started the synthesis from preparation of tetrasaccharide-Asn fragment **23** (Scheme 2-1). First, Fmoc group of disaccharide-Asn **28**[1] was removed with 15% Et_3N to give disaccharide acceptor **16**. Disaccharide donor **17** was prepared in the usual manner from β-mannosyl disaccharide **25**,[2] which was synthesized by microflow β-mannosylation. Allyl group of **25** was thus isomerized with Ir complex[3] and the resulting compound was oxidatively cleaved by I_2 and H_2O[4] to give **29**, which was converted to *N*-phenyltrifluoroacetimidate **17**.[5] Glycosyl donor **17** and acceptor **16** were then coupled to give tetrasaccharide-Asn fragment **14**.



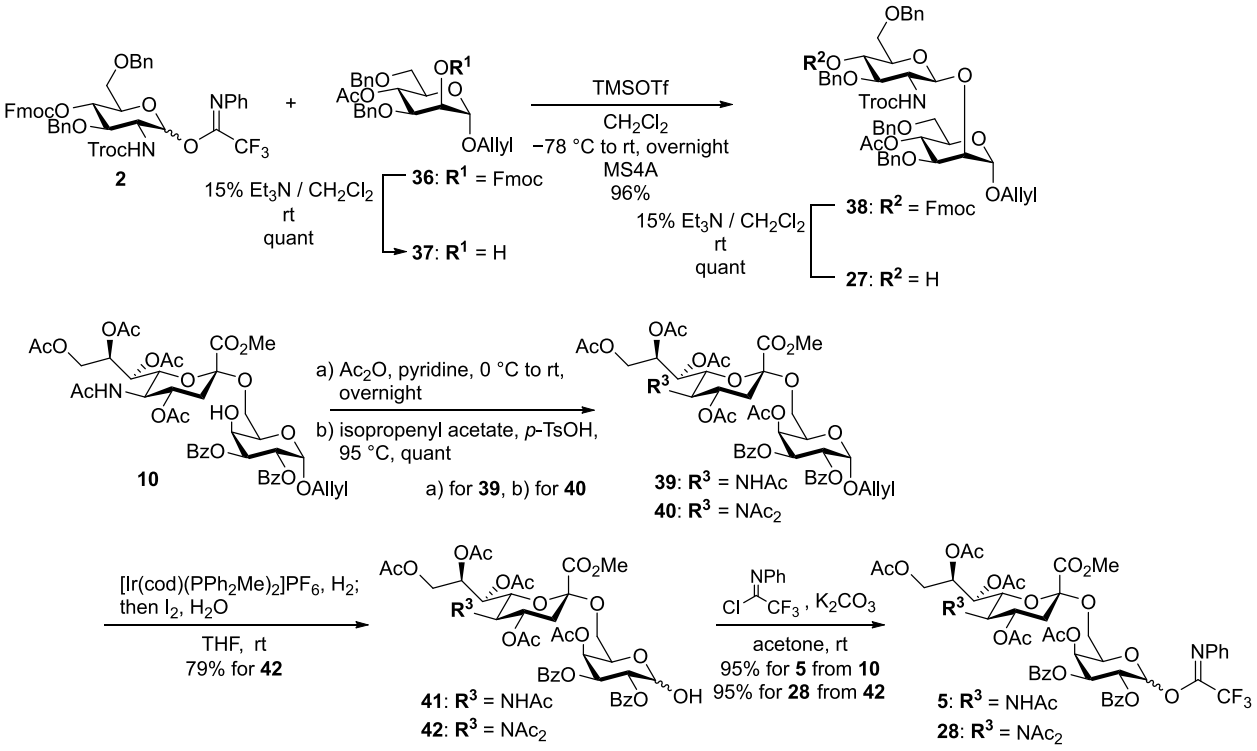
Scheme 2-1. Synthesis of tetrasaccharide-Asn fragment **14**.

2-2. Synthesis of Tetrasaccharide Fragment at Non-Reducing End

Tetrasaccharide containing a sialic acid residue was then synthesized by the coupling of the disaccharide acceptor **18** and the disaccharide donors **5** and **19** via (2+2) pathway. The acceptor **18** and donors **5** and **19** were prepared as shown in Scheme 2-2.

Fmoc group of protected mannose **30**[6] was removed and the resulting alcohol **31** was glycosylated with GlcNAc donor **2**[6] to produce disaccharide **32**.[7] The Fmoc group in **32** was cleaved to give disaccharide acceptor **18**.

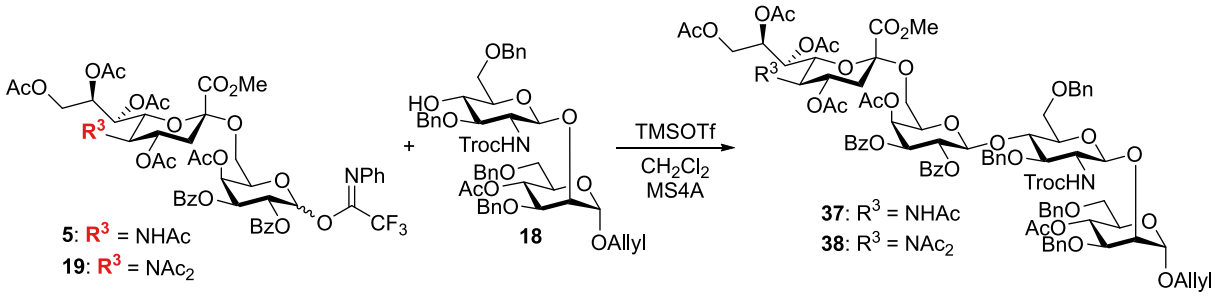
α-Sialyl disaccharide **22** was synthesized by stereoselective sialylation using microflow method or batch method under strict temperature control at −78°C according to the procedure that the author established.[8] Two kinds of donors **5** and **19** were prepared from **22** by acetylation under two different conditions; acetic anhydride in pyridine gave 4-*O*-acetate **33** whereas isopropenyl acetate with TsOH gave 4-*O*-acetyl diacetylimide **34**.[9] Both **33** and **34** were converted into glycosyl imidate **5**[6] and **19** via two-steps reactions.



Scheme 2-2. Synthesis of disaccharide acceptor **18** and disaccharide donors **5** and **19**.

Synthesis of the tetrasaccharide via (2+2) glycosylation was investigated by using donor **5** and **19** (Table 2-1). Donor **19** with NAc_2 group showed good reactivity and the desired tetrasaccharide **38** was obtained in 96% yield. On the other hand, donor **5** with NHAc group showed much lower reactivity. The reaction required more amount of activator TMSOTf and higher temperature to give tetrasaccharide **37** in 52% yield.

Table 2-1. Investigation of (2+2) glycosylation using two kinds of disaccharide donors.



Entry	R^3	donor	product	TMSOTf (eq)	temp	time	yield
1	NHAc	**5**	**37**	0.2 + 0.2	0 °C to rt	1.5 h + 1 h	52%
2	NAc_2	**19**	**38**	0.2	0 °C	20 min	96%

The difference in reactivity between donor **5** and **19** seemed to be attributed to an intermolecular hydrogen bonding of NHAc group. Kononov and co-workers reported that 5-NHAc sialic acid monosaccharide forms dynamic cluster-like structure in a solution to affect the reactivity and stereoselectivity in sialylation.[10] The author hypothesized that this effect may come from the intermolecular hydrogen bonding of NHAc group and hence also exists in the disaccharide. In order to confirm this hypothesis, ^{1}H NMR spectra of 5-NHAc donor **5** were measured under various concentrations (Figure 2-1a). The chemical shifts of 5-NHAc proton moved downfield at higher substrate concentration, indicating the existence of intermolecular hydrogen bonding. The intermolecular hydrogen bonding network may form the cluster-like structure of 5-NHAc sialic acid (Figure 2-1b) and hence reduce the reactivity of donor **5** by inhibiting molecular motion and intermolecular reaction.

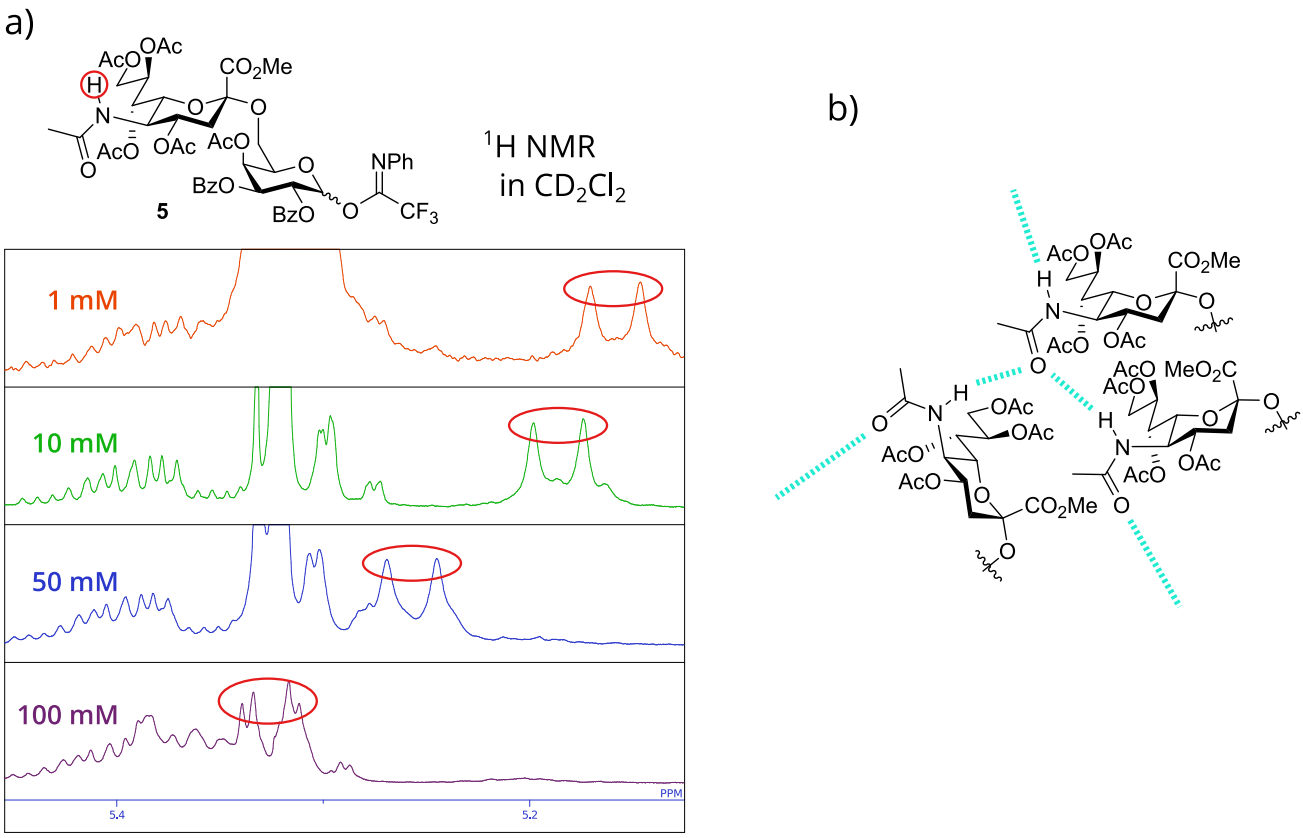
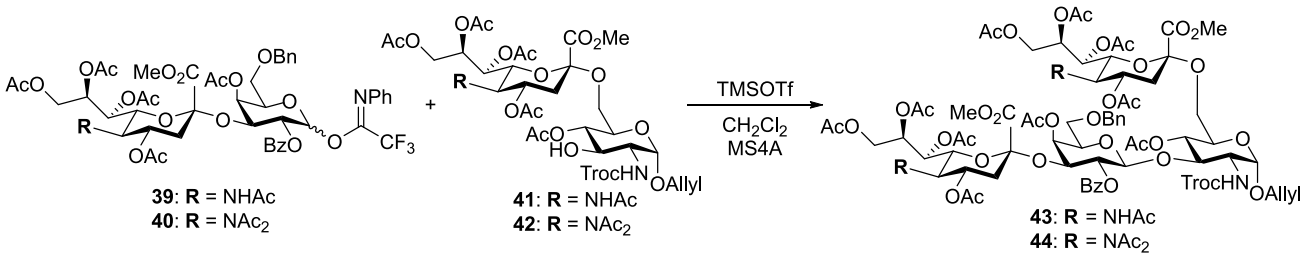


Figure 2-1. a) ^{1}H NMR of 5-NHAc disaccharide donor **5** under various concentrations.

b) Cluster-like structure of sialic acid by intermolecular hydrogen bonding.

Dr. Zhou in our laboratory also previously found the similar hydrogen bonding effect in the synthesis of the disialylated tetrasaccharide.[11] The glycosylation reaction of disaccharide donor **39** and acceptor **41**, both of which possessed 5-NHAc sialic acid, did not proceed. On the contrary, the glycosylation of donor **40** and acceptor **42** having 5-NAc$_2$ sialic acid efficiently proceeded to give the desired tetrasaccharide **44** quantitatively (Table 2-2).

Table 2-2. Effect of 5-substitution of sialic acid for disialyl tetrasaccharide motif.

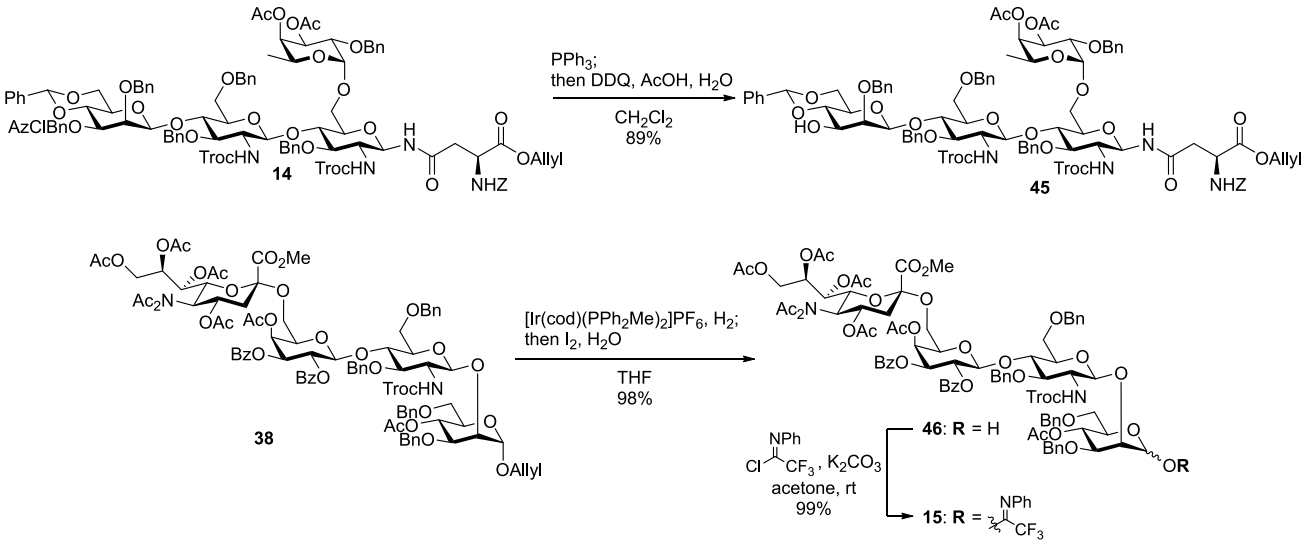


Entry	R	donor	acceptor	product	temp	yield
1	NHAc	**39**	**41**	**43**	−78 °C to rt	no reaction
2	NAc_2	**40**	**42**	**44**	rt	quant

The above results indicated that intermolecular hydrogen bonding in sialic acid residues greatly affects the reactivity of glycosylation. Glycosylation between larger oligosaccharide fragments should be more affected by intermolecular hydrogen bonding, considering the lower mobility of the larger molecules. Therefore, the use of 5-NAc_2 sialic acid should be efficient for the syntheses of the large oligosaccharides.

2-3. Preparation of Tetrasaccharide-Asn Acceptor and Tetrasaccharide Donor

With both reducing and non-reducing end tetrasaccharide fragments in hand, we then prepared tetrasaccharide-Asn acceptor **45** and tetrasaccharide donor **15** for the synthesis of the dodecasaccharide-Asn (Scheme 2-3). AzClBn group of tetrasaccharide-Asn fragment **14** was cleaved via formation of iminophosphorane with phosphine followed by DDQ oxidation to give tetrasaccharide-Asn acceptor **45**. On the other hand, 1-Allyl group of tetrasaccharide fragment **38** was converted into glycosyl imidate to obtain tetrasaccharide donor **15**.



Scheme 2-3. Preparation of tetrasaccharide-Asn acceptor **45** and tetrasaccharide donor **15**.

References

1) Tanaka, K.; Miyagawa, T.; Fukase, K. *Synlett* **2009**, 1571, DOI: 10.1055/s-0029-1217343.

2) Tanaka, K.; Mori, Y.; Fukase, K. *J. Carbohydr. Chem.* **2009**, *28*, 1, DOI: 10.1080/07328300802571129.

3) Oltvoort, J. J.; Van Boeckel, C. A. A.; De Koning, J. H.; Van Boom, J. H. *Synthesis* **1981**, 305, DOI: 10.1055/s-1981-29429.

4) Nashed, M. A.; Anderson, L. *J. Chem. Soc., Chem. Commun.* **1982**, 1274, DOI: 10.1039/c39820001274.

5) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405, DOI: 10.1016/S0040-4039(01)00157-5.

6) Tanaka, K.; Fujii, Y.; Tokimoto, H.; Mori, Y.; Tanaka, S.-i.; Bao, G.-m.; Siwu, E. R. O.; Nakayabu, A.; Fukase, K. *Chem. Asian J.* **2009**, *4*, 574, DOI: 10.1002/asia.200800411.

7) Minamoto, N. Master Thesis, **2014**.

8) Uchinashi, Y.; Nagasaki, M.; Zhou, J.; Tanaka, K.; Fukase, K. *Org. Biomol. Chem.* **2011**, *9*, 7243, DOI: 10.1039/c1ob06164j.

9) Demchenko, A. V.; Boons, G.-J. *Tetrahedron Lett.* **1998**, *39*, 3065, DOI: 10.1016/S0040-4039(98)00359-1.

10) a) Kononov, L. O.; Malysheva, N. N.; Kononova, E. G.; Orlova, A. V. *Eur. J. Org. Chem.* **2008**, 3251, DOI: 10.1002/ejoc.200800324; b) Kononov, L. O.; Malysheva, N. N.; Orlova, A. V.; Zinin, A. I.; Laptinskaya, T. V.; Kononova, E. G.; Kolotyrkina, N. G. *Eur. J. Org. Chem.* **2012**, 1926, DOI: 10.1002/ejoc.201101613.

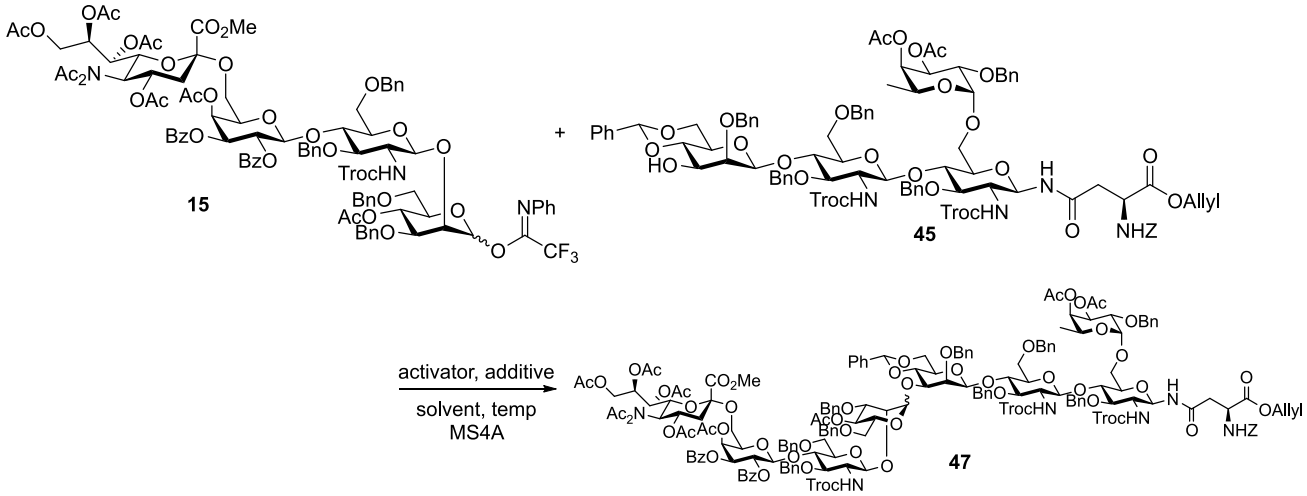
11) a) Zhou, J. Doctor Thesis, **2015**; b) Zhou, J.; Manabe, Y.; Tanaka, K.; Fukase, K. *Chem. Asian J.* accepted.

Chapter 3. Construction of Glycan Backbone by (4+4) and (4+8) Glycosylations

3-1. Investigation of (4+4) Glycosylation

Synthesis of octasaccharide-Asn **47** was carried out via (4+4) coupling by using tetrasaccharide donor **15** and tetrasaccharide-Asn acceptor **45** (Table 3-1). Reaction under 0 °C in CH_2Cl_2 gave the desired product in 56% yield with α:β = 3/1 (Entry 1). To improve the yield and the selectivity, the reaction solvent was investigated. In general, coupling between large fragments are difficult in comparison to coupling of small fragments, since both mobility of molecules and accessibility of glycosyl acceptor to oxocarbenium ion intermediate should be reduced. The author thought that the coordination of ether to the intermediate oxocarbenium ion should stabilize the cationic intermediate and prolong its lifetime to enable the attack of the large acceptor to the activated large donor before the degradation of the activated donor. Ether-type solvent cyclopentylmethylether (CPME) provided exceedingly high 91% yield (Entry 2), while reaction in nitrile-type solvent MeCN resulted in moderate yield and lower selectivity (Entry 3). More strongly coordinating THF reduced the stereoselectivity (Entry 4). Temperature had little effect on the selectivity (Entry 5). The selectivity was decreased, when $TMSClO_4$ was used as a promoter (Entry 6). TMSI was not strong enough to activate donor **15** (Entry 7). DMF was added to the reaction mixture to generate DMF adduct *in situ*,[1)] but only degradation of the donor occurred (Entry 8, 9).

Table 3-1. Investigation of (4+4) glycosylation.

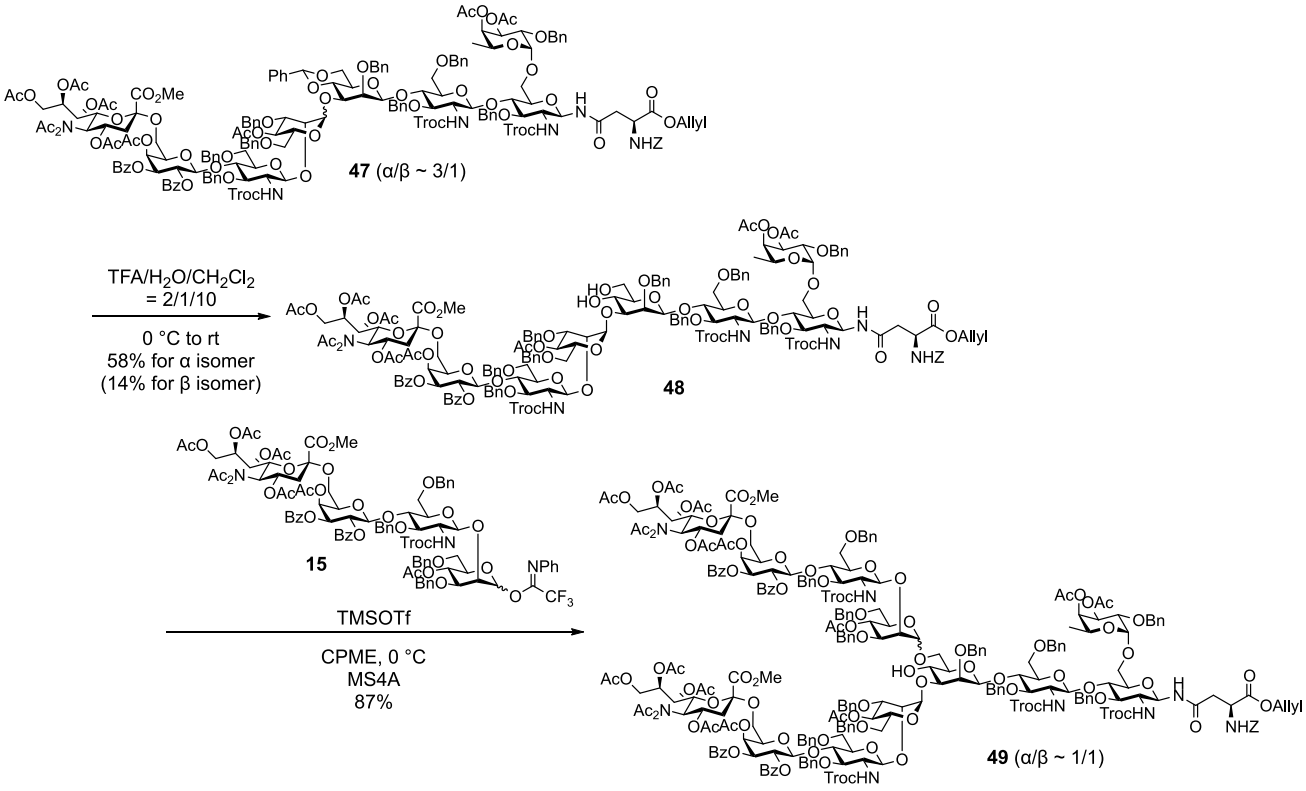


Entry	activator (eq)	solvent	temp	additive (eq)	yield	α/β
1	TMSOTf (0.5)	CH_2Cl_2	0 °C	—	56%	~ 3/1
2	TMSOTf (0.5)	CPME	0 °C	—	91%	~ 3/1
3	TMSOTf (1.0)	MeCN	0 °C	—	59%	~ 5/3
4	TMSOTf (0.5)	CPME/THF = 1/1	0 °C	—	68%	~ 5/3
5	TMSOTf (0.5)	CPME	rt	—	63%	~ 3/1
6	$TMSClO_4$[a)] (0.5)	CPME	0 °C	—	81%	~ 5/3
7	TMSI (0.5+2.0)	CPME	0 °C to rt	—	0%[b)]	—
8	TMSOTf (0.5)	CPME	0 °C to rt	DMF (5.0)	0%[c)]	—
9	TMSOTf (0.5)	CH_2Cl_2	0 °C to rt	DMF (5.0)	0%[c)]	—

a) generated by TMSCl / $AgClO_4$. b) no reaction. c) hydrolysis and β-elimination of **15** occured.

3-2. (4+8) Glycosylation for Protected Dodecasaccharide-Asn

Octasaccharide-Asn **47** was treated with TFA to cleave benzylidene acetal. After purification by column chromatography, α-isomer **48** was isolated in 58% yield. Glycosylation of donor **15** with **48** was carried out in CPME to obtain dodecasaccharide-Asn **49** in quite good 87% yield with α:β = 1/1 (Scheme 3-1). Both stereoisomers were inseparable but separated at later stage in the synthesis. By using 5-NAc_2 sialylated donor, the cluster formation of the donor owing to intermolecular hydrogen bonding should be suppressed. Coupling of large oligosaccharide fragments was hence achieved with such high efficiency. Low selectivity is attributable to the incompatibility of the donor and the acceptor. This issue is expected to be solved in the next generation synthesis.



Scheme 3-1. Synthesis of dodecasaccharide-Asn **49** by (4+8) glycosylation.

References

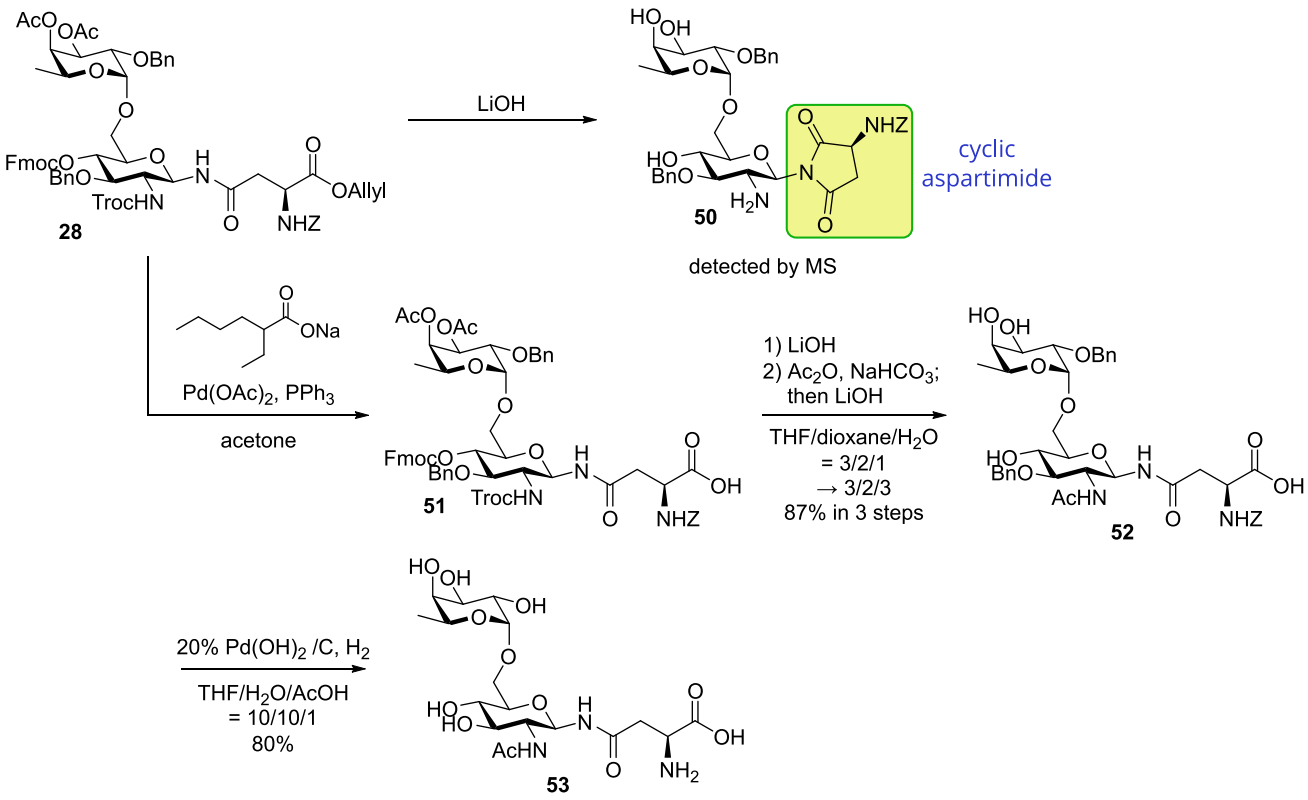
1) a) Lu, S.-R.; Lai, Y.-H.; Chen, J.-H.; Liu, C.-Y.; Mong, K.-K. T. *Angew. Chem. Int. Ed.* **2011**, *50*, 7315, DOI: 10.1002/anie.201100076; b) Lin, Y. H.; Ghosh, B.; Mong, K.-K. T. *Chem. Commun.* **2012**, *48*, 10910, DOI: 10.1039/C2CC35032G.

Chapter 4. Global Deprotection of Dodecasaccharide-Asn

4-1. Investigation of Deprotection Route Using Model Substrate

With protected *N*-glycan **49** in hand, global deprotection was then elucidated for the synthesis of target *N*-glycan **7**. Since the author employed a new strategy for the synthesis of *N*-glycan, investigation of the deprotection conditions is necessary. Present study is the first challenge to synthesize asparagine-linked glycans after construction of the protected asparagine-linked oligosaccharides. Treatment with strong bases must be avoided, since α position of L-asparagine is readily epimerized under strong basic condition. Treatment with strong acids should be also avoided because of the acid-labile fucosyl bond.

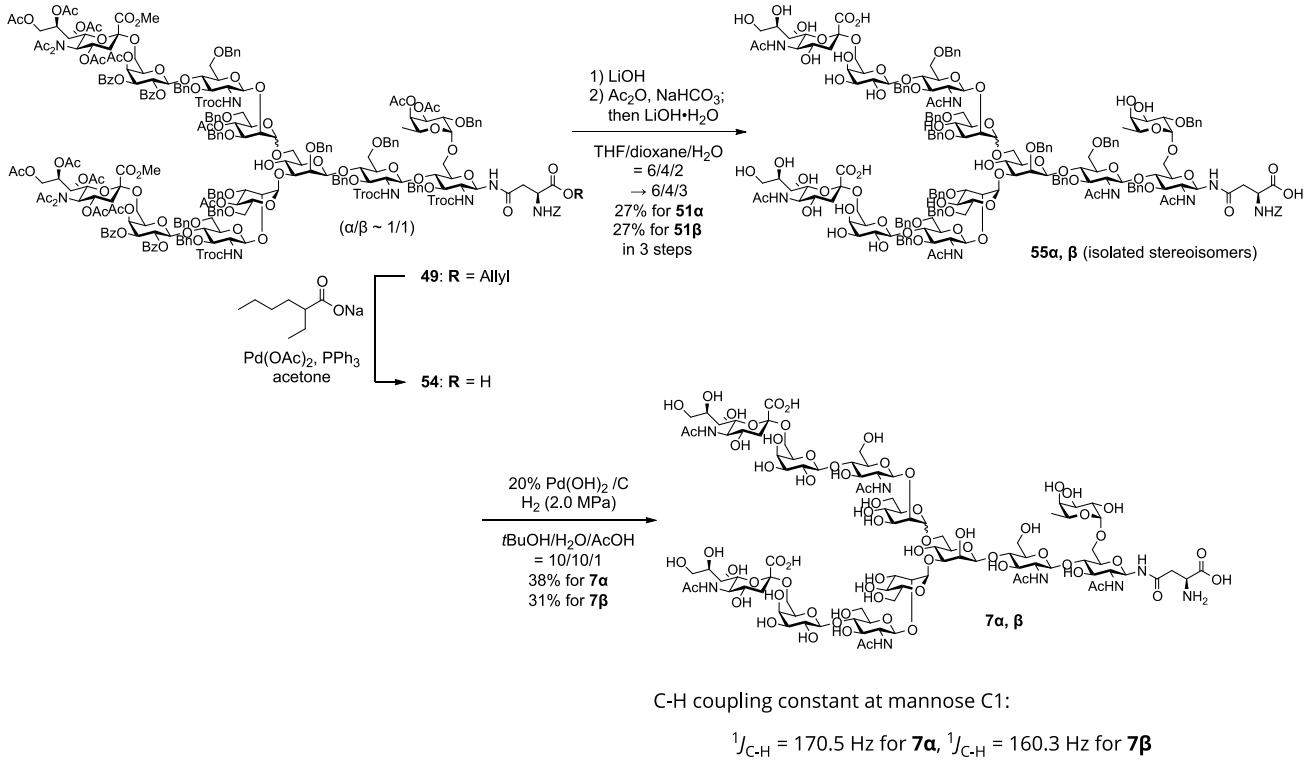
Fucose containing disaccharide-Asn **28** was employed as a model substrate to solve these problems (Scheme 4-1). Cleavage of *N*-Troc group with aqueous LiOH was found in our laboratory.[1)] The author , therefore, investigated the removal of all ester-type protecting groups and *N*-Troc group under a basic condition. However, cyclic aspartimide **50** was formed as a by-product with the desired product. Because this side reaction should occur via a nucleophilic attack of the nitrogen in the side chain to the allyl ester, the author expected that the selective cleavage of the allyl ester before the treatment with LiOH should solve this issue. Pd-catalyzed deallylation using sodium 2-ethylhexanoate as a scavenger[2)] gave carboxylic acid **51**. The product was then treated with aqueous LiOH, and the resulting amine was acetylated with Ac_2O to afford acetamide **52** without cyclization. Remaining *O*-Bn and *N*-Z groups were removed by hydrogenation to give deprotected disaccharide-Asn **53**. The deprotection route was thus successfully established.



Scheme 4-1. Global deprotection route of fucose containing disaccharide-Asn.

4-2. Deprotection of Dodecasaccharide-Asn

Deprotection of dodecasaccharide-Asn **49** was carried out according to the established route (Scheme 4-2). First, allyl ester of asparagine was cleaved with Pd catalyst. Resulting carboxylic acid **54** was treated with aqueous LiOH, and subsequent *N*-acetylation gave compound **55**. Compound **55** was purified by reverse-phase HPLC and two stereoisomers generated in (4+8) glycosylation were separated in this step. Finally, each stereoisomers were hydrogenated to obtain target *N*-glycan **7*α*** and the unnatural stereoisomer **7β**. Stereochemistry at 1-position of mannose was determined by NMR analysis, measuring $^{1}J_{C\text{-}H}$ value. Based on experimentally confirmed data (~170 Hz for *α* glycoside and ~160 Hz for β glycoside),[3] the isomer with 170.5 Hz $^{1}J_{C\text{-}H}$ at 1-position of mannose was identified as **7*α*** and the other isomer with 160.3 Hz $^{1}J_{C\text{-}H}$ was identified as **7β**.



Scheme 4-2. Global deprotection of target *N*-glycan.

References

1) Huang, C.-y.; Wang, N.; Fujiki, K.; Otsuka, Y.; Akamatsu, M.; Fujimoto, Y.; Fukase, K. *J. Carbohydr. Chem.* **2010**, *29*, 289, DOI: 10.1080/07328303.2010.512405.

2) Jeffrey, P. D.; McCombie, S. W. *J. Org. Chem.* **1982**, *47*, 587, DOI: 10.1021/jo00342a048.

3) a) Bock, K.; Pedersen, C. *J. Chem. Soc. Perkin Trans. 2* **1974**, 293, DOI: 10.1039/p29740000293; b) Wolfe, S.; Pinto, B. M.; Varma, V.; Leung, R. Y. N. *Can. J. Chem.* **1990**, *68*, 1051, DOI: 10.1139/v90-164.

Chapter 5. Conclusion

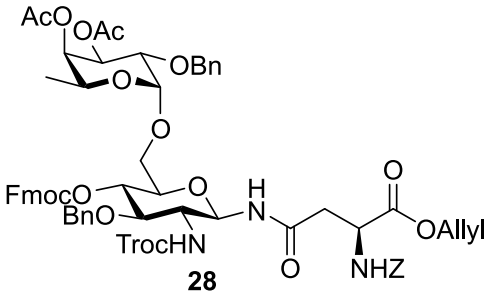
A chemical synthesis of core fucose containing complex-type *N*-glycan **7α** was achieved in this research. The author developed the universal route for various asparagine-linked *N*-glycans based on the several new synthetic strategies. Asparagine residue was introduced by *N*-glycosylation at the early step of the synthesis, whereas asparagine has been introduced to the final step of the synthesis by coupling of glycosyl amine with aspartic acid residue in the previous studies. Protection of 5-acetamide in sialic acid with additional acetyl group dramatically increased the reactivity of glycosylation in fragment coupling by avoiding intermolecular hydrogen bonding owing to 5-NHAc group. Fragment coupling strategy successfully reduced the total reaction steps. Especially, stepwise coupling of the stem tetrasaccharides to 3- and 6-positions of mannose in the tetrasaccharide asparagine was the key for the synthesis. Glycosylation with *N*-phenyltrifluoroacetimidate donors in ether was found to be suitable for the fragment coupling. High yields in fragment coupling were obtained under mild conditions, since ether works as a Lewis base to reduce the acidity of Lewis acid catalysts. However, low selectivity of the glycosylation in these fragment coupling is the issue to be solved in the future work. The present study enables the efficient synthesis of other *N*-glycans and the precise biological study using synthetic *N*-glycans.

Chapter 6. Experimental Section

General Procedures

^{1}H and ^{13}C NMR spectra were recorded in an indicated solvent with JEOL ECA 500 spectrometer, JEOL ECS 400 spectrometer, or Bruker 600 DRX spectrometer equipped with a cryoprobe, and analyzed with JEOL ALICE2® ver. 6, JEOL Delta® ver. 5, or Bruker Topspin™ ver. 3 software. For ^{1}H NMR analysis, the chemical shifts in $CDCl_3$ are given δ values from tetramethylsilane (TMS) as an internal standard. Acetone (δ = 2.22 ppm) or *t*BuOH (δ = 1.24 ppm) is used as an internal standard for the measurement in D_2O. CHD_2OD (δ = 3.30 ppm), CHD_2COCD_3 (δ = 2.05 ppm), CHD_2SOCD_3 (δ = 2.49 ppm), and $CHDCl_2$ (δ = 5.32 ppm) are used as references for the measurements in CD_3OD, acetone-D_6, DMSO-D_6, and CD_2Cl_2, respectively. ESI-Orbitrap mass spectra were obtained on Thermo Scientific LTQ Orbitrap™ XL spectrometer. MALDI-TOF mass spectra were obtained on Applied Biosystems Voyager™ spectrometer with a nitrogen laser (λ = 337 nm). Silica-gel column chromatography was carried out using Kieselgel 60 F_{254} (Merck Co., 0.043-0.063 mm) or Silica Gel 60N (Kanto Chemical Co., 40-50 μm or 63-210 μm) at medium pressure (1-4 kg cm^{-2}). HPLC analysis and purification were performed with SHIMADZU LCsolution system. Kieselgel 60 F_{254} (Merck Co.) plate was used for TLC analysis and compounds were visualized by UV (254 nm) or *p*-methoxybenzaldehyde (*p*-anisaldehyde, 0.03% in EtOH-H_2SO_4-AcOH buffer). Distilled CH_2Cl_2 was distilled from calcium hydride. Distilled THF was distilled after removing peroxide through Al_2O_3 column. Anhydrous THF and CPME were purchased from Kanto Chemical Co. Distilled water was purchased from Wako Pure Chemical Industries, Ltd. Unless otherwise noted, reactions in anhydrous solvent were carried out under argon atmosphere. MS4A were activated with a microwave oven and dried *in vacuo* three times before use. All other commercially available reagents and solvents were used as purchased.

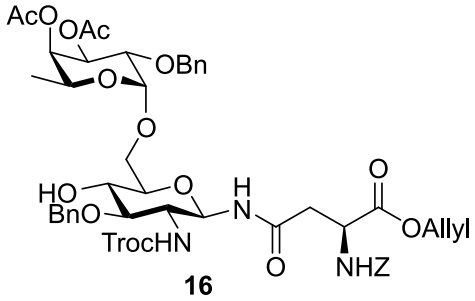
Chapter 2.



28

N^{α}-(Benzyloxycarbonyl)-N^{γ}-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-2-(2,2,2-trichloroethyloxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (28)

See Ref 1.



16

N^{α}-(Benzyloxycarbonyl)-N^{γ}-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (16)

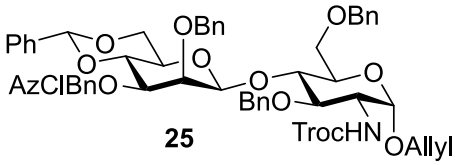
Disaccharide-Asn **28** (1.00 g, 0.784 mmol) was dissolved in CH_2Cl_2 (26.7 mL) and Et_3N (4.7 mL) was added to the solution. After stirring for 2 h under rt, the reaction mixture was diluted with toluene and concentrated *in vacuo*. The residue was co-evaporated four times with toluene to give a crude product. The crude product was purified by silica-gel column chromatography (toluene/EtOAc = 2/1 to 1/1) to obtain **16** (745 mg, 90%).

^{1}H NMR (500 MHz, $CDCl_3$); δ = 7.40-7.28 (m, 15H), 6.92 (d, 1H, *J* = 7.9 Hz), 5.91 (d, 1H, *J* = 8.7 Hz), 5.87-5.79 (m, 1H), 5.30 (dd, 1H, *J* = 10.4, 3.4 Hz), 5.28-5.24 (m, 2H), 5.18 (dd, 1H, *J* = 10.5, 1.1 Hz), 5.13-5.07 (m, 2H), 4.84 (d, 1H, *J* = 3.6 Hz), 4.81-4.77 (m, 2H), 4.75 (t, 1H, *J* = 10.9 Hz), 4.71 (d, 1H, *J* = 4.2 Hz), 4.67 (s,

1H), 4.63 (t, 1H, *J* = 11.2 Hz), 4.59-4.55 (m, 5H), 4.15 (q, 1H, *J* = 6.5 Hz), 3.90-3.85 (m, 2H), 3.83 (d, 2H, *J* = 4.7 Hz), 3.53-3.44 (m, 3H), 3.25 (dd, 1H, *J* = 10.2, 8.9 Hz), 2.85 (dd, 1H, *J* = 16.8, 3.3 Hz), 2.68 (dd, 1H, *J* = 16.4, 3.9 Hz), 2.13 (s, 3H), 2.00 (s, 3H), 1.08 (d, 3H, *J* = 6.4 Hz).

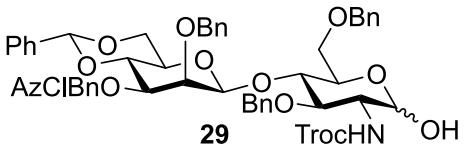
^{13}C NMR (125 MHz, $CDCl_3$); δ = 170.6, 170.4, 170.0, 156.1, 137.9, 137.6, 136.3, 131.6, 128.8, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 118.5, 98.2, 95.3, 80.1, 79.0, 74.8, 74.8, 73.9, 73.8, 73.8, 72.9, 71.5, 70.5, 68.5, 67.0, 66.2, 64.9, 55.1, 50.5, 37.7, 20.8, 20.7, 15.8.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{48}H_{56}Cl_3N_3O_{17}$ $[M+Na]^+$: 1074.2573, found: 1074.2589.



Allyl 4-*O*-(3-*O*-(4-azido-3-chlorobenzyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranoside (25)

See Ref 2.



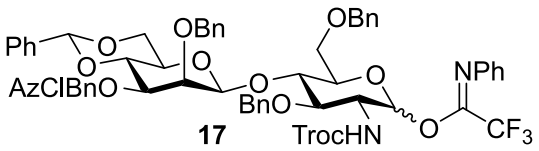
4-*O*-(3-*O*-(4-azido-3-chlorobenzyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (29)

A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (157 mg, 0.185 mmol) in anhydrous THF (24 mL) was stirred under H_2 atmosphere for 5 min to give an yellow solution. The solution was added to a solution of disaccharide allyl glycoside **25** (4.00 g, 3.70 mmol) in anhydrous THF (50 mL) and the mixture was stirred for 30 min under rt. The reaction solution was added H_2O (20 mL) and I_2 (1.88 g, 7.40 mmol) and stirred for additional 15 min. The reaction was quenched by adding 20% aqueous $Na_2S_2O_3$ and extracted by EtOAc. The organic layer was washed with sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting crude product was purified by silica-gel column

chromatography (toluene 100% to toluene/EtOAc = 3/1) to give corresponding hemiacetal **29** (3.72 g, 97%) as α/β mixture.

^{1}H NMR (500 MHz, $CDCl_3$) of major isomer; δ = 7.45-7.41 (m, 4H), 7.39-7.34 (m, 4H), 7.34-7.27 (m, 12H), 7.25-7.22 (m, 3H), 7.18 (dd, 1H, *J* = 8.2, 1.9 Hz), 7.05 (d, 1H, *J* = 8.2 Hz), 5.48 (s, 1H), 5.32 (t, 1H, *J* = 3.7 Hz), 5.05 (d, 1H, *J* = 7.2 Hz), 5.03 (d, 1H, *J* = 11.5 Hz), 4.84 (s, 2H), 4.72 (d, 1H, *J* = 11.7 Hz), 4.67-4.60 (m, 3H), 4.48-4.46 (m, 2H), 4.40 (d, 1H, *J* = 12.0 Hz), 4.08 (dd, 1H, *J* = 10.5, 4.8 Hz), 4.05 (t, 1H, *J* = 9.5 Hz), 3.97 (d, 1H, *J* = 3.0 Hz), 3.96 (s, 1H), 3.90 (td, 1H, *J* = 9.7, 3.0 Hz), 3.70 (td, 1H, *J* = 9.2, 3.2 Hz), 3.62-3.56 (m, 2H), 3.50 (t, 1H, *J* = 10.3 Hz), 3.33 (dd, 1H, *J* = 9.8, 2.9 Hz), 3.10 (td, 1H, *J* = 9.6, 4.9 Hz), 2.90 (d, 1H, *J* = 2.7 Hz).

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{50}H_{50}Cl_4N_4O_{12}$ $[M+Na]^+$: 1061.2077, found: 1061.2091.



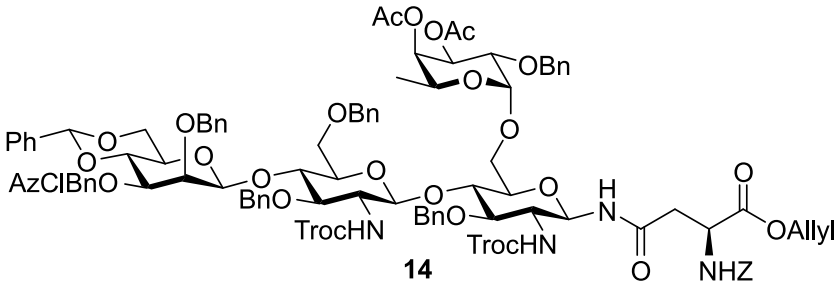
4-*O*-(3-*O*-(4-azido-3-chlorobenzyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl *N*-phenyltrifluoroacetimidate (17)

A solution of the hemiacetal **29** (100 mg, 0.0961 mmol) in acetone (1.9 mL) was added *N*-phenyltrifluoroacetimidoyl chloride (39.9 mg, 0.192 mmol) and K_2CO_3 (39.8 mg, 0.288 mmol). After stirring for 5 h under rt, insoluble materials were filtered and the filtrate was concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 30/1 to 10/1) was carried out to obtain **17** (103 mg, 88%) as α/β mixture.

^{1}H NMR (500 MHz, $CDCl_3$) of major isomer; δ = 7.45 (dd, 2H, *J* = 7.6, 1.9 Hz), 7.42 (dd, 2H, *J* = 7.6, 1.0 Hz), 7.39-7.35 (m, 4H), 7.32 (q, 8H, *J* = 6.9 Hz), 7.27 (d, 7H, *J* = 8.4 Hz), 7.20 (dd, 1H, *J* = 8.2, 1.8 Hz), 7.10 (tt, 1H, *J* = 7.4, 1.1 Hz), 7.06 (d, 1H, *J* = 8.2 Hz), 6.78 (d, 2H, *J* = 7.7 Hz), 6.32 (br s, 1H), 5.50 (s, 1H), 5.03 (d, 1H, *J* = 11.6 Hz), 4.88 (d, 1H, *J* = 11.9 Hz), 4.82 (d, 1H, *J* = 11.9 Hz), 4.75 (d, 1H, *J* = 12.1 Hz), 4.71 (d, 1H, *J* = 8.1 Hz), 4.67 (d, 1H, *J* = 3.3 Hz), 4.64 (d, 1H, *J* = 2.6 Hz), 4.63-4.60 (m, 1H), 4.51 (d, 1H, *J* = 12.7 Hz), 4.49 (s, 1H), 4.49 (s, 1H), 4.38 (d, 1H, *J* = 12.0 Hz), 4.12 (dd, 1H, *J* = 10.5, 4.8 Hz), 4.07 (t, 1H, *J* = 9.5 Hz), 4.07 (d, 1H, *J* =

8.9 Hz), 4.03 (s, 1H), 3.72 (d, 2H, *J* = 2.8 Hz), 3.65 (t, 1H, *J* = 9.8 Hz), 3.59-3.51 (m, 3H), 3.35 (dd, 1H, *J* = 9.9, 2.9 Hz), 3.13 (td, 1H, *J* = 9.7, 4.8 Hz).

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{58}H_{54}Cl_4F_3N_5O_{12}$ $[M+Na]^+$: 1232.2373, found: 1232.2384.



14

***N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\gamma}$-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-4-*O*-(4-*O*-(3-*O*-(4-azido-3-chlorobenzyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (14)**

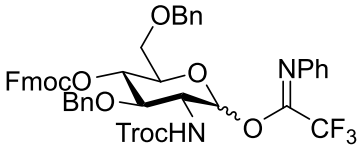
Disaccharide donor **17** (1.26 g, 1.04 mmol) and disaccharide-Asn acceptor **16** (1.00 g, 0.949 mmol) were lyophilized from benzene, added activated MS4A powder, and dissolved in dist. CH_2Cl_2 (19 mL). The solution was cooled to −20 °C and added TMSOTf (34.4 μL, 0.190 mmol), followed by 20 min stirring. After the reaction, sat. aqueous $NaHCO_3$ was added to the mixture at 0 °C and insoluble materials were filtered. The filtrate was poured into sat. aqueous $NaHCO_3$ and extracted by $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. The crude was purified by silica-gel column chromatography ($CHCl_3$/acetone = 20/1 to 8/1) to obtain **14** (1.94 g, 98%).

^{1}H NMR (500 MHz, $CDCl_3$); δ = 7.43-7.38 (m, 6H), 7.37-7.27 (m, 16H), 7.24-7.12 (m, 16H), 7.03 (d, 1H, *J* = 8.2 Hz), 6.91 (d, 1H, *J* = 7.4 Hz), 6.15 (d, 1H, *J* = 8.1 Hz), 5.90 (d, 1H, *J* = 8.8 Hz), 5.85-5.77 (m, 1H), 5.45 (s, 1H), 5.37 (dd, 1H, *J* = 10.5, 3.3 Hz), 5.24 (dq, 1H, *J* = 17.2, 1.4 Hz), 5.19 (dd, 1H, *J* = 3.1, 0.8 Hz), 5.16 (dq, 1H, *J* = 10.5, 1.2 Hz), 5.12 (d, 1H, *J* = 12.2 Hz), 5.04 (d, 1H, *J* = 6.0 Hz), 5.02 (d, 1H, *J* = 5.2 Hz), 4.98 (d, 1H, *J* = 2.3 Hz), 4.89-4.76 (m, 5H), 4.73-4.64 (m, 4H), 4.61-4.48 (m, 6H), 4.42 (d, 1H, *J* = 12.0 Hz), 4.39 (d, 1H, *J* = 7.0 Hz), 4.38 (d, 1H, *J* = 12.6 Hz), 4.34 (d, 1H, *J* = 11.5 Hz), 4.25 (d, 1H, *J* = 12.1 Hz), 4.23 (dd, 1H, *J* = 13.6, 5.5

Hz), 4.08 (dd, 1H, *J* = 12.1, 3.4 Hz), 4.06 (t, 1H, *J* = 9.2 Hz), 4.00 (t, 1H, *J* = 9.8 Hz), 3.98 (dd, 1H, *J* = 10.2, 5.2 Hz), 3.86-3.80 (m, 3H), 3.69 (d, 1H, *J* = 3.2 Hz), 3.68 (dd, 1H, *J* = 8.6, 2.5 Hz), 3.63 (d, 1H, *J* = 9.5 Hz), 3.54 (dd, 2H, *J* = 10.9, 2.9 Hz), 3.50 (dd, 2H, *J* = 9.8, 2.5 Hz), 3.43-3.39 (m, 3H), 3.32 (t, 1H, *J* = 9.5 Hz), 3.25 (dd, 1H, *J* = 9.8, 3.1 Hz), 3.04 (td, 1H, *J* = 9.6, 4.9 Hz), 2.83 (dd, 1H, *J* = 16.9, 3.4 Hz), 2.62 (dd, 1H, *J* = 16.5, 4.0 Hz), 2.05 (s, 3H), 1.78 (s, 3H), 1.00 (d, 3H, *J* = 6.4 Hz).

^{13}C NMR (100 MHz, $CDCl_3$); δ = 170.5, 170.5, 170.3, 169.9, 156.2, 156.1, 154.3, 139.1, 138.3, 138.1, 137.5, 137.1, 136.7, 136.2, 136.2, 131.6, 129.4, 129.0, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.2, 127.1, 126.7, 126.0, 124.9, 119.4, 118.5, 103.6, 102.0, 101.4, 97.2, 95.6, 95.2, 81.4, 81.0, 80.4, 78.5, 78.4, 78.0, 77.7, 77.3, 76.2, 75.9, 74.9, 74.8, 74.7, 74.7, 74.1, 73.7, 73.6, 73.4, 73.4, 71.7, 71.0, 69.6, 68.9, 68.4, 67.3, 67.0, 66.2, 64.9, 57.5, 55.9, 50.4, 37.6, 20.8, 20.6, 15.5.

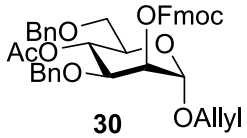
HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{98}H_{104}Cl_7N_7O_{28}$ [M+Na]$^+$: 2094.4647, found: 2094.4646.



2

3,6-di-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethyloxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl *N*-phenyltrifluoroacetimidate (2)

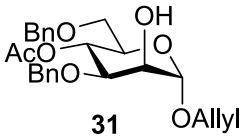
See Ref 3.



30

Allyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-*O*-(9-fluorenylmethyloxycarbonyl)-α-D-mannopyranoside (30)

See Ref 3.



31

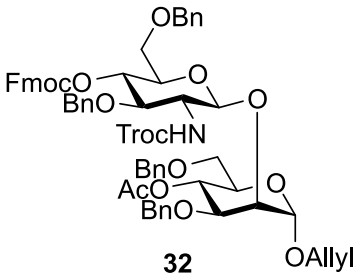
Allyl 4-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (31)

Protected mannose **30** (1.50 g, 2.33 mmol) was dissolved to CH_2Cl_2 (79 mL) and added Et_3N (14 mL). The mixture was stirred for 3 h under rt and diluted with toluene. The resulting solution was concentrated *in vacuo* and co-evaporated four times with toluene to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 5/1 to 3/1) was carried out to obtain pure product **31** (1.03 g, quant).

^{1}H NMR (500 MHz, $CDCl_3$); δ = 7.36-7.24 (m, 10H, aromatic), 5.95-5.87 (m, 1H, -CH_2-C<u>H</u>=CH_2), 5.29 (dq, 1H, *J* = 17.2, 1.6 Hz, -CH_2-CH=C<u>H</u>$_2$), 5.24 (t, 1H, *J* = 9.8 Hz, H-4), 5.20 (dq, 1H, *J* = 10.5, 1.3 Hz, -CH_2-CH-=C<u>H</u>$_2$), 4.95 (d, 1H, *J* = 1.8 Hz, H-1), 4.66 (d, 1H, *J* = 12.0 Hz, -C<u>H</u>$_2$Ph), 4.53 (d, 1H, *J* = 12.0 Hz, -C<u>H</u>$_2$Ph), 4.53 (s, 2H, -C<u>H</u>$_2$Ph), 4.21 (ddt, 1H, *J* = 13.0, 5.2, 1.6 Hz, -C<u>H</u>$_2$-CH=CH_2), 4.05 (m, 1H, H-2), 4.01 (ddt, 1H, *J* = 13.1, 6.3, 1.5 Hz, -C<u>H</u>$_2$-CH=CH_2), 3.86 (m, 1H, H-5), 3.81 (dd, 1H, *J* = 9.5, 3.5 Hz, H-3), 3.57 (dd, 1H, *J* = 10.8, 5.5 Hz, H-6), 3.53 (dd, 1H, *J* = 10.8, 3.5 Hz, H-6'), 2.60 (d, 1H, *J* = 2.0 Hz, 1H, -O<u>H</u>), 1.89 (s, 3H, Ac).

^{13}C NMR (125 MHz, $CDCl_3$); δ = 169.9, 138.0, 137.7, 133.6, 128.5, 128.3, 128.3, 127.9, 127.7, 127.6, 127.5, 117.6, 98.2, 77.1, 73.5, 71.8, 69.7, 69.6, 68.3, 68.2, 68.1, 20.8.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{25}H_{30}O_7$ $[M+Na]^+$: 465.1884, found: 465.1893.



32

Allyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-*O*-(3,6-di-*O*-benzyl-4-*O*-(9-fluorenylmethyloxycarbonyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-*α*-D-mannopyranoside (32)

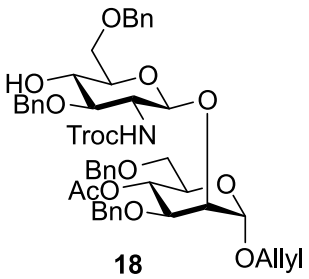
A mixture of lyophilized donor **2** (659 mg, 0.710 mmol), acceptor **31** (377 mg, 0.852 mmol), and activated MS4A powder were dissolved in dist. CH_2Cl_2 (7.1 mL). TMSOTf (25.0 μL, 0.142 mmol) was added at − 78 °C and stirred for 15 min. After being stirred overnight under rt, the reaction was quenched by sat. aqueous $NaHCO_3$ and insoluble materials were filtered. CH_2Cl_2 was evaporated and the residual mixture was poured into sat. aqueous $NaHCO_3$. The aqueous layer was extracted by EtOAc and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (toluene/EtOAc = 20/1 to 5/1) to give **32** (805 mg, 96%).

^{1}H NMR (500 MHz, $CDCl_3$); δ = 7.74 (dd, 2H, *J* = 7.6, 3.2 Hz, Fmoc aromatic), 7.56 (ddd, 2H, *J* = 22.1, 7.6, 0.8 Hz, Fmoc aromatic), 7.39-7.14 (m, 24H, aromatic), 5.92-5.84 (m, 1H, -CH_2-C<u>H</u>=CH_2), 5.42 (br s, 1H, N<u>H</u>Troc), 5.26 (t, 1H, *J* = 9.5 Hz, H-4), 5.23 (dq, 1H, *J* = 17.3, 1.7 Hz, -CH_2-CH=C<u>H</u>$_2$), 5.18 (dq, 1H, *J* = 10.5, 1.3 Hz, -CH_2-CH=C<u>H</u>$_2$), 5.07 (br d, 1H, *J* = 6.5 Hz, H-1′), 4.83 (s, 1H, H-1), 4.81 (t, 1H, *J* = 9.5 Hz, H-4′), 4.71 (d, 1H, *J* = 12.0 Hz, -C<u>H</u>$_2$Ph), 4.66 (d, 1H, *J* = 12.0 Hz, -C<u>H</u>$_2$Ph), 4.59 (d, 1H, *J* = 12.0 Hz, -C<u>H</u>$_2$Ph), 4.58 (s, 2H, -C<u>H</u>$_2$Ph), 4.52 (s, 2H, -C<u>H</u>$_2$Ph), 4.47 (d, 1H, *J* = 12.0 Hz, -C<u>H</u>$_2$Ph), 4.45 (d, 1H, *J* = 12.0 Hz, -NHCO$_2$C<u>H</u>$_2$CCl$_3$), 4.42 (d, 1H, *J* = 12.0 Hz, -NHCO$_2$C<u>H</u>$_2$CCl$_3$), 4.33 (br s, 1H, H-3′), 4.29 (d, 2H, *J* = 7.4 Hz, -COC<u>H</u>$_2$-fluorenyl), 4.18 (t, 1H, *J* = 3.2 Hz, H-2), 4.16 (ddt, 1H, *J* = 13.1, 6.3, 1.5 Hz, -C<u>H</u>$_2$-CH=CH_2), 4.10 (t, 1H, *J* = 7.3 Hz, Fmoc fluorenyl), 3.94 (ddt, 1H, *J* = 12.9, 6.0, 1.3 Hz, -C<u>H</u>$_2$-CH=CH_2), 3.84 (dd, 1H, *J* = 9.3, 3.3 Hz, H-3), 3.79 (m, 1H, H-5), 3.71 (m, 1H, H-5′), 3.60-3.51 (m, 4H, H-6, H-6′), 3.14 (br s, 1H, H-2′), 1.92 (s, 3H, Ac).

^{13}C NMR (125 MHz, $CDCl_3$); δ = 169.7, 154.3, 153.9, 143.3, 143.1, 141.3, 141.2, 138.2, 138.0, 137.8, 133.6,

129.0, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.6, 127.6, 127.1, 125.3, 125.1, 125.0, 120.0, 117.5, 95.5, 75.1, 74.2, 73.6, 73.4, 73.0, 72.8, 71.2, 70.5, 70.0, 69.8, 68.7, 68.3, 57.8, 46.7, 20.9.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{63}H_{64}Cl_3NO_{15}$ $[M+Na]^+$: 1202.3234, found: 1202.3234.



18

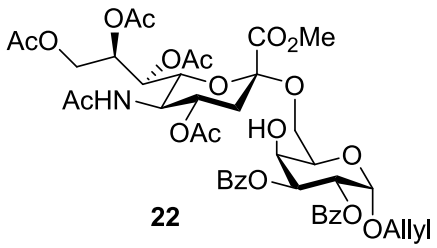
Allyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-α-D-mannopyranoside (18)

To a solution of protected disaccharide **32** (200 mg, 0.169 mmol) in CH_2Cl_2 (5.8 mL) was added Et_3N (1.0 mL) and the solution was stirred for 5.5 h under rt. The reaction solution was concentrated *in vacuo* and co-evaporated three times with toluene. The resulting crude product was purified by silica-gel column chromatography (toluene/EtOAc = 4/1 to 3/1) to give product **18** (162 mg, quant).

^{1}H NMR (400 MHz, acetone-D_6); δ = 7.37-7.19 (m, 20H), 6.96 (d, 1H, *J* = 8.6 Hz), 5.99-5.89 (m, 1H), 5.25 (dq, 1H, *J* = 17.3, 1.8 Hz), 5.15 (t, 1H, *J* = 9.8 Hz), 5.13 (dq, 1H, *J* = 10.9, 1.4 Hz), 4.99 (d, 1H, *J* = 1.4 Hz), 4.89-4.76 (m, 5H), 4.60 (d, 1H, *J* = 13.7 Hz), 4.57 (d, 1H, *J* = 10.2 Hz), 4.53 (s, 4H), 4.46 (d, 1H, *J* = 12.0 Hz), 4.30 (t, 1H, *J* = 2.3 Hz), 4.19 (ddt, 1H, *J* = 13.2, 5.2, 1.6 Hz), 3.98 (ddt, 1H, *J* = 13.2, 5.8, 1.5 Hz), 3.89 (dd, 1H, *J* = 10.7, 2.0 Hz), 3.83 (dd, 1H, *J* = 9.4, 3.3 Hz), 3.82 (ddd, 1H, *J* = 12.9, 6.2, 3.3 Hz), 3.77-3.69 (m, 2H), 3.65-3.50 (m, 5H), 1.92 (s, 3H).

^{13}C NMR (100 MHz, acetone-D_6); δ = 170.0, 155.2, 140.2, 139.8, 139.6, 135.2, 129.0, 129.0, 128.9, 128.8, 128.5, 128.4, 128.2, 128.1, 128.1, 128.0, 127.9, 117.0, 101.0, 97.8, 97.1, 82.9, 82.9, 76.6, 76.5, 76.0, 74.8, 74.7, 74.0, 73.9, 73.6, 72.1, 72.0, 71.3, 71.2, 71.1, 70.5, 69.3, 68.7, 58.0, 20.9.

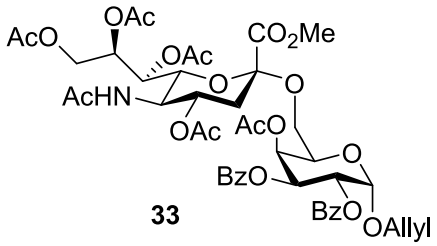
HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{48}H_{54}Cl_3NO_{13}$ $[M+Na]^+$: 980.2558, found: 980.2567.



22

Allyl 2,3-di-*O*-benzoyl-6-*O*-(Methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (22)

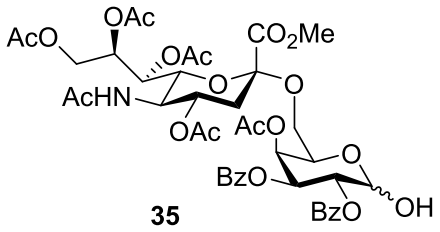
See Ref 4.



33

Allyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (33)

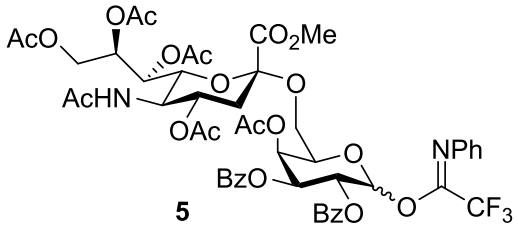
α-Sialyl disaccharide **22** (706 mg, 0.783 mmol) was dissolved in pyridine (15 mL) and Ac_2O (15 mL) was added to the solution. After overnight stirring under rt, the reaction was quenched by MeOH and the solution was concentrated *in vacuo*. The residue was dissolved in EtOAc and poured into H_2O. The aqueous layer was extracted by EtOAc and the organic layer was washed with brine three times, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and co-evaporated with toluene four times to give a crude product. The crude product was roughly purified by silica-gel column chromatography ($CHCl_3$/MeOH = 50/1 to 20/1). The resulting product was used for the next reaction without further purification.



35

4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-*α*-D-galactopyranose (35)

A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (133 mg, 0.157 mmol) in anhydrous THF (7.8 mL) was stirred for 5 min under H_2 atmosphere to give a yellow solution. The solution was added to a solution of the crude disaccharide **33** (0.783 mmol) in anhydrous THF (7.8 mL) under Ar atmosphere and the mixture was stirred overnight under rt. To the reaction solution were added H_2O (6 mL) and I_2 (398 mg, 1.57 mmol), and the mixture was stirred for 1.5 h under rt. The reaction was quenched by 20% aqueous $Na_2S_2O_3$ and THF was evaporated under reduced pressure. The residue was poured into sat. aqueous $NaHCO_3$ and the aqueous layer was extracted by EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. The crude product was roughly purified by silica-gel column chromatography ($CHCl_3$ only to $CHCl_3$/MeOH = 20/1). The product was used to the next reaction without further purification.



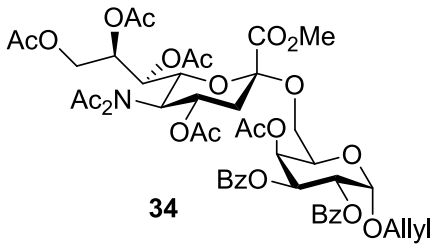
5

4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-*α*-D-galactopyranosyl *N*-phenyltrifluoroacetimidate (5)

To a solution of the crude disaccharide **35** (0.783 mmol) in acetone (7.8 mL) were added *N*-phenyltrifluoroacetimidoyl chloride (325 mg, 1.57 mmol) and K_2CO_3 (325 mg, 2.35 mmol). The mixture was stirred overnight under rt, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel

column chromatography (toluene/EtOAc = 1/3 to 1/10) to obtain the product **5** (804 mg, 95%) as α/β mixture.

^{1}H NMR (400 MHz, CD_2Cl_2) of major isomer; δ = 7.98 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.90 (dd, 2H, *J* = 8.3, 1.2 Hz), 7.61-7.52 (m, 2H), 7.45-7.38 (m, 5H), 7.30 (t, 1H, *J* = 7.8 Hz), 7.17 (t, 2H, *J* = 7.7 Hz), 7.04 (t, 1H, *J* = 7.4 Hz), 6.50 (d, 1H, *J* = 5.5 Hz), 5.83 (d, 2H, *J* = 2.1 Hz), 5.55-5.51 (m, 1H), 5.45-5.37 (m, 1H), 5.30 (dd, 1H, *J* = 2.1, 0.6 Hz), 5.19 (d, 1H, *J* = 9.6 Hz), 4.93-4.84 (m, 1H), 4.51 (t, 1H, *J* = 5.6 Hz), 4.26 (ddd, 1H, *J* = 21.1, 12.4, 2.9 Hz), 4.12-3.96 (m, 4H), 3.80 (s, 3H), 3.38 (dd, 1H, *J* = 10.0, 6.2 Hz), 2.61 (dd, 1H, *J* = 12.8, 4.7 Hz), 2.17 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (dd, 1H, *J* = 4.2, 3.6 Hz), 1.84 (s, 3H).



34

Allyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (34)

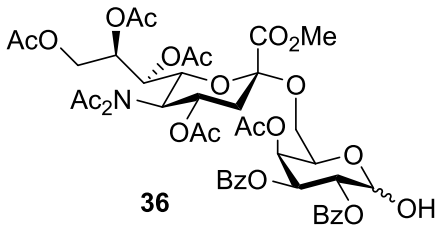
To α-sialyl disaccharide **22** (4.00 g, 4.44 mmol) was added isopropenyl acetate (220 mL) and *p*-TsOH (761 mg, 4.00 mmol). The mixture was stirred at 95 °C under reflux. After 2 h stirring, the reaction mixture was cooled to 0 °C and Et_3N (3 mL) was added. The mixture was concentrated *in vacuo* and co-evaporated twice with toluene to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 3/1 to 1/1) was carried out to obtain **34** (4.38g, quant).

^{1}H NMR (500 MHz, $CDCl_3$); δ = 7.98 (dd, 2H, *J* = 8.4, 1.2 Hz), 7.88 (dd, 2H, *J* = 8.4, 1.4 Hz), 7.53-7.47 (m, 2H), 7.37 (dt, 4H, *J* = 14.8, 6.7 Hz), 5.90-5.82 (m, 1H), 5.82 (dd, 1H, *J* = 10.9, 3.3 Hz), 5.73 (dd, 1H, *J* = 3.3, 1.1 Hz), 5.58 (dd, 1H, *J* = 10.7, 3.7 Hz), 5.51 (td, 1H, *J* = 10.5, 5.4 Hz), 5.35-5.30 (m, 3H), 5.17-5.15 (m, 2H), 4.94 (dd, 1H, *J* = 10.1, 1.8 Hz), 4.38-4.35 (m, 1H), 4.31 (ddt, 1H, *J* = 13.5, 4.5, 1.5 Hz), 4.29 (dd, 1H, *J* = 12.4, 2.9 Hz), 4.17 (t, 1H, *J* = 10.0 Hz), 4.15 (dd, 1H, *J* = 12.6, 5.2 Hz), 4.08 (ddt, 1H, *J* = 13.3, 5.9, 1.4 Hz), 3.94 (dd, 1H, *J* = 10.2, 6.3 Hz), 3.82 (s, 3H), 3.50 (dd, 1H, *J* = 10.2, 7.3 Hz), 2.73 (dd, 1H, *J* = 13.1, 5.4 Hz), 2.38 (s, 3H), 2.31

(s, 3H), 2.19 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.85 (dd, 1H, *J* = 13.2, 10.9 Hz).

^{13}C NMR (100 MHz, $CDCl_3$); δ = 174.5, 173.6, 170.5, 170.1, 169.9, 169.8, 169.6, 167.3, 166.0, 165.4, 133.5, 133.3, 133.1, 129.8, 129.5, 129.5, 129.3, 128.4, 128.3, 117.5, 98.7, 95.6, 77.2, 69.8, 69.0, 68.7, 68.6, 68.5, 68.3, 67.6, 67.1, 66.8, 62.5, 61.9, 57.1, 52.8, 38.7, 27.9, 25.9, 21.0, 21.0, 20.7, 20.7, 20.6.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{47}H_{55}NO_{22}$ [M+Na]$^+$: 1008.3113, found: 1008.3119.



36

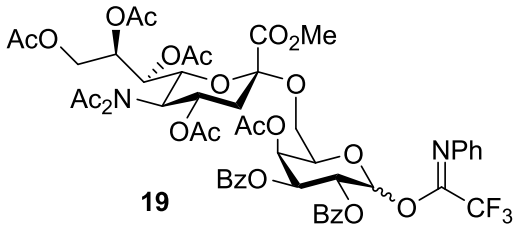
4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-D-galactopyranose (36)

A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (25.5 mg, 0.0301 mmol) in anhydrous THF (3.0 mL) was stirred for 5 min under H_2 atmosphere to give a yellow solution. The solution was added to a solution of allyl glycoside **34** (297 mg, 0.301 mmol) in anhydrous THF (3.0 mL) under Ar atmosphere and stirred for 1 h under rt. To the reaction solution were added H_2O (2 mL) and I_2 (153 mg), followed by 1 h stirring. 20% aqueous $Na_2S_2O_3$ was added to the solution and THF was evaporated. The aqueous layer was extracted by EtOAc. The organic solution was washed with sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude product was purified by silica-gel column chromatography ($CHCl_3$/acetone = 15/1 to 10/1) to give **36** (225 mg, 79%) as α/β mixture.

^{1}H NMR (500 MHz, $CDCl_3$) of major isomer; δ = 8.00 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.90 (dd, 2H, *J* = 8.4, 1.2 Hz), 7.52-7.48 (m, 2H), 7.39-7.35 (m, 4H), 5.91 (dd, 1H, *J* = 10.7, 3.4 Hz), 5.82 (dd, 1H, *J* = 3.4, 1.2 Hz), 5.68 (t, 1H, *J* = 3.2 Hz), 5.57 (ddd, 1H, *J* = 10.7, 3.6, 1.1 Hz), 5.54 (dd, 1H, *J* = 4.7, 1.7 Hz), 5.54-5.48 (m, 1H), 5.36 (td, 1H, *J* = 7.5, 2.5 Hz), 5.17 (dd, 1H, *J* = 6.9, 1.4 Hz), 5.02 (dd, 1H, *J* = 10.1, 1.5 Hz), 4.70 (ddd, 1H, *J* = 9.1, 5.3, 1.0 Hz), 4.62 (dd, 1H, *J* = 3.0, 1.3 Hz), 4.41 (dd, 1H, *J* = 12.1, 2.6 Hz), 4.11 (q, 1H, *J* = 10.0 Hz), 3.85 (s, 3H), 3.80 (dd, 1H, *J* = 11.2, 5.4 Hz), 3.57 (dd, 1H, *J* = 11.1, 9.4 Hz), 2.75 (dd, 1H, *J* = 13.2, 5.3 Hz), 2.38 (s, 3H), 2.33 (s,

3H), 2.30 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.89 (dd, 1H, *J* = 13.2, 10.9 Hz).

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{44}H_{51}NO_{22}$ [M+Na]$^+$: 968.2800, found: 968.2810.



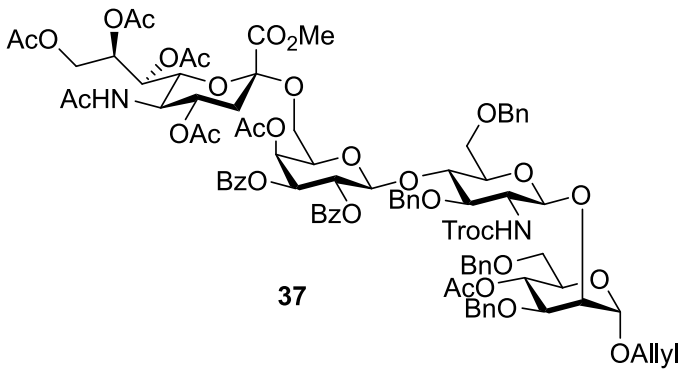
19

4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-D-galactopyranosyl *N*-phenyltrifluoroacetimidate (19)

To a solution of hemiacetal **36** (3.03 g, 3.20 mmol) in acetone (32 mL) were added *N*-phenylacetimidoyl chloride (1.33 g, 6.40 mmol) and K_2CO_3 (1.33 g, 9.60 mmol). The mixture was stirred for 30 min under rt and insoluble materials were filtered. The filtrate was concentrated *in vacuo* and purified by silica-gel column chromatography ($CHCl_3$/acetone = 30/1 to 15/1) to give product **19** (3.41 g, 95%) as α/β mixture.

^{1}H NMR (500 MHz, $CDCl_3$) of major isomer; δ = 7.98 (dd, 2H, *J* = 8.4, 1.2 Hz, Bz), 7.90 (dd, 2H, *J* = 8.3, 1.3 Hz, Bz), 7.57 (tt, 1H, *J* = 7.4, 1.4 Hz, Bz), 7.51 (tt, 1H, *J* = 7.4, 1.4 Hz, Bz), 7.43-7.36 (m, 4H, Bz), 7.14-7.08 (m, 2H, N<u>Ph</u>), 7.01 (t, 1H, *J* = 7.4 Hz, N<u>Ph</u>), 6.74 (d, 1H, *J* = 5.7 Hz, H-1′), 6.44 (d, 2H, *J* = 4.4 Hz, N<u>Ph</u>), 5.87-5.76 (m, 3H, H-2′, 3′, 4′), 5.52 (td, 1H, *J* = 11.0, 5.2 Hz, H-4), 5.35 (ddd, 1H, *J* = 8.0, 5.0, 2.6 Hz, H-8), 5.17 (dd, 1H, *J* = 8.4, 1.8 Hz, H-7), 4.94 (dd, 1H, *J* = 10.1, 1.8 Hz, H-6), 4.52 (t, 1H, *J* = 6.2 Hz, H-5′), 4.29 (dd, 1H, *J* = 12.5, 2.8 Hz, H-9a), 4.17 (t, 1H, *J* = 10.0 Hz, H-5), 4.14 (dd, 1H, *J* = 12.6, 5.0 Hz, H-9b), 4.03 (dd, 1H, *J* = 10.2, 6.2 Hz, H-6a′), 3.83 (s, 3H, CO_2<u>Me</u>), 3.53 (dd, 1H, *J* = 10.0, 7.4 Hz, H-6b′), 2.75 (dd, 1H, *J* = 13.0, 5.3 Hz, H-3$_{eq}$), 2.38 (s, 3H, *N*Ac), 2.31 (s, 3H, *N*Ac), 2.17 (s, 3H, *O*Ac), 2.17 (s, 3H, *O*Ac), 2.13 (s, 3H, *O*Ac), 2.02 (s, 3H, *O*Ac), 1.98 (s, 3H, *O*Ac), 1.86 (dd, 1H, *J* = 13.0, 10.9 Hz, H-3$_{ax}$).

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{52}H_{55}F_3N_2O_{22}$ [M+Na]$^+$: 1139.3096, found: 1139.3107.



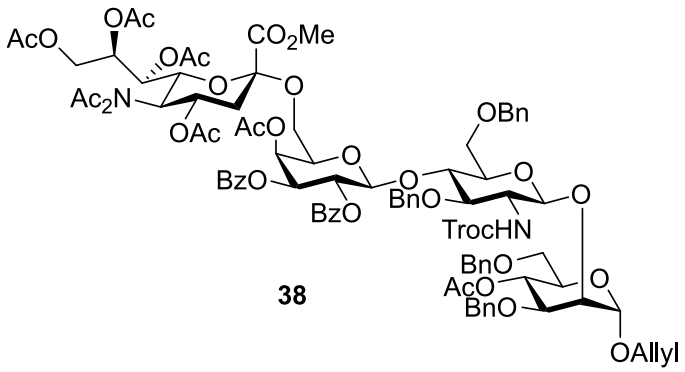
37

Allyl 4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-α-D-mannopyranoside (37)

C5-NHAc disaccharide donor **5** (20.0 mg, 0.0186 mmol) and disaccharide acceptor **18** (21.4 mg, 0.0223 mmol) were lyophilized from benzene and dissolved in dist. CH_2Cl_2 (0.36 mL) with activated MS4A powder. TMSOTf (67 μL, 0.372 mmol) was diluted in dist. CH_2Cl_2 (1.0 mL) with activated MS4A pellets. To the solution of **5** and **18** was added TMSOTf solution (10 μL, 3.72 μmol of TMSOTf) at 0 °C and stirred under the same temperature for 20 min. The reaction was allowed to warm up to rt and continued. After 1 h, another portion of TMSOTf solution (10 μL, 3.72 μmol of TMSOTf) was added and the mixture was stirred for 30 min. The reaction was quenched by adding sat. aqueous $NaHCO_3$ and insoluble materials were filtered. The filtrate was poured into sat. aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Silica-gel column chromatography (toluene/EtOAc = 1/1 to 2/3) was carried out to obtain **37** (17.7 mg, 52%).

^{1}H NMR (500 MHz, acetone-D_6); δ = 7.95 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.87 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.60-7.55 (m, 2H), 7.50 (dd, 2H, *J* = 8.0, 0.9 Hz), 7.43 (q, 4H, *J* = 8.0 Hz), 7.40-7.29 (m, 14H), 7.27-7.17 (m, 4H), 6.99 (d, 1H, *J* = 8.5 Hz), 6.87 (d, 1H, *J* = 9.7 Hz), 5.95-5.87 (m, 1H), 5.63 (dd, 1H, *J* = 3.5, 0.8 Hz), 5.60 (dd, 1H, *J* = 10.4, 8.0 Hz), 5.50 (dd, 1H, *J* = 10.5, 3.5 Hz), 5.44 (ddd, 1H, *J* = 8.5, 6.0, 3.0 Hz), 5.35 (dd, 1H, *J* = 8.2, 2.1 Hz), 5.22 (dq, 1H, *J* = 17.4, 1.8 Hz), 5.20 (d, 1H, *J* = 7.7 Hz), 5.13 (t, 1H, *J* = 9.8 Hz), 5.10 (dq, 1H, *J* = 10.4, 1.5 Hz),

5.09 (d, 1H, *J* = 10.8 Hz), 4.94 (br s, 1H), 4.86-4.76 (m, 4H), 4.63 (d, 1H, *J* = 11.7 Hz), 4.52 (d, 1H, *J* = 12.0 Hz), 4.52 (s, 2H), 4.40 (d, 1H, *J* = 12.1 Hz), 4.32 (dd, 1H, *J* = 12.7, 2.6 Hz), 4.30 (d, 1H, *J* = 12.2 Hz), 4.26 (t, 1H, *J* = 2.5 Hz), 4.21 (dd, 1H, *J* = 10.7, 2.2 Hz), 4.19-4.14 (m, 2H), 4.12-4.05 (m, 2H), 3.94 (ddt, 1H, *J* = 13.1, 5.7, 1.4 Hz), 3.92-3.86 (m, 2H), 3.80-3.77 (m, 5H), 3.73 (dd, 1H, *J* = 11.5, 2.7 Hz), 3.71 (d, 1H, *J* = 4.5 Hz), 3.59-3.49 (m, 2H), 3.44-3.41 (m, 2H), 2.54 (dd, 1H, *J* = 12.8, 4.8 Hz), 2.10 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.78 (s, 3H), 1.76 (dd, 4H, *J* = 13.6, 10.2 Hz).



38

Allyl 4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-*α*-D-mannopyranoside (38)

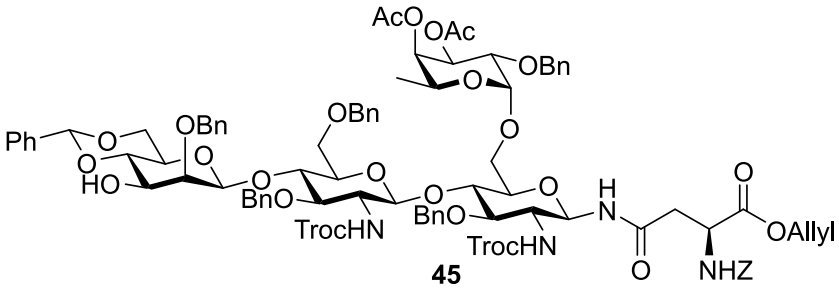
C5-NAc_2 disaccharide donor **19** (1.39 g, 1.24 mmol) and disaccharide acceptor **18** (1.30 g, 1.36 mmol) were lyophilized from benzene and dissolved in dist. CH_2Cl_2 (25 mL) with activated MS4A powder. To the solution was added TMSOTf (45 μL, 0.248 mmol) at 0 °C and the mixture was stirred under the same temperature for 20 min. Sat. aqueous $NaHCO_3$ was added to the reaction mixture and insoluble materials were filtered. The filtrate was poured into sat. aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Silica-gel column chromatography (toluene/EtOAc = 5/1 to 3/2) was carried out to give product **38** (2.25 g, 96%).

^{1}H NMR (400 MHz, acetone-D_6); δ = 7.94 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.88 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.61-7.54 (m, 2H), 7.51-7.36 (m, 10H), 7.34-7.30 (m, 9H), 7.29-7.12 (m, 5H), 6.98 (d, 1H, *J* = 8.7 Hz), 5.96-5.86 (m, 1H),

5.65 (dd, 1H, *J* = 3.5, 0.9 Hz), 5.62 (d, 1H, *J* = 14.9 Hz), 5.61 (dd, 1H, *J* = 7.4, 3.0 Hz), 5.54-5.48 (m, 2H), 5.36 (ddd, 1H, *J* = 8.3, 5.0, 2.3 Hz), 5.22 (dq, 1H, *J* = 17.2, 1.7 Hz), 5.19 (d, 1H, *J* = 8.0 Hz), 5.17 (dd, 1H, *J* = 5.2, 2.2 Hz), 5.13 (d, 1H, *J* = 9.6 Hz), 5.11 (ddd, 1H, *J* = 10.4, 3.2, 1.3 Hz), 5.09 (d, 1H, *J* = 10.9 Hz), 5.00 (dd, 1H, *J* = 10.1, 1.7 Hz), 4.95 (d, 1H, *J* = 1.1 Hz), 4.89-4.76 (m, 4H), 4.63 (d, 1H, *J* = 12.3 Hz), 4.56 (d, 1H, *J* = 12.1 Hz), 4.53 (s, 2H), 4.41 (d, 1H, *J* = 11.9 Hz), 4.38 (dd, 1H, *J* = 12.3, 3.5 Hz), 4.33 (d, 1H, *J* = 12.0 Hz), 4.32 (t, 1H, *J* = 10.2 Hz), 4.20-4.09 (m, 3H), 4.06 (dd, 1H, *J* = 7.6, 6.0 Hz), 3.95 (ddt, 1H, *J* = 13.1, 5.8, 1.5 Hz), 3.95 (dd, 1H, *J* = 10.2, 5.7 Hz), 3.86 (t, 1H, *J* = 10.1 Hz), 3.82-3.78 (m, 5H), 3.76-3.69 (m, 2H), 3.61-3.49 (m, 4H), 3.40 (ddd, 1H, *J* = 9.7, 4.1, 2.3 Hz), 2.69 (dd, 1H, *J* = 12.9, 5.2 Hz), 2.37 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H), 1.81 (dd, 1H, *J* = 12.8, 11.0 Hz).

^{13}C NMR (100 MHz, acetone-D_6); δ = 175.2, 174.6, 170.8, 170.7, 170.4, 170.1, 170.1, 170.0, 168.4, 165.7, 165.7, 155.1, 140.2, 139.7, 139.6, 139.5, 135.1, 134.3, 134.1, 130.3, 130.3, 130.3, 130.2, 129.7, 129.5, 129.3, 129.2, 129.0, 128.8, 128.8, 128.6, 128.4, 128.2, 128.1, 128.0, 128.0, 117.0, 100.9, 100.8, 99.8, 97.8, 97.0, 80.5, 77.4, 76.0, 75.5, 74.8, 74.5, 74.1, 73.9, 73.6, 72.9, 72.6, 71.4, 71.2, 71.1, 71.0, 70.5, 69.7, 69.6, 69.3, 68.7, 68.1, 67.1, 62.7, 58.0, 57.5, 53.3, 39.3, 28.0, 26.0, 21.2, 21.1, 20.9, 20.7, 20.7, 20.6.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{92}H_{103}Cl_3N_2O_{34}$ $[M+Na]^+$: 1907.5356, found: 1907.5353.



45

***N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\gamma}$-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-3-*O*-benzyl-4-*O*-(3,6-di-*O*-benzyl-4-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (45)**

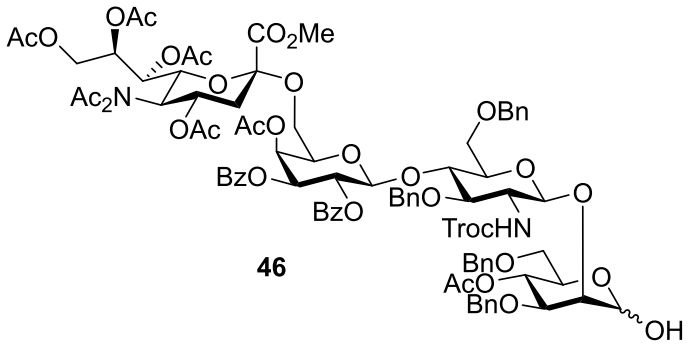
To a solution of protected tetrasaccharide-Asn **14** (509 mg, 0.245 mmol) in anhydrous CH_2Cl_2 (49 mL) was added PPh_3 (193 mg, 0.735 mmol) and the solution was stirred for 1 h under rt. AcOH (422 μL, 7.35

mmol), H_2O (132 μL, 7.35 mmol), and DDQ (195 mg, 0.858 mmol) were added to the reaction solution and stirred for another 20 min. The resulting mixture was diluted by $CHCl_3$ and remaining DDQ was reduced by adding 5% aqueous ascorbic acid. The aqueous layer was extracted with $CHCl_3$ twice and the gathered organic layer was washed by sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mixture was purified by silica-gel column chromatography ($CHCl_3$/acetone = 15/1 to 10/1) to give **45** (412 mg, 89%).

^1H NMR (500 MHz, $CDCl_3$); δ = 7.44-7.39 (m, 4H), 7.36-7.27 (m, 20H), 7.24-7.16 (m, 12H), 6.94 (d, 1H, *J* = 7.7 Hz), 6.15 (d, 1H, *J* = 8.7 Hz), 5.91 (d, 1H, *J* = 8.5 Hz), 5.85-5.78 (m, 1H), 5.39 (s, 1H), 5.35 (dd, 1H, *J* = 10.6, 3.4 Hz), 5.25 (dq, 1H, *J* = 17.2, 1.4 Hz), 5.18 (br s, 1H), 5.16 (dq, 1H, *J* = 10.4, 1.3 Hz), 5.12 (d, 1H, *J* = 12.3 Hz), 5.01 (dt, 4H, *J* = 20.1, 8.3 Hz), 4.88 (d, 1H, *J* = 13.2 Hz), 4.86 (d, 1H, *J* = 14.4 Hz), 4.79-4.65 (m, 6H), 4.59-4.55 (m, 6H), 4.50 (d, 1H, *J* = 11.8 Hz), 4.42 (d, 1H, *J* = 7.2 Hz), 4.33 (d, 1H, *J* = 13.7 Hz), 4.31 (d, 1H, *J* = 11.8 Hz), 4.22 (q, 1H, *J* = 6.5 Hz), 4.09 (q, 2H, *J* = 9.0 Hz), 4.00 (dd, 1H, *J* = 10.6, 4.8 Hz), 3.86-3.81 (m, 4H), 3.72 (d, 1H, *J* = 8.7 Hz), 3.65-3.60 (m, 4H), 3.52 (q, 1H, *J* = 9.0 Hz), 3.52-3.30 (m, 5H), 3.03 (td, 1H, *J* = 9.5, 4.8 Hz), 2.83 (dd, 1H, *J* = 16.5, 3.4 Hz), 2.62 (dd, 1H, *J* = 16.5, 4.2 Hz), 2.29 (d, 1H, *J* = 5.4 Hz), 2.04 (s, 3H), 1.72 (s, 3H), 1.00 (d, 3H, *J* = 6.5 Hz).

^{13}C NMR (100 MHz, $CDCl_3$); δ = 170.5, 170.5, 170.3, 169.8, 156.2, 156.1, 154.3, 139.1, 138.1, 137.3, 137.3, 137.1, 136.2, 131.6, 129.0, 129.0, 128.8, 128.6, 128.5, 128.5, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.8, 127.2, 127.2, 126.3, 118.5, 103.6, 101.9, 101.8, 97.1, 95.6, 95.2, 81.3, 81.0, 80.3, 79.1, 78.5, 77.8, 77.5, 77.3, 75.9, 75.4, 74.8, 74.8, 74.6, 73.8, 73.7, 73.5, 73.4, 71.7, 70.7, 69.6, 68.6, 68.4, 67.0, 66.9, 66.7, 66.2, 64.9, 57.4, 55.9, 50.4, 37.6, 20.7, 20.5, 15.5.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{91}H_{100}Cl_6N_4O_{28}$ $[M+Na]^+$: 1929.4553, found: 1929.4554.



46

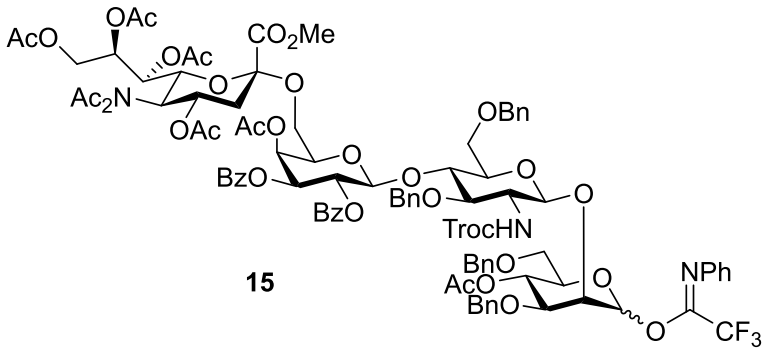
4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-D-mannopyranose (46)

A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (44.8 mg, 0.0530 mmol) in anhydrous THF (5 mL) was stirred for 10 min under H_2 atmosphere to give yellow solution. To a solution of allyl glycoside **38** (2.00 g, 1.06 mmol) in anhydrous THF (16 mL) was added the solution of activated Ir complex and stirred for 1.5 h under rt. H_2O (5 mL) and I_2 (538 mg, 2.12 mmol) were added to the reaction solution and the solution was stirred for another 10 min. The reaction was quenched by 20% aqueous Na_2SO_4 and THF was evaporated. The residual mixture was poured into 20% aqueous Na_2SO_4 and extracted with EtOAc. The organic layer was washed by sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a crude product. The crude was purified by silica-gel column chromatography (toluene/acetone = 6/1 to 4/1) to obtain **46** (1.93 g, 98%) as α/β mixture.

^1H NMR (400 MHz, acetone-D_6) of major isomer; δ = 7.94 (dd, 2H, *J* = 8.2, 1.1 Hz), 7.87 (dd, 2H, *J* = 8.2, 1.1 Hz), 7.57 (qt, 2H, *J* = 9.3, 1.5 Hz), 7.50 (d, 2H, *J* = 7.1 Hz), 7.46-7.38 (m, 7H), 7.36-7.30 (m, 10H), 7.28-7.12 (m, 5H), 6.96 (d, 1H, *J* = 8.7 Hz), 5.68 (d, 1H, *J* = 4.1 Hz), 5.64 (d, 1H, *J* = 3.5 Hz), 5.61 (s, 2H), 5.60 (d, 1H, *J* = 5.8 Hz), 5.54-5.47 (m, 2H), 5.36 (ddd, 1H, *J* = 7.8, 6.0, 3.2 Hz), 5.25 (br s, 1H), 5.21-5.08 (m, 4H), 5.00 (dd, 1H, *J* = 10.1, 1.6 Hz), 4.86-4.76 (m, 3H), 4.61 (d, 1H, *J* = 12.7 Hz), 4.57 (d, 1H, *J* = 12.3 Hz), 4.48 (s, 2H), 4.43-4.29 (m, 4H), 4.24 (t, 1H, *J* = 2.2 Hz), 4.16-4.04 (m, 3H), 4.01 (ddd, 1H, *J* = 10.0, 6.0, 3.8 Hz), 3.95 (dd, 1H, *J* = 9.7, 5.5 Hz), 3.89-3.85 (m, 2H), 3.82 (s, 3H), 3.76-3.69 (m, 2H), 3.60-3.46 (m, 3H), 3.41 (ddd, 1H, *J* =

9.8, 4.2, 2.2 Hz), 2.69 (dd, 1H, *J* = 12.8, 5.2 Hz), 2.37 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.81 (dd, 1H, *J* = 12.7, 10.9 Hz).

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{89}H_{99}Cl_3N_2O_{34}$ [M+Na]$^+$: 1867.5043, found: 1867.5054.



15

4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-D-mannopyranosyl *N*-phenyltrifluoroacetimidate (15)

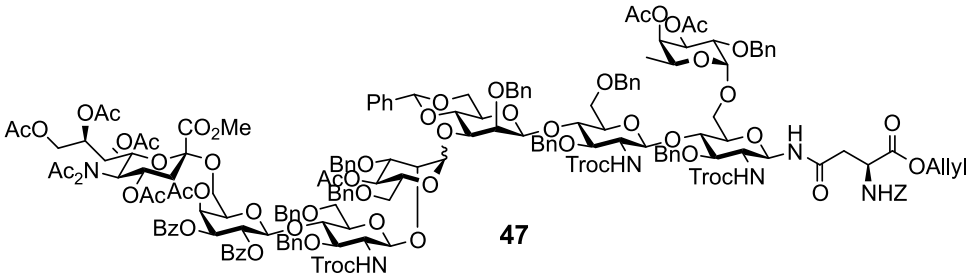
To a solution of hamiacetal **46** (3.45 g, 1.87 mmol) in acetone (37 mL) were added *N*-phenyltrifluoroacetimidoyl chloride (776 mg, 3.74 mmol) and K_2CO_3 (1.29 g, 9.35 mmol). The mixture was stirred for 1 h under rt and insoluble materials were filtered. The filtrate was concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/acetone = 7/1 to 5/1) to obtain **15** (3.72 g, 99%) as α/β mixture.

^{1}H NMR (500 MHz, acetone-D_6) of major isomer; δ = 7.96 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.88 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.61 (tt, 1H, *J* = 7.5, 1.4 Hz), 7.56 (tt, 1H, *J* = 7.4, 1.4 Hz), 7.51-7.36 (m, 11H), 7.35-7.19 (m, 16H), 7.05 (tt, 2H, *J* = 7.5, 1.1 Hz), 6.77 (d, 2H, *J* = 7.7 Hz), 6.28 (br s, 1H), 5.65 (dd, 1H, *J* = 3.5, 1.0 Hz), 5.61 (dd, 1H, *J* = 10.4, 7.9 Hz), 5.53-5.48 (m, 1H), 5.36 (ddd, 1H, *J* = 7.0, 6.0, 3.5 Hz), 5.23 (t, 1H, *J* = 9.6 Hz), 5.19 (d, 1H, *J* = 7.9 Hz), 5.17 (dd, 1H, *J* = 7.3, 1.7 Hz), 5.09 (d, 1H, *J* = 10.9 Hz), 5.00 (dd, 1H, *J* = 10.1, 1.7 Hz), 4.89-4.81 (m, 2H), 4.77 (d, 2H, *J* = 12.2 Hz), 4.76 (d, 2H, *J* = 10.7 Hz), 4.57 (d, 2H, *J* = 12.0 Hz), 4.54 (s, 2H), 4.48 (d, 1H, *J* = 11.7 Hz), 4.37 (dd, 1H, *J* = 12.0, 3.5 Hz), 4.35 (d, 1H, *J* = 12.2 Hz), 4.31 (d, 1H, *J* = 10.1 Hz), 4.14 (dd, 1H, *J* =

12.2, 6.0 Hz), 4.11 (dd, 1H, *J* = 9.6, 8.8 Hz), 4.07 (ddd, 1H, *J* = 8.0, 5.5, 0.9 Hz), 3.96-3.92 (m, 2H), 3.90-3.85 (m, 2H), 3.82 (s, 3H), 3.74-3.73 (m, 2H), 3.58 (d, 2H, *J* = 4.5 Hz), 3.52 (t, 1H, *J* = 8.9 Hz), 3.38 (dt, 1H, *J* = 9.5, 3.3 Hz), 2.69 (dd, 1H, *J* = 12.9, 5.2 Hz), 2.37 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.81 (dd, 1H, *J* = 12.5, 11.3 Hz).

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{97}H_{103}Cl_3F_3N_3O_{34}$ $[M+Na]^+$: 2038.5338, found: 2038.5328.

Chapter 3.

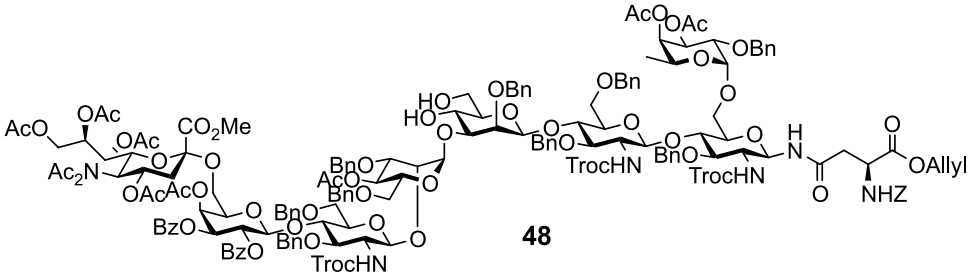


N^{α}-benzyloxycarbonyl-N^{γ}-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)- 3-*O*-benzyl-4-*O*-(3,6-di-*O*-benzyl-4-*O*-(3-*O*-(4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-D-mannopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (47)

Tetrasaccharide donor **15** (1.27 g, 0.628 mmol) and tetrasaccharide-Asn acceptor **45** (1.00 g, 0.523 mmol) were lyophilized from benzene and dissolved in anhydrous CPME (9.0 mL) with activated MS4A powder. TMSOTf (47.4 μL, 0.262 mmol) was diluted with anhydrous CPME (1.0 mL) with activated MS4A pellets. Both solutions were cooled to 0 °C and the TMSOTf solution was cannulated into the solution of **15** and **45**. The reaction solution was stirred for 20 min under 0 °C and sat. aqueous $NaHCO_3$ was added to the solution. Insoluble materials were filtered and the filtrate was poured into sat. aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc and the organic layer was washed by brine, dried

over Na_2SO_4, filtered, and concentrated *in vacuo*. Silica-gel column chromatography (toluene/acetone = 6/1 to 4/1) was carried out to obtain **47** as α/β mixture (1.79 g, 91%, α/β ~ 3/1).

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{180}H_{197}Cl_9N_6O_{61}$ $[M+2Na]^{2+}$: 1889.4739, found: 1889.4762.



48

***N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\gamma}$-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)- 3-*O*-benzyl-4-*O*-(3,6-di-*O*-benzyl-4-*O*-(3-*O*-(4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-*α*-D-mannopyranosyl)-2-*O*-benzyl-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (48)**

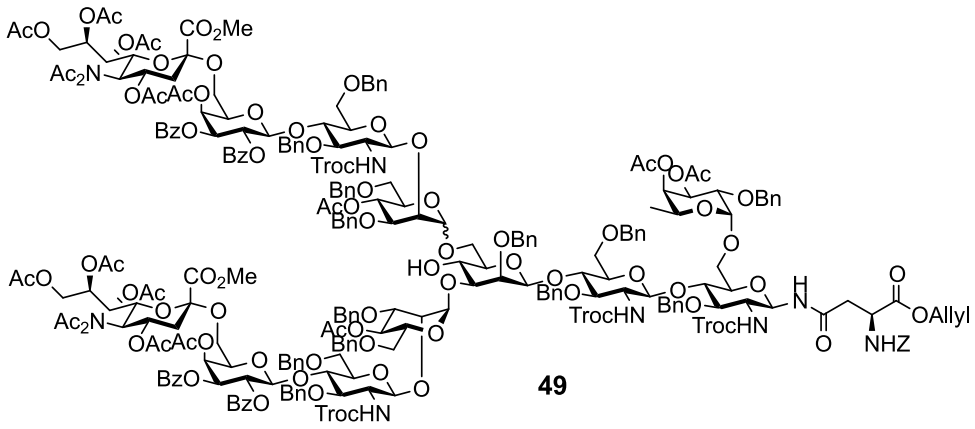
To a solution of octasaccharide-Asn **47** (1.73 g, 0.463 mmol) in CH_2Cl_2 (18 mL) was added H_2O (1.8 mL) and TFA (3.5 mL) at 0 °C. The solution was stirred for 1.5 h under rt and neutralized by sat. aqueous $NaHCO_3$. The mixture was poured into sat. aqueous $NaHCO_3$ and extracted twice with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography ($CHCl_3$/MeCN = 5/1 to 3/1) was carried out to obtain **48** (974 mg, 58%, α isomer) and β isomer at C-1^{E} (244 mg, 14%).

^{1}H NMR (500 MHz, acetone-D_6); δ = 7.95 (dd, 2H, *J* = 8.3, 1.1 Hz), 7.87 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.83 (d, 1H, *J* = 9.5 Hz), 7.61-7.55 (m, 2H), 7.49 (d, 2H, *J* = 7.1 Hz), 7.47-7.41 (m, 4H), 7.39-7.15 (m, 50H), 6.99 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 1H, *J* = 5.9 Hz), 6.88 (d, 1H, *J* = 9.2 Hz), 6.47 (d, 1H, *J* = 8.7 Hz), 5.94-5.86 (m, 1H), 5.63 (d, 1H, *J* = 3.6 Hz), 5.60 (dd, 1H, *J* = 10.3, 7.9 Hz), 5.49 (d, 1H, *J* = 10.5 Hz), 5.48 (dd, 1H, *J* = 10.5, 2.0 Hz), 5.36-5.29 (m, 4H), 5.22-5.00 (m, 13H), 4.99 (dd, 1H, *J* = 10.1, 1.7 Hz), 4.87-4.66 (m, 12H), 4.63-4.56 (m, 5H),

4.52-4.48 (m, 4H), 4.43 (d, 1H, *J* = 11.2 Hz), 4.39-4.24 (m, 7H), 4.13 (dd, 1H, *J* = 12.2, 6.1 Hz), 4.12-4.01 (m, 4H), 3.94-3.45 (m, 28H), 3.39 (dd, 1H, *J* = 11.5, 5.9 Hz), 3.24 (ddd, 1H, *J* = 9.7, 4.3, 2.3 Hz), 3.02 (ddd, 1H, *J* = 9.2, 5.6, 3.3 Hz), 2.81 (d, 2H, *J* = 5.7 Hz), 2.72 (t, 1H, *J* = 6.3 Hz), 2.68 (dd, 1H, *J* = 12.9, 5.2 Hz), 2.36 (s, 3H), 2.33 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H), 1.80 (dd, 1H, *J* = 11.2, 9.3 Hz), 1.77 (s, 3H), 1.02 (d, 3H, *J* = 6.4 Hz).

^{13}C NMR (125 MHz, acetone-D_6); δ = 175.2, 174.6, 171.6, 170.9, 170.9, 170.8, 170.8, 170.4, 170.3, 170.1, 170.1, 170.0, 168.4, 165.7, 165.7, 156.8, 155.8, 155.3, 155.3, 155.2, 140.6, 140.6, 140.3, 140.2, 139.7, 139.4, 139.4, 139.3, 138.0, 134.3, 134.2, 133.3, 130.4, 130.3, 130.3, 130.2, 129.6, 129.4, 129.3, 129.2, 129.2, 129.2, 128.9, 128.8, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.6, 127.6, 117.9, 102.3, 102.3, 101.4, 101.1, 101.1, 100.9, 97.5, 97.1, 97.0, 96.9, 82.0, 81.8, 81.8, 81.8, 80.6, 79.8, 79.7, 78.2, 77.7, 77.5, 77.2, 76.9, 76.3, 75.5, 75.4, 75.3, 75.0, 74.9, 74.8, 74.5, 74.4, 74.2, 73.9, 73.8, 73.5, 73.1, 72.9, 72.6, 72.4, 71.6, 71.4, 71.2, 71.0, 70.8, 69.7, 69.7, 69.4, 68.6, 68.2, 67.1, 66.9, 66.3, 66.1, 65.1, 63.0, 62.7, 58.8, 57.9, 57.6, 57.4, 57.4, 53.3, 51.5, 39.3, 38.2, 38.1, 30.2, 30.1, 28.0, 26.0, 21.2, 21.1, 20.9, 20.9, 20.7, 20.7, 20.6, 20.5, 16.1.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{173}H_{193}Cl_9N_6O_{61}$ $[M+2Na]^{2+}$: 1845.4583, found: 1845.4594.



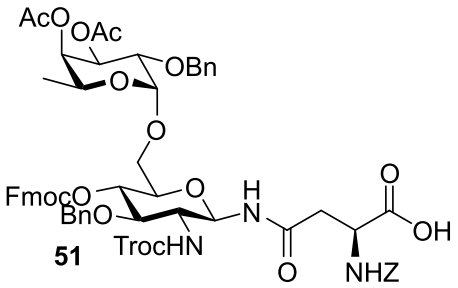
49

***N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\gamma}$-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)- 3-*O*-benzyl-4-*O*-(3,6-di-*O*-benzyl-4-*O*-(3,6-di-*O*-(4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-D-mannopyranosyl)-2-*O*-benzyl-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (49)**

Tetrasaccharide donor **15** (8.29 mg, 4.11 μmol) and octasaccharide-Asn acceptor **48** (10.0 mg, 2.74 μmol) were lyophilized from benzene and dissolved in anhydrous CPME (132 μL) with activated MS4A powder. TMSOTf (30 μL, 0.166 mmol) was diluted with anhydrous CPME (1.0 mL) with activated MS4A pellets. The TMSOTf solution (5.0 μL, 0.822 μmol of TMSOTf) was added to the solution of **15** and **48** at 0 °C and the mixture was stirred for 10 min under the same temperature. Sat. aqueous $NaHCO_3$ was added to the reaction mixture and insoluble materials were filtered. The filtrate was poured into sat. $NaHCO_3$ and the aqueous layer was extracted with EtOAc. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Silica-gel column chromatography was carried out to give **49** as α/β mixture (13.1 mg, 87%, α/β ~ 1/1). The mixture was used for the next reaction without further purification.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{262}H_{290}Cl_{12}N_8O_{94}$ $[M+3Na]^{3+}$: 1846.8032, found: 1846.8000.

Chapter 4.

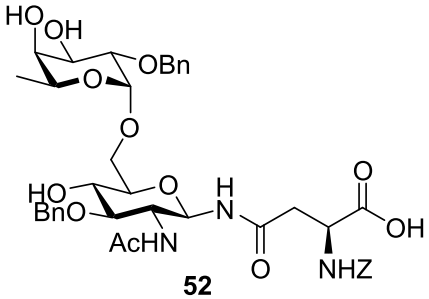


51

***N*$^{\alpha}$-(Benzyloxycarbonyl)-*N*$^{\gamma}$-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzyl-4-*O*-(9-fluorenylmethyloxycarbonyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine (51)**

To a solution of protected disaccharide-Asn **28** (100 mg, 0.0784 mmol), $Pd(OAc)_2$ (3.52 mg, 0.157 mmol), and PPh_3 (19.5 mg, 0.0745 mmol) in acetone (0.78 mL) was added sodium 2-ethylhexanoate (39.1 mg, 0.235 mmol). The mixture was stirred for 20 min under rt and diluted with EtOAc. Resulting solution was poured into brine and the aqueous layer was extracted with EtOAc. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. The crude product was used for the next reaction without further purification.

MALDI-TOF MS; *m*/*z* calcd for $C_{60}H_{62}Cl_3N_3O_{19}$ $[M+Na]^+$: 1256.3, found: 1256.7.



52

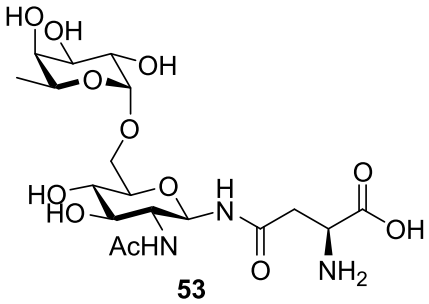
***N*$^{\alpha}$-(Benzyloxycarbonyl)-*N*$^{\gamma}$-(2-acetamino-6-*O*-(2-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (52)**

The crude **51** (0.0784 mmol) was dissolved in THF (1.3 mL) and dioxane (0.87 mL). 3M LiOHaq (0.44 mL) was added to the solution and the mixture was stirred for 1.5 h under rt. To the mixture were added H_2O (0.87 mL), $NaHCO_3$ (65.9 mg, 0.784 mmol), and Ac_2O (37 μL, 0.392 mmol). After additional 30 min

stirring, $NaHCO_3$ (65.9 mg, 0.784 mmol) and Ac_2O (37 μL, 0.392 mmol) were added to the reaction mixture and stirred for 1 h. $LiOH \bullet H_2O$ (32.9 mg, 0.784 mmol) was added to the mixture and stirred for additional 1 h. Resulting mixture was neutralized by 1M aqueous HCl and concentrated *in vacuo*. To the residue was added $CHCl_3$ and insoluble materials were filtered through Hyflo Super Cel®. The residue was washed by MeOH and the MeOH solution was concentrated *in vacuo* to give a crude product. The crude product was purified through a column of diaion™ HP20 resin (H_2O/MeOH = 3/1 to 1/2) to obtain pure product **52** (54.4 mg, 87%).

^{1}H NMR (500 MHz, DMSO-D_6); δ = 8.76 (d, 1H, *J* = 8.6 Hz), 8.04 (d, 1H, *J* = 9.0 Hz), 7.39-7.19 (m, 15H), 6.30 (d, 1H, *J* = 6.2 Hz), 5.28 (d, 1H, *J* = 5.7 Hz), 4.98 (t, 1H, *J* = 13.2 Hz), 4.95 (s, 1H), 4.94 (t, 1H, *J* = 9.3 Hz), 4.84 (d, 1H, *J* = 3.6 Hz), 4.78 (d, 1H, *J* = 11.5 Hz), 4.75 (br s, 1H), 4.61 (t, 1H, *J* = 13.9 Hz), 4.57 (d, 1H, *J* = 11.7 Hz), 4.52 (br s, 1H), 3.87 (d, 1H, *J* = 5.9 Hz), 3.83 (q, 1H, *J* = 6.8 Hz), 3.79 (d, 1H, *J* = 10.9 Hz), 3.74 (dd, 1H, *J* = 10.1, 2.9 Hz), 3.68 (q, 1H, *J* = 9.6 Hz), 3.54-3.48 (m, 4H), 3.38 (t, 1H, *J* = 8.2 Hz), 2.51-2.47 (m, 4H), 2.43 (dd, 1H, *J* = 14.8, 4.9 Hz), 1.74 (s, 3H), 1.07 (d, 3H, *J* = 6.4 Hz).

MALDI-TOF MS; *m*/*z* calcd for $C_{40}H_{49}N_3O_{14}$ $[M+Na]^+$: 818.3, found: 819.2.



53

***N*$^\gamma$-(2-Acetamino-2-deoxy-6-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosyl)-L-asparagine (53)**

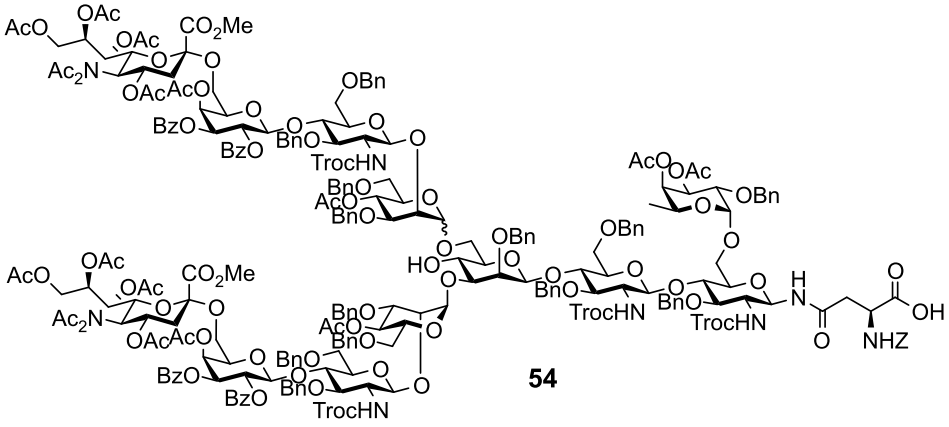
Disaccharide-Asn **52** (2.00 mg, 2.51 μmol) was dissolved in dist. H_2O (200 μL), dist. THF (200 μL), and AcOH (40 μL). A suspension of 20% $Pd(OH)_2$/C (20.0 mg) in dist. H_2O (200 μL)/dist. THF (200 μL) was added to the solution and the mixture was stirred under rt under H_2 atmosphere. After 1 h reaction, insoluble materials were filtered through Hyflo Super Cel® and the filtrate was concentrated *in vacuo* to reduce the amount of the solvent. Resulting solution was lyophilized to give the product **53** as a mixture

with acetate salt (2.15 mg). The yield was calculated by NMR (80%).

^{1}H NMR (500 MHz, D_2O); δ = 5.09 (d, 1H, *J* = 9.7 Hz, H-1), 4.90 (d, 1H, *J* = 3.9 Hz, H-1′), 4.12 (q, 1H, *J* = 6.5 Hz, H-5′), 3.97 (d, 1H, *J* = 10.7 Hz, H-6a), 3.91 (br s, 1H, Asn H-*α*), 3.88 (dd, 1H, *J* = 10.4, 3.3 Hz, H-3′), 3.84 (t, 1H, *J* = 10.0 Hz, H-2), 3.80 (d, 1H, *J* = 3.1 Hz, H-2′), 3.77 (dd, 1H, *J* = 10.3, 3.8 Hz, H-4′), 3.73 (dd, 1H, *J* = 11.6, 6.0 Hz, H-6b), 3.66 (dd, 1H, *J* = 9.2, 5.3 Hz, H-5), 3.61 (t, 1H, *J* = 9.6 Hz, H-3), 3.52 (t, 1H, *J* = 9.4 Hz, H-4), 2.89 (dd, 1H, *J* = 17.0, 2.5 Hz, Asn C$\underline{H_2}$CONHa), 2.81 (dd, 1H, *J* = 16.9, 6.7 Hz, Asn C$\underline{H_2}$CONHb), 2.02 (s, 3H, Ac), 1.21 (d, 3H, *J* = 6.6 Hz, H-6′).

^{13}C NMR (125 MHz, D_2O); δ = 175.52, 173.50, 173.14, 99.72, 78.78, 77.42, 74.80, 72.48, 70.34, 70.17, 68.86, 67.86, 67.41, 54.70, 51.57, 35.54, 23.81, 15.99.

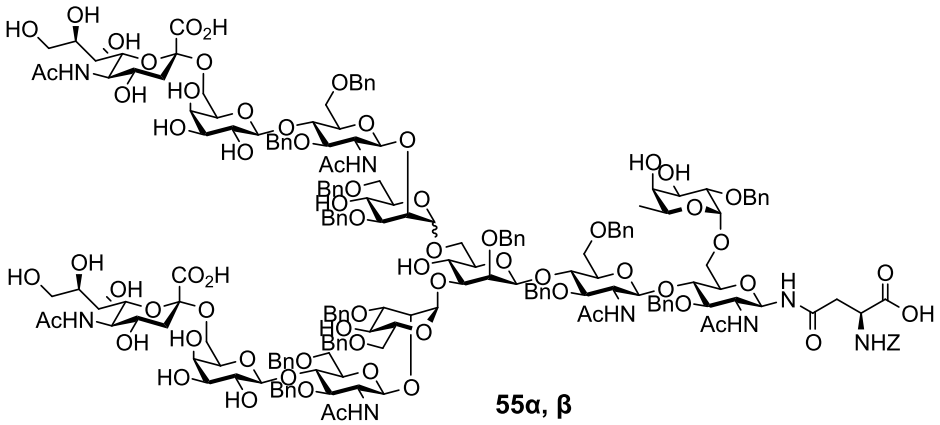
HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{18}H_{31}N_3O_{12}$ [M+Na]$^{+}$: 504.1800, found: 504.1806.



N^{α}-benzyloxycarbonyl-N^{γ}-(6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)- 3-*O*-benzyl-4-*O*-(3,6-di-*O*-benzyl-4-*O*-(3,6-di-*O*-(4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-D-mannopyranosyl)-2-*O*-benzyl-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine (54)

Solutions of $Pd(OAc)_2$ (1.23 mg, 5.46 μmol) in acetone (200 μL), PPh_3 (7.16 mg, 0.0273 mmol) in acetone (200 μL), and sodium 2-ethylhexanoate (30.2 mg, 0.182 mmol) in acetone (400 μL) were prepared. To a

solution of dodecasaccharide-Asn **49** (10.0 mg, 1.82 μmol) in acetone (122 μL) were added $Pd(OAc)_2$ solution (20 μL, 0.546 μmol of $Pd(OAc)_2$), PPh_3 solution (20 μL, 2.73 μmol of PPh_3), and sodium 2-ethylhexanoate solution (20 μL, 9.10 μmol of sodium 2-ethylhexanoate). The reaction solution was stirred for 2 h under rt, diluted with EtOAc, and poured into brine. The aqueous layer was extracted with EtOAc and the organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give crude product of **54**. The crude product was used for the next reaction without further purification.



55α, β

N^{α}-benzyloxycarbonyl-N^{γ}-(2-acetamide-4-*O*-(2-acetamide-4-*O*-(3,6-di-*O*-(2-*O*-(2-acetamide-4-*O*-(6-*O*-(5-acetamide-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-D-mannopyranosyl)-2-*O*-benzyl-β-D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-benzyl-6-*O*-(2-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine (55)

The crude product of carboxylic acid **54** was dissolved in THF (455 μL)/dioxane (303 μL) and 3M aqueous LiOH (152 μL) was added to the solution. The mixture was stirred overnight under rt to produce tetramine. To the reaction mixture were added H_2O (76 μL), $NaHCO_3$ (61.2 mg, 0.728 mmol), and Ac_2O (34.4 μL, 0.364 mmol) and stirred again. After 1 h reaction, another portion of $NaHCO_3$ (61.2 mg, 0.728 mmol) and Ac_2O (34.4 μL, 0.364 mmol) were put into the reaction mixture, followed by additional 1 h reaction. $LiOH \bullet H_2O$ (30.5 mg, 0.728 mmol) was added to the resulting mixture and the mixture was stirred for 2 h. The reaction mixture was neutralized by adding dry ice and concentrated *in*

vacuo. The residue was roughly purified by a column of diaion™ HP20 resin (H_2O/MeOH = 3/2 to 1/2) to give a crude product. HPLC purification (COSMOSIL 5C_{18}-AR-300 10 × 250 mm) was carried out to obtain pure **55*α*** (1.88 mg, 27%) and **55β** (1.88 mg, 27%). Eluting condition: H_2O + 0.1% TFA/MeCN + 0.1% TFA as mobile phase, 4 mL^{-1} isocratic flow of 53% MeCN, 7.1 min for **55*α***, 10.9 min for **55β**.

Analytical data for **55*α***:

^{1}H NMR (400 MHz, CD_3OD); δ = 7.42-7.17 (m, 54H), 7.15-7.05 (m, 16H), 5.20 (s, 1H), 5.10-5.01 (m, 4H), 4.97-4.91 (m, 4H), 4.87-4.67 (m, 11H), 4.61-4.48 (m, 9H), 4.46-4.36 (m, 7H), 4.34-4.28 (m, 3H), 4.08-3.87 (m, 17H), 3.85-3.69 (m, 22H), 3.67-3.34 (m, 32H), 3.17 (d, 1H, *J* = 7.0 Hz), 2.70-2.61 (m, 4H), 2.00 (s, 6H), 1.82 (s, 3H), 1.81 (s, 3H), 1.81 (s, 3H), 1.77-1.69 (m, 5H), 1.12 (d, 3H, *J* = 6.4 Hz).

^{13}C NMR (100 MHz, CD_3OD); δ = 175.3, 174.5, 174.0, 173.6, 173.5, 173.3, 172.6, 171.8, 171.8, 140.9, 140.6, 140.2, 140.2, 140.0, 139.8, 139.7, 139.7, 139.6, 139.5, 138.0, 129.6, 129.6, 129.5, 129.5, 129.5, 129.4, 129.3, 129.2, 129.2, 129.2, 129.1, 129.0, 129.0, 128.9, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 104.7, 104.4, 101.7, 101.5, 101.1, 100.9, 100.9, 100.8, 100.3, 100.2, 98.6, 98.3, 83.3, 81.8, 81.6, 81.3, 80.5, 79.8, 79.6, 78.6, 78.3, 78.0, 77.9, 77.6, 77.1, 76.9, 76.6, 76.4, 76.3, 76.1, 75.8, 75.6, 75.3, 75.1, 75.1, 74.9, 74.9, 74.5, 74.4, 74.3, 74.2, 74.1, 74.0, 73.9, 73.5, 73.2, 73.1, 73.0, 72.6, 72.2, 71.7, 71.6, 71.4, 71.2, 70.3, 70.1, 69.9, 69.8, 69.5, 68.8, 68.4, 67.8, 67.7, 67.5, 67.2, 66.8, 64.5, 64.5, 63.3, 57.4, 56.5, 56.3, 54.9, 51.9, 40.9, 40.5, 38.5, 33.0, 30.7, 23.7, 23.4, 23.4, 22.9, 22.7, 16.6.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{193}H_{238}N_8O_{70}$ $[M+3Na]^{3+}$: 1285.4995, found: 1285.4995.

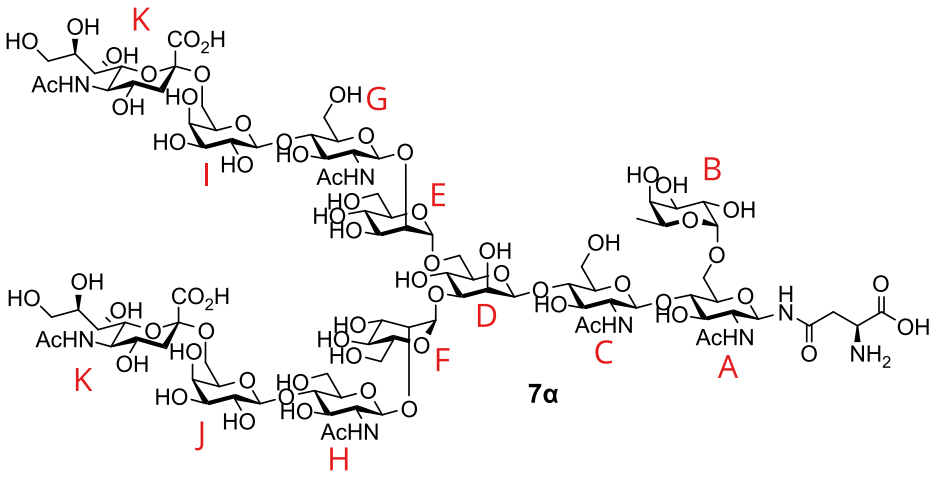
Analytical data for **55β**:

^{1}H NMR (400 MHz, CD_3OD); δ = 7.42-7.13 (m, 66H), 7.08-7.03 (m, 4H), 5.23 (d, 1H, *J* = 11.8 Hz), 5.23 (s, 1H), 5.09-4.93 (m, 7H), 4.89 (t, 1H, *J* = 2.2 Hz), 4.79-4.46 (m, 18H), 4.44-4.33 (m, 9H), 4.31-4.11 (m, 7H), 4.04-3.49 (m, 62H), 3.48-3.41 (m, 5H), 2.68-2.56 (m, 4H), 1.99 (s, 6H), 1.90 (s, 3H), 1.83 (s, 3H), 1.80 (s, 3H), 1.75-1.66 (m, 5H), 1.13 (d, 3H, *J* = 6.5 Hz).

^{13}C NMR (100 MHz, CD_3OD); δ = 175.3, 174.5, 174.0, 173.6, 173.5, 172.6, 171.8, 171.7, 171.6, 158.3, 141.1, 140.7, 140.3, 140.2, 140.0, 139.7, 139.7, 139.6, 139.5, 139.5, 138.0, 133.7, 133.1, 133.0, 130.0, 129.9, 129.7, 129.5, 129.4, 129.4, 129.3, 129.2, 129.1, 129.1, 128.9, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.1, 104.7,

104.5, 102.7, 102.2, 102.1, 101.8, 101.0, 100.9, 100.2, 100.0, 98.3, 83.7, 82.1, 81.7, 81.5, 81.3, 80.3, 79.6, 78.6, 78.4, 77.7, 77.5, 77.1, 76.7, 76.4, 76.3, 76.2, 75.9, 75.8, 75.7, 75.1, 75.0, 74.9, 74.8, 74.7, 74.6, 74.5, 74.5, 74.3, 74.2, 74.1, 73.9, 73.8, 73.1, 72.9, 72.6, 72.2, 71.6, 71.5, 71.4, 71.3, 71.1, 70.2, 70.1, 69.8, 69.5, 69.4, 68.8, 67.7, 67.3, 64.6, 64.3, 63.3, 63.2, 62.3, 62.1, 61.7, 56.9, 56.5, 55.2, 53.8, 51.8, 42.5, 40.8, 40.4, 38.4, 30.7, 24.0, 23.7, 23.4, 22.9, 22.7, 16.6.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{193}H_{238}N_8O_{70}$ $[M+3Na]^{3+}$: 1285.4995, found: 1285.5017.



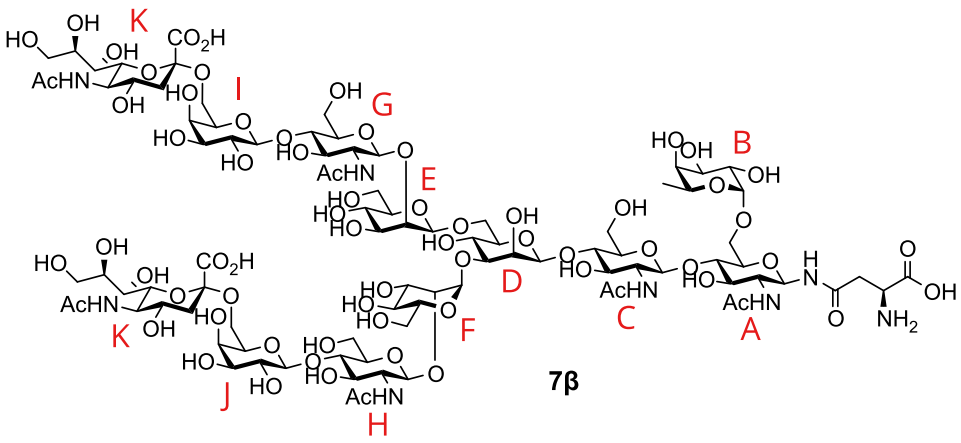
***N*ᵞ-(2-acetamide-4-*O*-(2-acetamide-4-*O*-(3,6-di-*O*-(2-*O*-(2-acetamide-4-*O*-(6-*O*-(5-acetamide-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl)-β-D-mannopyranosyl)-2-deoxy-β-D-glucopyranosyl)-2-deoxy-6-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosyl)-L-asparagine (7α)**

To a solution of dodecasaccharide-Asn **55α** (1.00 mg, 0.264 μmol) in *t*BuOH (63 μL)/dist. H_2O (63 μL)/AcOH (6.3 μL) was added a suspension of 20% $Pd(OH)_2$/C (8.00 mg) in *t*BuOH (63 μL)/dist. H_2O (63 μL)/AcOH (6.3 μL). The mixture was stirred under H_2 (2.0 MPa) atmosphere overnight under rt. After the reaction, insoluble materials were filtered through Hyflo Super Cel® and washed by dist. H_2O + 0.1% TFA and MeOH. The filtrate was concentrated *in vacuo* and lyophilized from H_2O to give a crude product. HPLC purification (Waters XBridge® Glycan BEH Amide 4.6 × 250 mm) was carried out to obtain *N*-glycan **7α**. The yield was calculated by NMR analysis (38%). Eluting condition: MeCN/aqueous 25 mM NH_4OAc as mobile phase, 1 mL min^{-1} isocratic flow of 45% aqueous solution, 13.5 min.

^{1}H NMR (600 MHz, D_2O); δ = 5.03 (s, 1H), 4.96 (d, *J* = 9.3 Hz, 1H), 4.83 (s, 1H), 4.77 (d, *J* = 3.5 Hz, 1H), 4.67 (s, 1H), 4.58 (d, *J* = 7.99 Hz, 1H), 4.50 (d, *J* = 7.24 Hz, 2H), 4.34 (d, *J* = 8.2 Hz, 2H), 4.15 (s, 1H), 4.09 (s, 1H), 4.04-3.98 (m, 2H), 3.92-3.84 (m, 2H), 3.84-3.37 (m, 34H), 2.82-2.66 (m, 3H), 2.02-1.77 (m, 18H), 1.67-1.57 (m, 1H), 1.09 (d, *J* = 5.47 Hz, 3H).

^{13}C NMR (150 MHz, D_2O); δ = 180.1, 174.81, 173.1, 174.6, 174.48, 103.35 ($^{1}J_{CH}$ = 160.8 Hz, C-$1^{I/J}$), 100.83 ($^{1}J_{CH}$ = 160.7 Hz, C-1^{C}), 100.5 ($^{1}J_{CH}$ = 159.7 Hz, C-1^{D}), 99.8, 99.4 ($^{1}J_{CH}$ = 169.3 Hz, C-1^{F}), 99.25 ($^{1}J_{CH}$ = 168.3 Hz, C-1^{B}), 99.2 ($^{1}J_{CH}$ = 160.4 Hz, C-$1^{G/H}$), 96.77 ($^{1}J_{CH}$ = 170.5 Hz, C-1^{E}), 80.55, 80.4, 79.7, 77.92 ($^{1}J_{CH}$ = 155.3 Hz, C-1^{A}), 76.07, 76.02, 74.39, 74.3, 74.22, 73.63, 73.42, 73.3, 73.27, 72.7, 72.4, 72.39, 72.1, 72.04, 71.99, 71.9, 71.7, 70.67, 70.1, 69.4, 69.3, 69.3, 68.3, 68.26, 68.2, 68.0, 67.27, 67.2, 66.8, 66.3, 65.9, 63.26, 62.6, 61.54, 61.5, 60.13, 59.9, 54.68, 54.56, 53.45, 51.6, 51.19, 48.2, 40.0, 36.0, 15.0.

HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{94}H_{154}N_8O_{68}$ $[M+2H]^{2+}$: 1242.4492, found: 1242.4505.



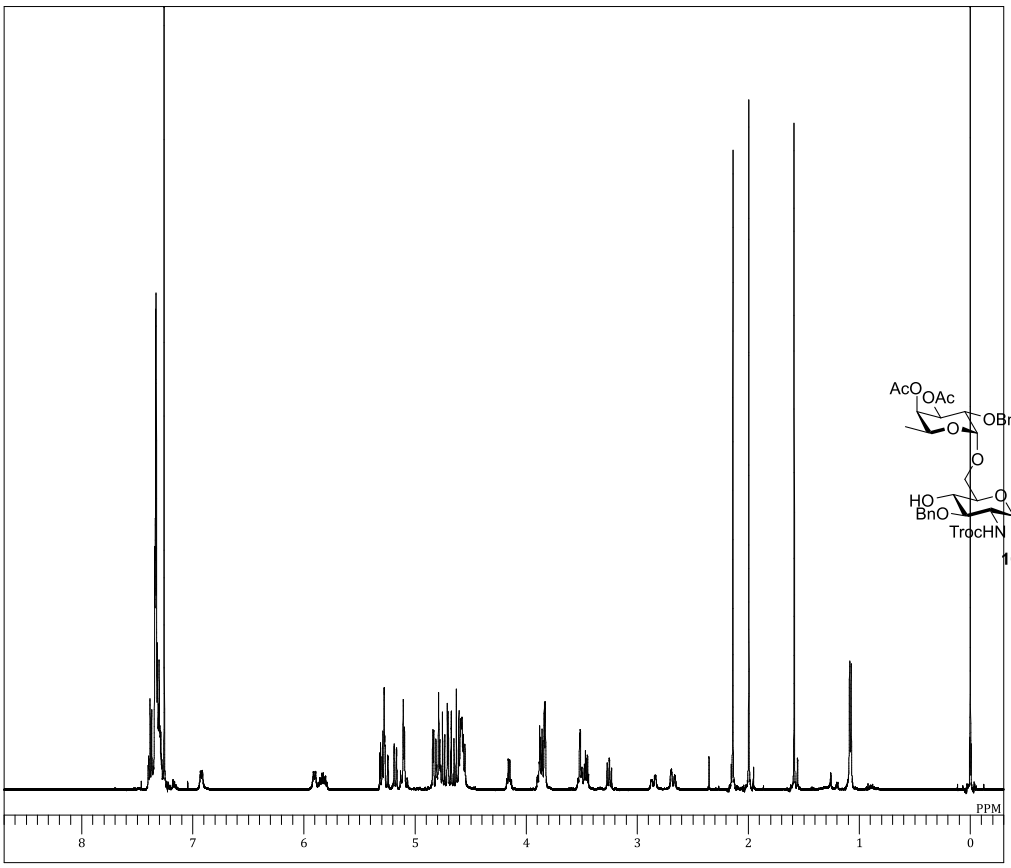
N^{γ}-(2-acetamide-4-*O*-(2-acetamide-4-*O*-(3-*O*-(2-*O*-(2-acetamide-4-*O*-(6-*O*-(5-acetamide-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl)-6-*O*-(2-*O*-(2-acetamide-4-*O*-(6-*O*-(5-acetamide-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-2-deoxy-β-D-glucopyranosyl)-2-deoxy-6-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosyl)-L-asparagine (7β)

To a solution of dodecasaccharide-Asn **55β** (1.00 mg, 0.264 μmol) in *t*BuOH (63 μL)/dist. H_2O (63 μL)/AcOH (12.6 μL) was added a suspension of 20% $Pd(OH)_2/C$ (10.0 mg) in *t*BuOH (63 μL)/dist. H_2O (63 μL). The mixture was stirred under H_2 (2.0 MPa) atmosphere overnight under rt. After the reaction, insoluble materials were filtered through Hyflo Super Cel® and washed by dist. H_2O + 0.1% TFA. The filtrate was concentrated *in vacuo* and lyophilized from H_2O to give a crude product. The crude product was purified by HPLC (Waters XBridge® Glycan BEH Amide 4.6 × 250 mm) to obtain product **7β**. The yield was calculated by NMR analysis (31%). Eluting condition: MeCN/25 mM aqueous NH_4OAc as mobile phase, 1 mL min^{-1} isocratic flow of 45% aqueous solution, 11.4 min.

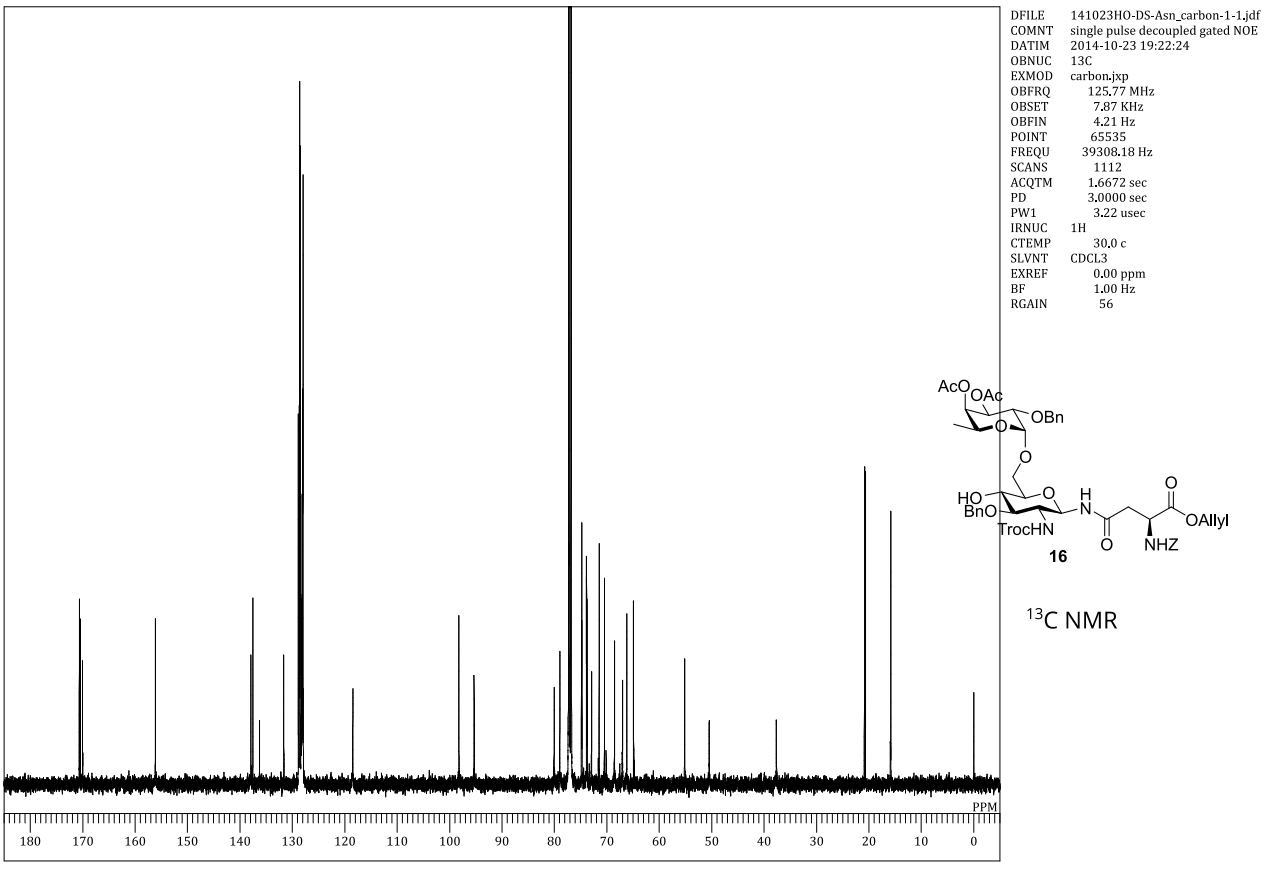
^1H NMR (600 MHz, D_2O); δ = 5.05 (s, 1H), 4.96 (d, *J* = 10.6 Hz, 1H), 4.79 (d, *J* = 7.81 Hz, 1H), 4.78 (d, *J* = 3.7 Hz, 1H), 4.68 (s, 1H), 4.59 (s, 1H), 4.57 (d, *J* = 7.14 Hz, 1H), 4.49 (d, *J* = 7.48 Hz, 1H), 4.34 (d, *J* = 7.6 Hz, 1H), 4.32(d, *J* = 7.6 Hz, 1H), 4.18-4.16 (m, 2H), 4.10-4.05 (m, 2H), 4.05-3.97 (m, 2H), 3.92-3.84 (m, 2H), 3.84-3.37 (m, 41H), 3.32(t, *J* = 9.66 Hz, 1H), 3.20 (m, 1H), 2.49-2.66 (m, 3H), 2.02-1.77 (m, 18H), 1.67-1.57 (m, 1H), 1.09 (d, *J* = 5.47 Hz, 3H).

^{13}C NMR (150 MHz, D_2O); δ = 180.1, 174.8, 174.6, 174.5, 173.6, 103.49 ($^1J_{CH}$ = 161.4 Hz, C-$1^{I/J}$), 100.90 ($^1J_{CH}$ = 160.3 Hz, C-1^E) , 100.9 ($^1J_{CH}$ = 161.0Hz, C-1^C), 100.52 ($^1J_{CH}$ = 163.7 Hz, C-1^H), 100.35 ($^1J_{CH}$ = 160.7 Hz, C-1^D), 100.0, 99.24 ($^1J_{CH}$ = 169.0 Hz, C-1^F), 99.2 ($^1J_{CH}$ = 160.3 Hz, C-1^G), 92.24 ($^1J_{CH}$ = 169.2 Hz, C-1^B), 80.51, 80.36, 80.2, 79.3, 78.30, 78.04 ($^1J_{CH}$ = 156.4 Hz, C-1^A), 76.22, 76.21, 75.47, 75.18, 74.7, 74.6, 74.4, 74.4, 73.62, 73.35, 72.78, 72.47, 72.35, 71.99, 71.98, 71.9, 71.89, 71.8, 71.6, 70.62, 69.88, 69.39, 69.3, 68.36, 68.2, 68.16, 68.1, 68.0, 67.11, 67.05, 66.6, 66.5, 65.6, 63.16, 61.58, 61.4, 61.37, 60.09, 60.0, 54.9, 54.89, 54.55, 53.47, 51.8, 51.8, 39.9, 38.3, 15.3.

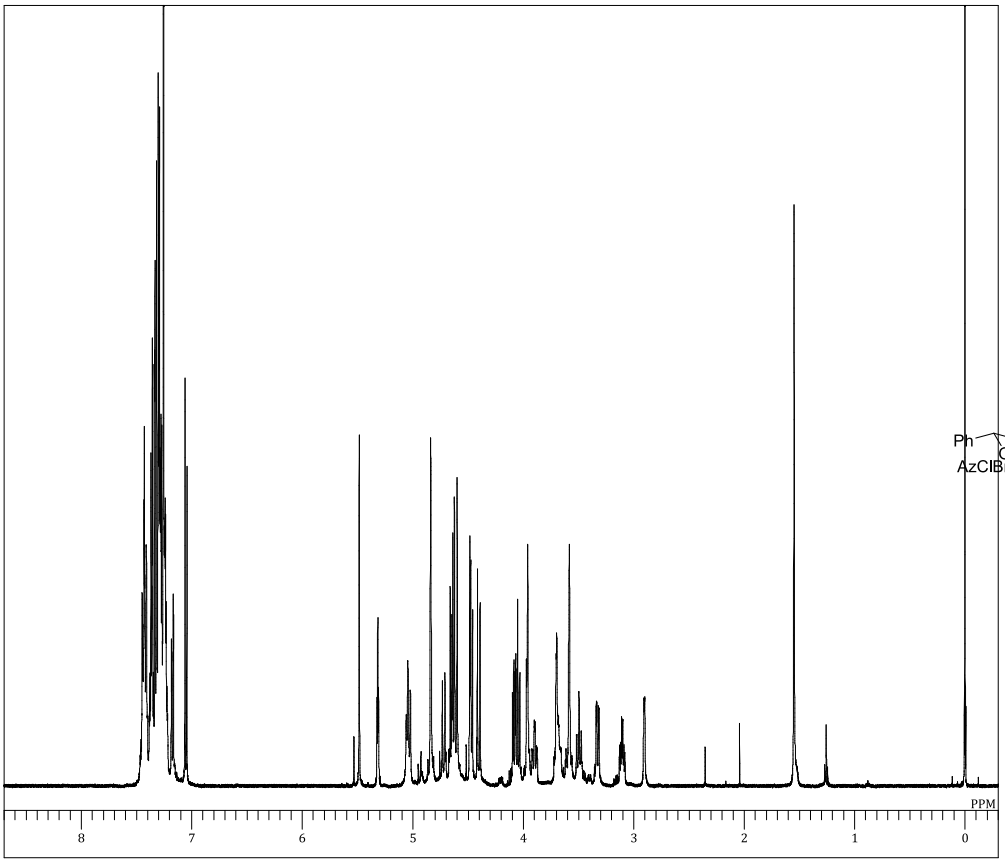
HR ESI-Orbitrap MS; *m*/*z* calcd for $C_{94}H_{154}N_8O_{68}$ $[M+2H]^{2+}$: 1242.4492, found: 1242.4492.



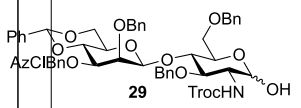
^{1}H NMR



^{13}C NMR

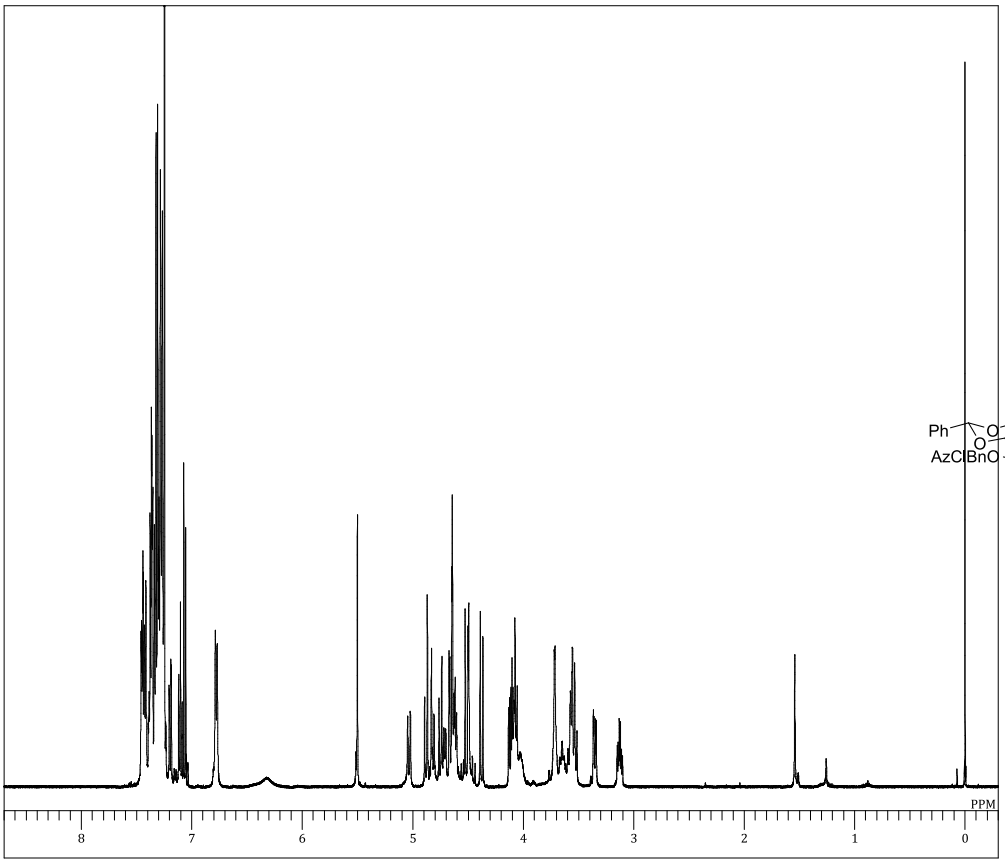


DFILE 130104bMan-GlcN-OH_proton-1-1.jdf
COMNT single_pulse
DATIM 2013-01-04 10:42:12
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 65535
FREQU 9384.38 Hz
SCANS 24
ACQTM 6.9835 sec
PD 5.0000 sec
PW1 6.70 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT CDCL3
EXREF 0.00 ppm
BF 0.05 Hz
RGAIN 44

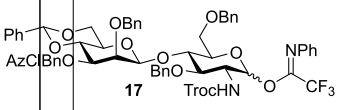


29

^{1}H NMR

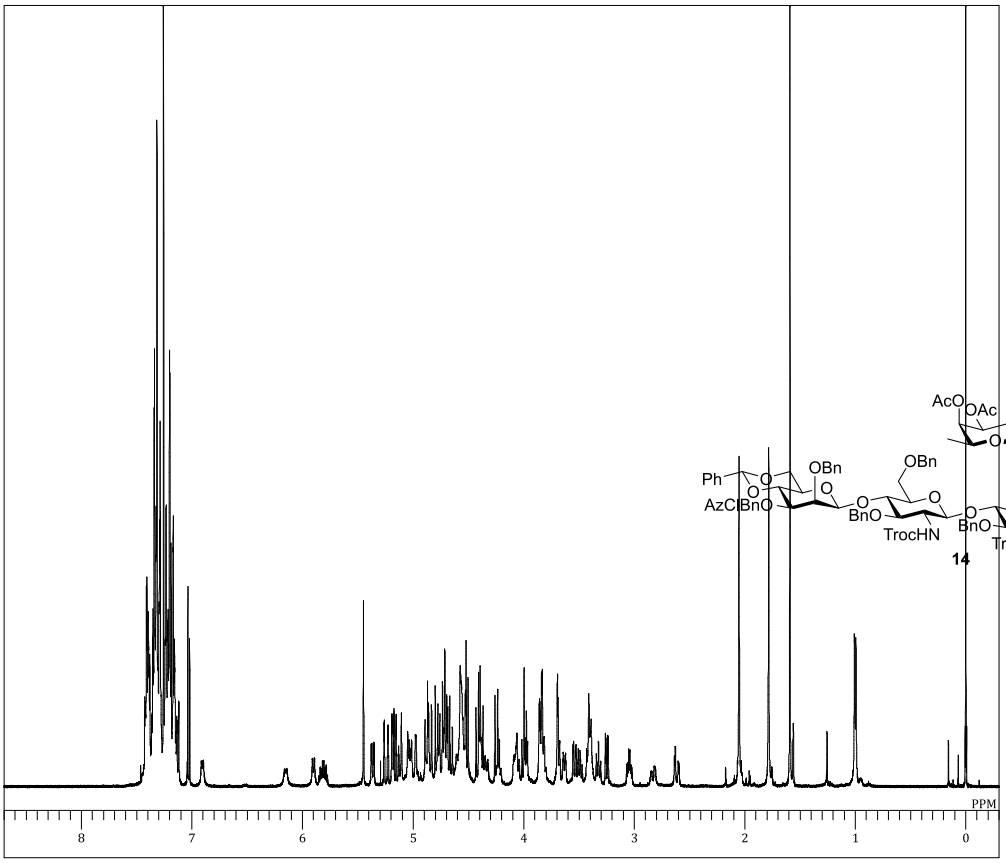


DFILE 130105bMan-GlcN-imi_proton-1-1.jdf
COMNT single_pulse
DATIM 2013-01-05 13:19:48
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 65535
FREQU 9384.38 Hz
SCANS 16
ACQTM 6.9835 sec
PD 5.0000 sec
PW1 6.70 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT CDCL3
EXREF 0.00 ppm
BF 0.02 Hz
RGAIN 36

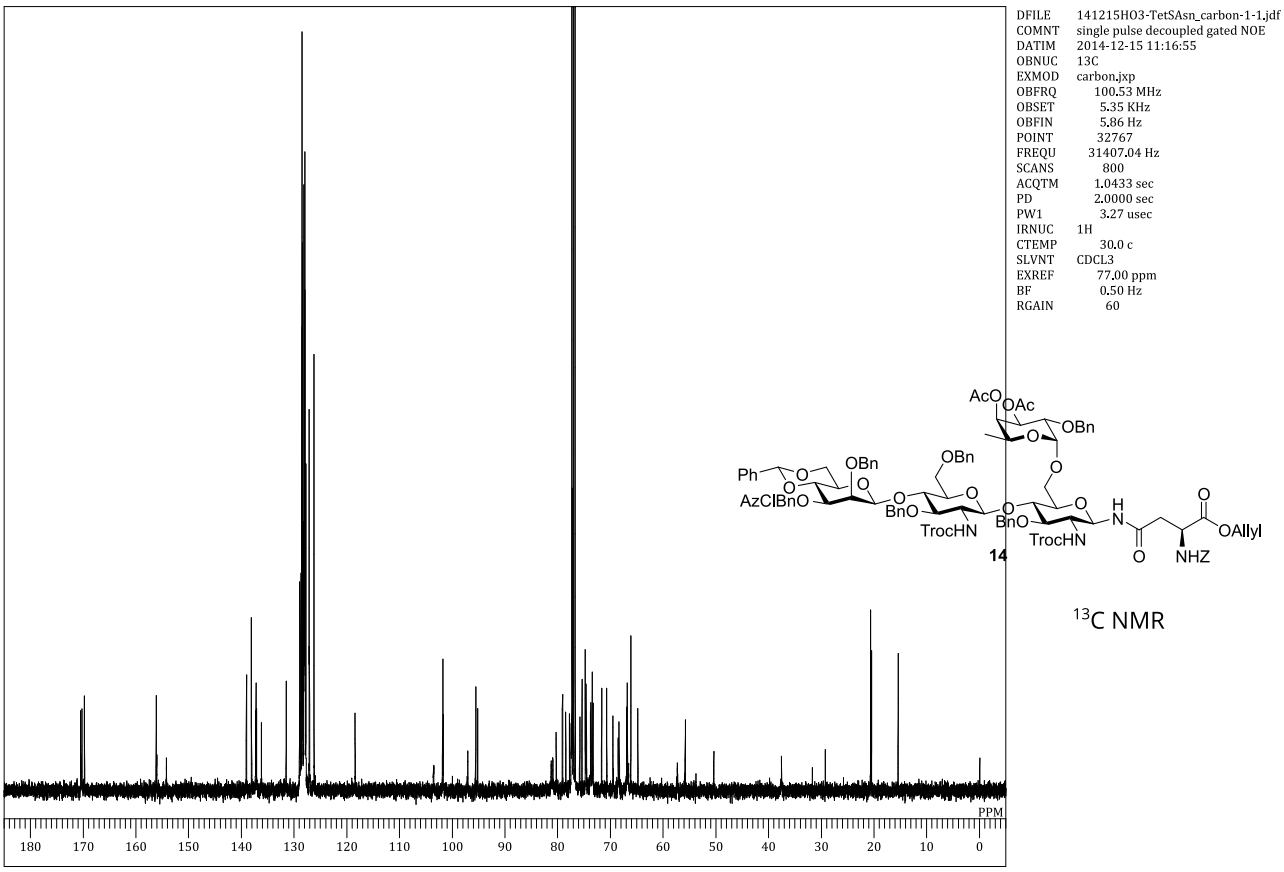


17

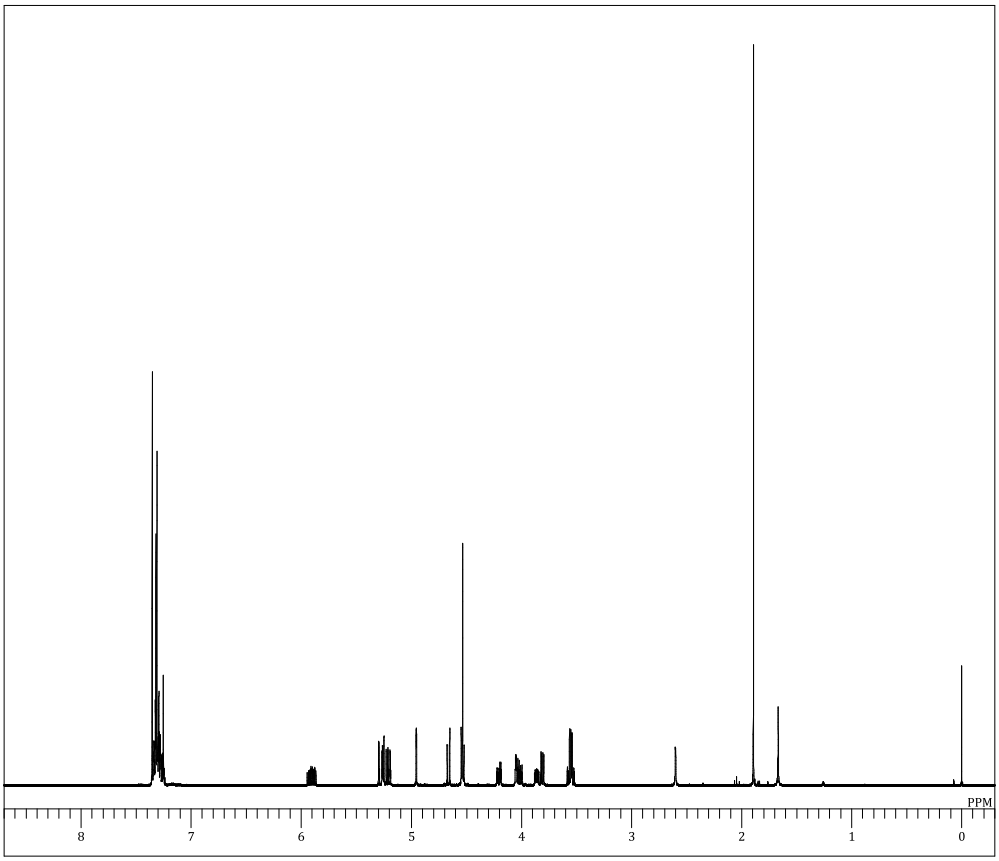
^{1}H NMR



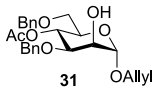
^{1}H NMR



^{13}C NMR

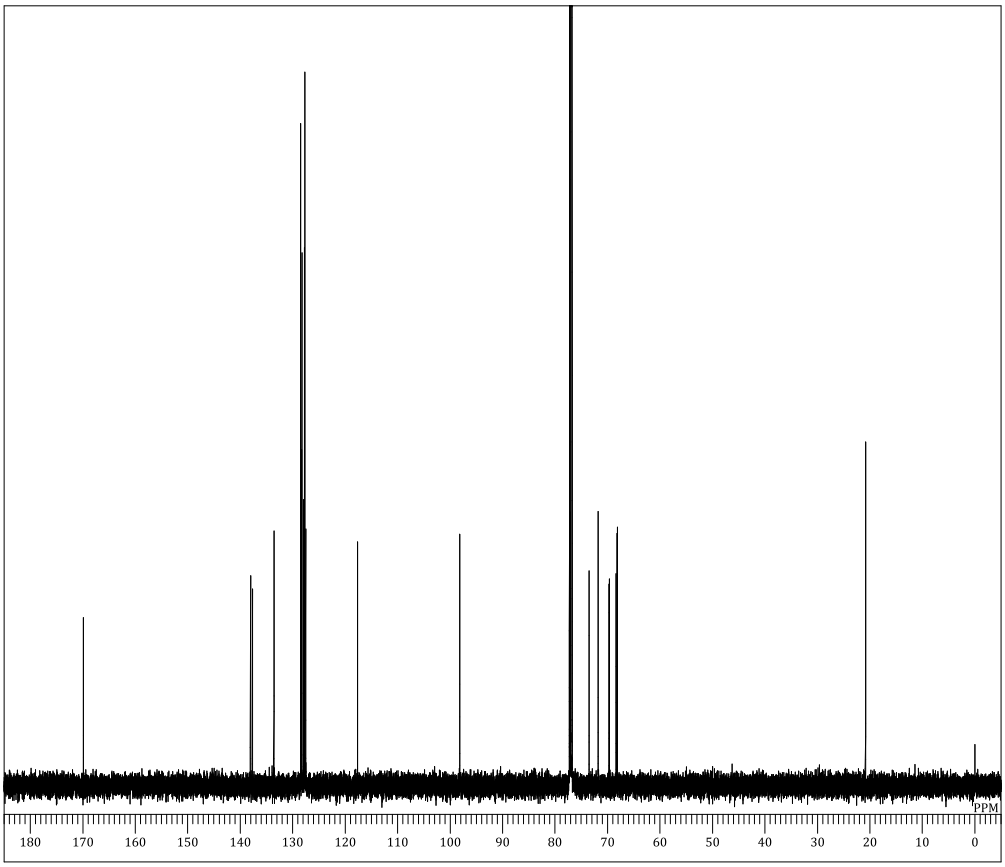


DFILE 37_120928 2-48_proton-1-1.jdf
COMNT single_pulse
DATIM 2012-09-28 18:08:15
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 65535
FREQU 9384.38 Hz
SCANS 8
ACQTM 6.9835 sec
PD 5.0000 sec
PW1 6.70 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT CDCL3
EXREF 0.00 ppm
BF 0.05 Hz
RGAIN 36

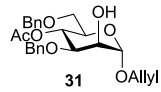


31

^{1}H NMR

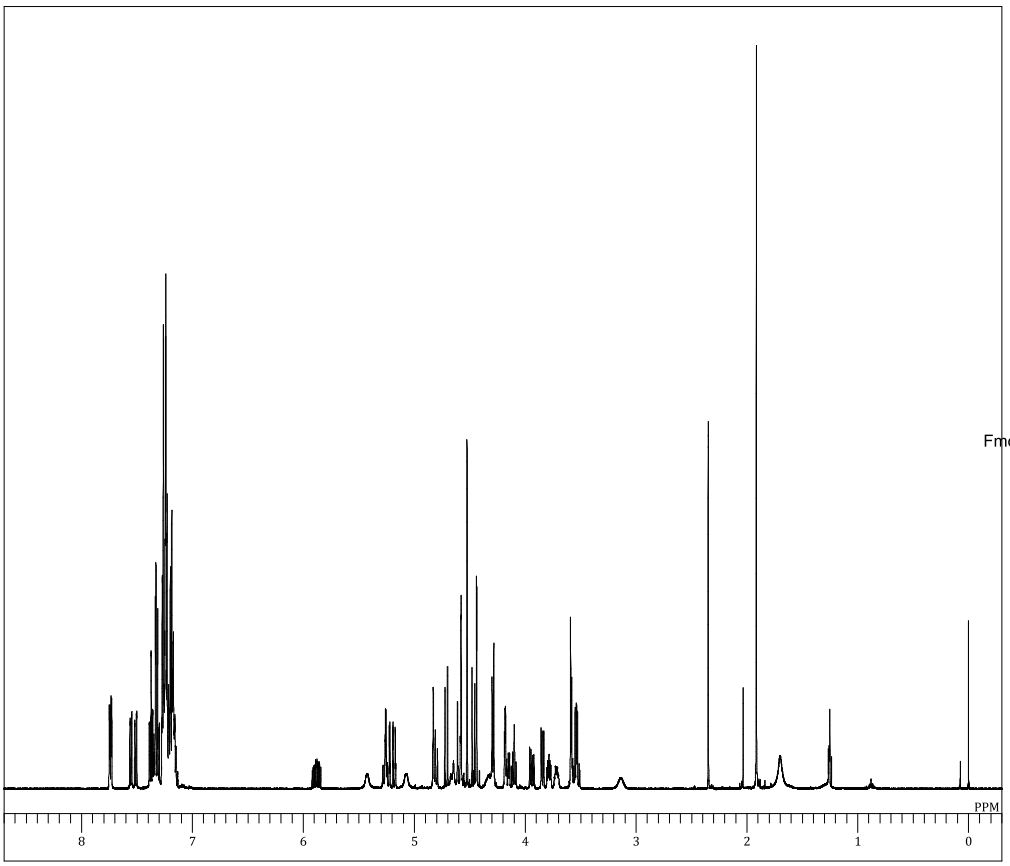


DFILE 37_120928 2-48_Carbon-1-1.jdf
COMNT single pulse decoupled gated NOE
DATIM 2012-09-28 18:16:51
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 65535
FREQU 39308.18 Hz
SCANS 154
ACQTM 1.6672 sec
PD 30.0000 sec
PW1 3.13 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT CDCL3
EXREF 77.00 ppm
BF 0.30 Hz
RGAIN 58

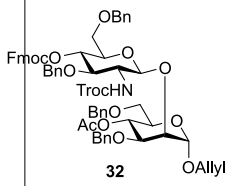


31

^{13}C NMR

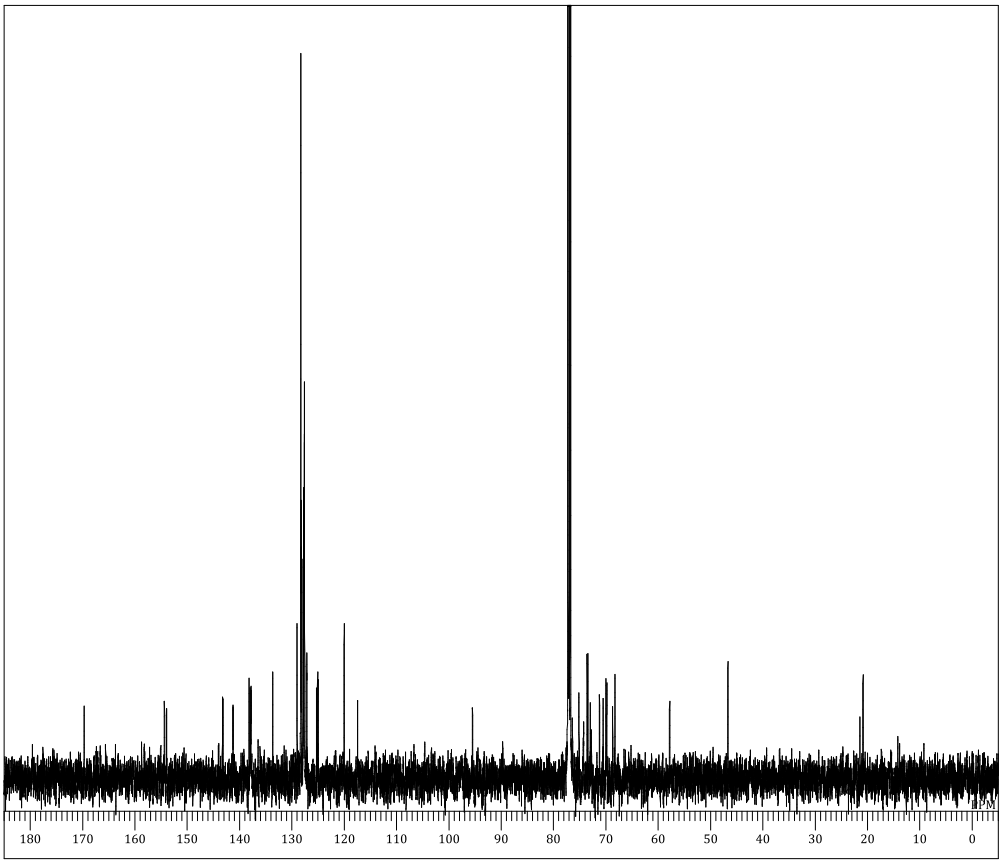


DFILE	38_120929 2-50 b-isomer_proton-1-1.jdf
COMNT	single_pulse
DATIM	2012-09-29 18:38:33
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65535
FREQU	9384.38 Hz
SCANS	16
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	3.00 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	CDCL3
EXREF	0.00 ppm
BF	0.02 Hz
RGAIN	30

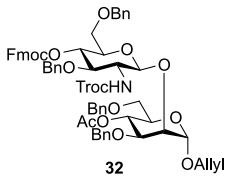


32

^{1}H NMR

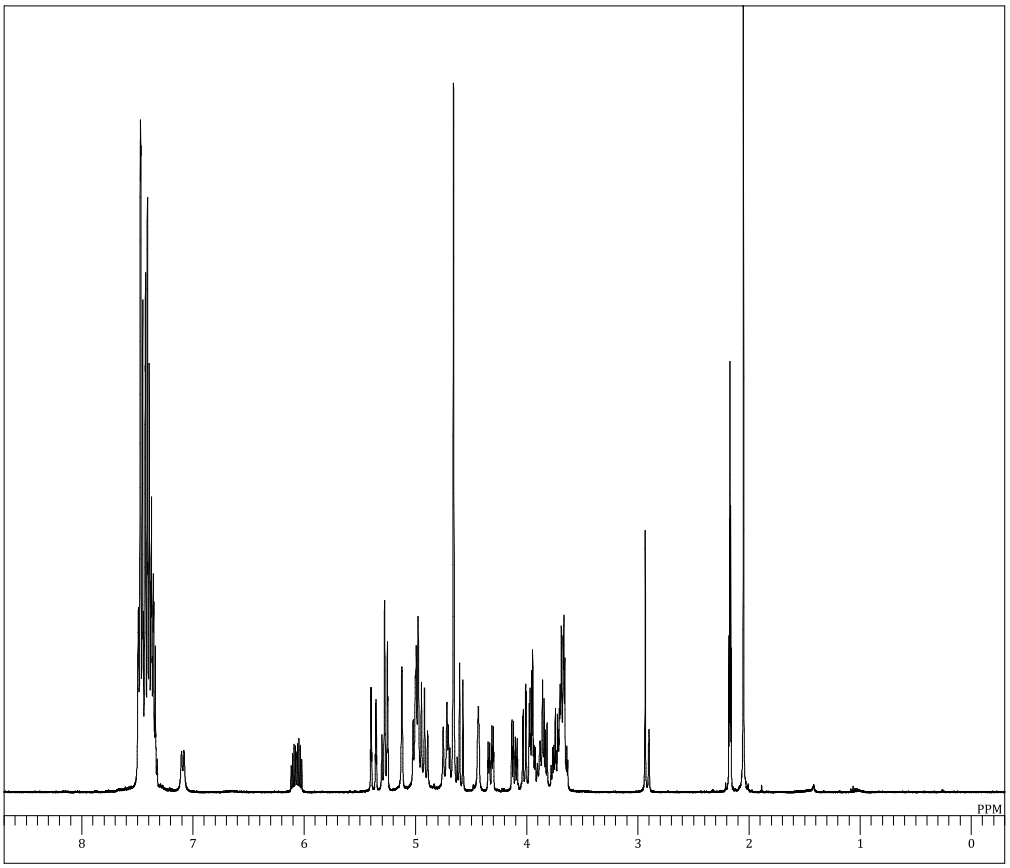


DFILE	38_120929 2-50 b-isomer_Carbon-1-1.jdf
COMNT	single pulse decoupled gated NOE
DATIM	2012-09-29 18:49:18
OBNUC	13C
EXMOD	carbon.jxp
OBFRQ	125.77 MHz
OBSET	7.87 KHz
OBFIN	4.21 Hz
POINT	65535
FREQU	39308.18 Hz
SCANS	173
ACQTM	1.6672 sec
PD	30.0000 sec
PW1	5.67 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	CDCL3
EXREF	77.00 ppm
BF	2.00 Hz
RGAIN	54



32

^{13}C NMR



DFILE	150227HO-GlcN-Man_proton-1-1.jdf
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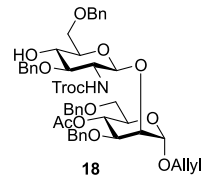
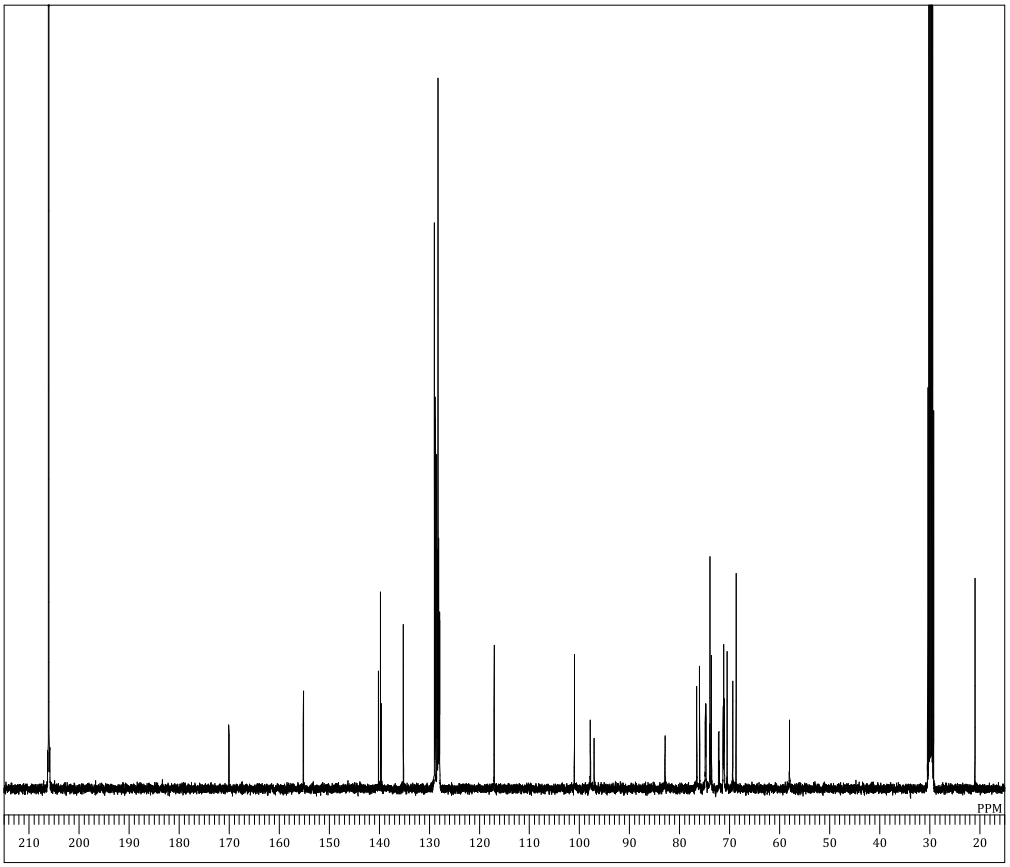
DATIM	2015-02-27 16:18:34
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	399.78 MHz
OBSET	4.19 KHz
OBFIN	7.29 Hz
POINT	32767
FREQU	7503.00 Hz
SCANS	4
ACQTM	4.3673 sec
PD	5.0000 sec
PW1	5.20 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	ACETN
EXREF	2.05 ppm
BF	0.05 Hz
RGAIN	30

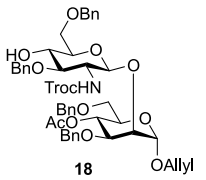


18

^{1}H NMR

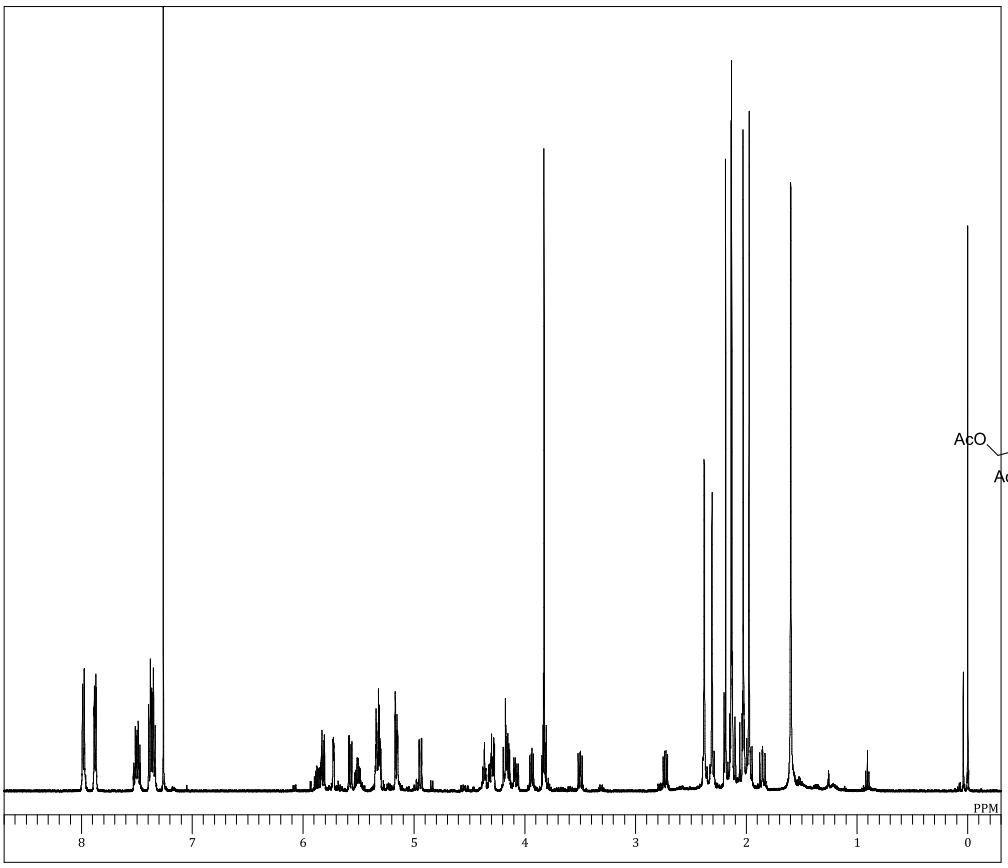


DFILE	150227HO-GlcN-Man_carbon-1-1.jdf
COMNT	single pulse decoupled gated NOE
DATIM	2015-02-27 16:20:33
OBNUC	13C
EXMOD	carbon.jxp
OBFRQ	100.53 MHz
OBSET	5.35 KHz
OBFIN	5.86 Hz
POINT	32767
FREQU	31407.04 Hz
SCANS	659
ACQTM	1.0433 sec
PD	2.0000 sec
PW1	3.27 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	ACETN
EXREF	29.80 ppm
BF	1.00 Hz
RGAIN	60



18

^{13}C NMR



DFILE	130611Ac2Sia-Gal_proton-1-1.jdf
COMNT	single_pulse
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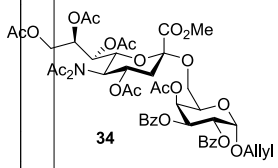
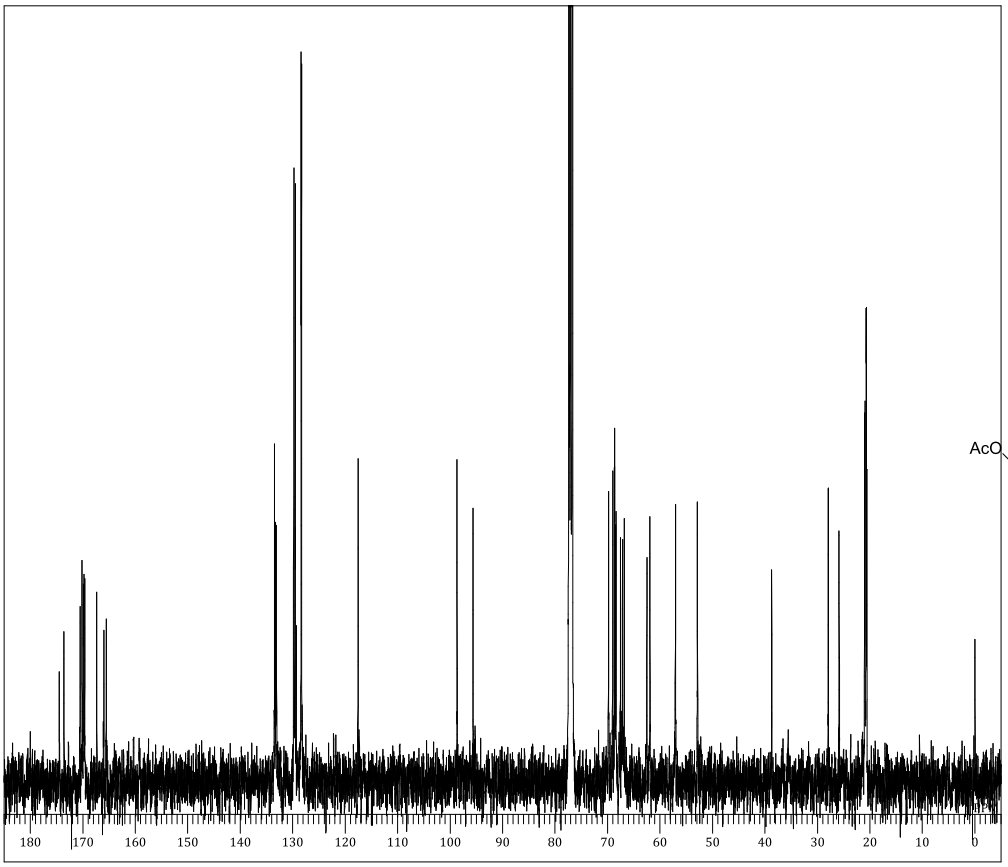
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65535
FREQU	9384.38 Hz
SCANS	24
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	6.70 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	CDCL3
EXREF	0.00 ppm
BF	0.05 Hz
RGAIN	42

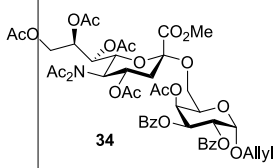


34

^{1}H NMR

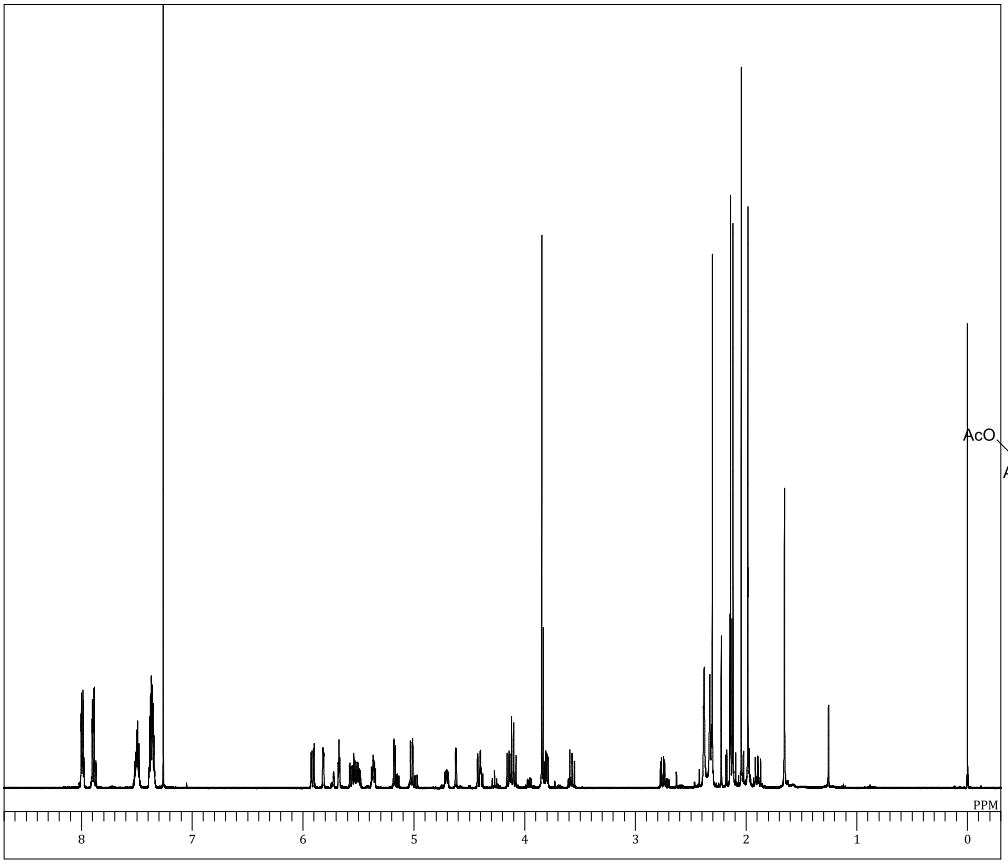


DFILE	150303Ac2Sia-Gal_carbon-1-1.jdf
COMNT	single pulse decoupled gated NOE
DATIM	2015-03-03 21:31:48
OBNUC	13C
EXMOD	carbon.jxp
OBFRQ	100.53 MHz
OBSET	5.35 KHz
OBFIN	5.86 Hz
POINT	32767
FREQU	31407.04 Hz
SCANS	1145
ACQTM	1.0433 sec
PD	3.0000 sec
PW1	3.27 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	CDCL3
EXREF	77.00 ppm
BF	2.00 Hz
RGAIN	60

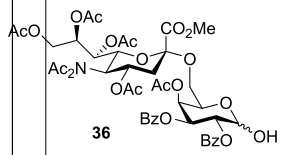


34

^{13}C NMR

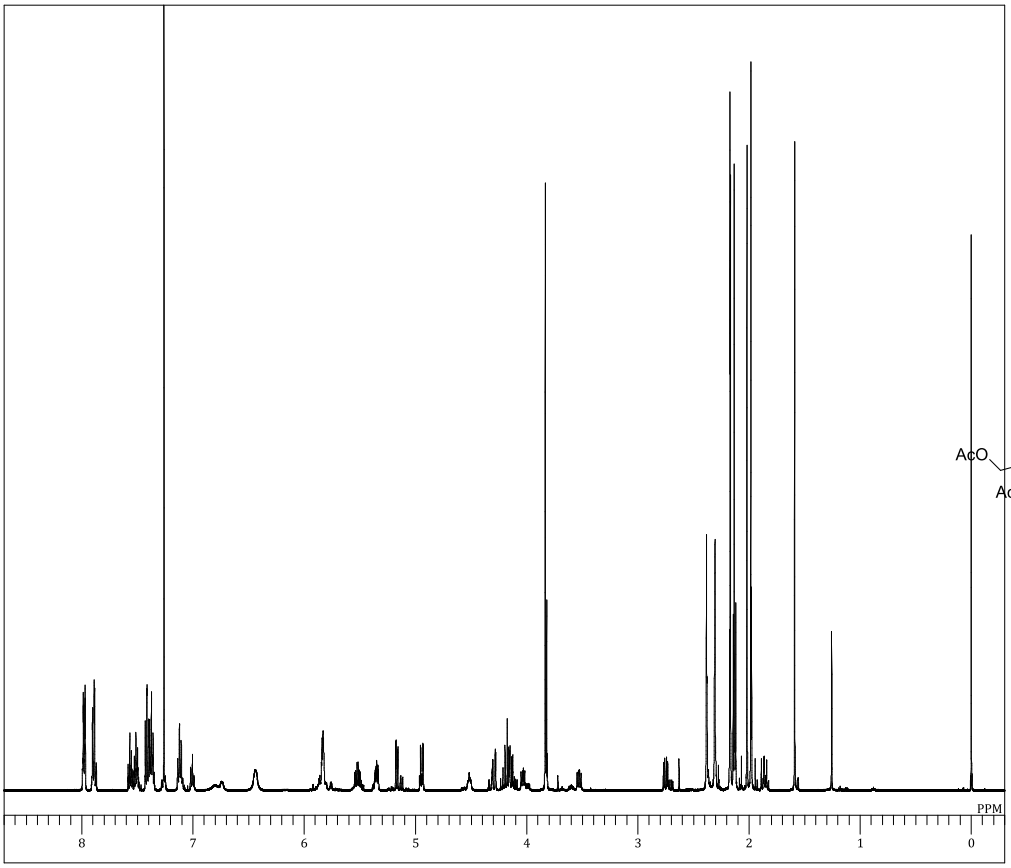


DFILE	130612Ac2Sia-Gal-OH_proton-1-1.jdf
COMNT	single_pulse
DATIM	2013-06-12 21:10:25
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65535
FREQU	9384.38 Hz
SCANS	24
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	3.00 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	CDCL3
EXREF	0.00 ppm
BF	0.02 Hz
RGAIN	38

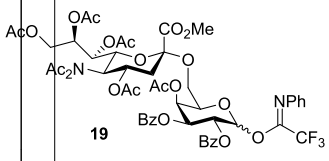


36

^{1}H NMR

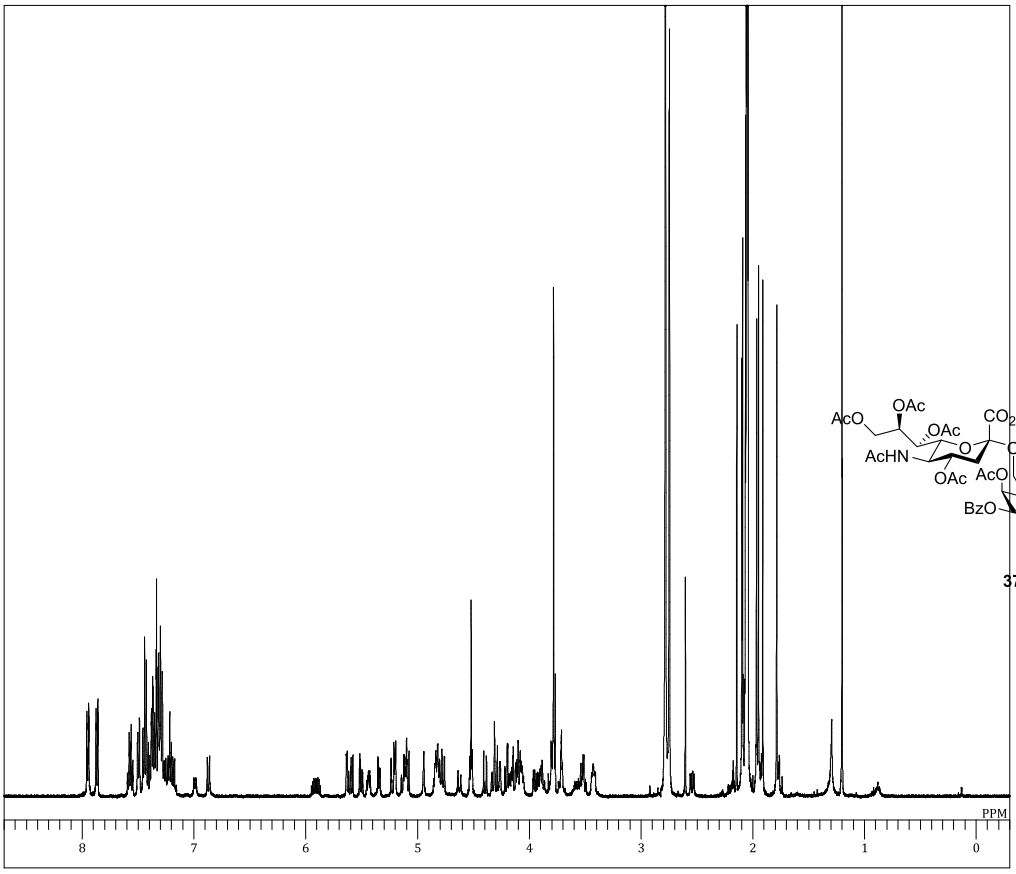


DFILE	130614Ac2Sia2-6Gal-imi_proton-1-1.jdf
COMNT	single_pulse
DATIM	2013-06-14 10:42:50
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65535
FREQU	9384.38 Hz
SCANS	24
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	3.00 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	CDCL3
EXREF	0.00 ppm
BF	0.02 Hz
RGAIN	40

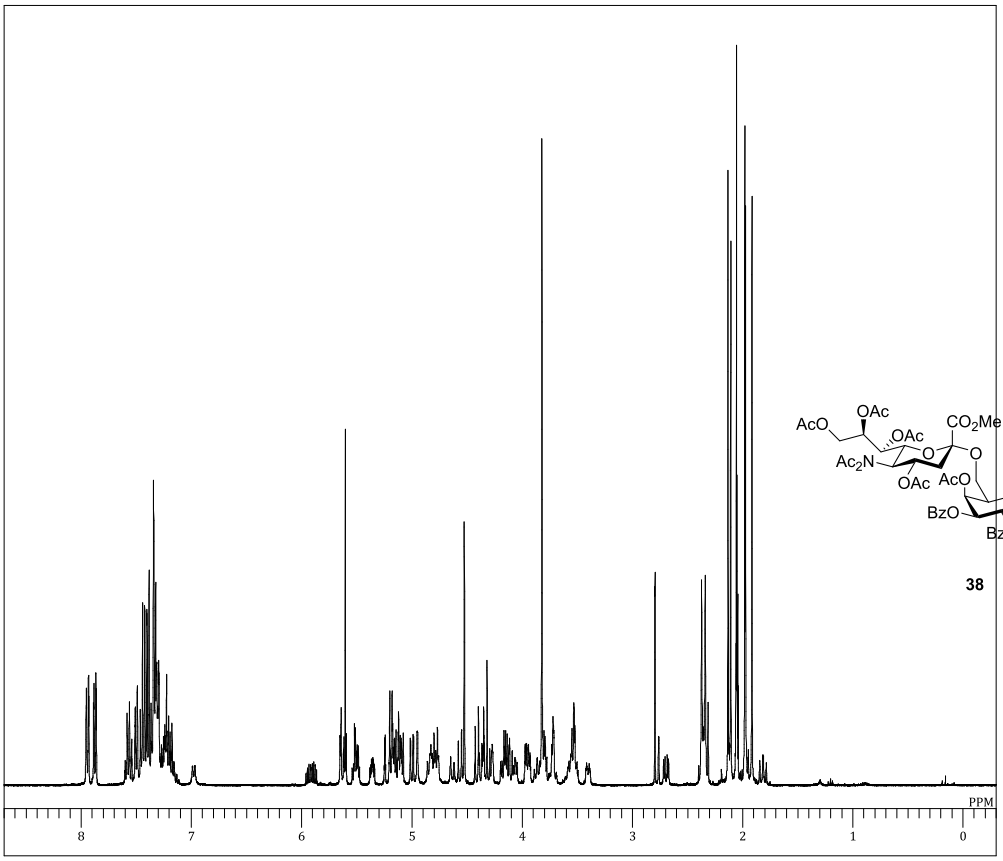


19

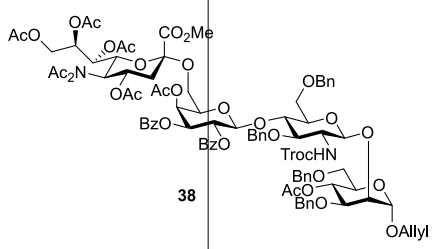
^{1}H NMR


DFILE 130719monoAcTetS_proton-1-1.jdf
COMNT single_pulse
DATIM 2013-07-19 19:29:35
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 65535
FREQU 9384.38 Hz
SCANS 40
ACQTM 6.9835 sec
PD 5.0000 sec
PW1 3.00 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT ACETN
EXREF 2.05 ppm
BF 0.20 Hz
RGAIN 54
AcO
OAc
OAc
CO2Me
AcHN
OAc
AcO
BzO
BzO
BnO
OBn
TrocHN
BnO
AcO
BnO
OAllyl
37
PPM
8
7
6
5
4
3
2
1
0

^{1}H NMR

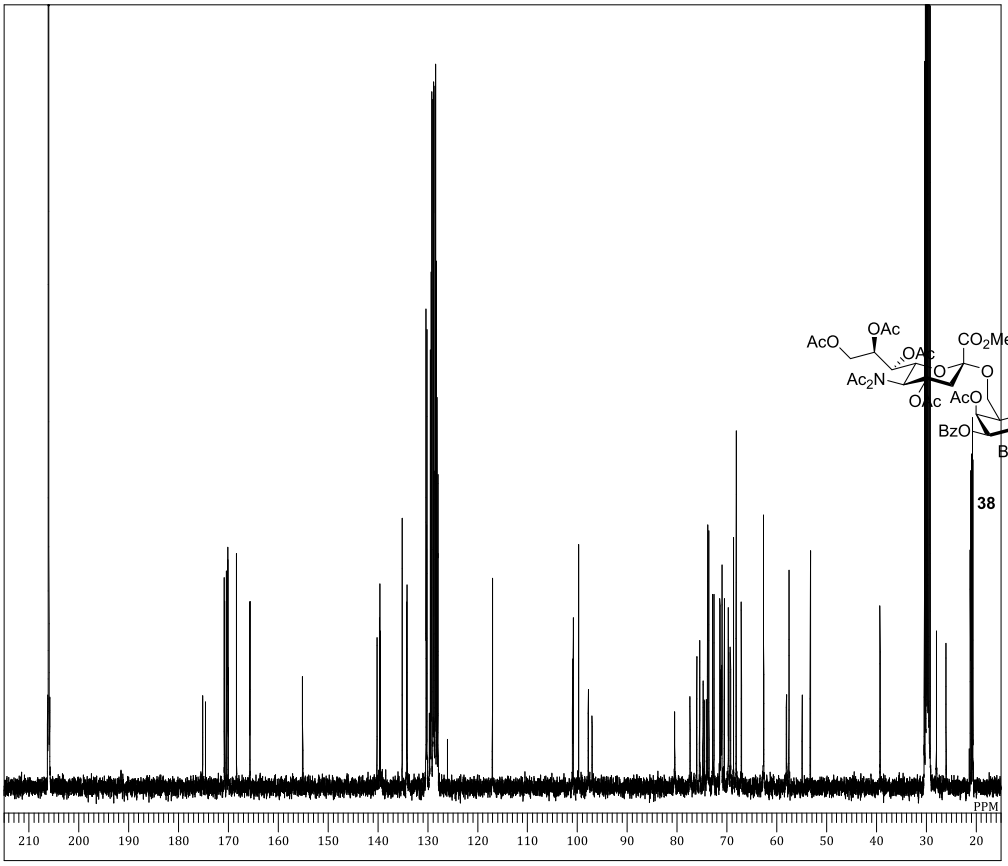


DFILE 141217Ac2TetS_proton-1-1.jdf
COMNT single_pulse
DATIM 2014-12-17 14:14:01
OBNUC 1H
EXMOD proton.jxp
OBFRQ 399.78 MHz
OBSET 4.19 KHz
OBFIN 7.29 Hz
POINT 32767
FREQU 7503.00 Hz
SCANS 4
ACQTM 4.3673 sec
PD 5.0000 sec
PW1 5.20 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT ACETN
EXREF 2.05 ppm
BF 0.05 Hz
RGAIN 26

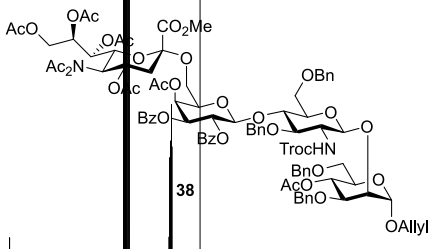


38

^{1}H NMR

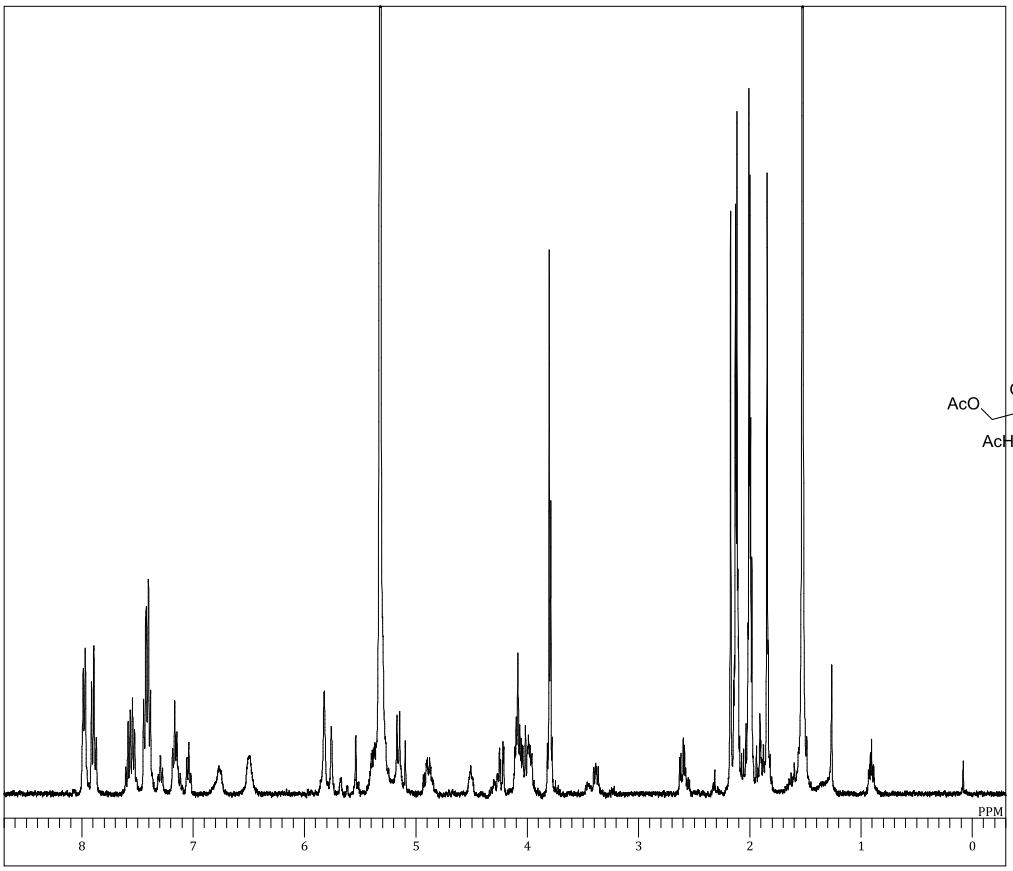


DFILE 141217Ac2TetS_carbon-1-1.jdf
COMNT single pulse decoupled gated NOE
DATIM 2014-12-17 14:16:29
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 100.53 MHz
OBSET 5.35 KHz
OBFIN 5.86 Hz
POINT 32767
FREQU 31407.04 Hz
SCANS 800
ACQTM 1.0433 sec
PD 2.0000 sec
PW1 3.27 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT ACETN
EXREF 29.80 ppm
BF 1.00 Hz
RGAIN 60

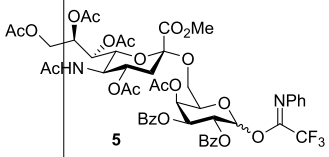


38

^{13}C NMR



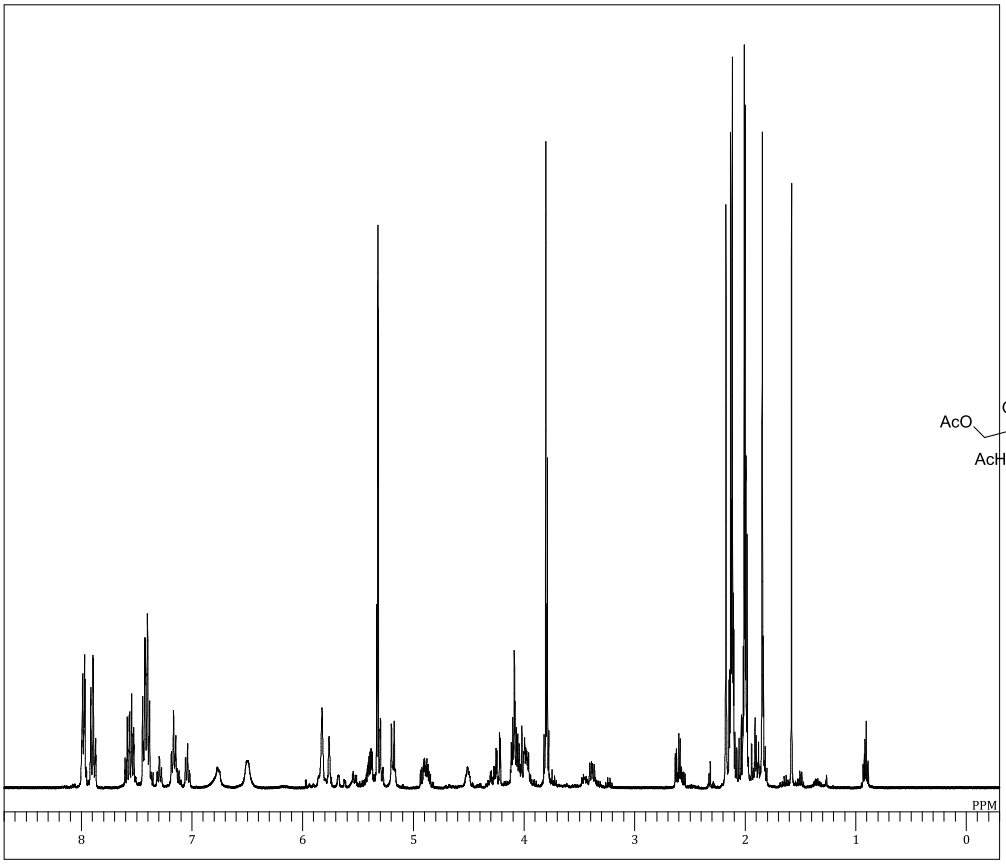
DFILE 131102Sia-Gal-imi_1mM_proton-1-1.jdf
COMNT single_pulse
DATIM 2013-11-02 18:40:10
OBNUC 1H
EXMOD proton.jxp
OBFRQ 399.78 MHz
OBSET 4.19 KHz
OBFIN 7.29 Hz
POINT 32767
FREQU 7503.00 Hz
SCANS 32
ACQTM 4.3673 sec
PD 5.0000 sec
PW1 6.00 usec
IRNUC 1H
CTEMP 25.0 c
SLVNT CD2CL2
EXREF 5.32 ppm
BF 0.70 Hz
RGAIN 40



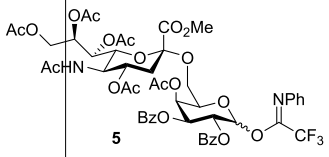
5

^{1}H NMR

1 mM in CD_2Cl_2



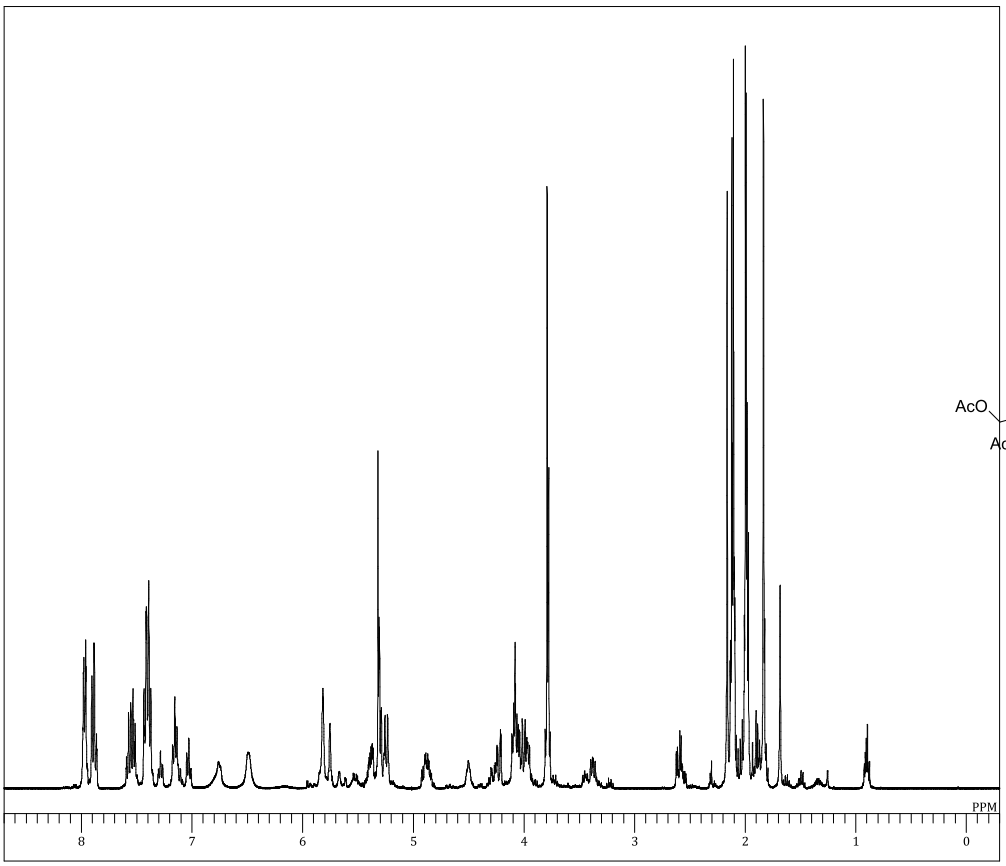
DFILE 131102Sia-Gal-imi_10mM_proton-1-1.jdf
COMNT single_pulse
DATIM 2013-11-02 18:54:25
OBNUC 1H
EXMOD proton.jxp
OBFRQ 399.78 MHz
OBSET 4.19 KHz
OBFIN 7.29 Hz
POINT 32767
FREQU 7503.00 Hz
SCANS 16
ACQTM 4.3673 sec
PD 5.0000 sec
PW1 6.00 usec
IRNUC 1H
CTEMP 25.0 c
SLVNT CD2CL2
EXREF 5.32 ppm
BF 0.10 Hz
RGAIN 34



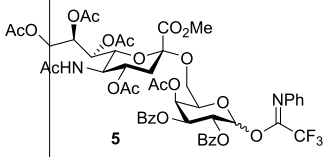
5

^{1}H NMR

10 mM in CD_2Cl_2



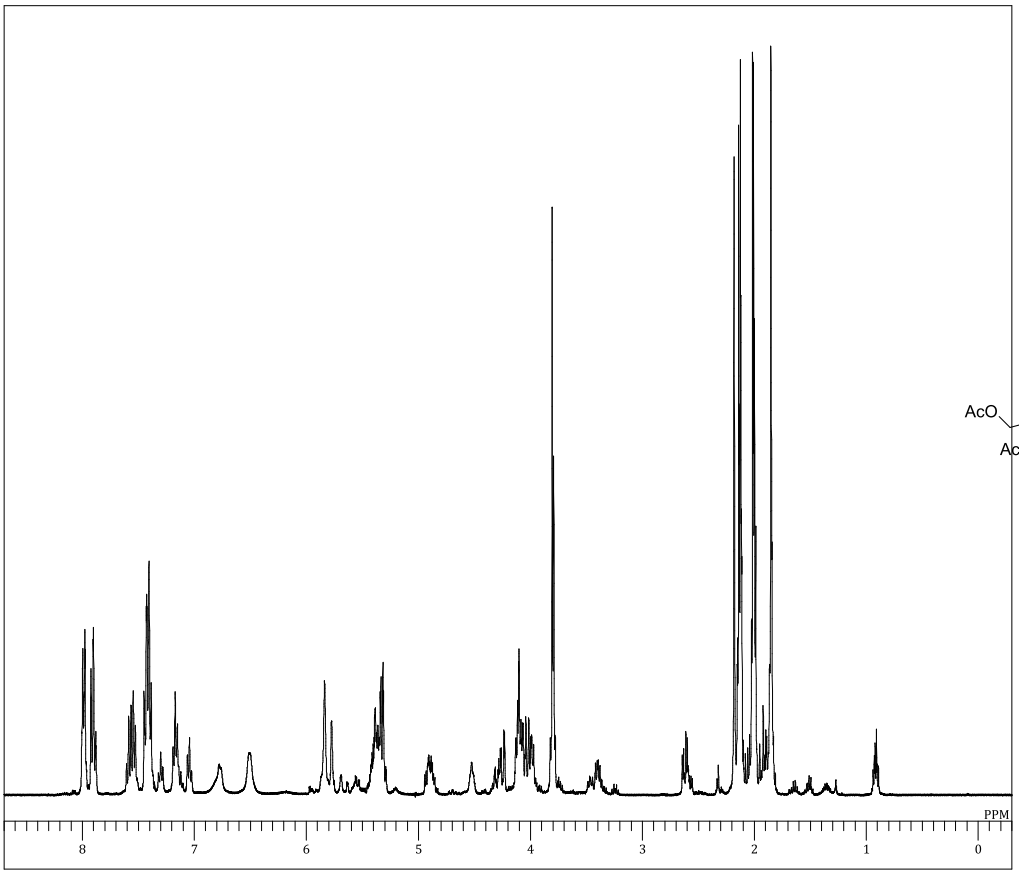
DFILE 131114Sia-Gal-imi_50mM_proton-1-1.jdf
COMNT single_pulse
DATIM 2013-11-14 22:22:38
OBNUC 1H
EXMOD single_pulse.jxp
OBFRQ 399.78 MHz
OBSET 4.19 KHz
OBFIN 7.29 Hz
POINT 32767
FREQU 7503.00 Hz
SCANS 4
ACQTM 4.3673 sec
PD 5.0000 sec
PW1 10.89 usec
IRNUC 1H
CTEMP 25.0 c
SLVNT CD2CL2
EXREF 5.32 ppm
BF 0.05 Hz
RGAIN 26



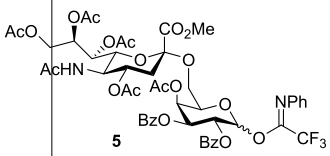
5

^{1}H NMR

50 mM in CD_2Cl_2



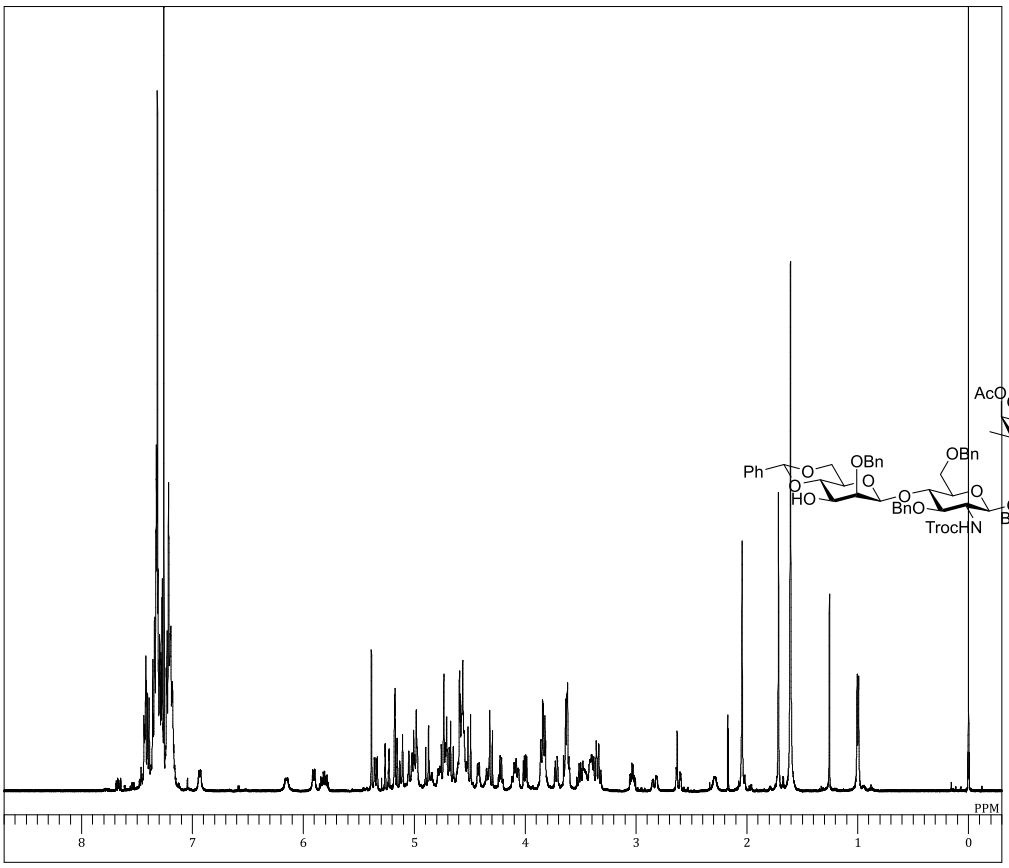
DFILE 131102Sia-Gal-imi_100mM_proton-1-1.jdf
COMNT single_pulse
DATIM 2013-11-02 19:25:18
OBNUC 1H
EXMOD single_pulse.jxp
OBFRQ 399.78 MHz
OBSET 4.19 KHz
OBFIN 7.29 Hz
POINT 32767
FREQU 7503.00 Hz
SCANS 4
ACQTM 4.3673 sec
PD 5.0000 sec
PW1 11.04 usec
IRNUC 1H
CTEMP 25.0 c
SLVNT CD2CL2
EXREF 5.32 ppm
BF 0.01 Hz
RGAIN 22



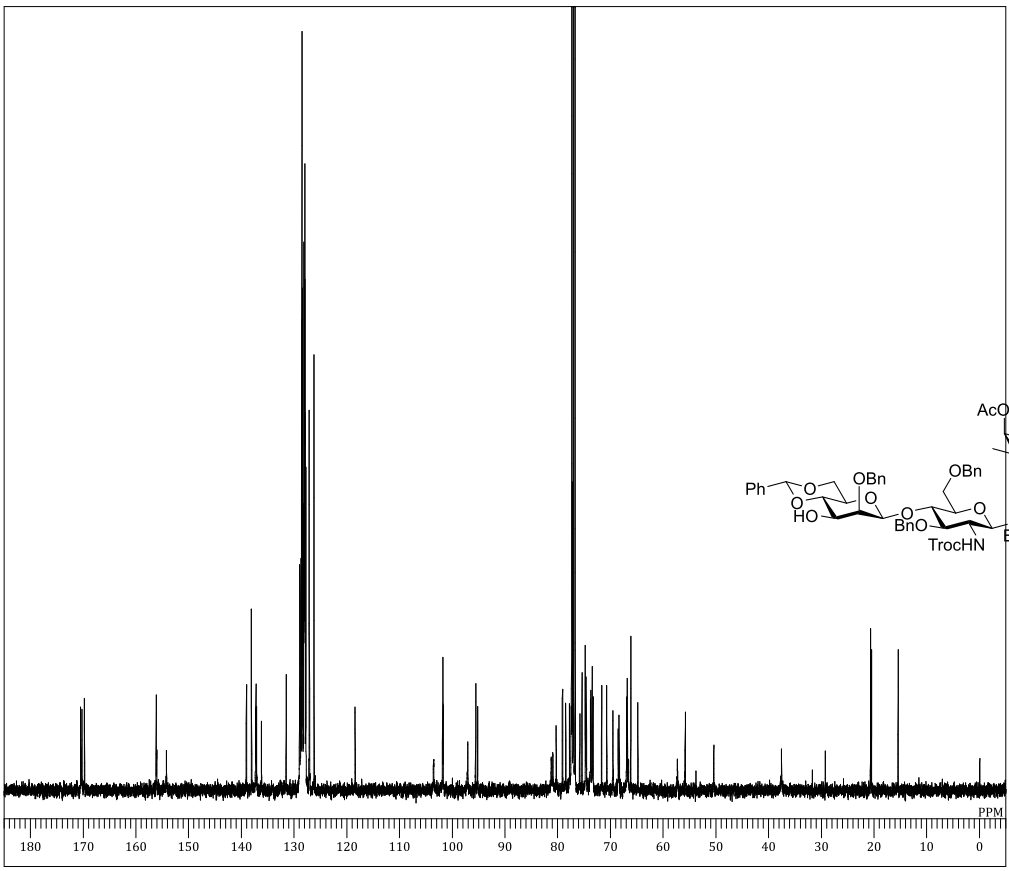
5

^{1}H NMR

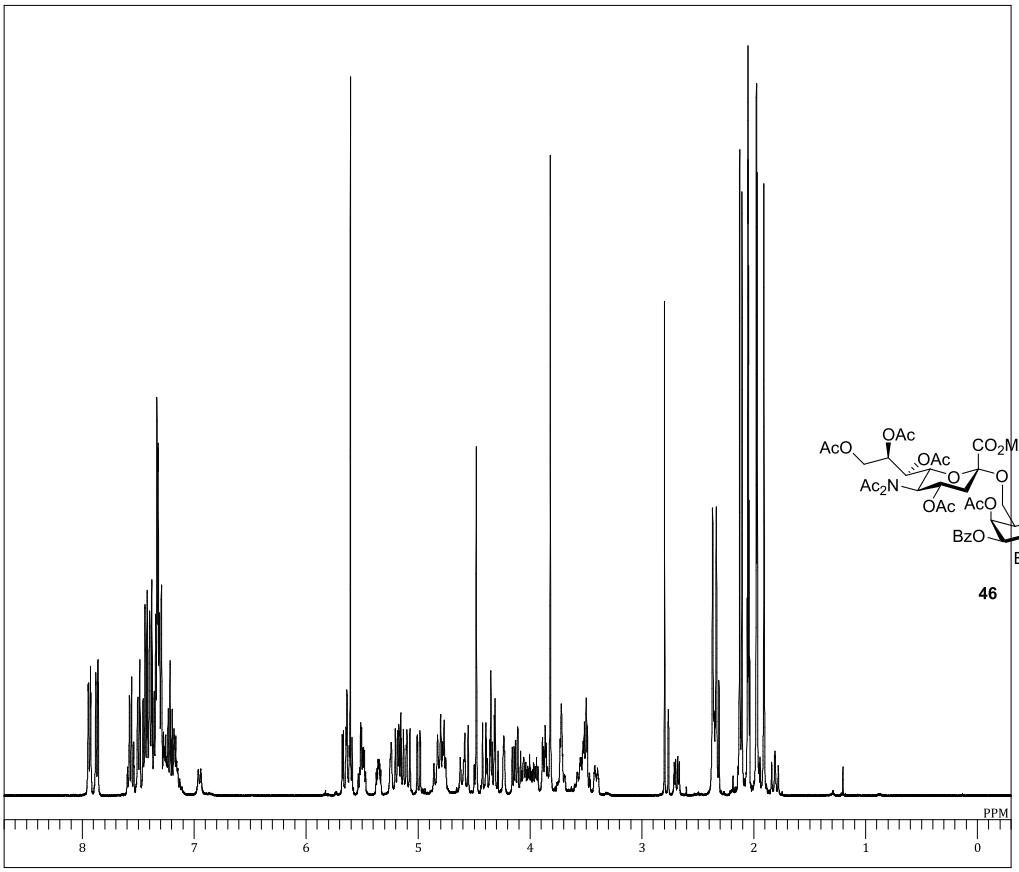
100 mM in CD_2Cl_2



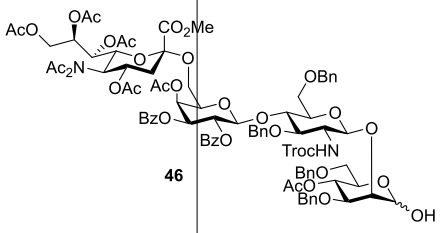
^{1}H NMR



^{13}C NMR

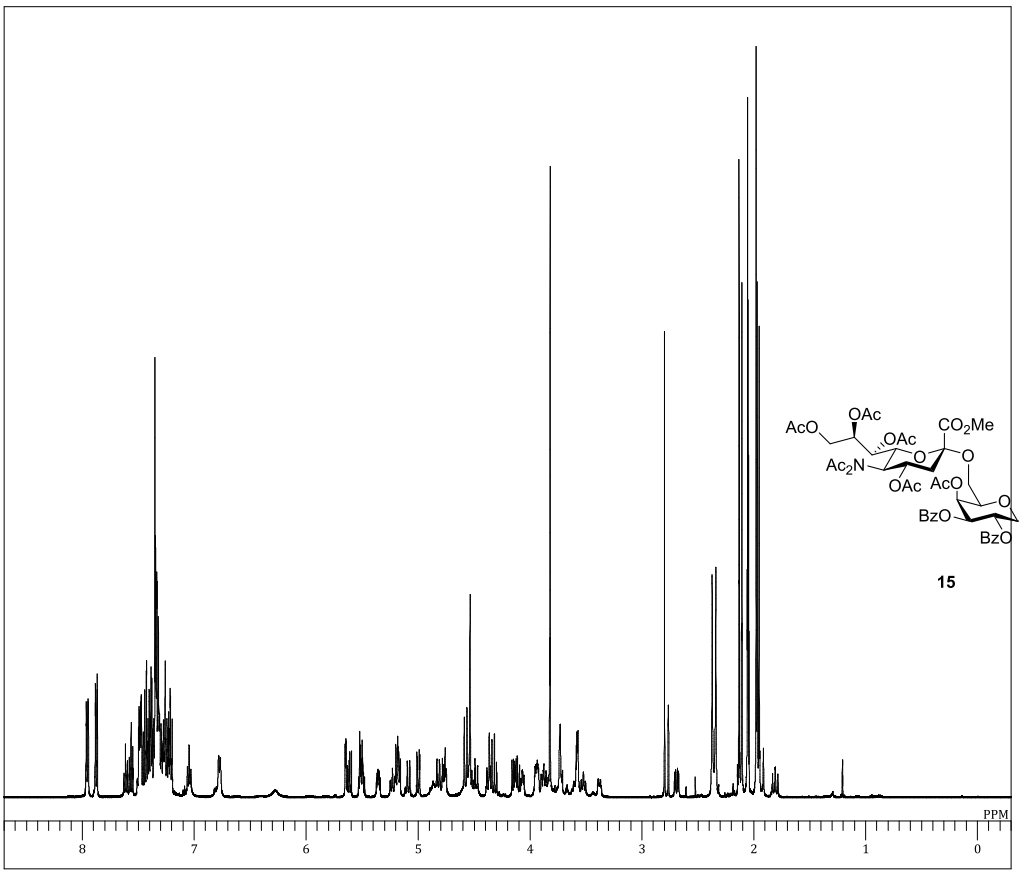


DFILE 141218Ac2TetS-OH_proton-1-1.jdf
COMNT single_pulse
DATIM 2014-12-18 19:13:20
OBNUC 1H
EXMOD proton.jxp
OBFRQ 399.78 MHz
OBSET 4.19 KHz
OBFIN 7.29 Hz
POINT 32767
FREQU 7503.00 Hz
SCANS 4
ACQTM 4.3673 sec
PD 5.0000 sec
PW1 5.20 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT ACETN
EXREF 2.05 ppm
BF 0.05 Hz
RGAIN 30

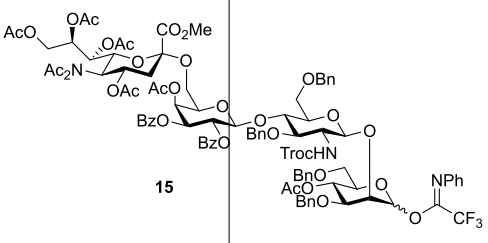


46

^{1}H NMR

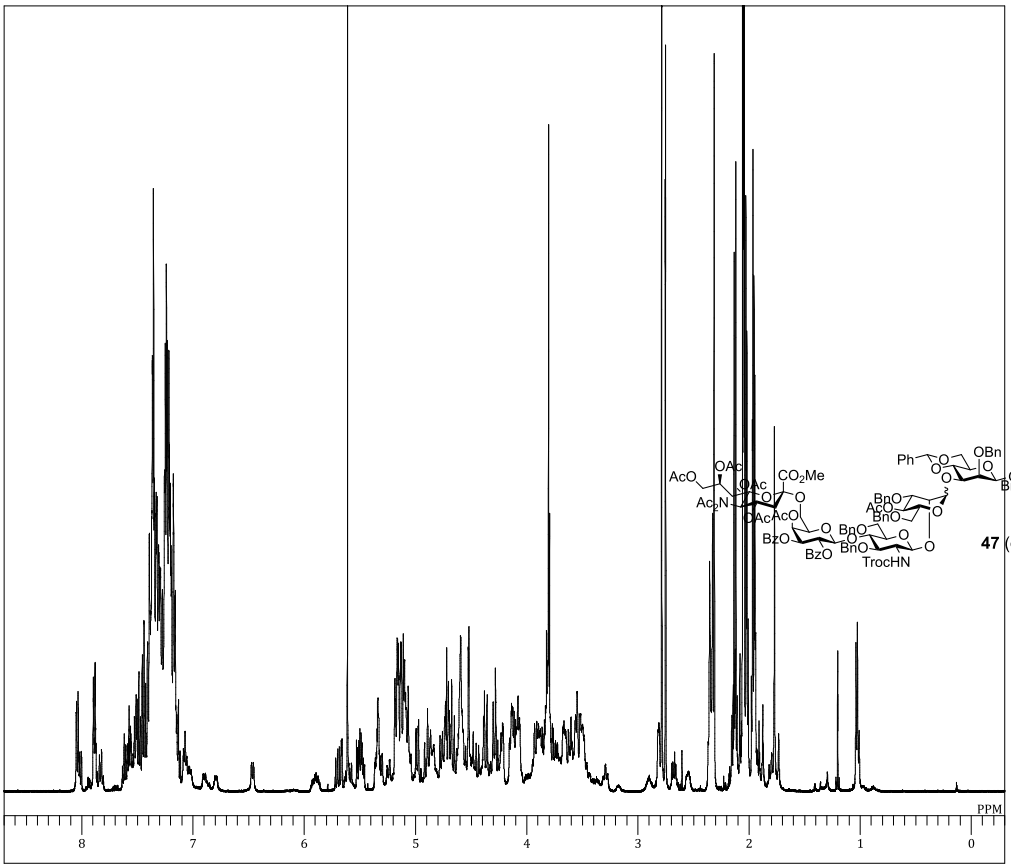


DFILE 150331Ac2TetS-imi_proton-1-1.jdf
COMNT single_pulse
DATIM 2015-03-31 15:16:14
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 65535
FREQU 9384.38 Hz
SCANS 4
ACQTM 6.9835 sec
PD 5.0000 sec
PW1 3.20 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT ACETN
EXREF 2.05 ppm
BF 0.05 Hz
RGAIN 26



15

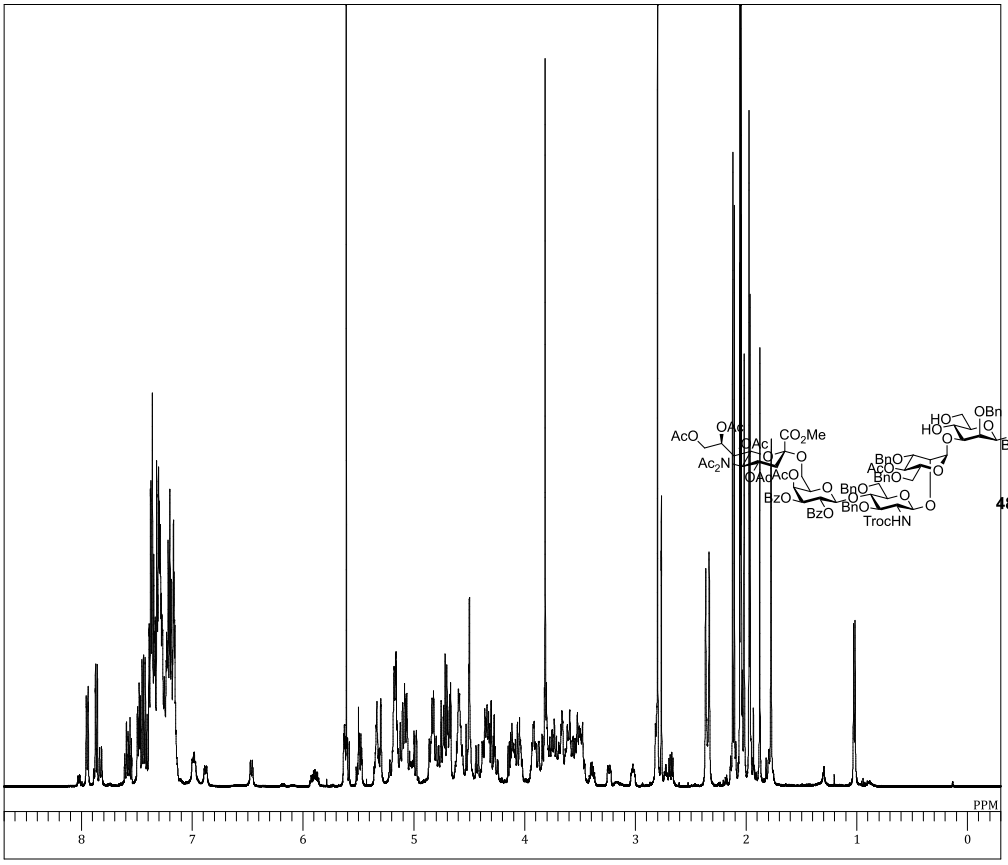
^{1}H NMR



DFILE	141224TetS1-3TetSN_proton-1-1.jdf
COMNT	single_pulse
DATIM	2014-12-24 12:21:12
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65535
FREQU	9384.38 Hz
SCANS	24
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	4.50 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	ACETN
EXREF	2.05 ppm
BF	0.20 Hz
RGAIN	40

47 (α/β ~ 3/1)

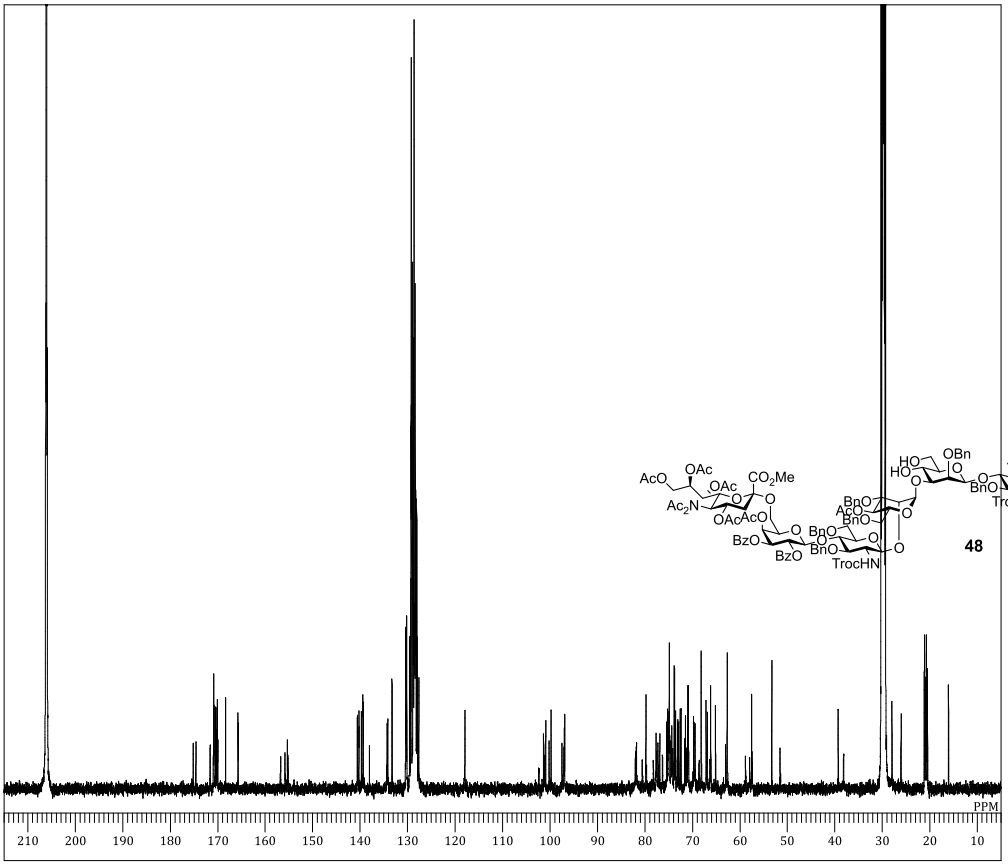
^{1}H NMR



DFILE	150105diHO46-OSN-a_proton-1-1.jdf
COMNT	single_pulse
DATIM	2015-01-05 11:19:08
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65535
FREQU	9384.38 Hz
SCANS	20
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	4.50 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	ACETN
EXREF	2.05 ppm
BF	0.20 Hz
RGAIN	36

48

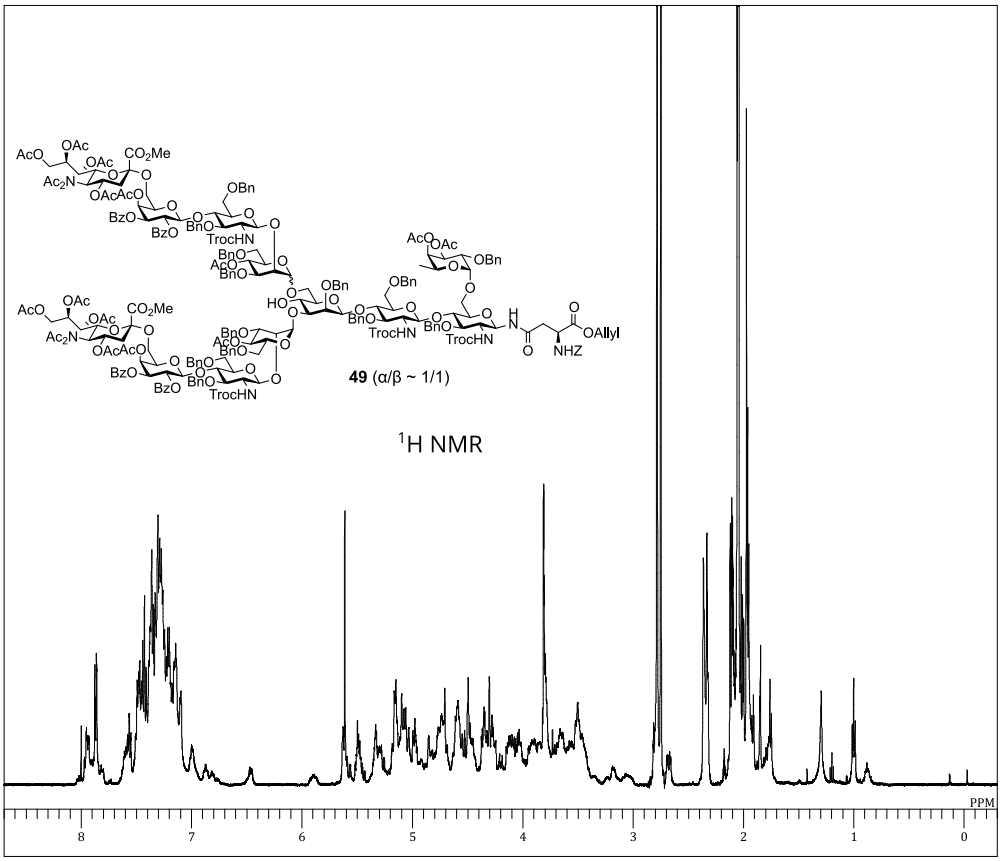
^{1}H NMR



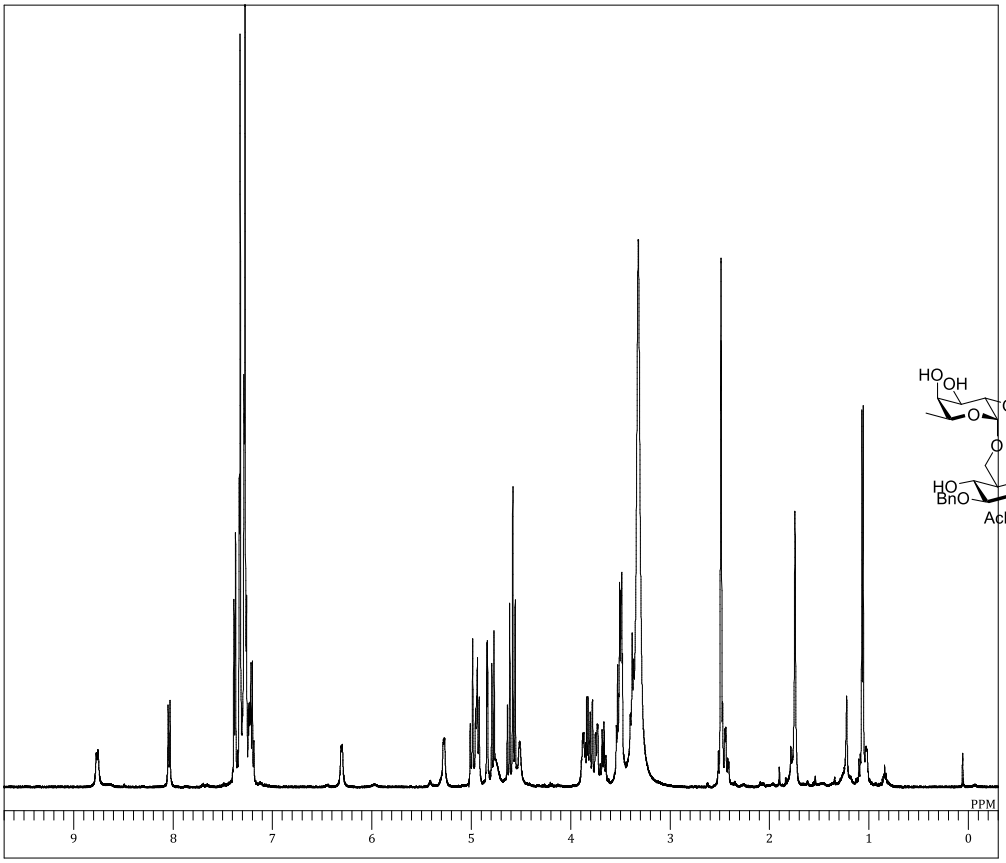
DFILE	151221HO46-OSN_carbon-1-1.jdf
COMNT	single pulse decoupled gated NOE
DATIM	2015-12-21 21:23:57
OBNUC	13C
EXMOD	carbon.jxp
OBFRQ	125.77 MHz
OBSET	7.87 KHz
OBFIN	4.21 Hz
POINT	65535
FREQU	39308.18 Hz
SCANS	7000
ACQTM	1.6672 sec
PD	4.0000 sec
PW1	4.33 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	ACETN
EXREF	29.80 ppm
BF	2.00 Hz
RGAIN	56

48

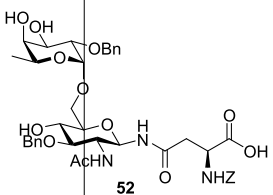
^{13}C NMR



DFILE	140108TetS1-6OSN_proton-1-1.jdf
COMNT	single_pulse
DATIM	2014-01-08 10:47:14
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65535
FREQU	9384.38 Hz
SCANS	36
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	5.60 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	ACETN
EXREF	2.05 ppm
BF	0.30 Hz
RGAIN	44

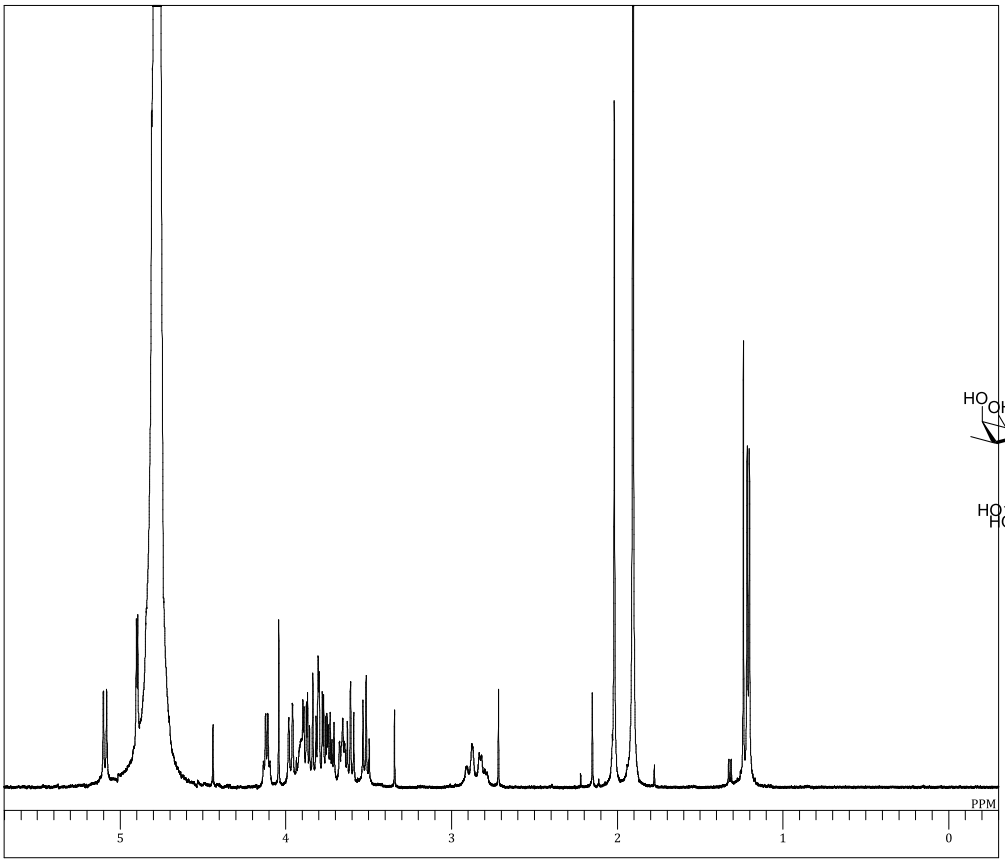


DFILE	110831DSAsn-NAc-1.jdf
COMNT	single_pulse
DATIM	2011-08-31 13:40:28
OBNUC	1H
EXMOD	single_pulse.ex2
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	65536
FREQU	9384.38 Hz
SCANS	24
ACQTM	6.9835 sec
PD	5.0000 sec
PW1	3.80 usec
IRNUC	1H
CTEMP	30.0 c
SLVNT	DMSO
EXREF	2.49 ppm
BF	0.02 Hz
RGAIN	38

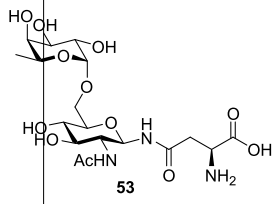


52

^{1}H NMR

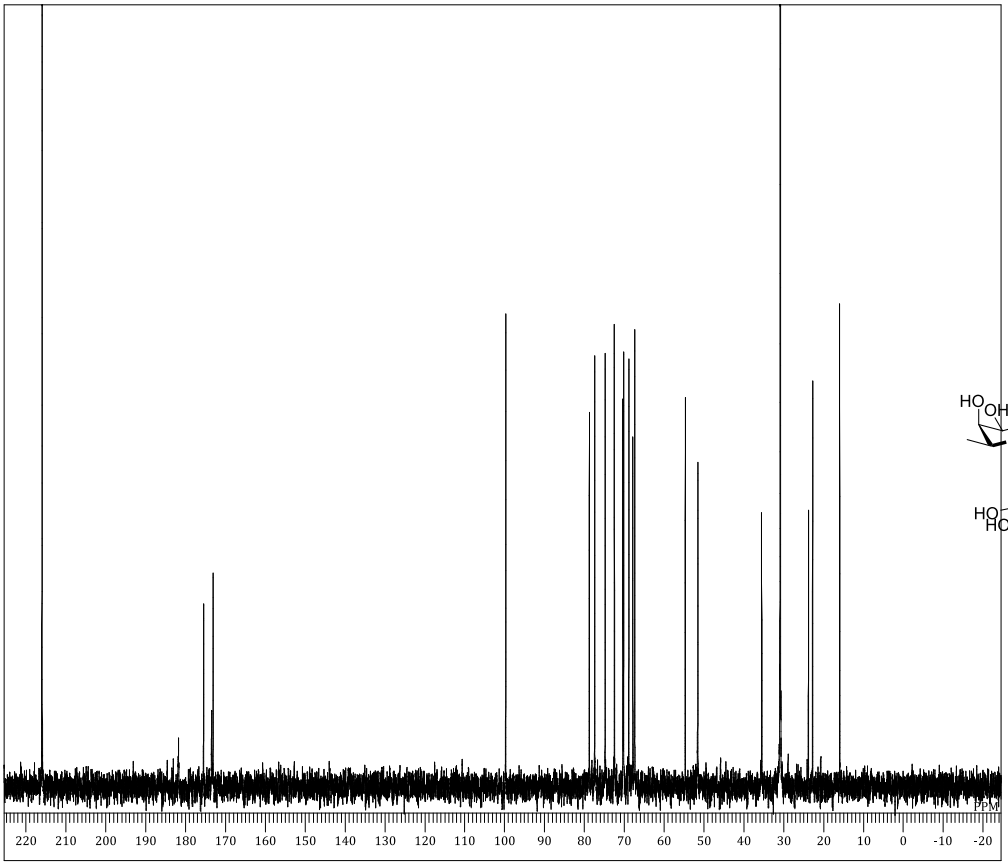


DFILE 150727DS-Asn-dep_proton-1-1.jdf
COMNT single_pulse
DATIM 2015-07-27 12:12:23
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 65535
FREQU 9384.38 Hz
SCANS 48
ACQTM 6.9835 sec
PD 5.0000 sec
PW1 5.25 usec
IRNUC 1H
CTEMP 25.0 c
SLVNT D2O
EXREF 1.24 ppm
BF 0.30 Hz
RGAIN 38

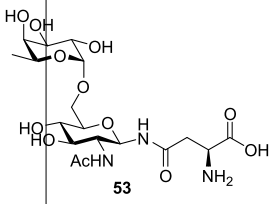


53

^{1}H NMR

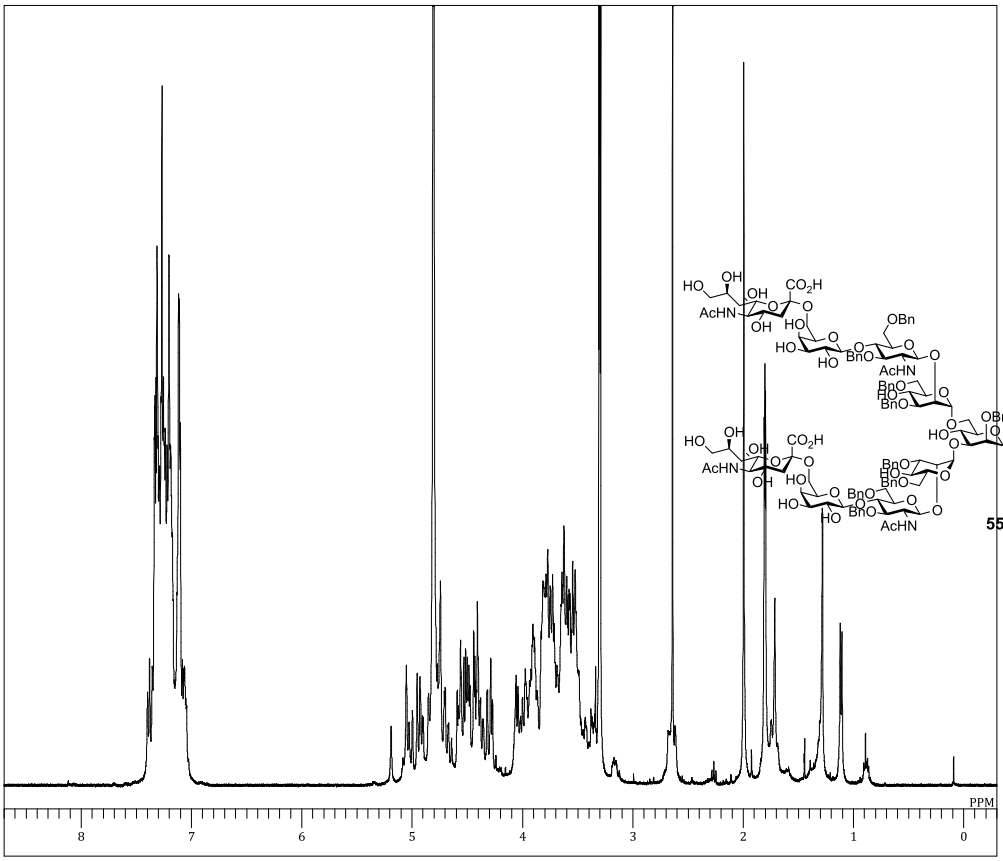


DFILE 120110DS-Asn-dep-carbon-1.jdf
COMNT single pulse decoupled gated NOE
DATIM 2012-01-11 08:26:19
OBNUC 13C
EXMOD single_pulse_dec
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 65536
FREQU 39308.18 Hz
SCANS 12388
ACQTM 1.6672 sec
PD 1.5000 sec
PW1 5.00 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT D2O
EXREF 30.89 ppm
BF 3.00 Hz
RGAIN 50

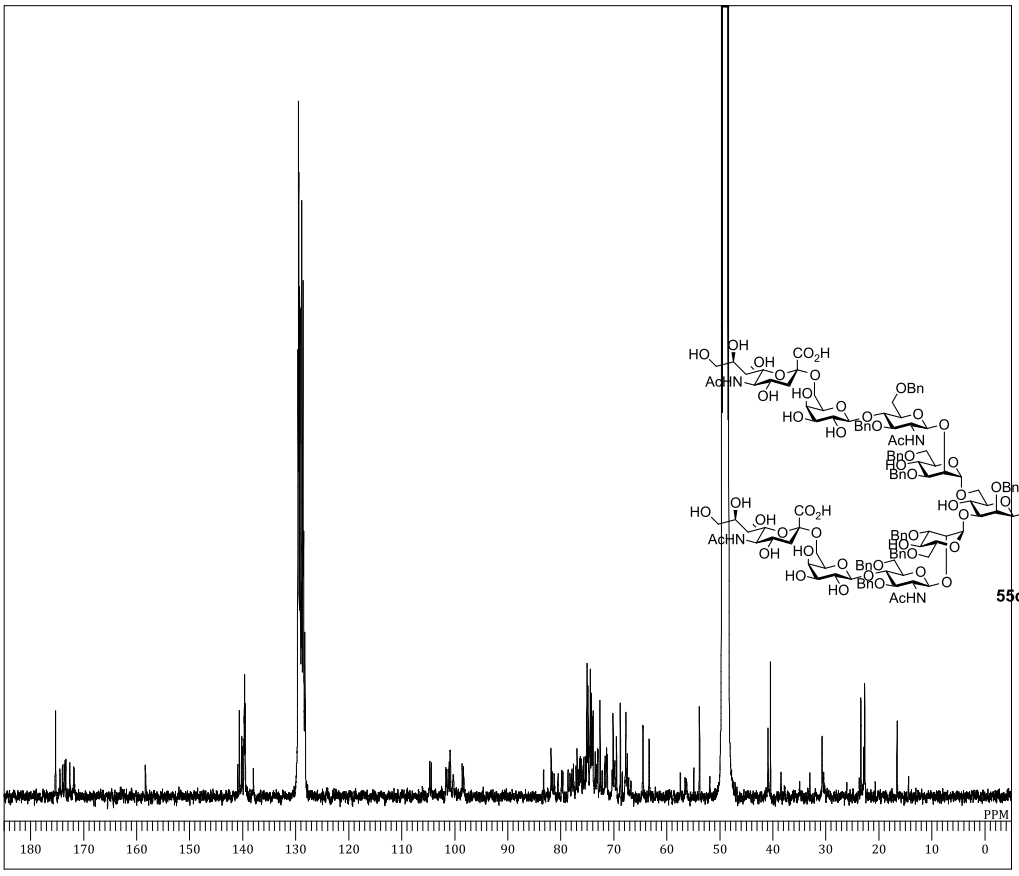


53

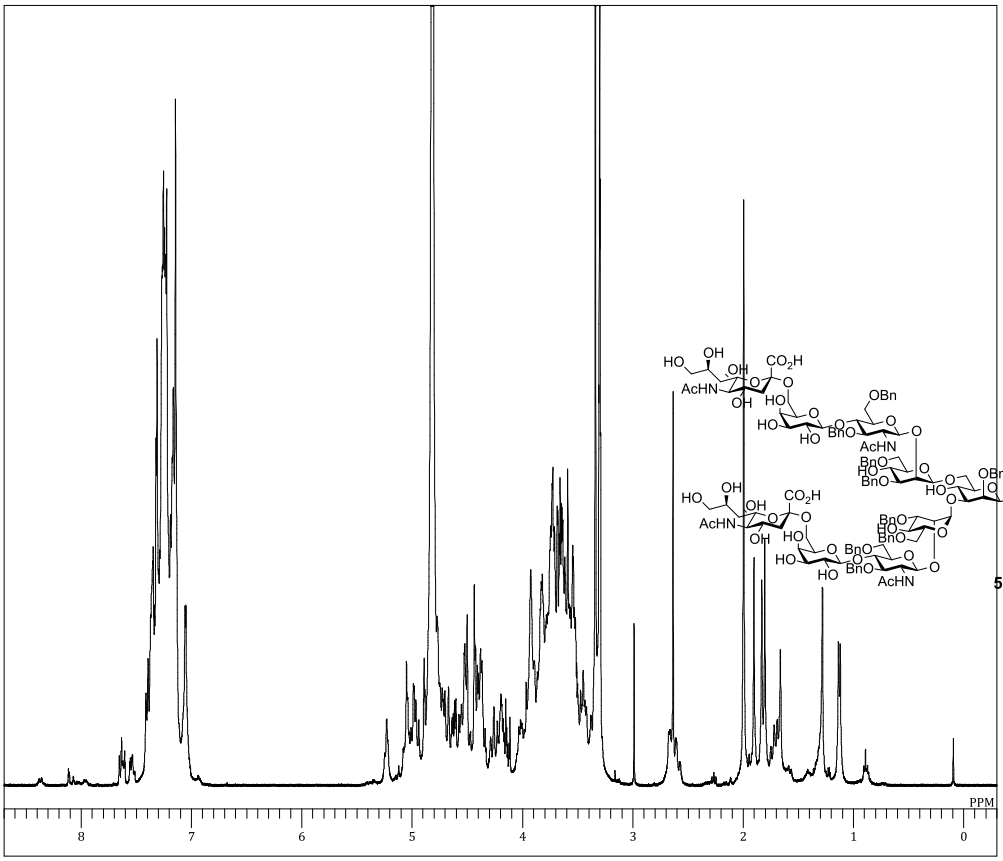
^{13}C NMR



^{1}H NMR



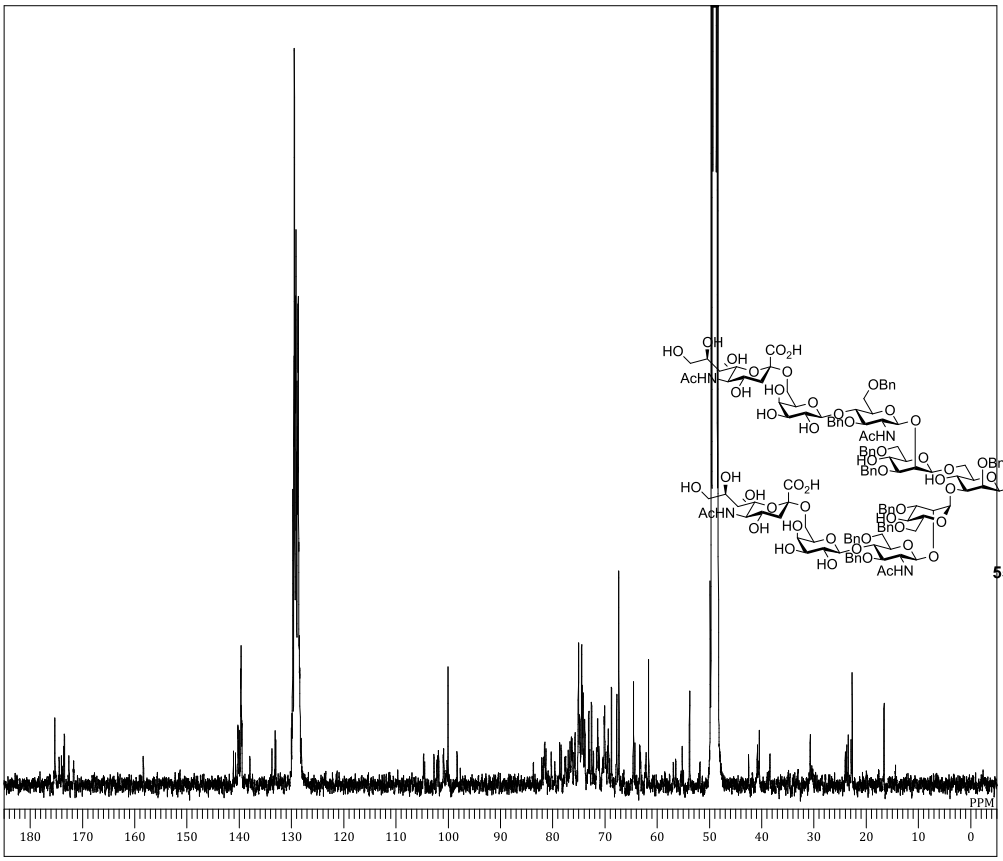
^{13}C NMR



DFILE 150223DodSN-sapon-a_proton-1-1.jdf
COMNT single_pulse
DATIM 2015-02-23 19:18:57
OBNUC 1H
EXMOD proton.jxp
OBFRQ 399.78 MHz
OBSET 4.19 KHz
OBFIN 7.29 Hz
POINT 32767
FREQU 7503.00 Hz
SCANS 4
ACQTM 4.3673 sec
PD 5.0000 sec
PW1 5.20 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT CD3OD
EXREF 3.30 ppm
BF 0.20 Hz
RGAIN 30

55β

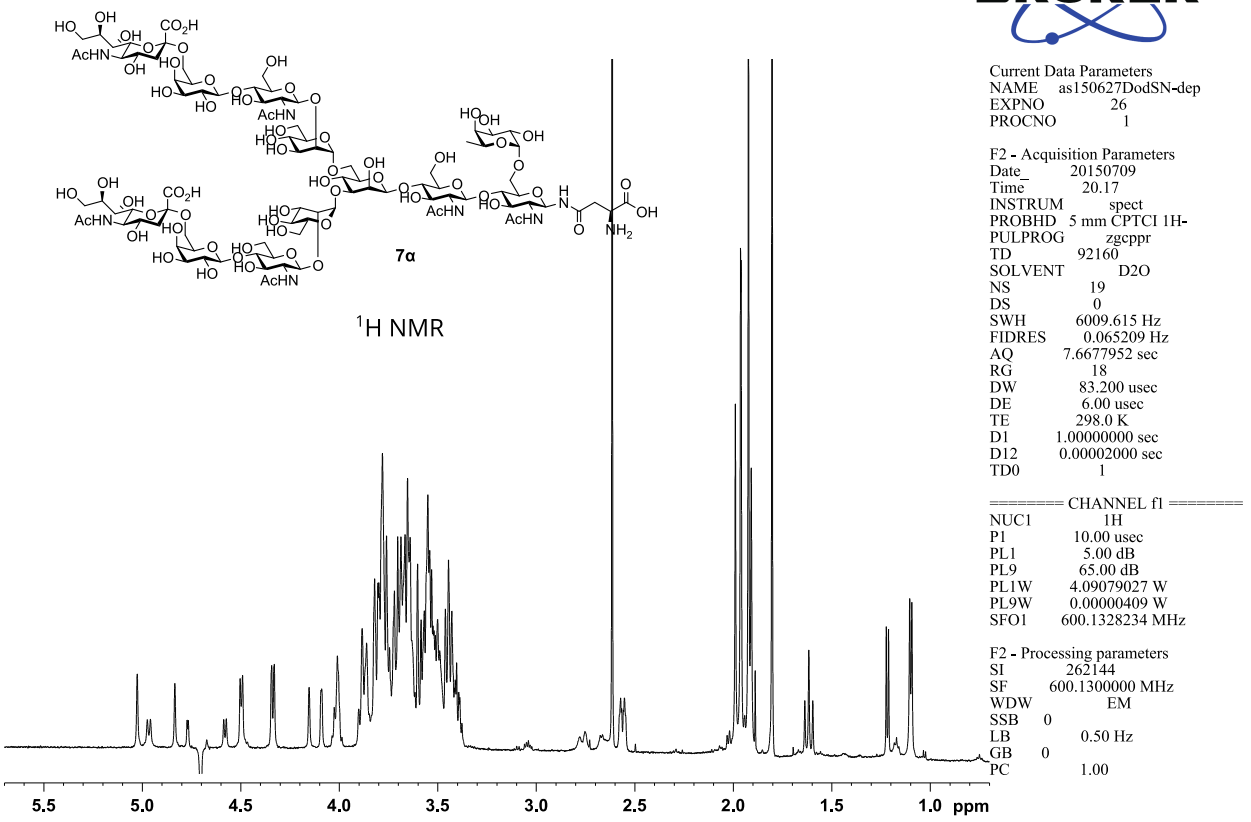
^{1}H NMR



DFILE 150224DodSN-sapon-a_carbon-1-1.jdf
COMNT single pulse decoupled gated NOE
DATIM 2015-02-24 23:44:25
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 100.53 MHz
OBSET 5.35 KHz
OBFIN 5.86 Hz
POINT 32767
FREQU 31407.04 Hz
SCANS 8150
ACQTM 1.0433 sec
PD 3.0000 sec
PW1 3.27 usec
IRNUC 1H
CTEMP 30.0 c
SLVNT CD3OD
EXREF 49.00 ppm
BF 3.00 Hz
RGAIN 60

55β

^{13}C NMR

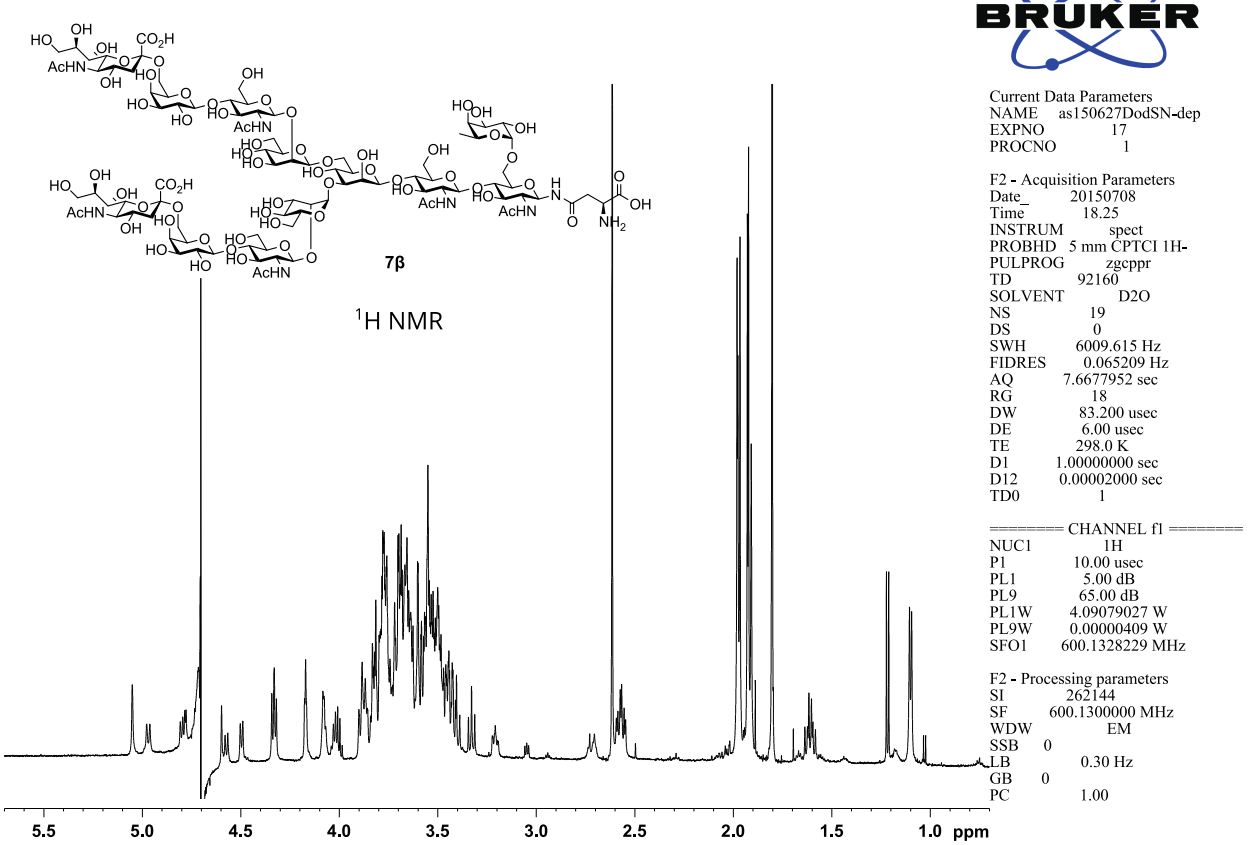


Current Data Parameters
NAME as150627DodSN-dep
EXPNO 26
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150709
Time 20.17
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgcppr
TD 92160
SOLVENT D2O
NS 19
DS 0
SWH 6009.615 Hz
FIDRES 0.065209 Hz
AQ 7.6677952 sec
RG 18
DW 83.200 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
D12 0.00002000 sec
TD0 1

========== CHANNEL f1 ==========
NUC1 1H
P1 10.00 usec
PL1 5.00 dB
PL9 65.00 dB
PL1W 4.09079027 W
PL9W 0.00000409 W
SFO1 600.1328234 MHz

F2 - Processing parameters
SI 262144
SF 600.1300000 MHz
WDW EM
SSB 0
LB 0.50 Hz
GB 0
PC 1.00



Current Data Parameters
NAME as150627DodSN-dep
EXPNO 17
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150708
Time 18.25
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgcppr
TD 92160
SOLVENT D2O
NS 19
DS 0
SWH 6009.615 Hz
FIDRES 0.065209 Hz
AQ 7.6677952 sec
RG 18
DW 83.200 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
D12 0.00002000 sec
TD0 1

========== CHANNEL f1 ==========
NUC1 1H
P1 10.00 usec
PL1 5.00 dB
PL9 65.00 dB
PL1W 4.09079027 W
PL9W 0.00000409 W
SFO1 600.1328229 MHz

F2 - Processing parameters
SI 262144
SF 600.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

References

1) Tanaka, K.; Miyagawa, T.; Fukase, K. *Synlett* **2009**, 1571, DOI: 10.1055/s-0029-1217343.

2) Tanaka, K.; Mori, Y.; Fukase, K. *J. Carbohydr. Chem.* **2009**, *28*, 1, DOI: 10.1080/07328300802571129.

3) Tanaka, K.; Fujii, Y.; Tokimoto, H.; Mori, Y.; Tanaka, S.-i.; Bao, G.-m.; Siwu, E. R. O.; Nakayabu, A.; Fukase, K. *Chem. Asian J.* **2009**, *4*, 574, DOI: 10.1002/asia.200800411.

4) Uchinashi, Y.; Nagasaki, M.; Zhou, J.; Tanaka, K.; Fukase, K. *Org. Biomol. Chem.* **2011**, *9*, 7243, DOI: 10.1039/c1ob06164j.

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Masahiro Nagasaki