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Synthesis of a Sialic Acid Containing Complex-Type *N*-Glycan on a Solid Support

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Abstract: A new solid-phase synthesis of *N*-linked glycans featuring 1) highly stereoselective β-mannosylation and microfluidic α-sialylation and 2) efficient glycosylation of the *N*-phenyltrifluoroacetimidate units on JandaJel resin is reported. Reagent concentration effects by a fluorous solvent are effectively applied, and the use of these methods results in the first synthesis of a sialic acid containing complex-type *N*-glycan on a solid support.

Keywords: carbohydrates · glycoproteins · glycosylation · microfluidics · solid-phase synthesis

Introduction

Among the various types of oligosaccharide structures, asparagine-linked oligosaccharides (*N*-glycans) are the most prominent in terms of diversity and complexity.[1] It is becoming clear that they are involved in a variety of important physiological events, such as cell–cell recognition, adhesion, signal transduction, quality control, and circulatory residence of proteins.[1] However, isolating large quantities of structurally pure *N*-glycans from natural sources is difficult. Thus, chemical synthesis provides an attractive opportunity to evaluate their biological functions. Although a number of new chemical or combined methods employing biological technology have been actively investigated,[2] an efficient approach to these complex oligosaccharides has yet to be established in terms of 1) selectivity in the glycosyl bond formations, namely, β-mannosylation and α-sialylation, and 2) a nontedious purification process during each step of glycosylation and deprotection. A solid-supported protocol is the most attractive and practical approach to the library-directed synthesis of these diverse structures of natural and nonnatural *N*-glycans.[3] Schmidt and co-workers recently reported the first library-directed synthesis of *N*-glycans on solid supports.[4] Although the nonreducing end sialic acid residue was not introduced to these oligosaccharides, a small library of complex-type *N*-glycans, which consisted of 17 structures, was elegantly constructed.[4d] Seeberger and co-workers have also been investigating automated oligosaccharides synthesis on solid supports;[5] a core pentasaccharide structure of complex-type *N*-glycans was prepared.[5a]

We have been investigating a variety of solid-phase and solid-supported methods,[6] such as chemical fishing, catch-and-release,[6a–c] or separation based on affinity separation (SAS)[6d–g] in combination with microfluidic glycosylation technology,[6g,7] and these methods have been successfully applied to the library synthesis of oligosaccharides. While we continuously strive to develop efficient solid-phase methods, our interests in elucidating unknown biological functions of mammalian *N*-glycans as well as sialic acid containing oligosaccharides[8] have motivated us to establish a practical solid-phase synthesis for complex-type *N*-glycans. Herein, we report an efficient solid-phase protocol that can readily deal with the structural diversity to the *N*-glycans. The first chemical synthesis of the sialic acid containing complex-type *N*-glycan on solid supports was achieved based on the established method.

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Results and Discussion

The initial target of our strategy was sialic acid-containing *N*-glycan **1**[1] (see structure in Scheme 4) with asymmetrical branching chains, which is difficult to obtain from natural sources. To prepare target *N*-glycan **1** as well as other diverse structures of this family efficiently, we designed frag-

ments **a**–**d** (Figure 1) by using ideas similar to Schmidt's precedent,[4] namely, two challenging glycosyl bond formations (i.e., β-mannosylation and α-sialylation) were constructed in advance in the solution-phase[9] or under microfluidic conditions.[7b] Then, suitably protected mono- and disaccharide donors **a**–**d**, activated as the corresponding glycosyl *N*-phenyltrifluoroacetimidates,[10] could stereoselectively be glycosylated on solid supports with the aid of neighboring group participation (Figure 1). *N*-Phenyltrifluoroacetimidate was selected as the leaving group because of its higher stability than the corresponding trichloroacetimidate, which makes it suited for the longer storage of fragments **a**–**d**. An *O*-Fmoc group was used as a temporary protecting group for the sugar extension on the solid phase, while an azidochlorobenzyl (AzClBn) group[6a–c] was applied at the C-3 hydroxy group of the branching mannose. These temporary protecting groups allowed *N*-glycans to be prepared with diverse structures at the C-3″ and C-6″ hydroxy groups. A trichloroethoxycarbonyl (Troc) group was applied to protect the nitrogen atom on glucosamine because this protecting group not only ensured glycosylation selectivity by the neighboring participation, but also conferred high reactivity as the glycosyl acceptor.[11] After a sugar extension on solid supports, alcoholysis of the *p*-xylyleneglycol ester linkage by NaOBn provided *N*-glycans protected with hydrogenolysis-sensitive groups (benzyl on hydroxy groups, benzyloxycarbonyl on the glycosaminyl amino group, and 4-hydroxymethylbenzyl at the anomeric hydroxy group).

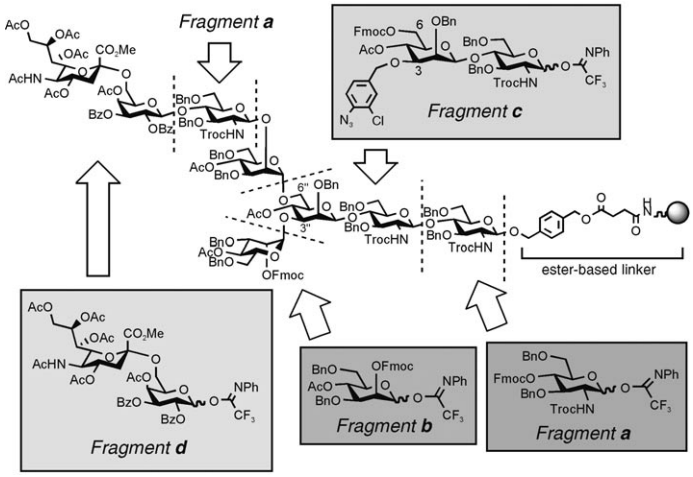
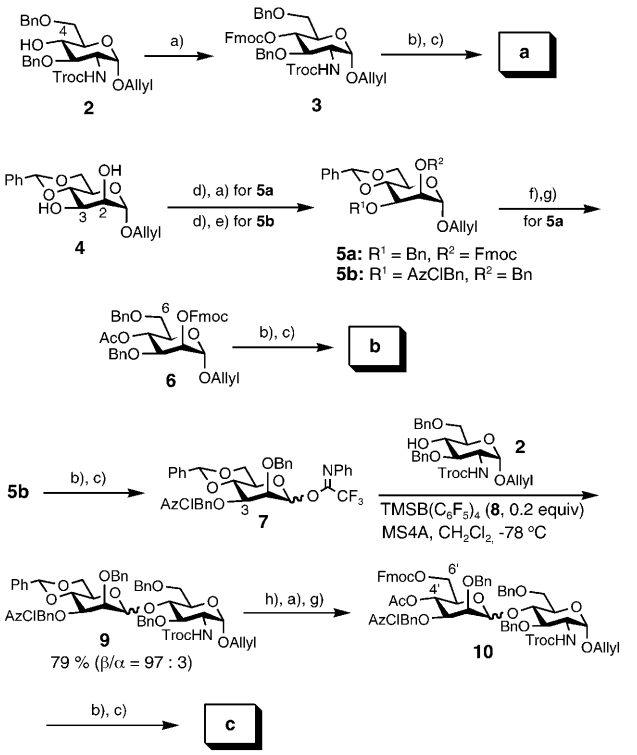


Figure 1. Synthetic strategy of *N*-glycan library on solid supports.

Scheme 1 shows the synthesis for fragments **a**–**c**. Glucosaminyl fragment **a** was prepared from known **2**[9] by Fmoc protection of the C-4 hydroxy group followed by conversion of the 1-*O*-allyl group into an *N*-phenyltrifluoroacetimidate in quantitative yield in three steps. Mannosyl fragment **b** was synthesized from **4**[9] by selective benzylation of the C-3 hydroxy group; **4** was treated with dibutyltin(IV) oxide in toluene to provide the intermediary cyclic tin acetal, which

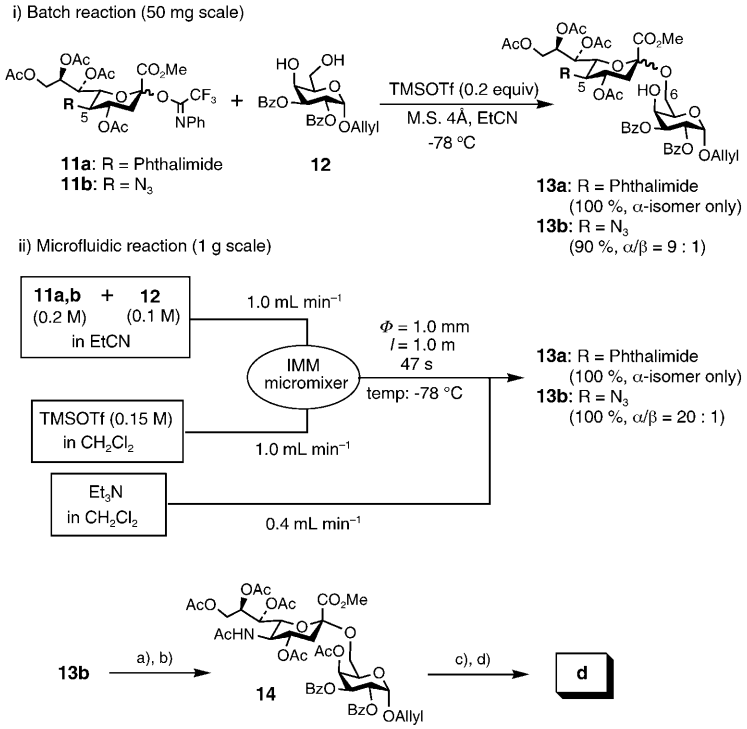


Scheme 1. Synthesis of imidate fragments **a**–**c**. Reagents and conditions: a) FmocCl, pyridine, CH_2Cl_2, RT, quant. for **3**, 88% for **5a**, 95% for **10**; b) "Ir" complex, H_2, THF, RT, then I_2, H_2O, RT; c) *N*-phenyltrifluoroacetimidoyl chloride, Na_2CO_3 or K_2CO_3 acetone, RT, quant. for **a**, 86% for **b**, 81% for **7**, 97% for **c** (2 steps); d) Bu_2SnO, toluene, 120 °C, 3 h, then, CsF, TBAI, BnBr or AzClBnBr, RT, 73% for R^1=Bn, 87% for R^1=AzClBn; e) BnBr, NaH, DMF, RT, 95%; f) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, RT, 82% or microfluidic conditions;[12] g) Ac_2O, pyridine, RT, 83% for **6**, quant. for **10**; h) 1 M HCl, MeOH, 60 °C, 92%.

Abstract in Japanese:

非還元末端シアル酸を有する複合型 *N*-結合型糖タンパク質糖鎖の固相合成に初めて成功した。既に我々の研究室において開発した、嵩高いルイス酸を用いる高立体選択的β-マンノシル化反応、およびマイクロフローシステムを活用した実用的α-シアリル化反応、さらにベンジリデンアセタールのマイクロフロー還元的開環反応を用いて、*N*-phenyltrifluoroacetimidate を脱離基とする単糖および２糖フラグメント a-d を効率的に調製した。次いで、これらフラグメントを用いて固相上でのグリコシル化反応を種々検討した結果、ジオキシブタン架橋ポリスチレンを基本構造とする *JandaJel*™を固相担体に用いた場合に、グリコシル化反応が定量的に進行することが判明した。固相上で糖鎖が伸長した際には反応性の低下が見られたが、この場合にはフルオラス溶媒の添加による、”固相濃縮効果”を利用することにより回避することができた。このように見出した *JandaJel*™固相担体に対して順次グリコシル化反応を繰り返すことによって、高収率で非還元末端シアル酸を含む複合型 *N*-結合型糖鎖８糖の合成に成功した。

was subsequently treated with benzyl bromide in the presence of CsF and TBAI. After the C-2 hydroxy group was protected with Fmoc group, **5a** was obtained in 64% in two steps. Reductive opening of the 4,6-*O*-benzylidene acetal in **5a** by treating with triethylsilane (Et_3SiH) in the presence of $BF_3{\cdot}OEt_2$ selectively gave the C-6 benzyloxy function, from which **6** was obtained by acetylation (68% in two steps, 1 g scale). However, reductive opening of the acetal on a large scale produced a significant amount of the diol by-product through the concomitant acid-mediated hydrolysis of the acetal. These problems were circumvented under microfluidic reaction conditions, which our group previously developed;[12] **6** was continuously and reproducibly obtained in nearly quantitative yields even on a 10 g scale. Finally, the deprotection of the anomeric allyloxy group followed by the imidate formation provided fragment **b** in 86% in two steps.



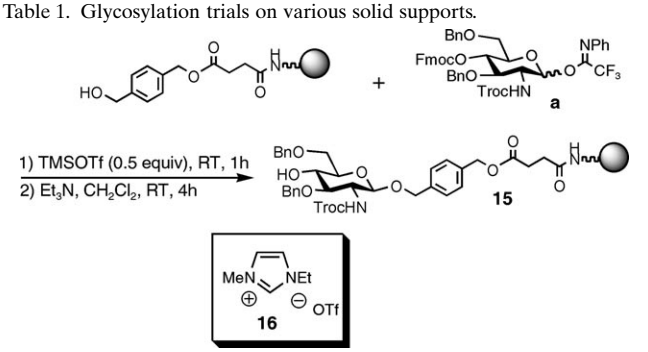
Scheme 2. Synthesis of imidate fragment **d** by microfluidic α-sialylation. Reagents and conditions: a) PPh_3, MeCN, RT, quant; b) Ac_2O, pyridine, 70 °C, 87%; c) "Ir" complex, H_2, THF, RT, then I_2, H_2O, RT; d) *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3 acetone, RT, 82% for 2 steps.

The synthesis of fragment **c** was achieved according to our highly β-selective mannosylation method,[9] which utilized the trimethylsilyl tetrakis(pentafluorophenyl)borate, $TMSB(C_6F_5)_4$,[9b] as a Lewis acid/cation trap activator (Scheme 1). Thus, mannosyl donor **7** with azidochlorobenzyl group at the C-3 hydroxy group, which was prepared from **5b** via common mannosyl fragment **4**, was glycosylated with *N*-Troc-glucosaminyl acceptor **2** in the presence of 20 mol% of $TMSB(C_6F_5)_4$ **8** and 4 Å molecular sieves (4 Å M.S.) in CH_2Cl_2 at −78 °C to provide **9** in 79% with excellent β-selectivity (β/α = 97:3). Hydrolysis of the benzylidene acetal (92%), sequential protection of C-6′ and C-4′ hydroxy groups by Fmoc and acetyl groups (95% in two steps), and conversion of 1-*O*-allyl into the imidate leaving group (97% in two steps) provided fragment **c**.

The most critical endeavor was α-sialylation toward fragment **d** (Scheme 2). We recently discovered the fixed-dipole moment effects of the C-5 *N*-phthalimide group in sialyl donor **11a**,[7a] and realized the quantitative and completely α-selective sialylation under microfluidic conditions.[7b] However, the selective deprotection of the *N*-phthalyl group in the presence of the C-1 methoxycarbonyl in **13a** was troublesome on the 1–2 g reaction scale. As an alternative to the *N*-phthalyl function, we employed the C-5 azide group in sialyl donor **11b** because this azide group should direct similar fixed-dipole moment effects,[13] but should be easier to convert into naturally occurring nitrogen substituents of neuraminic acids, namely, *N*-acetyl or *N*-glycolyl groups. As anticipated, sialylation between **11b** and galactosyl acceptor **12**[7] in the presence of TMSOTf as an activator and 4 Å M.S. in propionitrile provided **13b** in 90% yield with good α-selectivity (α/β = 9:1). Furthermore, applying the continuous microfluidic sialylation, which was developed by our group for **11a**,[7b] improved both the yield and α-selectivity; **13b** was obtained quantitatively with near total α-selectivity (α/β = 20:1). The α and β stereoisomers were easily separated by chromatography on silica-gel, and the pure α-isomer was readily converted into the *N*-acetyl derivative **14** in 95% yield by treatment with PPh_3 and subsequent *N*-acetylation. Finally, **14** was converted into imidate **d** in 82% yield using the general procedure. Hence, we successfully prepared fragments **a**–**d** on a 5–10 g scale.

Prior to the glycosylation trials on the solid supports using imidates **a**–**d** towards the *N*-glycan synthesis, detailed glycosylation conditions as well as the effects of the co-solvents were optimized by using *N*-Troc glucosaminyl fragment **a** (Table 1, entries 1–4). Initially, we examined polystyrene supports (co-polymer of styrene with 1% of divinylbenzene), which are currently commonplace in solid-phase synthesis. The loading yields of the glucosaminyl moiety were calculated after Et_3N-induced deprotection of the Fmoc group and subsequent UV/Vis analysis of the resulting dibenzofulvene (absorbance intensity at 301 nm). When polystyrene loaded with a *p*-xylyleneglycol ester linker (0.5 mequiv g^{-1})[4,6c] was treated with 2.5 equivalents of fragment **a** in the presence of TMSOTf (0.5 equiv) in CH_2Cl_2 at room

Table 1. Glycosylation trials on various solid supports.

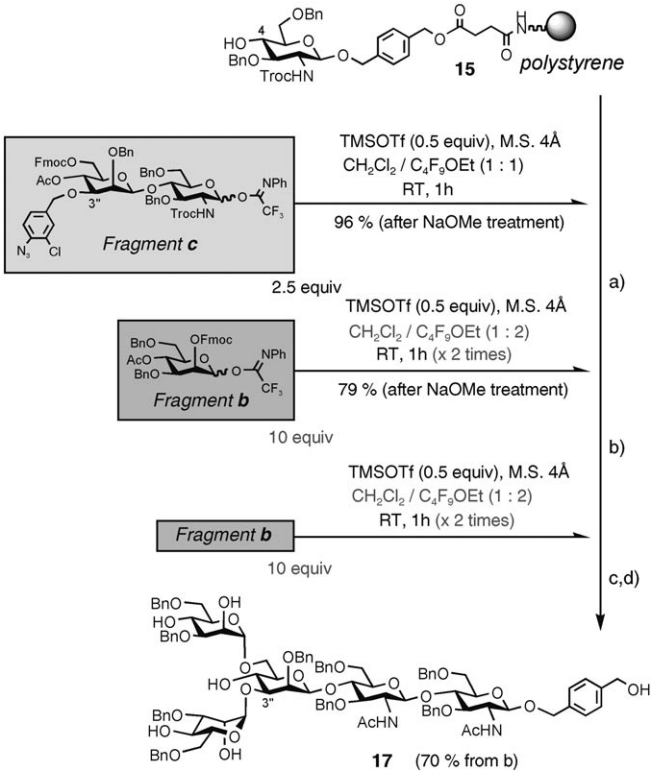


Entry	Resin[a]	Solvents	Yield [%][b]
1	polystyrene	CH_2Cl_2	13
2	polystyrene	CH_2Cl_2/ionic liquid **16** (3:1)	16
3	polystyrene	CH_2Cl_2/C_4F_9OEt (3:1)	19
4	polystyrene	CH_2Cl_2/C_4F_9OEt (1:1)	23
5	ArgoPore	CH_2Cl_2	30
6	ArgoPore	CH_2Cl_2/C_4F_9OEt (1:1)	39
7	JandaJel	CH_2Cl_2	100
8	JandaJel	CH_2Cl_2/C_4F_9OEt (1:1)	100

[a] *p*-Xylyleneglycol linker was introduced to each amino group-loaded resin in the presence of HOBt and DIC in DMF (loading amount: 0.5 mequiv g^{-1}). [b] Estimated from UV/Vis spectrum of dibenzofulvene at 301 nm in CH_2Cl_2.

temperature for 1 h (Table 1, entry 1), 13% of the xylylene linker was glycosylated. Interestingly, the addition of a 1/3 volume of ionic liquid **16** relative to CH_2Cl_2 slightly increased the loading yield (16%, Table 1, entry 2). Furthermore, the addition of a fluorous solvent (C_4F_9OEt) showed a similar effect (19%, Table 1, entry 3), and when a 1:1 mixed solvent of C_4F_9OEt and CH_2Cl_2 was applied, the efficiency of glycosylation was enhanced twofold (23%) relative to the reaction performed in neat CH_2Cl_2 (Table 1, entry 1 vs. 4). The profound effects of these co-solvents can be rationalized by the reagent concentration effects on the solid supports. That is, glycosyl donor **a** (or the corresponding oxocarbenium ion equivalent) might efficiently be accumulated onto the polystyrene supports because of favorable hydrophobic interactions in the presence of an ionic liquid or fluorous solvent additives such that the glycosylation reactivity may be enhanced. These reagent concentration effects have been applied to the solid-phase synthesis of peptides and other reactions,[14] but to the best of our knowledge, this is the first observation for glycosylation.

After successfully activating the glycosyl acceptor on the solid supports towards glycosylation, we investigated the solid-phase synthesis of core pentasaccharide **17** of complex-type *N*-glycans (Scheme 3). The success of each glycosylation step was evaluated by TLC, ESI-MS, and HPLC after cleaving the oligosaccharides from the resin by treatment with sodium methoxide (cleavage products obtained as *N*-methoxycarbonyl derivatives). Thus, the C-4 hydroxy group of the glucosaminyl fragment introduced on the supports (**15**) was further glycosylated with Manβ(1-4)GlcNTroc imidate **c** under identical conditions established in Table 1 (donor; 2.5 equiv, TMSOTf; 0.5 equiv, 4 Å M.S., CH_2Cl_2/



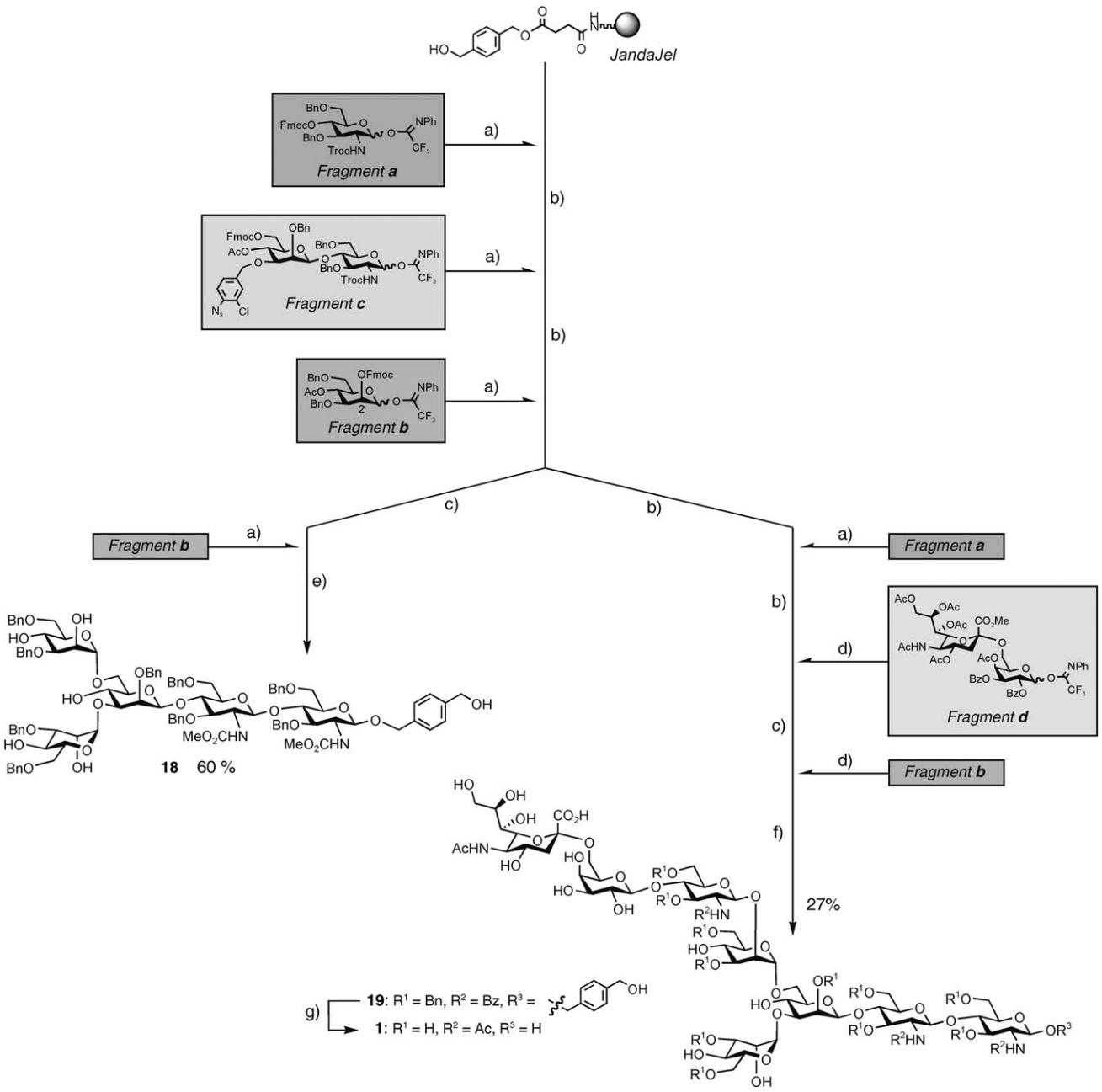
Scheme 3. Solid-phase synthesis of pentasaccharide **17** on polystyrene supports. Reagents and conditions: a) 15% Et_3N, CH_2Cl_2, RT, 4 h; b) PBu_3, CH_2Cl_2, 1 h, then DDQ/AcOH/H_2O (1:1:1), THF, RT, 2 h; c) $(Bu_3Sn)_2$, microwave (50 W, 20 psi, 200 °C), DMF, 40 min, then Ac_2O, THF, 1 h; d) 1 M NaOMe, MeOH/THF (1:1), RT, 1 h.

C_4F_9OEt = 1:1, room temperature) to provide the corresponding trisaccharide in 96% yield (Scheme 3). However, Fmoc-deprotection and subsequent continuous glycosylation with mannose fragment **b** under the same conditions resulted in only 10% of the desired tetrasaccharide, and most of the starting trisaccharide was recovered. After optimizing the conditions, this glycosylation could only be achieved in a reasonable yield (79%) when using 10 equivalents of the donor and excess fluorous solvent (twice the volume relative to CH_2Cl_2), as well as repeating the procedure (double glycosylation). Furthermore, subsequent glycosylation with fragment **b** after deprotection of the AzClBn group on solid supports (PBu_3–H_2O, then DDQ–AcOH),[6a–c] also necessitated this rather inefficient glycosylation operation; pentasaccharide **17** was obtained in 70% yield from the cleavage of the AzClBn group by radical-mediated *N*-Troc deprotection under microwave irradiation on a solid support.[14e]

Disappointed by these glycosylation results on polystyrene supports, we reinvestigated solid supports to achieve efficient glycosylation even during the late stage of the glycosylation on the solid supports, namely, when the hydroxy groups are glycosylated as the size of the oligosaccharide acceptors increases. It was found that the dioxybutane-linked polystyrene, JandaJel,[15] meets our requirements (Table 1, entries 5–8). Although the use of macroporous polystyrene, ArgoPore,[6b,c,14a,b,16] achieved a threefold increase in the re-

activity relative to that of the regular polystyrene-based resin (30% and 39% without and with fluorous solvent additive, Table 1, entries 5 and 6, respectively), JandaJel afforded quantitative glycosylation without the addition of a fluorous co-solvent (Table 1, entries 7 and 8). The flexible dioxybutane-linked polystyrene structure as well as the accumulation of Lewis acid by coordinating to the oxygen inside this resin might explain the high glycosylation efficiency, although the detailed reactivity on JandaJel supports needs further to be evaluated.

Based on the discovery of JandaJel as an effective solid-phase platform for glycosylation, the assembly of the *N*-glycan structures was investigated (Scheme 4). By simply repeating the sequence of glycosylation (donor; 2.5 equiv, TMSOTf; 0.5 equiv, and reaction performed only in CH_2Cl_2) and deprotection (15% Et_3N in CH_2Cl_2 for Fmoc, and PBu_3/H_2O-DDQ/AcOH for AzClBn) in the glycosylation order of **a**-**c**-**b**-**b**, pentasaccharide **18** was obtained in 60% total yield (from the first glycosylation) after cleavage from the resin by NaOMe treatment. TLC analysis of the intermediary tri-, tetra-, and pentasaccharides revealed that each



Scheme 4. Solid-phase synthesis of *N*-glycans **18** and **1** on JandaJel supports. Reagents and conditions: a) donor (2.5 equiv), TMSOTf (0.5 equiv), 4 Å M.S., CH_2Cl_2, RT, 1 h; b) 15% Et_3N, CH_2Cl_2, RT, 4 h; c) PBu_3, CH_2Cl_2, 1 h, then DDQ/AcOH/H_2O (1:1:1), THF, RT, 2 h; d) donor (2.5 equiv), TMSOTf (0.5 equiv), 4 Å M.S., CH_2Cl_2/C_4F_9OEt (1:1), RT, 1 h; e) 1 M NaOMe, MeOH/THF (1:1), RT, 1 h; f) 1 M NaOBn, BnOH/THF (1:2), RT, 3 h, then 3 M NaOH, RT, 12 h; g) 20% $Pd(OH)_2/C$, H_2, Ac_2O/MeOH (1:1), RT, 9 h.

glycosylation step proceeded in nearly quantitative yield. Neither a fluorous solvent nor a large excess of glycosyl donors was required.

Encouraged by these results, we then examined the synthesis of octasaccharide **1**, which contains a sialic acid residue, by sequentially glycosylating fragments **a**-**c**-**b**-**a**-**d**-**b** (Scheme 4). Although glycosylation was quite successful up to the fourth glycosylation with glycosaminyl imidate **a**, the unreacted pentasaccharide acceptor was mainly recovered during the fifth glycosylation with Neuα(2-6)Gal imidate **d**. To circumvent the retarded reactivity of the acceptor hydroxy groups on the extended oligosaccharide structures on the solid supports, the reagent concentration effect shown in Table 1 was effectively applied. Thus, a mixed solvent of CH_2Cl_2/C_4F_9OEt (1:1) was employed in the fifth and the final glycosylation with fragments **d** and **b**; after treating the resin with NaOBn, protected octasaccharide **19** was obtained in 27% total yield (calculated from the first introduction of the fragment **a** to the resin), after gel filtration followed by the removal of the strongly UV/Vis absorbing polystyrene derivatives by HPLC, produced during the cleavage from the JandaJel resin. Finally, **19** was treated with acetic anhydride in the presence of 20% $Pd(OH)_2$ on carbon under a hydrogen atmosphere to give octasaccharide **1** in 74% yield. The ^{1}H NMR spectrum showed good match with an authentic *N*-glycan sample with asparagine residue, and hence, the first solid-phase synthesis of the sialic acid-containing *N*-glycan was achieved.[17]

Conclusions

In conclusion, we developed an efficient solid-phase method of *N*-glycan synthesis and successfully applied it to the first chemical synthesis of a complex-type *N*-glycan with a nonreducing end sialic acid. Our solid-phase protocol was successful because of 1) the highly selective β-mannosylation and α-sialylation either in the solution phase or under microfluidic conditions, which led to the large scale preparation of *N*-phenyltrifluoroacetimidate fragments **a**–**d**, 2) the nearly quantitative glycosylation on JandaJel supports, and 3) the fluorous-solvent-assisted reagent concentration effects on the solid-phase glycosylation. Because a variety of natural and nonnatural *N*-glycans could be easily prepared by glycosylating imidates **a**–**d** or their slightly structural variants (such as fucosylated congener of the glucosamino fragment **a**), the present protocol might be applicable to a general *N*-glycan synthesis, even in an automated synthesis. A library synthesis of the mammalian *N*-glycans and their biofunctional studies, including the PET biodistribution,[18] is currently in progress in our laboratory.

Experimental Section

General procedure for glycosylation, deprotection of Fmoc and azidochlorobenzyl (AzClBn) groups on JandaJel, and reaction monitoring (cleavage from the resin): Glycosylation: A solution of fragments **a**–**d** (125 μmol, 2.0 mL) in CH_2Cl_2 (or with fluorous co-solvent) was added to a resin (50 μmol of the acceptor hydroxy group on the resin) at room temperature, and the resulting mixture was shaken at this temperature for 10 min. TMSOTf (4.5 μL, 25 μmol) was added, and the resulting suspension was shaken at this temperature for 1 h. The solution was removed, and the resin was washed sequentially with CH_2Cl_2 and MeOH (each 1.5 mL, 1.5 min, 5 sets), and dried in vacuo.

Fmoc deprotection: The resin (50 μmol of the protected hydroxy group on the resin) was suspended in CH_2Cl_2 (1.0 mL) and 15% Et_3N solution in CH_2Cl_2 (2.0 mL) was added. The resulting mixture was shaken at room temperature for 4 h. The solution was removed the resin was washed with DMF (1.5 mL, 1.5 min, 14 times) and then sequentially with CH_2Cl_2 and Et_2O (each 1.5 mL, 2.0 min, 10 sets), and dried in vacuo for 5 h to prepare for subsequent glycosylation.

Azidochlorobenzyl (AzClBn) deprotection; To the resin (50 μmol of the protected hydroxy group on the resin) suspended in CH_2Cl_2 (2.0 mL) was added tributylphosphine (43.1 μL, 173 μmol) at room temperature, and the resulting mixture was shaken at this temperature for 1 h. The solution was filtered off and the resin was washed with CH_2Cl_2 (each 1.5 mL, 2.0 min, 4 times). To the resin again suspended in THF (2.0 mL) were added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 39.2 mg, 173 μmol), AcOH (99.2 μL, 1.73 mmol), and H_2O (31.2 μL, 1.73 mmol) at room temperature and the resulting mixture was shaken at this temperature for 2 h. After the solution was filtered off, the resin was washed sequentially with DMF, MeOH, and CH_2Cl_2 (each 1.5 mL, 2.0 min, 5 sets), and dried in vacuo for 5 h to prepare for subsequent glycosylation.

Reaction monitoring (cleavage from the resin by NaOMe treatments): A 28% solution of NaOMe in MeOH (500 μL) was added to a suspension of the resin (50 μmol of the oligosaccharide on the resin) in THF (1.0 mL) and MeOH (1.0 mL) at room temperature, and the resulting mixture was shaken at this temperature for 1 h. The resin was washed with THF (1.5 mL, 2.0 min, 5 times), and the resulting solution was neutralized by ion-exchange resin, dowex H^+ to provide the crude products. The residue was purified by thin layer chromatography on silica gel (6% MeOH in chloroform) to give the corresponding *N*-methoxycarbonyl derivatives as the colorless solids. The efficiency of the reaction was evaluated by TLC, HPLC (column; $5C_{18}$-AR300 (nacalai tesque) 4.6×250 mm, eluent; MeCN in H_2O), and ESI- or MALDI-TOF-MS; mono-GlcN *m/z* calcd for $C_{30}H_{35}NNaO_8$ $[M+Na]^+$: 560.2, found: 560.2; Manβ(1–4)GlcNβ-(1–4)GlcN *m/z* calcd for $C_{72}H_{80}ClN_5NaO_{19}$ $[M+Na]^+$: 1377.9, found: 1377.4; Manα(1–6)Manβ(1–4)GlcNβ(1–4)GlcN *m/z* calcd for $C_{92}H_{102}ClN_5NaO_{24}$ $[M+Na]^+$: 1720.3, found: 1720.6; Manα(1–6)[Manα(1–3)]Manβ(1–4)GlcNβ(1–4)GlcN **18** *m/z* calcd for $C_{105}H_{121}N_2O_{29}$ $[M+H]^+$: 1873.8, found: 1873.9; GlcNβ(1–2)Manα(1–6)Manβ(1–4)GlcNβ(1–4)GlcN *m/z* calcd for $C_{114}H_{127}ClN_6NaO_{30}$ $[M+Na]^+$: 2117.8, found: 2117.6; methyl ester of Neuα(2–6)Galβ(1–4)GlcNβ(1–2)Manα(1–6)Manβ(1–4)GlcNβ(1–4)GlcN *m/z* calcd for $C_{132}H_{157}ClN_7O_{43}$ $[M+H]^+$: 2563.0, found: 2562.8.

Protected octasaccharide (**19**): To a suspension of octasaccharide-loaded JandaJel resin (theoretical amount of oligosaccharide loaded on the resin; 30.2 μmol) in dry THF (1.0 mL) was added an 1 M NaOBn solution in BnOH (500 μL, 500 μmol) at room temperature, and the resulting mixture was shaken at this temperature for 3 h. Subsequently, aqueous 3 M NaOH (500 μL, 1.5 mmol) was added and the resulting suspension was shaken overnight at room temperature. The resin was washed with THF (1.5 mL, 2.0 min, 5 times), and the resulting solution was neutralized by ion-exchange resin, dowex H^+ (500W×8). After the excess benzyl alcohol was removed by size-partitioning gel filtration through a column filled with sephadex LH-20 (eluent: MeOH), the protected octasaccharide **19** (a white solid, 38.4 mg) was further separated from the strongly UV/Vis absorbing polystyrene derivatives by HPLC, produced during the cleavage from JandaJel resin (23.9 mg, 27% overall yield; column: nacalai tesque $5C_{18}$-AR300, 4.6×250 mm; MeCN in H_2O (50–100% gradient over 60 min); retention time of **19**: 14.4 min): MALDI-TOF-MS *m/z* calcd for $C_{162}H_{185}N_4O_{48}$ $[M+H]^+$: 2955.2, found: 2955.4. Owing to the very low solubility of **19** in a variety of the NMR solvents, only broad, but characteristic proton signals of the oligosaccharide with benzyl type-protection groups could be observed in CD_3CN; ^{1}HNMR (600 MHz;

CD_3CN): $\delta = 1.82$ (s, 3H), 1.88 (dd, 1H, $J = 12.0$ Hz, 3-Hax^{Neu}), 1.99 (s, 3H×3), 2.69–2.87 (brdm, including 3-Heq^{Neu}), 3.19–4.11 (m, 22H), 4.16–5.42 (m, 32H), 5.59–5.97 (m, 6H), 7.07–7.62 (m, 67H), 7.85–8.03 ppm (m, 7H).

Octasaccharide (**1**): To a solution of the protected octasaccharide **19** (2.0 mg, 680 nmol) in MeOH (150 μL) and Ac_2O (150 μL) was added 20% $Pd(OH)_2$ on carbon (1.7 mg), and the resulting mixture was stirred under a hydrogen atmosphere for 9 h at room temperature. After the catalyst was filtered off and washed with 20% MeCN in H_2O, the solution was lypophilized to give **1** (1.1 mg, 74%): MALDI-TOF-MS *m/z* calcd for $C_{59}H_{98}N_4NaO_{44}$ $[M+Na]^+$: 1589.5, found: 1590.5, *m/z* calcd for $C_{59}H_{96}N_4NaO_{43}$ $[M-H_2O+Na]^+$: 1571.5, found: 1571.9, *m/z* calcd for $C_{59}H_{97}N_4O_{43}$ $(M-H_2O+H]^+$: 1549.6, found: 1548.8, *m/z* calcd for $C_{59}H_{95}N_4O_{42}$ $(M-2H_2O+H]^+$: 1531.5, found: 1531.7, and *m/z* calcd for $C_{48}H_{79}N_3NaO_{35}$ (M-sialic acid-H_2O+Na)$^+$: 1279.4, found: 1279.0; ^{1}H NMR (500 MHz; D_2O): $\delta = 1.64$ (dd, 1H, $J = 12.0$, 12.0 Hz), 1.95 (s, 6H), 1.99 (s, 6H), 2.60 (dd, 1H, $J = 11.9$, 4.6 Hz), 3.40–3.93 (m, 46H), 3.99 (brs, 1H), 4.04 (brs, 1H), 4.18 (brs, 1H), 4.37 (d, 1H, $J = 7.9$ Hz), 4.52 (brs, 1H), 4.70 (brs, 0.5H), 4.86 (s, 1H), 5.03 ppm (s, 1H).

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