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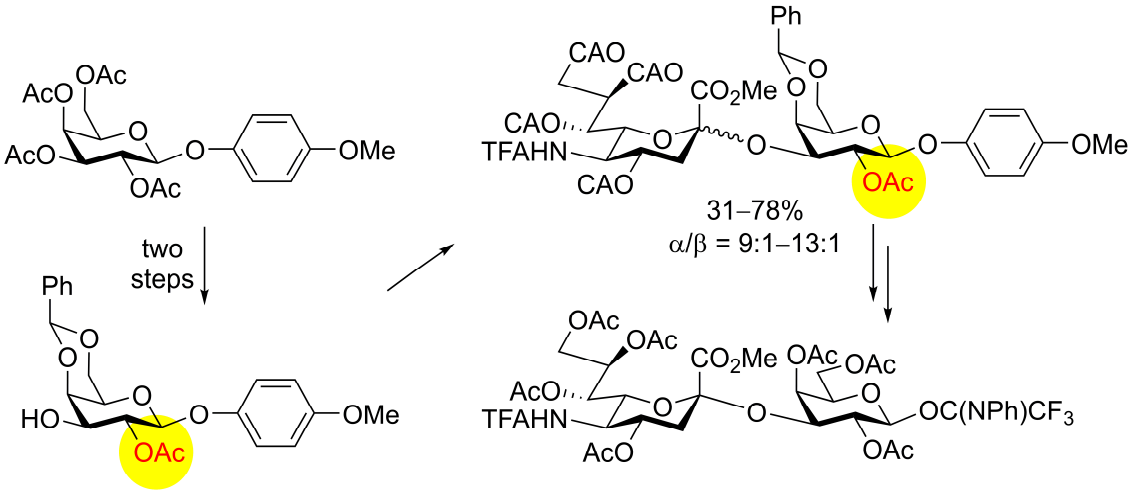

The use of the novel glycosyl acceptor and supramer analysis in the synthesis of sialyl-α(2-3)-galactose building block

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Abstract

A new glycosyl acceptor to be used in sialylation was designed as a 3-hydroxy derivative of 4-methoxyphenyl β-D-galactopyranoside with 2-*O*-acetyl group and O-4 and O-6 protected as benzylidene acetal. Two alternative syntheses of this compound were compared. Sialylation of 3-OH group of the glycosyl acceptor with O-chloroacetylated *N*-trifluoroacetylneuraminic acid phenyl thioglycoside (NIS, TfOH, MeCN, MS 3 Å, –40 °C) was studied in a wide concentration range (2–150 mmol·L^{-1}). The outcome of sialylation generally followed the predictions of supramer analysis of solutions of sialyl donor in MeCN, which was performed by polarimetry and static light scattering and revealed two concentration ranges differing in solution structure and the structures of supramers of glycosyl donor. The optimized conditions of sialylation (*C* =50 mmol·L^{-1}) were used to synthesize protected Neu-α(2-3)-Gal disaccharide (78%, α:β = 13:1), which was then converted to sialyl-α(2-3)-galactose imidate building block useful for the synthesis of complex sialo-oligosaccharides.

Keywords: Neuraminic acid; Glycosylation; Sialylation; Concentration; Reactivity; Supramer approach

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1. Introduction

Sialic acid-containing glycoconjugates are ubiquitously and abundantly present at the surface of all cell types in various organisms and involved in a wide range of biological phenomena ranging from cell–cell adhesion and mobility to recognition by viruses and bacteria [1-7]. Tremendous efforts have been made in order to develop efficient methods for stereoselective synthesis of α-sialosides by chemical glycosylation (sialylation), and, therefore, more efficient assembly of sialo-oligosaccharides and sialo-conjugates. Unfortunately, reliable introduction of sialic acid residues into oligosaccharides remains quite a difficult problem and poor predictability and reproducibility of yield and stereoselectivity are still typical of the sialylation reaction (for leading references see the latest reviews [8-10]; a comprehensive list of references can be found in a recent publication [11]). For this reason, a detour is commonly used that during sialo-oligosaccharide assembly utilizes disaccharide building blocks, which contain sialic acid residue at the non-reducing end and are prepared either by chemical or enzymatic methods (see [12-26] and references cited therein). The main advantage of such an approach is the minimization of a number of difficult sialylation steps. Since the sialyl-α(2-3)-galactose disaccharide fragment is often found at the non-reducing end of sialo-glycans, especially appealing is the use of sialyl-α(2-3)-galactose [Neu-α(2-3)-Gal] building blocks with removable protective group at the N-5 of sialic acid residue which can be used in the divergent synthesis of various *N*-acyl (including *N*-acetyl and *N*-glycolyl) derivatives of sialo-oligosaccharides with the terminal sialic acid residue connected to O-3 of galactose [13-15, 18-22, 24-26].

Clearly, for the preparation of sialyl-α(2-3)-galactose block two monosaccharide units are required: a sialyl donor and a galactosyl acceptor. As in other glycosylations [27], each combination of sialyl donor and glycosyl acceptor, which in case of sialylation may have one or several (up to four) hydroxy groups [28], is unique, the outcome of sialylation depending on many variables [11, 29-34] including the nature of protective groups on both partners [22, 23,

35] and concentration of reagents [30, 32, 33, 36]. While most studies on sialylation extensively optimize the nature of glycosyl donors, only limited attention has been given to the design of efficient nucleophiles for use in sialylation reactions. Not only should the reactivity of a glycosyl acceptor match that of glycosyl donor [37], but, for practical reasons, the former also needs to be preparatively accessible in good overall yield, preferably in minimal number of steps.

In this communication, we describe a short synthesis of a novel readily available glycosyl acceptor with one hydroxy group at C-3 of galactose, demonstrate its utility in sialylation reaction and accomplish an efficient synthesis of the sialyl-α(2-3)-galactosyl imidate building block.

2. Results and discussion

2.1. Synthesis of glycosyl acceptor

2.1.1. Design of glycosyl acceptor

The new glycosyl acceptor was designed as a 3-hydroxy derivative of 4-methoxyphenyl β-D-galactopyranoside **6** with 2-*O*-acetyl group and O-4 and O-6 protected as benzylidene acetal. Galactose-based glycosyl acceptors with 4,6-*O*-benzylidene groups have already been used in sialylation [14, 15, 20, 38]. Unlike benzoyl group, acetyl group only mildly deactivates the vicinal hydroxy group,[2,3] and we expected glycosyl acceptor **6** to be reactive enough to be readily glycosylated with conventional sialyl donors rather than with more sophisticated sialyl donors [20, 44-46] that often require very low temperatures. The presence of participating 2-*O*-

[2] For a discussion on this issue, see [39]. Benzoyl group is more electron-withdrawing than acetyl group since benzoic acid is somewhat more acidic (pK_a 4.17) than acetic acid (pK_a 4.76). This can lead to decreased nucleophilicity of the vicinal hydroxy group at C-3.

[3] Note that all neighboring acyl groups (including acetyl group at O-2) deactivate 3-OH of galactose often leading to diminished yields of silaylation [40]. For this reason, the use of O-benzylated glycosyl acceptors in sialylation is a more common practice [38, 41-43]. This issue is rather complex and a balanced discussion of the influence of the nature and position of protective groups in glycosyl acceptor on the outcome of sialylation is clearly beyond the scope of this publication.

acetyl group would ensure 1,2-*trans*-stereochemistry in subsequent glycosylations with prospective sialyl-α(2-3)-galactose building block [12-14, 16, 18, 19]. 4-Methoxyphenyl aglycon has been used to protect anomeric position in glycosyl acceptor during sialylation step [25, 32] and then cleaved under oxidative conditions to give hemiacetal thus allowing transformation to a variety of disaccharide glycosyl donors [18, 21].

2-O-Substituted 4,6-*O*-benzylidene-β-D-galactopyranosides cannot be prepared by selective protection of O-2 in 4,6-*O*-benzylidene-protected 2,3-diequatorial diols [4]. A feasible approach to such derivatives can rely on introduction of benzylidene group into a 3,4,6-triol with the desired protective group at O-2.

Scheme 1 here

*2.1.2. Preparation of 2-O-acetyl derivative **5** in one step by selective deacetylation*

We have recently reported that 2-*O*-acetyl aryl glucopyranosides can be prepared in one-step from per-O-acetylated aryl glycosides with β-*gluco*-configuration by selective O-deacetylation under acidic conditions [48, 49]. This method is also applicable for aryl glycosides with β-*galacto*-configuration [49]. Indeed, acid-catalyzed deacetylation (aq HCl, EtOH, $CHCl_3$) [49] of the known 4-methoxyphenyl 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (**1**) [50] readily afforded previously unknown 2-*O*-acetyl galactoside **5** in a good yield (55%) (Scheme 1) along with tetraol **2** [50, 51] (40%), which can be re-acetylated to give **1** and then recycled in the deacetylation reaction thus making this route to **5** even more attractive.

*2.1.3. Preparation of 2-O-acetyl derivative **5** in four steps via acetal **4***

[4] Note that migration of acyl groups from O-3 to O-2 under basic conditions can be used for the preparation of 2-*O*-acyl-4,6-*O*-benzylidene-β-D-galactopyranose derivatives [47].

For comparison, a more traditional approach to 2-O-substituted β-D-galactopyranosides was also explored. A reaction of tetraol **2** [50, 51], prepared from tetraacetate **1** [50] by Zemplén deacetylation [52], with 2,2-dimethoxypropane in the presence of CSA under conditions of thermodynamic control [53, 54] gave the known isopropylidene acetal **3** [55, 56] with free hydroxy group at C-2, which was further acetylated to give fully protected derivative **4**. Cleavage of acetal groups with trifluoroacetic acid (TFA) [56] gave 2-*O*-acetyl glycoside **5** (Scheme 1) identical to that prepared by one-step procedure (see section 2.1.2). Although the four-step procedure gave slightly higher overall yield of the target compound **5** (63%) it requires much more time (more than 5 days) and effort in comparison to the one-step synthesis described in section 2.1.2.

*2.1.4. Preparation of glycosyl acceptor **6***

The prepared galactoside **5** with one acetyl group at O-2 then was converted by reaction with $PhCH(OMe)_2$ in the presence of TsOH in MeCN to 4,6-*O*-benzylidene derivative **6** (83%) (Scheme 1) that had only one free hydroxy group at C-3 making it a prospective glycosyl acceptor in various glycosylation reactions. Importantly, alcohol **6** is highly soluble in MeCN commonly used for sialylation thus making this compound suitable as glycosyl acceptor[5] that was demonstrated in this study (see section 2.2).

2.2. Sialylation

2.2.1. Design of sialyl donor

[5] Note that although the corresponding derivative with benzoyl group at O-2 is known [47], it has never been used as glycosyl acceptor in sialylation to the best of our knowledge. Similar 2-*O*-benzoyl-4,6-*O*-benzylidene derivatives of galactose with different aglycons have been used as glycosyl acceptors in sialylation [20, 46].

As discussed in section 1, a "good" sialyl donor should contain a cleavable protective group at the N-5 of sialic acid residue that would allow subsequent introduction of the desired *N*-acyl substituent (*e.g.*, *N*-acetyl or *N*-glycolyl). Sialyl donors with *N*-trifluoroacetyl (TFA) protective group have successfully been used in a number of syntheses of naturally occurring sialyl derivatives (see [11] and references cited therein). For this reason, *N*-TFA-sialyl donor **7** [11] with O-chloroacetyl (CA) groups was chosen for testing ability of galactose derivative **6** to act as glycosyl acceptor (Scheme 2). Since glycosylation of primary hydroxy group of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with sialyl donor **7** gave high yield (86%) and good stereoselectivity (α:β = 11:1) [11] it was reasonable to test this sialyl donor in sialylation of a secondary hydroxy group of derivative **6** (it is known that outcome of sialylation with the same glycosyl donor may dramatically depend on the nature of glycosyl acceptor [8-10]).

Scheme 2 here

2.2.2. Supramer analysis of solutions of sialyl donor

2.2.2.1. Basics of the supramer approach

Since concentration of reagents is known to affect glycosylation outcome, one has to choose concentrations of glycosyl donor and glycosyl acceptor. This can be done either by analogy to previous examples of similar reactions or using some rationalization that could suggest possibly optimal conditions, the latter approach being more appealing. For this reason, before performing actual glycosylation experiments we studied, like previously [30, 32, 33, 36], solutions of sialyl donor **7** in MeCN[6] with different concentrations by polarimetry [34, 57, 58][7] and light scattering [32, 34, 59].[8] This so called supramer analysis [36] of solutions is an integral

[6] Sialylation reaction was performed in MeCN. See Experimental (section 4.6) for details.
[7] Polarimetry is extremely sensitive to changes in solution structure (see [36] the detailed discussion).
[8] Glycosyl donor **7** has earlier been used in sialylation only at a single "regular" concentration (50 mmol·L^{-1}) [11], its solutions have never been studied by supramer analysis.

part of an approach, recently proposed by us [11, 29-36, 57-65] (see [33, 34] for the reviews), which explicitly accounts for the structure of a reaction solution and is based on the hypothesis that in many cases the real reactive species in solution are non-covalently-bonded supramolecular aggregates, supramers, rather than isolated molecules of reagents.[9] Changes in solute concentration may influence structure of the corresponding supramers in a step-wise manner [34, 36]. The concentration ranges, where supramers of similar structures hence chemical properties exist, are separated by critical concentrations from other concentration ranges, where differently arranged supramers featured by altered chemical properties (reactivity, selectivity) are formed [34, 36]. The supramer approach was shown to be useful for explanation, prediction and discovery of a series of unexpected phenomena and allowed the development of highly efficient and stereoselective glycosylation reactions with sialyl donors that lead to formation of Neu-α(2-3)-Gal [32, 33] and Neu-α(2-6)-Gal [31, 33] glycosidic linkages found in many natural sialo-oligosaccharides of biological and medical significance.

2.2.2.2. Measurement of optical rotation of solutions of sialyl donor

Analysis of concentration dependence of specific optical rotation (SR, $[\alpha]_D$) of freshly prepared solutions of sialyl donor **7** in MeCN (Fig. 1, *a*) revealed the existence of *two* ranges of concentrations: high concentration range (C = 25–150 mmol·L^{-1}), where the SR values almost do not depend on concentration ($[\alpha]_D$ = –(130–135) deg·dm^{-1}·cm^3·g^{-1}), and low concentration range (C = 2–5 mmol·L^{-1}), where the SR values are noticeably different ($[\alpha]_D$ = –(145–147) deg·dm^{-1}·cm^3·g^{-1}). The SR values measured at 10 mmol·L^{-1} were scattered and for this reason this concentration cannot be unambiguously included in neither range (see error bars in Fig. 1, *a*); it should be rather considered as a border-line concentration between the two ranges, *i.e.* critical concentration.

[9] For the detailed discussion of the foundations of the supramer approach and supramer analysis of solutions as well as the relevant references not cited here see [36].

According to the supramer approach (section 2.2.2.1), which associates the observed differences in SR values of solutions with changes in solution structure (see [36] for the details), this observation suggests *two different types of supramers* to be present in the high (C = 25–150 mmol·L^{-1}) and low (C = 2–5 mmol·L^{-1}) concentration ranges. Basing on previous experience [32, 36], we may expect *two different reactivity patterns* in these two concentration ranges. Only the experiment may reveal, in which concentration range sialyl donor **7** would perform better both in terms of the product yield and sialylation stereoselectivity (see section 2.2.3).

Figure 1 here

2.2.2.3. Measurement of light scattering of solutions of sialyl donor

Our previous studies [32, 57, 59] suggest that *in some cases* different types of supramers, which exist in different concentration ranges (as follows from supramer analysis of solutions by polarimetry, see section 2.2.2.2), can be distinguished also by static or dynamic light scattering (SLS and DLS, respectively). Analysis of intensities of scattered light (SLS) in solutions of sialyl donor **7** was performed in terms of solvent quality, which is an approach normally used for polymers [66-69] and was reported for analysis of structure of solutions of a low-molecular-mass substance [57]. The measured values of scattering intensities were used for construction of the Debye plot (Fig. 1, *b*) in which the slope (equal to the second virial coefficient A_2) [57, 66, 67] indicates the thermodynamic quality of solvent.

Interestingly, the solvent quality changes dramatically with concentration of sialyl donor **7** in MeCN (Fig. 1, *b*). Thus, for solutions with concentrations in the low concentration range (C = 2–5 mmol·L^{-1}) the second virial coefficient $A_2 < 0$ (negative slope) suggesting features of poor solvent characterized by attraction between the solute particles leading to increased solute aggregation and compaction of supramers, whereas for solutions with concentrations in the high

concentration range (C = 25–150 mmol·L^{-1}) A_2 ~ 0 (horizontal line) typical of theta (θ) solvent suggesting that different, less dense, supramers of the solute are formed in this range (assuming the generalizations established for solutions of polymers are applicable in this case too). It is important that these changes from poor to theta solvent correlate well with changes in SR values as the critical point in Fig. 1, *b* (at *ca.* 10–25 mmol·L^{-1}) corresponds to that in Fig. 1, *a* (at 10 mmol·L^{-1}). Even more important is the finding that the high concentration range (C = 25–150 mmol·L^{-1}) corresponds to theta solvent. This means that aggregation and solvation effects are balanced (ideal conditions) and we can expect featureless glycosylation in the high concentration range (C = 25–150 mmol·L^{-1}). In other words, glycosylation performed in this concentration range is expected to provide high yield of disaccharide[10] independently of concentration used.[11] At the same time, glycosylation in the low concentration range (C = 2–5 mmol·L^{-1}) is expected to be low-yielding as the solute molecules are expected to form *tight* supramers (with limited accessibility of the molecules of glycosyl donor **7** located in the supramer core) similar to dense collapsed polymer globules usually formed in poor solvents.

2.2.3. Results of sialylation experiments at different concentrations

Having established the existence of two concentration ranges featured by the presence of different supramers of glycosyl donor, we performed a series of glycosylation experiments of with equimolar amounts of sialyl donor **7** and glycosyl acceptor **6** (Scheme 2) under standard conditions (NIS, TfOH, MeCN, MS 3 Å, –40 °C) at various concentrations that belong to these ranges (Table 1, Fig. 1, *c*).[12] In accordance with the results of supramer analysis of solutions of

[10] Since the molecules of glycosyl donor **7**, incorporated in relatively *loose* supramers that are formed under theta conditions, are accessible for other reagents.

[11] One may speculate that under theta conditions the outcome of glycosylation (including stereoselectivity) would be determined mainly by molecular structure of reagents and follow generalization already found [8-10, 27], while solution structure effects would play minimal role.

[12] We intentionally used equimolar amounts of glycosyl donor **7** and glycosyl acceptor **6** since this experimental design allows easy monitoring the reaction course and correct estimation of time required for the reaction to complete. The use of excess of a sialyl donor is quite a common practice; in such cases, higher yields of

sialyl donor **7** in MeCN (see section 2.2.2), the outcome of sialylation differed considerably when the sialylation reaction was performed at concentrations belonging to the different concentrations ranges. Not surprisingly, the yield of disaccharide **8** formed at concentrations that belong to the high concentration range (C = 25–150 mmol·L^{-1}), *i.e.* under theta conditions, was high (68–78%) and almost did not change with concentration; all reactions were finished within 1 h (Table 1, Fig. 1, *c*). On the contrary, sialylation at concentration 5 mmol·L^{-1}, which belongs to the low concentration range (C = 2–5 mmol·L^{-1}),[13] *i.e.* in poor solvent, was sluggish and gave much lower yield of disaccharide **8** (31%) after 28 h at –40 °C. This remarkable retardation of glycosylation in dilute solution[14] is in accordance (Fig. 1) with prediction made based on the results of supramer analysis of solutions of sialyl donor **7** in MeCN by SLS (see section 2.2.2.3), which suggests that molecules of glycosyl donor **7** located in the core of *tight* supramers, which are formed in dilute solutions (C = 2–5 mmol·L^{-1}) (see section 2.2.2.3), have limited accessibility hence cannot efficiently participate in glycosylation reaction.

Stereoselectivity of sialylation was good (α:β = 9:1 – 13:1) in all cases studied (Table 1) although the α/β ratio of disaccharide **8** had no clear relation to the concentration unlike previously studied examples [32, 36]. Pure α- and β-isomers of disaccharide **α-8** and **β-8** were isolated by silica gel chromatography and extensively characterized (see section 4.6).

Table 1 here

2.2.4. Comparison of results of supramer analysis and glycosylation experiments with previously reported results

glycosylation products are usually obtained in line with general consensus that the competing elimination from a sialyl donor is the main reason for diminished yields in sialylation.

[13] Only one concentration from this range was used as an example. Practically useful glycosylations are normally performed at reasonably high concentrations (50–500 mmol·L^{-1}). Glycosylations in very dilute solutions, although sometimes reported to be advantageous (see [36] for relevant references), are impractical since exceptionally large volumes of solvent are required when the reactions are performed at the millimolar scale.

[14] Similar observations have been reported [32, 36].

Since this is the third example of the use supramer analysis for rational selection of concentrations at which glycosylation is performed, it is reasonable to compare these three cases. Although sialylation with two different glycosyl donors in MeCN was studied only in two (Ref. [32] and this study) of three cases (the third one was arabinofuranosylation in 1,2-dichloroethane [36]), it clear that there are obvious similarities as well as differences.

In all three cases, the glycosylation yields were virtually identical at concentrations greater than a critical concentration found by polarimetry. At these critical concentrations, a plot of SR of solutions of a glycosyl donor (in the solvent used for glycosylation) against concentration experienced a discontinuity – either a minimum [32] or levelling off (Ref. [36] and this study). According to the supramer approach (see section 2.2.2.2, [36, 57] and references cited therein) identical SR values (levelling off at concentrations exceeding critical values) at different concentrations (for the same compound dissolved in the same solvent) suggest similar structures of supramers present at these concentrations, hence similar chemical properties of the solute. For this reason, in these cases (Ref. [36] and this study) we limited ourselves to the study of solutions of glycosyl donor only.[15] However, in the case of a minimum of SR [32] this reasoning was no longer valid. We could no longer ignore the presence of glycosyl acceptor in the reaction mixture and performed additional experiments although this required extra efforts. Using polarimetry and DLS for equimolar mixtures of glycosyl donor and glycosyl acceptor dissolved in MeCN, we were able to reveal formation of hetero-supramers that incorporated the molecules of both glycosyl donor and glycosyl acceptor [32]. Notably, these hetero-supramers were present only at concentrations above the mentioned critical concentration, which was detected for solutions of glycosyl donor, *i.e.* in the concentration range where glycosylation yield did not depend on concentration. Interestingly, in all three cases studied glycosylation yield significantly dropped while the reaction time increased in the low-concentration range (below the critical

[15] Recall that the main aim of using supramer analysis is the rational selection of concentrations for glycosylation. In the mentioned cases the choice of the concentrations was based on data for solutions of glycosyl donor only. As experience suggests this simplified approach can provide effective means for optimization of glycosylation while saving unnecessary efforts required for a more thorough study.

concentration) suggesting lower reactivity of supramers of glycosyl donor formed at these concentrations.

A possibility to use light scattering for studying solutions requires sufficient intensity of scattered light (SLS and DLS) and adequate contrast (difference of refractive indices) between the dispersed particles and the medium (DLS). Unfortunately, these conditions are not always met.[16] It is for this reason, we were able to use light scattering only in two of three cases. We were lucky to use DLS in the first studied case [32] to get information on the size of supramers present in reaction solutions. In this study, however, while optical contrast was small preventing observation of a correlation function of scattered light (DLS), the intensity of scattered light was sufficient for reliable SLS experiments (see section 2.2.2.3). These experiments for the first time allowed us to gain insight into the inner structure of supramers, discriminating tight and loose supramers that differed in their reactivity (see sections 2.2.2.3 and 2.2.3).

To summarize, the use of supramer analysis of solutions of either glycosyl donor or mixtures of glycosyl donor and acceptor (in the reaction solvent) can provide a rational basis for selection of concentrations at which glycosylation is performed.

2.3. Preparation of the sialyl-α(2-3)-galactose imidate building block

The obtained sialyl-α(2-3)-galactose disaccharide block **α-8** has one obvious zest: it can be further functionalized at galactose O-4 or O-6 after selective removal of benzylidene group (see compound **10** in Scheme 3)[17] to give a variety of more complex building blocks which can be then elaborated in a way similar that described below.[18] However, in the present study we elected to convert pure disaccharide **α-8** to a simpler sialyl-α(2-3)-galactose *N*-

[16] These features of light scattering make polarimetry a method of choice for supramer analysis of reaction solutions.
[17] For example, galactose 4,6-diols can be used as glycosyl acceptors.
[18] This direction of research is currently being explored in our laboratories.

phenyltrifluoroacetimidate building block **14** since imidates are widely used for the preparation of oligosaccharides [70] (Scheme 3). Cleavage of benzylidene group under acidic conditions gave diol **10**, all O-acyl groups were removed by treatment with methanolic MeONa to give heptaol **11**, which was acetylated to give heptaacetate **12** (63% over 3 steps) identical to that described by us earlier [18]. Methoxyphenyl anomeric protective group was then oxidatively cleaved from O-acetylated disaccharide **12** to give hemiacetal **13** and imidoyl group was successively introduced to obtain the target sialyl-α(2-3)-galactose imidate building block **14** (66% over 2 steps).

3. Conclusions

In conclusion, a new glycosyl acceptor to be used in sialylation was designed as a 3-hydroxy derivative of 4-methoxyphenyl β-D-galactopyranoside **6** with 2-*O*-acetyl group and O-4 and O-6 protected as benzylidene acetal. Two alternative syntheses of compound **6** were compared. Sialylation of 3-OH group of the glycosyl acceptor with O-chloroacetylated *N*-trifluoroacetylneuraminic acid phenyl thioglycoside **7** (NIS, TfOH, MeCN, MS 3 Å, –40 °C) was studied in a wide concentration range (2–150 mmol·L^{-1}). The outcome of sialylation generally followed the predictions of supramer analysis of solutions of sialyl donor **7** in MeCN, which was performed by polarimetry and static light scattering and revealed two concentration ranges differing in solution structure and the structures of supramers of glycosyl donor **7**. The optimized conditions of sialylation (C =50 mmol·L^{-1}) were used to synthesize protected Neu-α(2-3)-Gal disaccharide **8** (78%, α:β = 13:1), which was then converted to sialyl-α(2-3)-galactose imidate building block **14** useful for the synthesis of complex sialo-oligosaccharides.

4. Experimental

4.1. General Methods

Glycosylation reactions were carried out under argon atmosphere with anhydrous solvents. The reactions were performed with the use of commercial reagents (Aldrich, Fluka, Acros Organics). Anhydrous solvents were purified and dried (where appropriate) according to standard procedures. Ethanol used for partial deacetylation of compound **1** contained 5% of water. MeCN for glycosylation reactions was distilled under argon over P_2O_5 and then over CaH_2 and stored over molecular sieves (MS) 3 Å under argon. Powdered MS 3 Å (Fluka) were activated before the reactions by heating at 220 °C in high vacuum for 24 h and then for 6 h just before use in glycosylation. Gel permeation chromatography was performed in toluene on a column (570 × 25 mm) packed with Bio Beads S-X3 gel (200–400 mesh, Bio-Rad) using a differential refractive index detector (Knauer). Column chromatography was performed on silica gel 60 (40–63 μm, Merck). Thin-layer chromatography was carried out on silica gel 60 F_{254} plates on aluminum foil (Merck). Spots of compounds were visualized under UV light (254 nm) and by heating the plates (at *ca.* 150 °C) after immersion in a 1:10 (v/v) mixture of 85% aqueous H_3PO_4 and 95% EtOH. HPLC analysis was carried out on an Agilent Compact LC instrument equipped with a 150 × 4.6 mm Eclipse Plus C-18 (5 μm) column eluted with a gradient of MeCN (from 0% to 100%) in H_2O (that contained 0.1% trifluoroacetic acid) in 20 min at 0.4 mL/min flowrate and UV detection (220 nm); sample volume was 20 μL. ^1H, ^{13}C NMR spectra were registered for solutions in $CDCl_3$ or CD_3OD on a Bruker AM-300 instrument (300.13 and 75.48 MHz for ^1H and ^{13}C respectively) or on a Bruker AVANCE 600 spectrometer (600.13 and 150.9 MHz for ^1H and ^{13}C, respectively). The ^1H NMR chemical shifts are referred to the residual signal of $CHCl_3$ (δ_H 7.27 ppm), CHD_2OD (δ_H 3.31 ppm), the ^{13}C NMR shifts – to the central line of $CDCl_3$ signal (δ_C 77.00 ppm), CD_3OD signal (δ_C 49.00 ppm). Assignments of the signals in the NMR spectra were performed using 2D-spectroscopy (COSY, HSQC, and HMBC) and DEPT-135 experiments. High resolution mass spectra (electrospray ionization, HRESIMS) were

recorded in a positive ion mode on a Bruker micrOTOF II mass spectrometer for 2×10^{-5} M solutions in MeCN. Optical rotations were measured using a JASCO P-2000 automatic digital polarimeter (Japan).

4.2. Experimental procedure for optical rotation measurements

Optical rotation values were measured with a Jasco P-2000 automatic digital polarimeter at 20 °C in a jacketed glass cell (10 cm length). Special precautions were made to ensure the stability of the instrument and the temperature within the measuring compartment of the instrument and the cell, which was maintained with an accuracy of ±0.1 °C. After the instrument was warmed up for at least 1 h (as experience suggests, after this period the temperature within the instrument remains stable for at least 8–10 h of continuous work) the instrument readings were verified against the quartz standards (α = +21.267 and –21.248).

When studying the concentration dependence of the specific rotation, three solutions were independently prepared for each concentration. Samples were prepared by dissolving the weighed amount of compound **7** in anhydrous MeCN using a 2 mL (or 10 mL for concentration 2 mmol·L^{-1}) volumetric flask immediately before the measurements. The solutions were filtered through a 0.45 μm filter (PTFE, diameter 0.13 mm, Chromafil, Macherey Nagel; separate filters were used for different samples) directly to the measuring cell with a jacket connected a Huber CC-K6 (Exclusive) thermostat. Only after stabilization of temperature of the solution in the cell (typically after 10 min; judged by the readings of built-in Pt100 temperature probe and stabilization of the values of optical rotation) sampling of the data began. The optical rotation was measured continuously (TimeCourse program supplied the Jasco P-2000 polarimeter) for 30 min (1800 sample points with 1 s integration time each). The data obtained were averaged and the corresponding mean value and standard deviation were calculated for each concentration. These data are plotted in Fig. 1, *a* as specific rotation values that were calculated using concentrations (*c*) of the solutions expressed in traditional polarimetric units (g/100 mL) unless

otherwise explicitly stated; in Fig. 1 molar concentrations are shown, which are identical to those indicated in Table 1.

4.3. Experimental procedure for light scattering measurements

For static light scattering (SLS) experiments, three solutions of compound **7** in anhydrous MeCN were independently prepared for each concentration as described in section 4.2 immediately before measurements. Each sample was filtered three times through a 0.45 μm filter, (PTFE, diameter 0.13 mm, Chromafil, Macherey Nagel; separate filters were used for different samples). SLS measurements were performed with an ALV Correlation Goniometer System 5000/6010 (Langen, Germany) at 150° scattering angle using Pyrex cells (1 cm in diameter) and a He–Ne laser (632.8 nm, 23 mW) as the light source. The temperature of the scattering cell was maintained at 20 ± 0.2 °C. Intensities of scattered light were measured by photon counting and are expressed in Hertz. For each solution, scattering intensities were averaged over 20 independent measurements (the total collection time was 20 min for each experiment). Solvent scattering I_0 (I_0 = 7.5 ± 0.1 kHz) was subtracted from total solution scattering I to obtain the excess intensity of scattered light ($I - I_0$). Then the resulting three values for each concentration were averaged and the corresponding mean value and standard deviation were calculated for each concentration. These data were used to construct a Debye plot (Fig. 1, *b*) in which the slope (equal to the second virial coefficient A_2) [66, 67] indicates the thermodynamic quality of solvent (in the polymer sense) [57, 66-69].

*4.4. 4-Methoxyphenyl 2-O-acetyl-β-D-galactopyranoside (**5**)*

4.4.1. Preparation in one step

Crystalline 4-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**1**) [50] (454 mg, 1 mmol) was dissolved in $CHCl_3$ (1 mL) and 95% EtOH (3 mL) was added followed by

36% HCl (1 mL, d = 1.18 g·mL^{-1}). The homogenous reaction mixture was kept at 30 °C for 10 h until HPLC analysis showed that the peak of the target monoacetate **5** (t_R = 9.8 min) became the major one. Anion-exchange resin Amberlite AB-17 (HCO_3^-) was added until pH 7 was achieved then filtered off, the resin was washed with 95% EtOH (20 mL) and the combined filtrate was concentrated under reduced pressure to give the residue that was purified by silica gel column chromatography (gradient $CHCl_3$–95% EtOH, 9:1 → 3:1) to give 4-methoxyphenyl-β-D-galactopyranoside (**2**) [50, 51] as white crystals (114 mg, 40%), which could be recycled, and the target triol **5** obtained as white crystals after crystallization from EtOAc–petroleum ether (180 mg, 55%).

*4.4.2. Preparation in four steps via acetal **4***

Crystalline 4-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**1**) [50] (1.59 g, 3.5 mmol) was suspended in anhydrous MeOH and 0.1 M methanolic MeONa (5 μL, 0.5 μmol) was added and the mixture was stirred at ~20 °C for 18 h, then neutralized with cation-exchange resin Amberlyst 15 (H^+). The resin was filtered off, washed with MeOH (20 mL) and the combined filtrate was concentrated under reduced pressure, the residue was dried *in vacuo* to give crude tetraol **2** [50, 51] (1 g, ~100%; R_f = 0.38, $CHCl_3$–EtOH, 4:1). A mixture of tetraol **2** (1 g, 3.5 mmol) and (1*S*)-(+)-camphorsulfonic acid (17.5 mg, 75 mmol) was dried *in vacuo* for 2 h and 2,2-dimethoxypropane (105 mL, 850 mmol) was added under argon [53, 54]. The reaction mixture was stirred at ~20 °C for 3 days and then quenched with Et_3N (7 mL, 0.05 mol), the mixture was stirred for 30 min, concentrated under reduced pressure, toluene was added and then concentrated (2 × 10 mL). The residue, which contained mainly compound **3** [55, 56] (R_f = 0.67, $CHCl_3$–EtOAc, 5:1), was dissolved in anhydrous pyridine (4 mL, 0.05 mol) followed by addition of Ac_2O (8 mL, 0.085 mol) and stirred at ~20 °C for 38 h. Methanol (8 mL) was added, the mixture was stirred for 15 min, concentrated under reduced pressure, toluene was added and then concentrated (2 × 10 mL). The residue, which contained mainly compound **4** (R_f = 0.33,

petroleum-ether–EtOAc, 7:3), was dissolved in CH_2Cl_2 (20 mL) and the solution was cooled to 0 °C (ice–water bath) and a 10:1 (v/v) mixture of trifluoroacetic acid–water (3.3 mL) was added. The reaction mixture was stirred at ~20 °C for 1 h until TLC showed full conversion of the starting **4** (R_f = 0.90, $CHCl_3$–EtOH, 5:1) to a lower running spot (R_f = 0.50, $CHCl_3$–EtOH, 5:1) and then concentrated under reduced pressure, toluene was added and then concentrated (2 × 10 mL). The residue was purified by silica gel column chromatography (gradient $CHCl_3$–95% EtOH, 9:1 → 3:1) to give triol **5** as a white solid (0.723 g, 63% over three steps).

*4.4.3. Data for 4-methoxyphenyl 2-O-acetyl-β-D-galactopyranoside (**5**)*

Mp 124–125 °C (EtOAc–petroleum ether). $[\alpha]_D^{24}$ + 11.8 (*c* 1.0, 95% EtOH). R_f = 0.50 ($CHCl_3$–95% EtOH, 5:1). ^{1}H NMR (300 MHz, CD_3OD, δ, ppm, *J*, Hz): 2.10 (s, 3H, CH_3CO), 3.68 (ddd, 1H, $J_{4,5}$ 1.0, $J_{5,6a}$ 5.1, $J_{5,6b}$ 6.8, H-5), 3.74 (s, 3H, $C_6H_4OC\underline{H}_3$), 3.75 (dd, 1H, $J_{3,4}$ 3.5, $J_{2,3}$ 9.9, H-3), 3.77 (dd, 1H, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 11.3, H-6a), 3.82 (dd, 1H, $J_{5,6b}$ 6.8, $J_{6a,6b}$ 11.3, H-6b), 3.94 (dd, 1H, $J_{4,5}$ 1.0, $J_{3,4}$ 3.5, H-4), 4.89 (d, 1H, $J_{1,2}$ 8.0, H-1), 5.25 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.9, H-2), 6.80–6.88 (m, 2H, OC_6H_4O), 6.92–7.01 (m, 2H, OC_6H_4O). ^{13}C NMR (75 MHz, CD_3OD, δ, ppm): 21.0 ($\underline{C}H_3CO$), 56.1 ($C_6H_4O\underline{C}H_3$), 62.3 (C-6), 70.4 (C-4), 73.1 (C-3), 73.8 (C-2), 77.1 (C-5), 102.1 (C-1), 115.6, 119.2, 153.0, 156.8 (OC_6H_4O), 172.2 ($CH_3\underline{C}O$). HRESIMS: found *m/z* 367.0788 $[M + K]^+$. Calcd for$C_{15}H_{20}O_8K$: 367.0790.

*4.5. 4-Methoxyphenyl 4,6-O-benzylidene-2-O-acetyl-β-D-galactopyranoside (**6**)*

4-Methoxyphenyl 2-*O*-acetyl-β-D-galactopyranoside (**5**) (0.895 g, 2.7 mmol) was dissolved in anhydrous MeCN (100 mL) and *p*-toluenesulphonic acid monohydrate (23 mg, 0.12 mmol) was added followed by $PhCH(OMe)_2$ (809 μL, 5.4 mmol). The reaction mixture was stirred at ~20 °C for 18 h, Et_3N (50 μL, 0.36 mmol) was then added and the reaction mixture was concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (gradient toluene–95% EtOH, 15:1 → 3:1) gave the title compound **6** as a white

solid (0.944 g, 83%). Mp 104–106 °C. $[\alpha]_D^{24}$ –28.4 (*c* 0.97, $CHCl_3$). R_f = 0.48 (toluene–95% EtOH, 9:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 2.15 (s, 3H, CH_3CO), 2.60 (d, 1H, $J_{OH\text{-}3,3}$ 11.0, OH-3), 3.57 (ddd, 1H, $J_{4,5}$ 0.6, $J_{5,6b}$ 1.3, $J_{5,6a}$ 1.6, H-5), 3.78 (s, 3H,$C_6H_4OC\underline{H}_3$), 3.81 (ddd, 1H, $J_{3,4}$ 3.7, $J_{2,3}$ 9.9, $J_{3\text{-}OH,3}$ 11.0, H-3), 4.10 (dd, 1H, $J_{5,6a}$ 1.6, $J_{6a,6b}$ 12.4, H-6a), 4.26 (dd, 1H, $J_{4,5}$ 0.6, $J_{3,4}$ 3.7, H-4), 4.37 (dd, 1H, $J_{5,6b}$ 1.3, $J_{6a,6b}$ 12.4, H-6b), 4.90 (d, 1H, $J_{1,2}$ 8.0, H-1), 5.33 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.9, H-2), 5.57 (s, 1H, C<u>H</u>Ph), 6.78–6.88 (m, 2H, OC_6H_4O), 6.97–7.06 (m, 2H, OC_6H_4O), 7.35–7.44 (m, 3H, Ph), 7.54 (dd, 2H, *J* 3.1, *J* 6.6, Ph). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 20.9 ($\underline{C}H_3CO$), 55.6 ($C_6H_4O\underline{C}H_3$), 66.7 (C-5), 68.9 (C-6), 71.7 (C-3), 72.0 (C-2), 75.4 (C-4), 100.6 (C-1), 101.5 (<u>C</u>HPh), 114.5, 119.0 (OC_6H_4O), 126.5, 128.3, 129.3, 137.3 (Ph), 151.3, 155.6 (OC_6H_4O), 170.4 ($CH_3\underline{C}O$). HRESIMS: found *m/z* 439.1362 $[M + Na]^+$. Calcd for $C_{22}H_{24}O_8Na$: 439.1303.

4.6. Typical glycosylation procedure

A mixture of thoglycoside sialyl donor **7** [11] (77.5 mg, 0.1 mmol) and alcohol **6** (41.6 mg, 0.1 mmol) was dried *in vacuo* for 2 h, then anhydrous MeCN (see Table 1 for the concentrations used) was added under argon. Freshly activated powdered MS 3 Å (Fluka; 100 mg per 1 mL of MeCN) were added to the resulting solution. The suspension was stirred under argon at ~20 °C overnight (15 h), then cooled to –40 °C (MeCN–solid CO_2 bath). Solid NIS (53.4 mg, 0.3 mmol) (dried *in vacuo* for 2 h) was added under argon followed by neat TfOH (2 μL, 0.02 mmol). Persistent iodine color became visible in 1–2 min. The reaction mixture was stirred under argon at –40 °C until TLC showed complete consumption (see Table 1 for reaction times) of the starting thioglycoside **7** (R_f = 0.78, toluene–acetone, 5:1).[19] Saturated aq $NaHCO_3$ (2 mL) was added and the reaction mixture was diluted with CH_2Cl_2 (20 mL), stirred for 5 min, filtered through Celite pad, the solids were additionally washed with CH_2Cl_2 (100 mL). The combined

[19] Complete consumption of the starting thioglycoside **7** (40–60 min for experiments performed at 50–150 mmol·L^{-1} concentrations, entries 2–5 in Table 1) was accompanied by appearance of green color of the reaction mixture. Note that reaction performed in dilute solution (5 mmol·L^{-1}, entry 1 in Table 1) did not turn green, iodine color persisted.

filtrate was washed with satd aq $NaHCO_3$ (50 mL), 1.2 M aq $Na_2S_2O_3$ (2 × 50 mL), water (2 × 50 mL), dried over Na_2SO_4, filtered through a cotton wool plug, concentrated under reduced pressure, toluene was added and then concentrated (2 × 10 mL). The residue was dried *in vacuo*, dissolved in toluene (2 mL) and separated by gel permeation chromatography on Bio-Beads S-X3 (toluene). The first eluted fraction contained disaccharide **8**, which was analyzed by NMR spectroscopy to give anomeric ratio values (α:β, see Table 1; for determination of ratio of anomers of disaccharide **8** integral intensities of signals of α-H-3eq and β-H-3eq of sialic acid residue were used). The disaccharide fraction was chromatographed on a silica gel 60 column (gradient toluene → toluene–acetone, 5:1) to give pure α- and β-isomers of disaccharide **8** as white solids. For the yields obtained at different concentrations, see Table 1 and Fig. 1, *c*.

*4.6.1. Data for methyl [4-methoxyphenyl 2-O-acetyl-4,6-O-benzylidene-3-O-(4,7,8,9-tetra-O-chloroacetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-β-D-galacto-nonulopyranosyl)-β-D-galactopyranosid]onate (**α-8**).*

$[\alpha]_D^{24}$ + 4.9 (*c* 1.0, $CHCl_3$). R_f = 0.30 (toluene–acetone, 5:1). ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 1.88 (dd~t, 1H, $J_{3eq,3ax}$= $J_{3ax,4}$ 12.8, H-3ax^{II}), 2.21 (s, 3H, CH_3CO), 2.84 (dd, 1H, $J_{3eq,4}$ 4.6, $J_{3eq,3ax}$ 12.8, H-3eq^{II}), 3.67 (ddd~tq, 1H, $J_{4,5}$ 1.0, $J_{5,6a}$=$J_{5,6b}$ 1.5, H-5^{I}), 3.72 (s, 3H,CO_2CH_3), 3.78 (s, 3H, $C_6H_4OC\underline{H}_3$), 3.86 (dd, 1H, $J_{4,5}$ 1.0, $J_{3,4}$ 3.7, H-4^{I}), 3.97 (d, 1H, *J* 14.9, CH_2Cl), 4.01 (d, 1H, *J* 14.9, CH_2Cl), 4.02 (s, 2H, CH_2Cl), 4.09 (dd, 1H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.4, H-6a^{I}), 4.11 (d, 1H, *J* 14.1, CH_2Cl), 4.12–4.15 (m, 2H, H-5^{II}, H-6^{II}), 4.14 (dd, 1H, $J_{8,9a}$ 5.8, $J_{9a,9b}$ 12.7, H-9a^{II}), 4.15 (d, 1H, *J* 14.1, CH_2Cl), 4.21 (d, 1H, *J* 15.7, CH_2Cl), 4.35 (dd, 1H, $J_{5,6b}$ 1.5, $J_{6a,6b}$ 12.4, H-6b^{I}), 4.45 (d, 1H, *J* 15.7, CH_2Cl), 4.46 (dd, 2H, $J_{3,4}$ 3.7, $J_{2,3}$ 10.1, H-3^{I}), 4.54 (dd, 1H, $J_{8,9b}$ 2.4, $J_{9a,9b}$ 12.7, H-9b^{II}), 5.00 (ddd, 1H, $J_{3eq,4}$ 4.6, $J_{3ax,4}$ 12.8, $J_{4,5}$ 9.8, H-4^{II}), 5.09 (d, 1H, $J_{1,2}$ 8.0, H-1^{I}), 5.39 (s, 1H, C$\underline{H}$Ph), 5.42 (dd, 2H, $J_{6,7}$ 2.4, $J_{7,8}$ 9.5, H-7^{II}), 5.42 (dd, 2H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1, H-2^{I}), 5.72 (ddd, 1H, $J_{8,9b}$ 2.4, $J_{8,9a}$ 5.8, $J_{7,8}$ 9.5, H-8^{II}), 6.64 (d, 1H, $J_{5,NH}$ 9.1, NH), 6.81–6.85 (m, 2H, OC_6H_4O), 7.01–7.06 (m, 2H, OC_6H_4O), 7.33–7.41 (m, 3H, Ph), 7.51 (dd, 2H,

J 1.7, *J* 7.6, Ph). ^{13}C NMR (151 MHz, $CDCl_3$, δ, ppm): 21.1 ($CO\underline{C}H_3$), 38.0 (C-3^{II}), 40.2, 40.2, 40.5, 41.6 (CH_2Cl), 49.8 (C-5^{II}), 53.4 ($CO_2\underline{C}H_3$), 55.7 ($C_6H_4O\underline{C}H_3$), 63.4 (C-9^{II}), 66.1 (C-5^{I}), 68.1 (C-2^{I}), 68.7 (C-8^{II}), 69.1 (C-6^{I}), 69.3 (C-7^{II}), 69.8 (C-4^{II}), 71.4 (C-6^{II}), 72.4 (C-3^{I}), 73.5 (C-4^{I}), 96.8 (C-2^{II}), 100.6 (C-1^{I}), 101.1 (<u>C</u>HPh), 113.3 (q, *J* 287.3, CF_3), 114.5, 119.0 (OC_6H_4O), 126.3, 128.2, 130.9, 137.5 (Ph), 151.4, 155.5 (OC_6H_4O), 158.0 (q, *J* 39.4, <u>C</u>OCF_3), 166.5, 167.0, 167.2, 167.4 ($ClCH_2\underline{C}O$), 168.7 (C-1^{II}), 169.7 ($CH_3\underline{C}O$). HRESIMS: found *m/z* 1102.1155 [M + Na]$^+$. Calcd for $C_{38}H_{42}F_3NO_{16}Na$: 1102.1055.

*4.6.2. Data for methyl [4-methoxyphenyl 2-O-acetyl-4,6-O-benzylidene-3-O-(4,7,8,9-tetra-O-chloroacetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-β-D-galacto-nonulopyranosyl)-β-D-galactopyranosid]onate (**β-8**).*

$[\alpha]_D^{29}$ + 14.3 (*c* 1.0, $CHCl_3$). R_f = 0.45 (toluene–acetone, 5:1). ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 1.90 (dd, 1H, $J_{3ax,4}$ 11.6, $J_{3ax,3eq}$ 13.0, H-3ax^{II}), 2.14 (s, 3H, CH_3CO), 2.64 (dd, 1H, $J_{3eq,4}$ 4.7, $J_{3eq,3ax}$ 13.0, H-3eq^{II}), 3.72 (ddd~dd, 1H, $J_{5,6b}$ 1.4, $J_{5,6a}$ 2.7, H-5^{I}), 3.78 (s, 3H, $C_6H_4OC\underline{H}_3$), 3.88 (s, 3H, CO_2CH_3), 3.92 (d, 1H, *J* 14.7, CH_2Cl), 3.96 (d, 1H, *J* 14.7, CH_2Cl), 4.04–4.09 (m, 2H, CH_2Cl), 4.08–4.20 (m, 8H, C-3^{I}, 2× CH_2Cl, H-6a^{I}, H-9a^{II}, H-5^{II}), 4.37 (dd, 2H, $J_{5,6b}$ 1.4, $J_{6a,6b}$ 12.7, H-6b^{I}), 4.38 (dd, 1H, $J_{6,7}$ 2.7, $J_{6,5}$ 10.5, H-6^{II}), 4.48 (dd, 1H, $J_{4,5}$ 0.9, $J_{3,4}$ 3.9, H-4^{I}), 4.96 (d, 1H, $J_{1,2}$ 8.1, H-1^{I}), 5.34 (dd, 1H, $J_{7,8}$ 2.0, $J_{6,7}$ 2.7, H-7^{II}), 5.39 (ddd, 1H, $J_{3eq,4}$ 4.7, $J_{4,5}$ 10.4, $J_{3ax,4}$ 11.6, H-4^{II}), 5.45 (dd, 1H, $J_{8,9b}$ 2.6, $J_{9a,9b}$ 12.4, H-9b^{II}), 5.50 (dd, 1H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.0, H-2^{I}), 5.58 (ddd, 1H, $J_{7,8}$ 2.0, $J_{8,9b}$ 2.6, $J_{8,9a}$ 9.5, H-8^{II}), 5.70 (s, 1H, PhC<u>H</u>), 6.80–6.86 (m, 3H, OC_6H_4O, NH), 6.99–7.03 (m, 2H, OC_6H_4O), 7.41–7.49 (m, 3H, <u>Ph</u>), 7.60–7.63 (m, 2H, <u>Ph</u> (H-2, H-6)). ^{13}C NMR (151 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 20.9 ($\underline{C}H_3CO$), 37.9 (C-3^{II}), 40.1, 40.4, 40.5, 40.6 (CH_2Cl), 49.5 (C-5^{II}), 53.2 ($CO_2\underline{C}H_3$), 55.6 ($C_6H_4O\underline{C}H_3$), 63.8 (C-9^{II}), 66.3 (C-5^{I}), 69.1 (C-6^{I}), 69.3, 69.4 (C-2^{I}, C-4^{II}), 70.8 (C-7^{II}), 72.4 (C-6^{II}), 73.6 (C-8^{II}), 74.9 (C- (C-2, C-6)), 75.5 (C-3^{I}), 100.3 (C-2^{II}), 100.5 (C-1^{I}), 101.1 (Ph<u>C</u>H), 114.5, 119.1 (OC_6H_4O), 126.2 (<u>Ph</u> (C-2, C-6)), 128.7 (Ph (C-3, C-5)), 129.8 (Ph (C-4)), 137.5 (Ph (C-1)), 151.2 (OC_6H_4O (C-1)),

155.7 (OC_6H_4O (C-4)), 158.0 (q, *J* 37.4, $CF_3\underline{C}O$), 166.0 (C-1^{II}), 166.4, 167.0, 167.6, 167.8 ($ClCH_2\underline{C}O$), 169.4 ($CH_3\underline{C}O$).[20] HRESIMS: found *m/z* 1102.1121 $[M + Na]^+$. Calcd for $C_{38}H_{42}F_3NO_{16}Na$: 1102.1055.

*4.7. Methyl [4-methoxyphenyl 2,4,6-tri-O-acetyl-3-O-(4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-nonulopyranosyl)-β-D-galactopyranosid]onate (**12**).*

A solution of α-isomer of disaccharide **α-8** (53.8 mg, 0.05 mmol) in 80% aq AcOH (1 mL), was heated at 70 °C for 8 h until TLC showed full conversion of the starting **α-8** (R_f 0.54, $CHCl_3$–95% EtOH, 9:1) to a lower running spot (**10**, R_f 0.50, $CHCl_3$–95% EtOH, 9:1). The reaction mixture was concentrated under reduced pressure, toluene was added and then concentrated (2 × 10 mL) to give crude **10** (38.7 mg, 78%) which was used without further purification.

Crude **10** (25 mg, 0.025 mmol) was dissolved in anhydrous MeOH (2 mL) and 1 M methanolic MeONa (10 μL, 0.01 mmol) was added and the mixture was stirred at ~20 °C for 48 h. Then AcOH (10 μL) was added and the mixture was stirred for 15 min, then concentrated under reduced pressure, toluene was added and then concentrated (2 × 10 mL) to give crude **11** (16.1 mg, 99%; R_f 0.47, $CHCl_3$–MeOH, 3:1), which was used without further purification.

Crude **11** (15 mg, 0.023 mmol) was dissolved in anhydrous pyridine (200 μL, 2.49 mmol) and Ac_2O (100 μL, 1 mmol) and stirred at ~20 °C for 15 h. Methanol (100 μL) was added and the mixture was stirred for 15 min, then concentrated under reduced pressure, toluene was added and then concentrated (2 × 10 mL). The residue was purified by silica gel column chromatography (gradient toluene– acetone, 9:1 → 3:1) to give fully O-acetylated disaccharide **12** as a white solid (18 mg, 82%; 63% over three steps). $[\alpha]_D^{24}$ + 2.0 (*c* 1.0, $CHCl_3$). R_f 0.44 (toluene–acetone, 5:1). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 1.75 (dd~t, 1H, $J_{3ax,3eq}$=

[20] The signal of CF_3 group could not be detected due to low signal-to-noise ratio.

$J_{3ax,4}$12.7, H-3ax^{II}), 1.96, 2.01, 2.06, 2.09, 2.13, 2.19, 2.25 (all s, 21H, CH_3CO), 2.67 (dd, 1H, $J_{3ax,3eq}$12.7, $J_{3eq,4}$ 4.6, H-3eq^{II}), 3.78 (s, 3H, $C_6H_4OC\underline{H}_3$), 3.83 (dd, 1H, $J_{6,7}$ 2.4, $J_{5,6}$ 10.7, H-6^{II}), 3.88 (s, 3H, CO_2CH_3), 3.93–4.05 (m, 3H, H-5^{II}, H-5^{I}, H-9a^{II}), 4.08–4.16 (m, 2H, H-6^{I}), 4.38 (dd, 1H, $J_{8,9b}$ 2.0, $J_{9a,9b}$ 12.5, H-9b^{II}), 4.68 (dd, 1H, $J_{3,4}$ 3.1, $J_{2,3}$ 10.0, H-3^{I}), 4.99 (d, 1H, $J_{3,4}$ 3.1, H-4^{I}), 5.05 (ddd, 1H, $J_{4,3eq}$ 4.6, $J_{4,5}$ 10.6, $J_{4,3ax}$ 12.7, H-4^{II}), 5.10 (d, 1H, $J_{1,2}$ 8.0, H-1^{I}), 5.29 (dd, 1H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0, H-2^{I}), 5.35 (dd, 1H, $J_{6,7}$ 2.4, $J_{7,8}$ 9.0, H-7^{II}), 5.56 (ddd~td, 1H, $J_{8,9b}$ 2.0, $J_{7,8}$ = $J_{8,9a}$ 9.0, H-8^{II}), 6.43 (d, 1H, $J_{5,NH}$ 9.4, NH), 6.83 (d, 2H, *J* 9.0, OC_6H_4O), 7.01 (d, 2H, *J* 9.0, OC_6H_4O).^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 20.4, 20.6, 20.6 (2C), 20.7, 20.9, 21.4 ($\underline{C}H_3CO$), 37.4 (C-3^{II}), 49.9 (C-5^{II}), 53.3 ($CO_2\underline{C}H_3$), 55.7 ($C_6H_4O\underline{C}H_3$), 62.0 (C-9^{II}), 62.3 (C-6^{I}), 67.0 (C-4^{I}), 67.5 (C7^{II}), 67.9 (C-8^{II}), 68.6 (C-4^{II}), 69.7 (C-2^{I}), 70.8 (C-3^{I}), 71.3 (C-6^{II}), 71.5 (C-3^{I}), 96.9 (C-2^{II}), 100.2 (C-1^{I}), 114.5 (OC_6H_4O), 115.3 (q, *J* 288.3, CF_3), 118.5 (OC_6H_4O), 151.4 (OC_6H_4O), 155.5 (OC_6H_4O), 157.7 (q, *J* 38.1, $\underline{C}OCF_3$), 167.7 (C-1^{II}), 169.4, 169.6, 170.3 (2C), 170.5, 170.6, 170.7 ($CH_3\underline{C}O$). HRESIMS: found *m/z* 962.2502 $[M + Na]^+$. Calcd for$C_{39}H_{48}F_3NO_{22}Na$: 962.2518.

*4.8. Methyl [2,4,6-tri-O-acetyl-1-O-(N-phenyltrifluoroacetylimidoyl)- 3-O-(4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-nonulopyranosyl)-β-D-galactopyranose]onate (**12**).*

Solution of $(NH_4)_2Ce(NO_3)_6$ (197 mg, 0.361 mmol) in 4:1 (v/v) MeCN–H_2O mixture (0.8 mL) was slowly added to a cold (0 °C, ice–water bath) solution of glycoside **12** (67.6 mg, 0.072 mmol) in 4:1 (v/v) MeCN–H_2O mixture (1.5 mL) and then the reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into aq $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (2 × 50 mL). Combined extracts were washed with brine (50 mL), water (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give hemiacetal **13** (50.4 mg, 84%) as an α/β-mixture which was used without further purification.

Crude hemiacetal **13** (36 mg, 0.043 mmol) was dried *in vacuo* for 2 h, dissolved in CH_2Cl_2 (1.5 mL) then $ClC(NPh)CF_3$ [71] (10.3 μL, 0.064 mmol) and Cs_2CO_3 (21 mg, 0.064 mmol) were added at 0 °C (ice–water bath) under argon. The reaction mixture was stirred at 0 °C (ice–water bath) for 1 h and at ~20 °C for 15 h until TLC showed full conversion of the starting **13** (R_f 0.42, toluene–acetone, 5:1) to a higher running spot (R_f 0.36, toluene–acetone, 5:1) and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (gradient toluene–acetone (+0.1% Et_3N), 9:1 → 3:1; the eluent was made basic by addition of Et_3N (100 μL per 100 mL of eluent)) to give imidate **β-14** as a white solid (34 mg, 78%; 66% over 2 steps). $[\alpha]_D^{25}$ + 0.3 (*c* 0.96, $CHCl_3$). R_f 0.36 (toluene–acetone, 5:1). ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 1.75 (dd~t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12.7, H-3ax^{II}), 1.97, 2.02, 2.11, 2.14, 2.18, 2.26 (all s, 21H, CH_3CO), 2.66 (dd, 1H, $J_{3eq,4}$ 4.7, $J_{3ax,3eq}$ 12.7, H-3eq^{II}), 3.82 (dd, 1H, $J_{6,7}$ 2.7, $J_{5,6}$ 10.7, H-6^{II}), 3.89 (s, 3H, CO_2CH_3), 3.97–4.00 (m, 1H, H-5^{I}), 4.00 (ddd~dd, 1H, $J_{4,5} = J_{5,NH}$ 10.1, $J_{5,6}$ 10.7, H-5^{II}), 4.01 (dd, 1H, $J_{8,9a}$ 6.3, $J_{9a,9b}$ 12.4, H-9a^{II}), 4.06 (dd, 1H, $J_{5,6a}$ 7.6, $J_{6a,6b}$ 11.3, H-6a^{I}), 4.12 (dd, 1H, $J_{5,6b}$ 5.8, $J_{6a,6b}$ 11.3, H-6b^{I}), 4.43 (dd, 1H, $J_{8,9b}$ 2.6, $J_{9a,9b}$ 12.4, H-9b^{II}), 4.73 (dd, 1H, $J_{3,4}$ 3.4, $J_{2,3}$ 9.1, H-3^{I}), 4.99 (d, 1H, $J_{3,4}$ 3.4, H-4^{I}), 5.03 (ddd, 1H, $J_{3eq,4}$ 4.7, $J_{4,5}$ 10.1, $J_{3ax,4}$ 12.7, H-4^{II}), 5.29 (dd~t, 1H, $J_{1,2} = J_{2,3}$ 9.1, H-2^{I}), 5.34 (dd, 1H, $J_{6,7}$ 2.7, $J_{7,8}$ 8.5, H-7^{II}), 5.57 (ddd, 1H, $J_{8,9b}$ 2.6, $J_{8,9a}$ 6.3, $J_{7,8}$ 8.5, H-8^{II}), 5.85 (br.s, 1H, H-1^{I}), 6.41 (d, 1H, $J_{5,NH}$ 10.1, NH), 6.86 (d, 2H, *J* 7.7, Ph), 7.10–7.14 (m, 1H, Ph), 7.31 (dd, 2H, *J* 7.5, *J* 8.4, Ph). ^{13}C NMR (151 MHz, $CDCl_3$, δ, ppm): 20.4, 20.6 (2C), 20.6, 20.7, 21.4 ($\underline{C}H_3CO$), 37.4 (C-3^{II}), 49.9 (C-5^{II}), 53.4 ($CO_2\underline{C}H_3$), 61.6 (C-6^{I}), 62.4 (C-9^{II}), 67.1 (C-4^{I}), 67.3 (C-7^{II}), 68.1 (C-8^{II}), 68.5 (C-4^{II}), 68.8 (C-2^{I}), 71.3 (C-3^{I}), 71.5 (C-6^{II}), 71.6 (C-5^{I}), 95.1 (C-1^{I}), 96.9 (C-2^{II}), 119.4 (PhN (C-2, C-6)), 124.4 (PhN (C-4)), 128.7 (PhN (C-3, C-5)), 143.4 (PhN (C-1)), 157.7 (q, *J* 30.3, $\underline{C}OCF_3$), 167.6 (C-1^{II}), 169.3, 169.4, 170.2, 170.3, 170.6, 170.6, 170.7 ($CH_3\underline{C}O$).[21] HRESIMS: found *m/z* 1027.2389 $[M + Na]^+$. Calcd for $C_{40}H_{46}F_6N_2O_{21}Na$: 1027.2392.

[21] Signals of $CF_3C{=}N$ and CF_3 groups could not be detected due to low signal-to-noise ratio.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/***.

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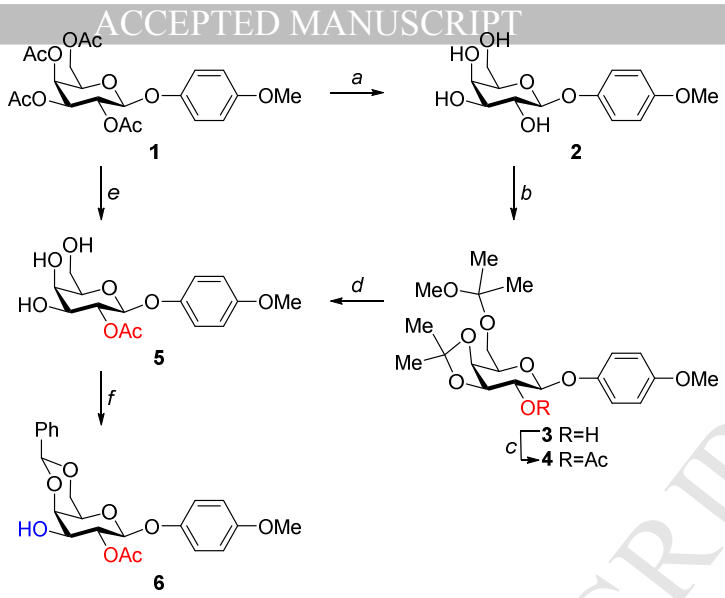
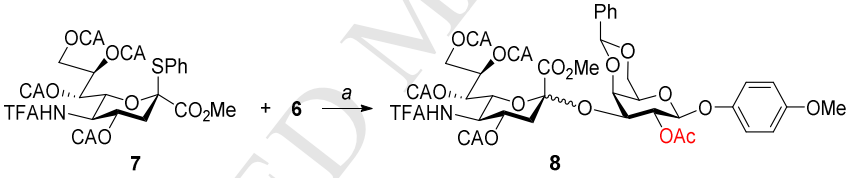
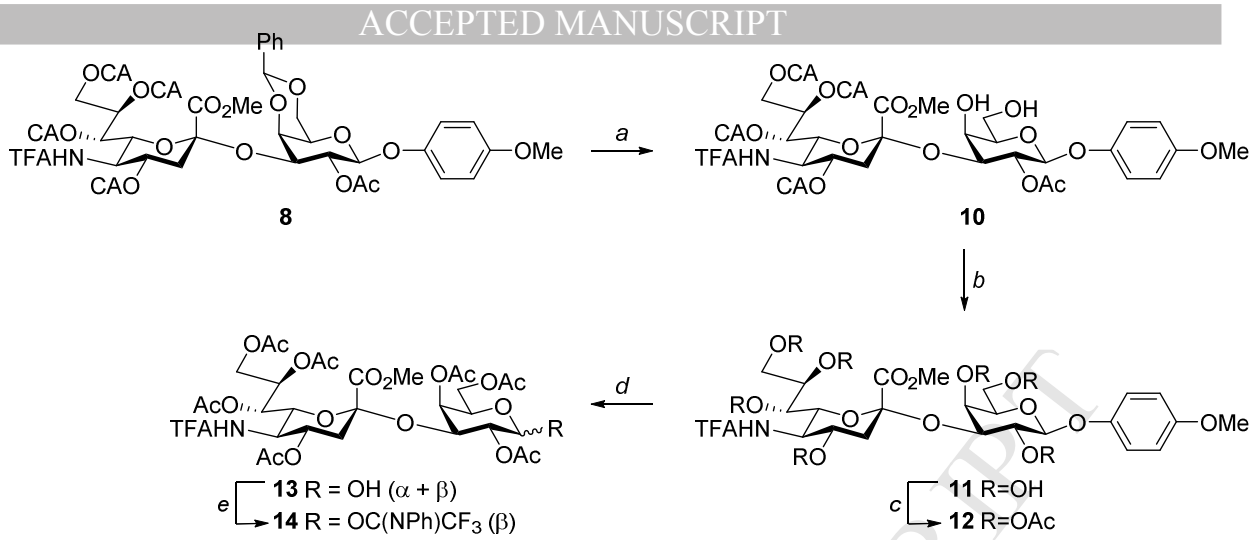


Scheme 1. Preparation of glycosyl acceptor **6** by two alternative routes. *Reagents and conditions*: *a*. MeONa, MeOH, ~20 °C. *b*. $Me_2C(OMe)_2$, CSA, ~20 °C. *c*. Ac_2O, Py, ~20 °C. *d*. TFA, H_2O, CH_2Cl_2, 0 °C (63% over 4 steps). *e*. aq HCl, EtOH, $CHCl_3$, 30 °C (55% of **5** and 40% of **2**). *f*. $PhCH(OMe)_2$, $TsOH \cdot H_2O$, MeCN, ~20 °C (83%).



Scheme 2. Glycosylation of alcohol **6** with sialyl donor **7**. *Reagents and conditions*: *a*. NIS, TfOH, MeCN, MS 3 Å, –40 °C. See Table 1 for other conditions, yields of **8** and α/β ratios obtained.



Scheme 3. Preparation of glycosyl imidate **14**. *Reagents and conditions*: *a.* AcOH, H_2O, 70 °C. *b.* MeONa, MeOH, ~20 °C. *c.* Ac_2O, Py, ~20 °C (63% over 3 steps). *d.* $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O (4:1), 0 °C. *e.* $ClC(NPh)CF_3$, Cs_2CO_3, CH_2Cl_2, 0°C → ~20 °C (66% over 2 steps).

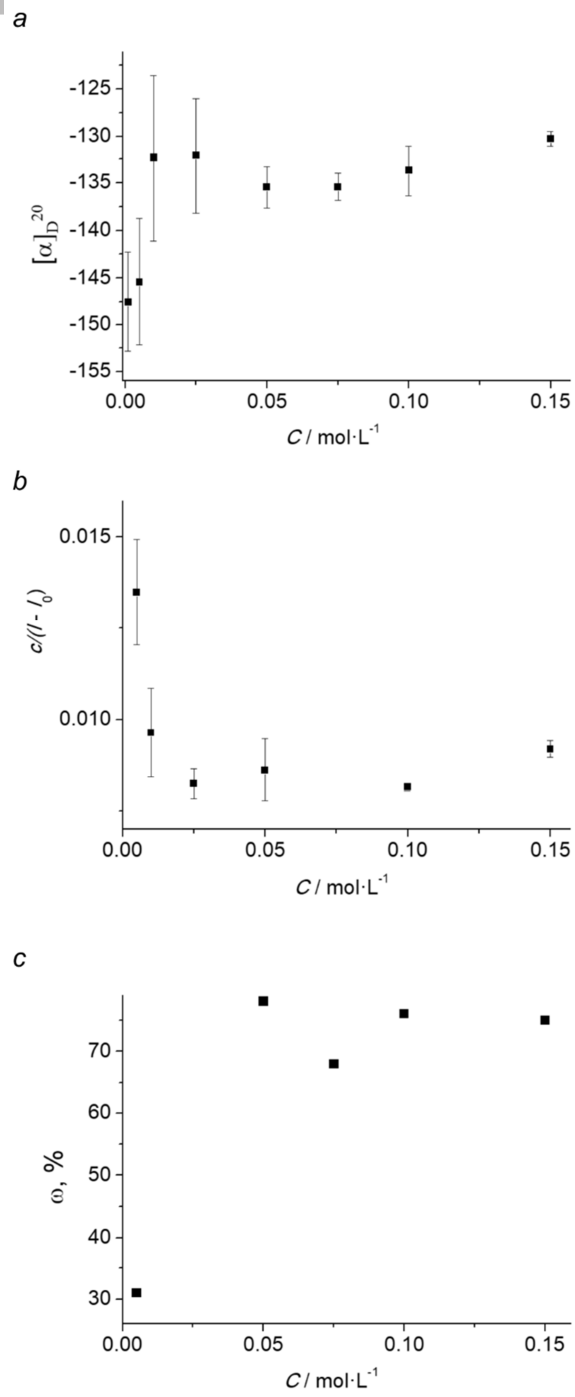


Fig. 1. *a*. Concentration dependence of specific rotation ($[\alpha]_D^{20}$) of freshly prepared solutions of sialyl donor **7** in MeCN. *b*. Static light scattering data for freshly prepared solutions of sialyl donor **7** in MeCN; Debye plot is shown in which the slope (equal to the second virial coefficient A_2) indicates the quality of solvent. *c*. The yield of disaccharide **8** obtained by sialylation of glycosyl acceptor **6** with sialyl donor **7** in MeCN (see Table 1 and Scheme 2).

Table 1
Results of sialylation reaction at different concentrations.[a]

Entry	Concentration (mmol·L^{-1})[b]	Reaction time (min)	Yield (%)	Anomeric ratio (α:β)[c]
1	5	1680[d]	31	11:1
2	50	60	78	13:1
3	75	40	68	9:1
4	100	45	76	10:1
5	150	45	75	9:1

[a] 1 equiv. of glycosyl donor **7**, 1 equiv. of glycosyl acceptor **6**, NIS, TfOH, MeCN, MS 3 Å, –40 °C (Scheme 2). Reaction was quenched after complete consumption of glycosyl donor (TLC control). Disaccharide fraction was isolated by gel permeation chromatography on BioBeads S-X3 (toluene) and analyzed by ^{1}H NMR to give anomeric ratio (α:β). Individual anomers of disaccharides were then separated by silica gel chromatography.

[b] Concentration of glycosyl donor **7** is shown.

[c] ^{1}H NMR data for the disaccharide fraction isolated by gel permeation chromatography on BioBeads S-X3 (toluene).

[d] Reaction performed at 5 mmol·L^{-1} was finished after 28 h.

Highlights

- One-step synthesis of *p*-methoxyphenyl 2-*O*-acetyl-β-D-galactopyranoside accomplished
- A new 3-OH galactosyl acceptor to be used in sialylation was synthesized
- Supramer analysis of solutions of sialyl donor was performed by polarimetry and SLS
- Sialylation at optimized concentration gave Neu-Gal disaccharide (78%, α:β = 13:1)
- Sialyl-α(2-3)-galactose imidate building block was prepared in good yield