Carbohydrate Chemistry

Divergent Synthesis of Sialylated Glycan Chains: Combined Use of Polymer Support, Resin Capture–Release, and Chemoenzymatic Strategies**

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As key constituents of cell-surface glycoproteins and glycosphingolipids, sialic acid containing glycan chains are involved in a variety of important physiological events such as cell–cell recognition, adhesion, and signal transduction.[1] It has been reported that the increased levels of sialic acid and sialyltransferase expression in tumor cells are closely related to their metastatic potential and malignancy progression.[2] Sialic acid residues of cell-surface glycoconjugates are also recognized by virulent proteins such as influenza virus hemagglutinins, which initiate the viral invasion into the host cell cytoplasm.[3] In cell-mediated immunity, the rolling of leukocytes is dependent on the levels of endothelial sialic acid.[4] Modulation of the recognition events exerted by sialylated glycans, therefore, seems promising for therapeutic purposes.[5]

Among the types of glycoprotein structures, asparagine-linked complex-type oligosaccharides (*N*-glycans) are most prominent[6] in terms of diversity as well as complexity. These *N*-glycans are often terminated with a sialic acid (Neu5Ac) residue attached to a penultimate galactose (Gal) moiety through an α(2,3) or α(2,6) linkage (Scheme 1 a). The synthesis of sialylated *N*-glycans has been reported by several groups.[7] Most notably, Unverzagt and co-workers developed chemoenzymatic routes to afford a variety of biantennary glycans.[7d,e,8] More recently, Kajihara and co-workers reported the systematic preparation of biantennary complex-type glycans and glycopeptides, starting from glycopeptides that are isolated from egg yolks as the common

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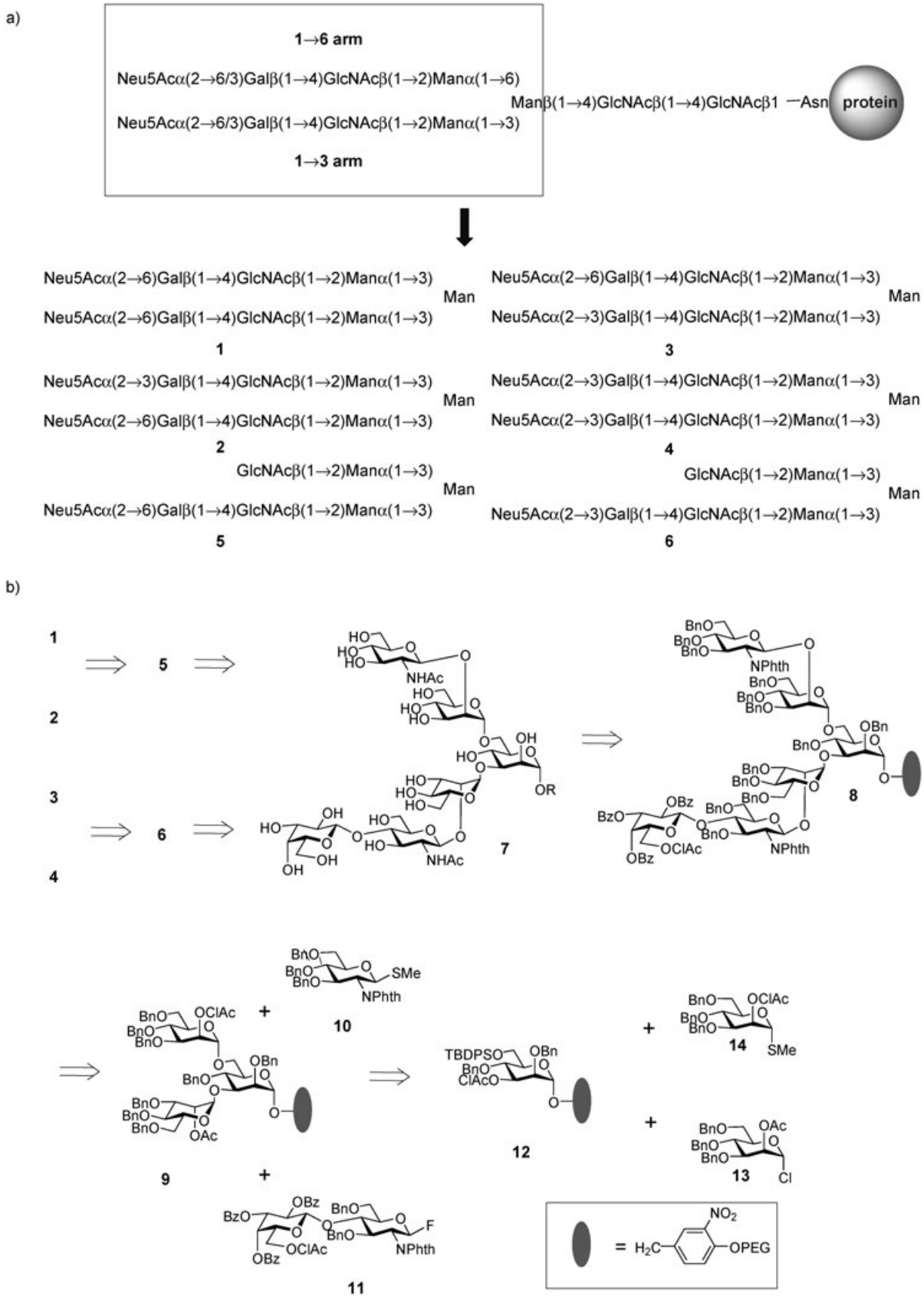


Scheme 1. a) Structures of sialylated complex-type *N*-glycans; b) retrosynthetic analysis for polysaccharides **1**–**6**. Bn = benzyl, Bz = benzoyl, Gal = galactose, GlcNAc = *N*-acetylglucosamine, Man = mannose, Neu5Ac = sialic acid, PEG = poly(ethylene glycol), Phth = phthalyl, TBDPS = *tert*-butyldiphenylsilyl.

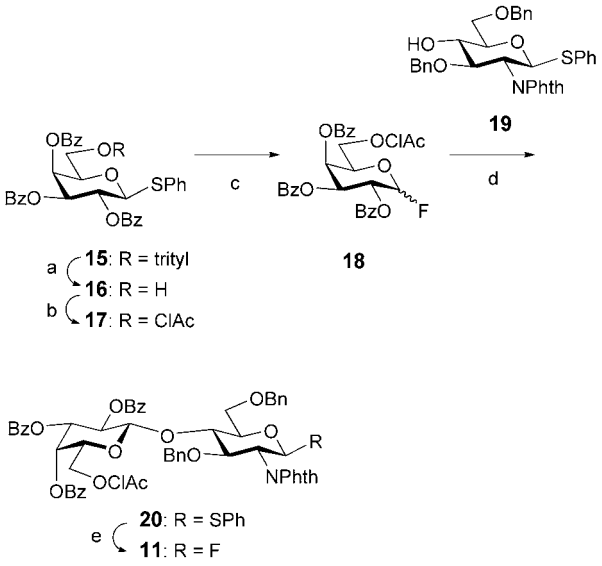
precursor.[9] In terms of chemical synthesis, the preparation of (2,6)-[7a] and (2,3)-linked disialylated *N*-glycans[7b] have been reported.

Polymer-supported synthesis is considered to be a promising technology that should facilitate, and ultimately automate, the synthesis of oligosaccharides. To date, however, there are only a few reported syntheses of branched complex structures such as sialylated *N*-glycans.[10] As part of our efforts to develop methodologies for the efficient construction of glycan chains based on soluble polymer-support technology,[11] we set out to synthesize disialylated compounds **1**–**4** as well as monosialylated saccharides **5** and **6** in a divergent manner. Low molecular weight (average $M_W \approx 750$) monomethyl polyethylene glycol (PEG) was chosen as the polymer support. For purification, the PEG-supported product can be adsorbed on silica gel, washed to remove the non-PEG supported materials (e.g. excess donor), and then retrieved by elution with polar solvents.[11e] With a unique resin capture–release purification, which uses a chloroacetyl group as the purification handle, fully assembled oligomers can be distinguished from shorter products. A nitro-modified Wang-resin-type linker, which has been shown to endure most of the typical glycosylation conditions,[12] was used.

The synthetic plan for the preparation of target glycans **1**–**6** is depicted in Scheme 1b. With hexasaccharide **7** as the common precursor, the scheme involves the use of glycosyltransferases to introduce the terminal Neu5Ac residues and penultimate Gal of the (1,6) branch. An initial glycosylation with either (2,6)- or (2,3)-sialyltransferase should provide monosialylated heptasaccharide **5** or **6**, which can then serve as substrates of sequential galactosylation–sialylation to afford **1**–**4**. Thus, simply by changing the type of glycosyltransferase, all positional isomers of $Neu5Ac_2Gal_2GlcNAc_2$-Man_3, **1** (2,6/2,6), **2** (2,3/2,6), **3** (2,6/2,3), and **4** (2,3/2,3), as well as monosialylated **5** and **6**, can be prepared from the single precursor **7**. Compound **8** was designed as a protected hexasaccharide that would be assembled from Man_3 **9**, GlcNAc **10**, and LacNAc **11** derivatives.

As shown in Scheme 2, lactosamine fluoride **11** was prepared from galactosyl donor **15**[13] and glucosamine component **19**.[14] Removal of the trityl group of **15** afforded **16**, which was reprotected with a chloroacetyl (ClAc) group to give **17**. Conversion of the phenylthio group to fluoride was carried out by using *N*-bromosuccinimide (NBS) and diethylaminosulfur trifluoride (DAST)[15] to afford **18** in nearly quantitative yield ($\alpha/\beta = 46{:}54$). Coupling with **19** was then conducted through activation with $[Cp_2HfCl_2]$ and AgOTf[16] in toluene to afford **20**, which was then converted into fluoride **11**.

The synthesis of the trimannose core is depicted in Scheme 3. Phenylthiomannoside **21**[17] was silylated and chloroacetylated to give **22** in 93% yield. The nitro-modified Wang-resin-type linker was introduced with **23**[18] under activation with *N*-iodosuccinimide (NIS) and triflic acid (TfOH),[19] to afford **24** in 92% yield. Removal of the allyl group afforded phenol **25**, to which PEG was introduced under standard Mitsunobu conditions to provide **12**. Following the selective deprotection with hydrazinedithiocarbonate (HDTC),[20] the resultant **26** was glycosylated sequentially

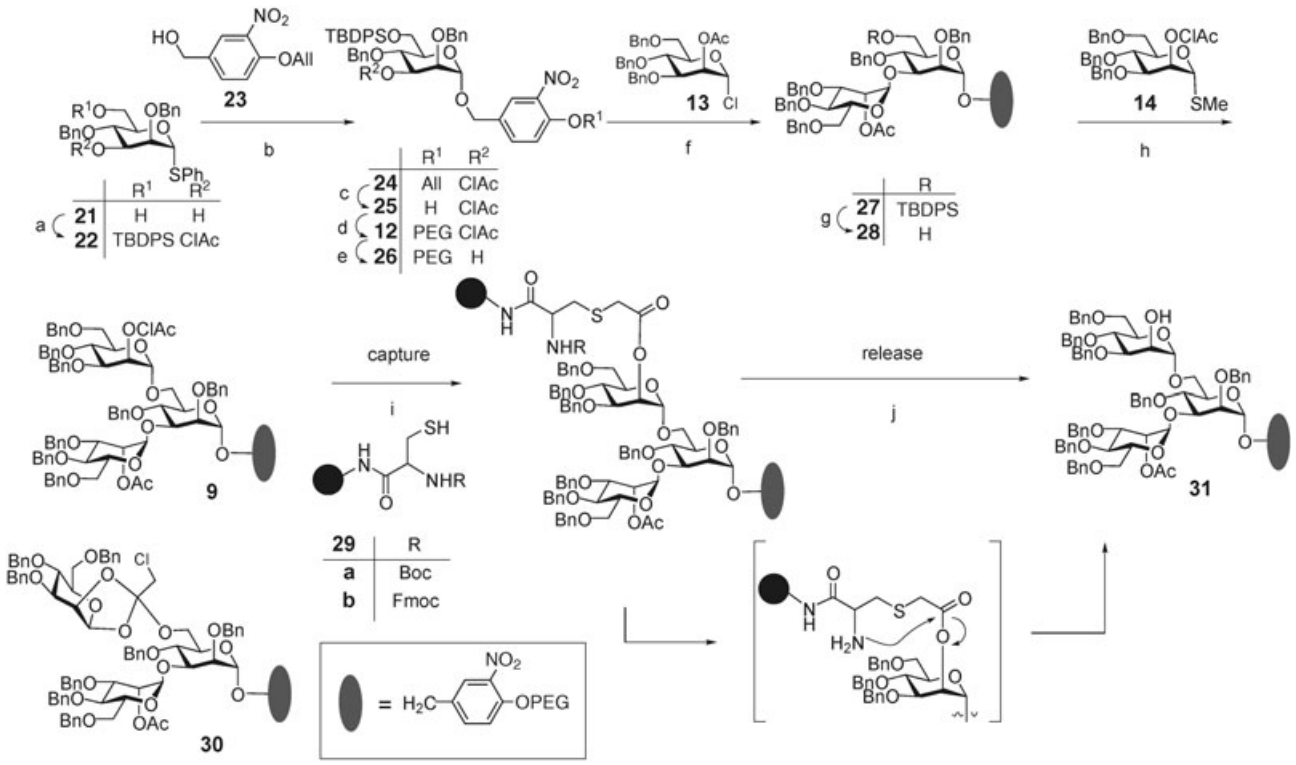


Scheme 2. Reagents and conditions: a) TFA, Et_3SiH, CH_2Cl_2, −40 °C, 96%; b) $(ClAc)_2O$, pyridine, CH_2Cl_2, 96%; c) NBS, DAST, CH_2Cl_2, 98%; d) AgOTf, $[Cp_2HfCl_2]$, molecular sieves (4 Å), toluene, 45 °C, 85%; e) NBS, DAST, CH_2Cl_2, 84%. TFA = trifluoroacetic acid, Cp = cyclopentadienyl.

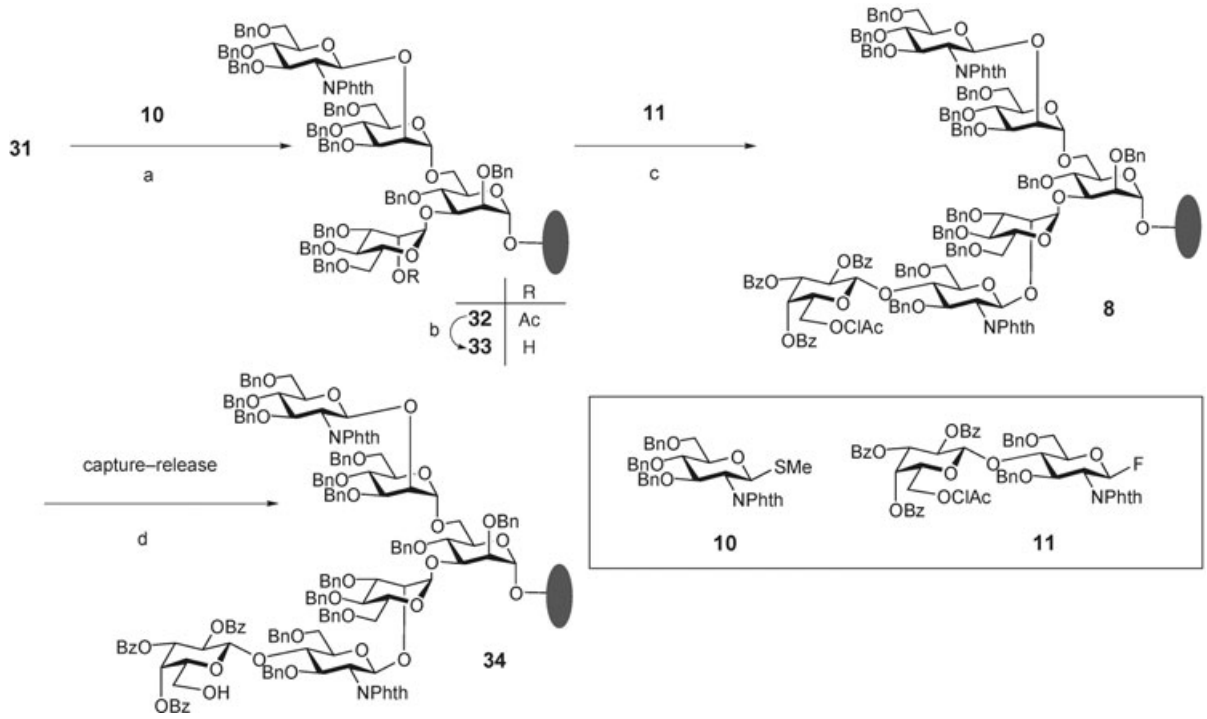
with 2-*O*-Ac- and 2-*O*-ClAc-protected donors **13** and **14**. The initial glycosylation with chloride **13**[21] proceeded smoothly under standard conditions to afford disaccharide **27** with excellent purity in 98% yield,[22] then desilylation produced **28**. Subsequent glycosylation with methylthiomannoside **14**,[11d] when conducted through activation with NIS and TMSOTf (0.2–0.4 equiv) in CH_2Cl_2 (−20 °C), gave a mixture of the desired product **9** [$\delta_H = 5.18$ (d, $J = 1.7$ Hz), 4.99 ppm (d, $J = 1.7$ Hz)] and the corresponding orthoester **30** [$\delta_H =$ 5.25 (d, $J = 2.4$ Hz), 5.19 ppm (s)] in variable ratios (1:5–1:0.7). In contrast, the use of a stoichiometric amount of TfOH with NIS at low temperatures drastically suppressed formation of the orthoester and provided **9** as the major product. Capture–release purification was then carried out by using Merrifield resin loaded with *N*-protected (Boc or Fmoc) Cys (**29a**,**b**)[11d] in the presence of excess *i*Pr_2NEt to specifically capture **9**. We found that Boc-protected **29a** was more expedient than the Fmoc-protected **29b** for the chemoselective reaction. Notably, orthoester **30** did not react under these conditions and remained in the solution phase, along with unreacted **9**. Removal of the Boc group was carried out with 10% TFA in CH_2Cl_2. Treatment with 10% piperidine in THF initiated the cyclo-release to afford product **31** (60% overall yield from **12**).

The construction of hexasaccharide **8** is shown in Scheme 4. For the elongation of the α(1,6) branch, thioglycoside **10**[23] was used as the donor to afford **32**,[22] which was deacetylated to give **33**. Reaction with LacNAc donor **11** with $[Cp_2HfCl_2]$ and AgOTf gave hexasaccharide **8** in 92% yield, which was subjected to the same capture–release purification used for **31** to afford **34** in 81% yield (from **31**).

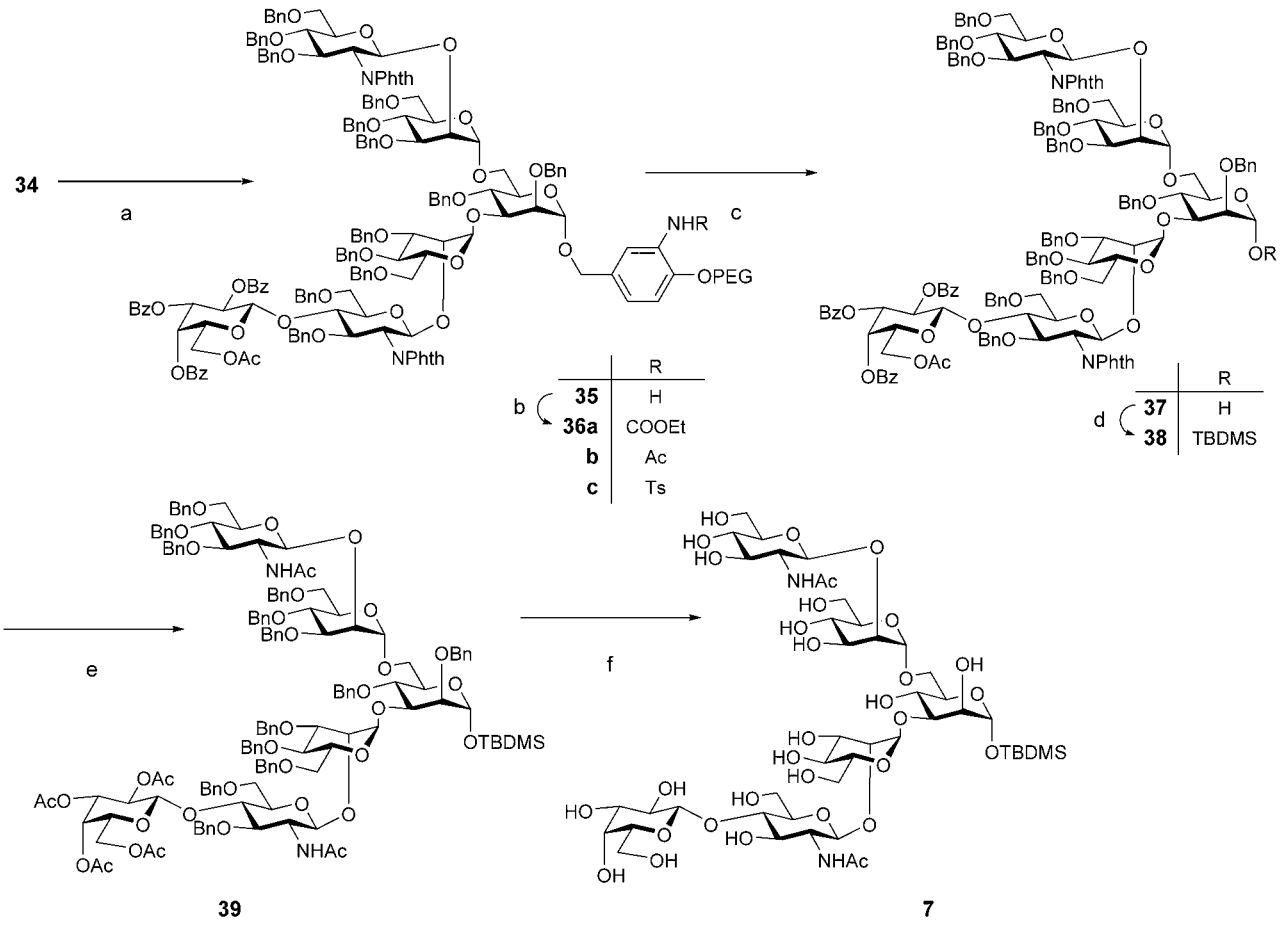
Cleavage from the linker and global deprotection are depicted in Scheme 5. After acetylation of **34**, the nitro group was reduced to an amine function by using $Mo(CO)_6$.[24]



Scheme 3. Reagents and conditions: a) 1) TBDPSCl, DMAP, Et_3N, DMF; 2) $(ClAc)_2O$, pyridine, CH_2Cl_2, 93%; b) NIS, TfOH, molecular sieves (4 Å), CH_2Cl_2, 4°C, 92%; c) $[Pd(PPh_3)_4]$, Et_3SiH, AcOH, toluene, 98%; d) HO-PEG-OMe, DEAD, Ph_3P, THF, 94%; e) HDTC (0.42 M), CH_3CN, 98%; f) **13**, AgOTf, CH_2Cl_2, molecular sieves (4 Å), −20→0°C, 98%; g) HF-pyridine, THF, 98%; h) **14**, NIS, TfOH, molecular sieves (4 Å), −40°C, 94%; i) iPr_2NEt, CH_3CN/CH_2Cl_2; j) 1) 10% TFA in CH_2Cl_2, 2) 10% piperidine, THF, 60% from **12**. All = allyl, DEAD = diethyl azodicarboxylate, DMAP = 4-(*N*,*N*-dimethylamino)pyridine, DMF = dimethylformamide.



Scheme 4. Reagents and conditions: a) **10**, NIS, TfOH, molecular sieves (4 Å), CH_2Cl_2, −10→0°C, 92%; b) NaOMe (0.05 M) in MeOH/THF, 96%; c) **11**, AgOTf, $[Cp_2HfCl_2]$, toluene, molecular sieves (4 Å), −20→0°C, 92%; d) 1) **29**, iPr_2NEt, DMF, 80°C, 2) TFA (10%) in CH_2Cl_2, 3) piperidine (10%) in THF, 88% from **31**.

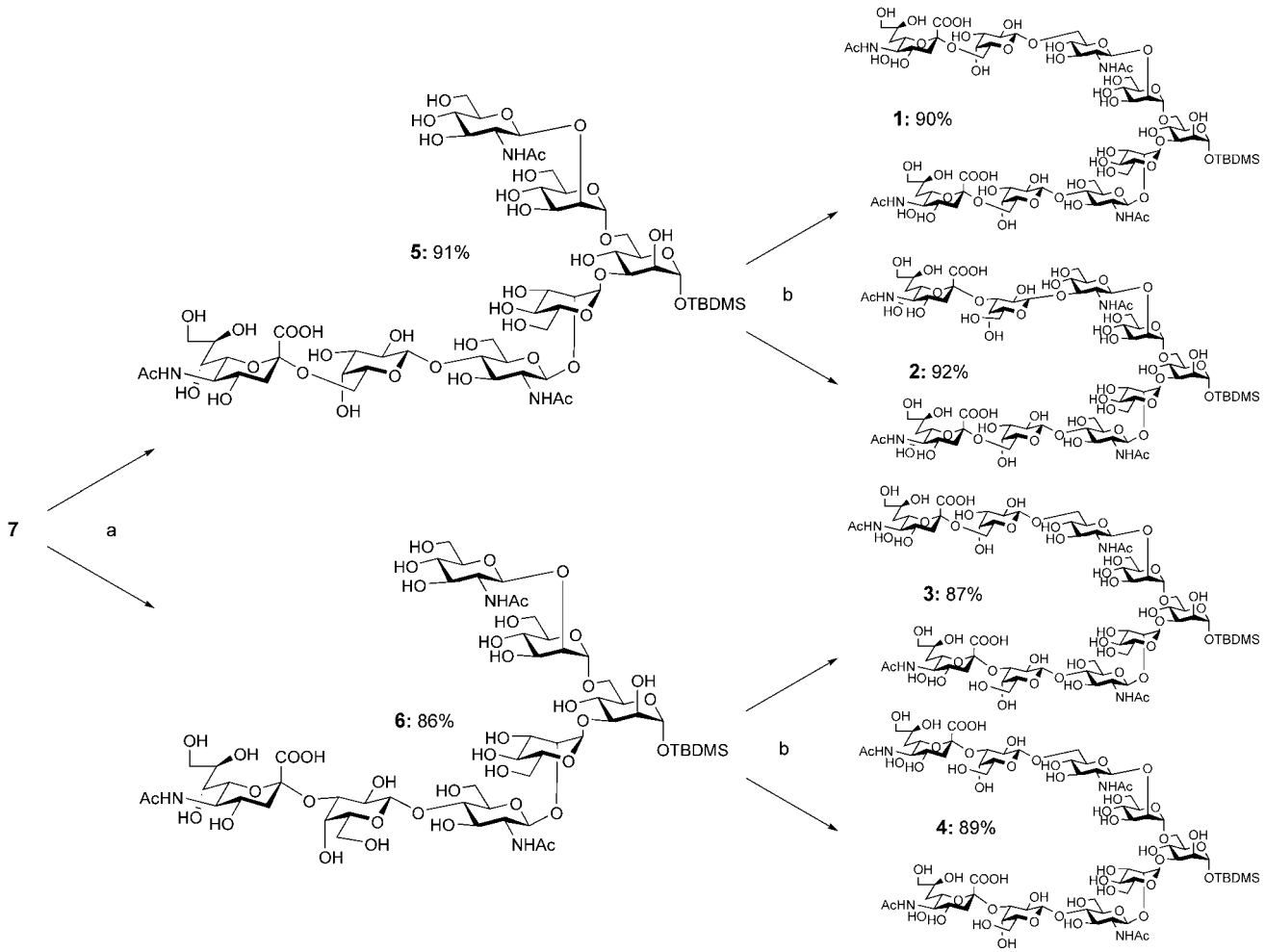


Scheme 5. Reagents and conditions: a) 1) Ac_2O, pyridine, 2) $Mo(CO)_6$, EtOH, dichloroethane; b) EtOCOCl, iPr_2NEt, CH_2Cl_2; c) TFA (15%) in CH_2Cl_2, 88%; d) BTBSA, TBAF (0.05 equiv) in THF-NMP; e) 1) ethylenediamine, *n*BuOH, 95°C; 2) Ac_2O, pyridine, 56%; f) 1) NaOMe (0.05 M) in MeOH, 93%; 2) $Pd(OH)_2/C$ (20%), MeOH, H_2O, 92%. Ts = tosyl.

Amine **35** was then converted into ethyl carbamate **36a**, then treated with 15% TFA in CH_2Cl_2 to give **37** in 88% yield (from **34**). Interestingly, the corresponding acetyl **36b** and tosyl **36c** derivatives were more resistant to cleavage under these conditions. The liberated hydroxy group was protected as a *tert*-butyldimethylsilyl (TBDMS) ether with *N*,*O*-bis(*tert*-butyldimethylsilyl)acetamide (BTBSA) and a catalytic amount of tetrabutylammonium fluoride (TBAF)[25] in *N*-methylpyrrolidinone (NMP) to yield **38**. Following conversion of the phthalimide **38** to the acetamide **39**, the acetyl and benzyl groups were removed to afford **7**.

As several types of sialyltransferases are commercially available, and chemical sialylation is technically demanding, enzymatic glycosylation is an attractive strategy for the preparation of oligosaccharides that contain sialic acids.[26] Hexasaccharide **7** possesses a terminal LacNAc residue, which is an excellent substrate for sialyltransferases. In fact, **7** was smoothly converted into **5** and **6** with α(2,6)-*N*-sialyltransferase (EC 2.4.99.1, Toyobo) and α(2,3)-*N*-sialyltransferase (EC 2.4.99.5, Calbiochem), respectively, then subjected to sequential galactosylation/sialylation[27] to afford **1**–**4** (Scheme 6). As an example, heptasaccharide **5** was treated with β(1,4)-galactosyltransferase (EC 2.4.1.22, Toyobo) and UDP-galactose in $MnCl_2$ containing a cacodylate buffer; upon completion of the galactose transfer, α(2,6)- or α(2,3)-sialyltransferase and CMP-Neu5Ac were added to the reaction mixture to give nonasaccharides **1** and **2** in quantitative yields (CMP = cytidine-5′-monophosphoryl). Similarly, heptasaccharide **6** was used to afford nonasaccharides **3** and **4** in high yields. During the enzymatic transformations, the TBDMS group served as a hydrophobic tag that allowed product isolation by using reversed-phase solid-phase extraction cartridges.[28]

In summary, the α(2,3)- or α(2,6)-sialylated biantennary glycans **1**–**6**, which correspond to the branched portions of typical complex-type *N*-glycans, were systematically synthesized with a polymer–resin hybrid strategy and enzymatic glycosylation. By using low molecular weight (average $M_W \approx 750$) monomethyl polyethylene glycol as the polymer support, the purification procedures were simple and involved only loading on silica gel, washing to remove the non-PEG-supported materials (e.g. excess donor), then eluting with polar solvents. Introduction of sialic acid on the nonreducing ends, and of the penultimate galactose moiety on the (1,6) branch was successful with commercially available glycosyltransferases and the appropriate sugar nucleotides. The common precursor **7** was synthesized by using a soluble polymer support strategy, in which Merrifield resin loaded



Scheme 6. Enzymatic transformations of **7** to **1**–**6**: a) α2,6- or α2,3-sialyltransferase, CMP-Neu5Ac, alkaline phosphatase, BSA, cacodylate buffer (50 mM); b) 1) β1,4-galactosyltransferase, UDP-galactose, BSA, alkaline phosphatase, $MnCl_2$, cacodylate buffer (50 mM); 2) α2,6- or α2,3-sialyltransferase, CMP-Neu5Ac. UDP = uridine diphosphate.

with Boc-Cys was used for the capture–release protocol to facilitate product purification. Hexasaccharide **7** was diverged to monosialylated **5** and **6** and disialylated **1**–**4** saccharides by enzymatic glycosylation with α(2,3)- or α(2,6)-sialyltransferase and β(1,4)-galactosyltransferase.

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