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Reactivity-Based One-Pot Synthesis of the Tumor-Associated Antigen N3 Minor Octasaccharide for the Development of a Photocleavable DIOS-MS Sugar Array**

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Human milk contains a variety of biologically important oligosaccharides which reflect the lactating mother's Lewis blood group.^[1] Antigens N3, known as N3 major and N3 minor, are examples of such milk-derived octasaccharides (Scheme 1).^[2] N3 major, difucosyllacto-*N*-hexose (**1**), is the major component of N3 antigens containing Le^a and Le^x moieties. N3 minor, difucosyllacto-*N*-neohexaose (**2**), contains two Le^x units and a lactose unit. The trisaccharide Le^x is one of the carbohydrate determinants of blood groups and is known to be a tumor-related antigen.^[3] The N3 antigens containing one or two Le^x units were also overexpressed in various tumors, including gastrointestinal cancer cells. Although the synthesis of **1** and **2** has been reported,^[4,5] little is known regarding the function of **2**. We describe here the synthesis of **2** by using the one-pot strategy based on anomeric reactivity,^[6] and the use of this glycan as a model to develop photocleavable arrays on porous silicon suitable for direct characterization by mass spectrometry.

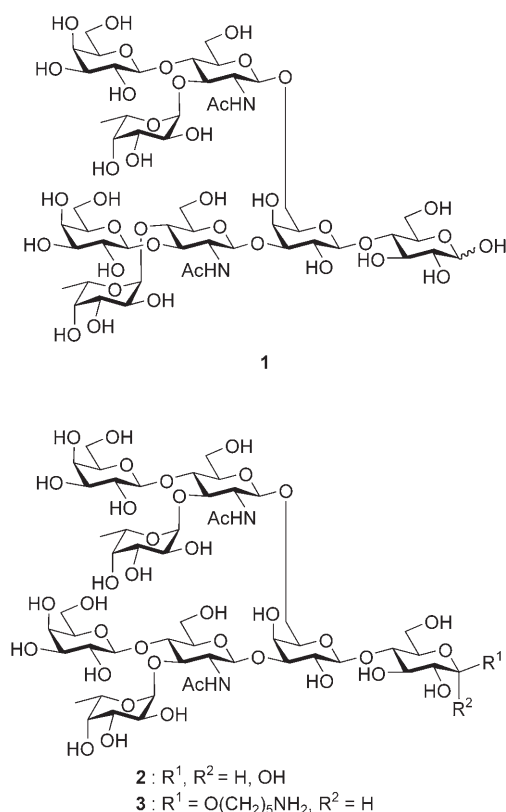
Carbohydrate arrays are becoming powerful tools in glycobiology and glycomics.^[7] We have recently reported the formation of a covalent array of complex oligosaccharides on glass slides^[8] and microtiter plates for high-throughput analysis of sugar–protein interactions.^[9] The microplate

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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



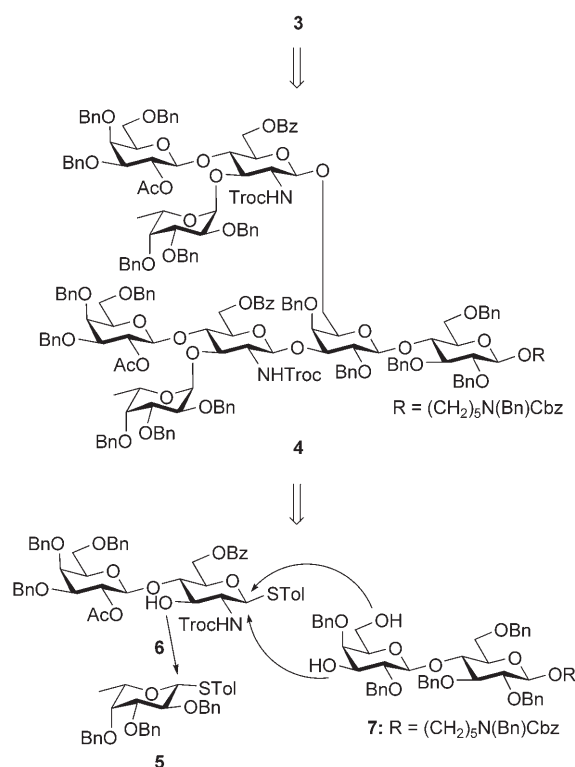
Scheme 1. The structures of antigens N3 **1**, **2**, and the amine-containing analogue **3**.

methodology incorporates a cleavable disulfide linker which allows characterization and quantitative analysis of the array. However, chemical cleavage of the disulfide bonds is not ideal for the glass-slide format. Furthermore, microtiter plate arrays require larger amounts of glycans than glass-slide arrays do, although microtiter plate arrays have advantages in certain applications.

Desorption/ionization on silicon mass spectrometry (DIOS-MS) is an ionization method that uses a porous silicon surface to generate gas-phase ions of small molecules (<3000 Da) without a matrix.^[10] With this technique, the process of sample manipulation is minimized and it only requires very small volumes for analysis. To test the feasibility of this system for sugar arrays, we prepared the amine-containing antigen N3 minor **3** and printed it on the surface of a modified porous silicon with a photocleavable linker, which could be cleaved by a laser ($\lambda = 337$ nm) in the mass spectrometric analysis to detect the carbohydrate on the porous silicon. A similar photocleavable linker on beads was used by Gerdes and Waldmann previously.^[11]

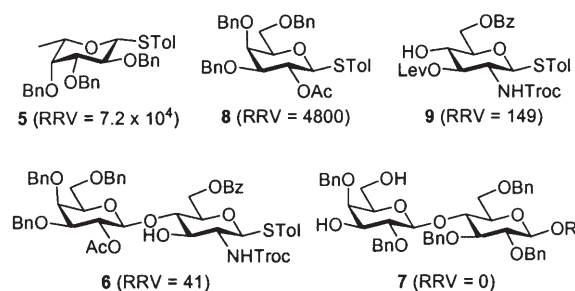
We used the programmable one-pot synthesis strategy to prepare antigen N3 minor **3**, since the method is rapid and has been used in the synthesis of many complex oligosaccharides.^[12–16] The methodology is based on the use of designer thioglycoside building blocks with defined relative reactivity values (RRVs) that are collected in the Optimizer database.^[6]

Our retrosynthetic plan of the antigen N3 minor octasaccharide **3** is shown in Scheme 2. The target molecule **3** can be prepared through a global deprotection of the fully protected



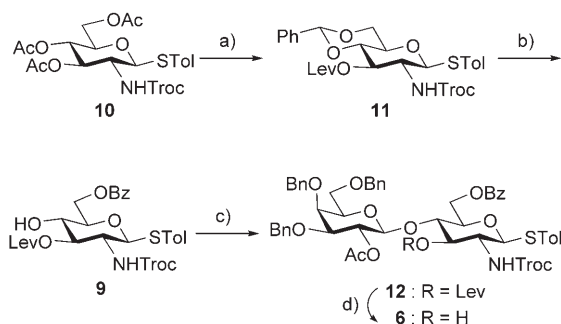
Scheme 2. Retrosynthetic analysis of the antigen N3 minor glycoside **3**. Bn = benzyl, Bz = benzoyl, Cbz = benzyloxycarbonyl, Tol = *p*-methylphenyl, Troc = 2,2,2-trichloroethoxycarbonyl.

oligosaccharide **4**, which could be assembled by one-pot glycosylation with three key building blocks: fucosyl unit **5**, lactosaminyl disaccharide unit **6**, and lactosyl unit **7**. Since the reactivities of these three building blocks decrease progressively, they can react sequentially in a single flask. The fucosyl building block **5** ($RRV = 7.2 \times 10^4$) and the reducing end unit **7** ($RRV = 0$) have the highest and the least reactivity, respectively. The lactosaminyl disaccharide **6** ($RRV = 41$), with a reactivity falling between **5** and **7**, was prepared from galactosyl donor **8** and acceptor **9** (Scheme 3).



Scheme 3. Relative reactivity values (RRV) of building blocks **5–9**. Lev = levulinoyl.

The preparation of perbenzylated fucosyl thioglycoside **5** and galactosyl thioglycoside **8** followed literature procedures.^[6,17] The synthesis of lactosaminyl disaccharide building block **6** is illustrated in Scheme 4. Thioglycoside **10**,^[6]

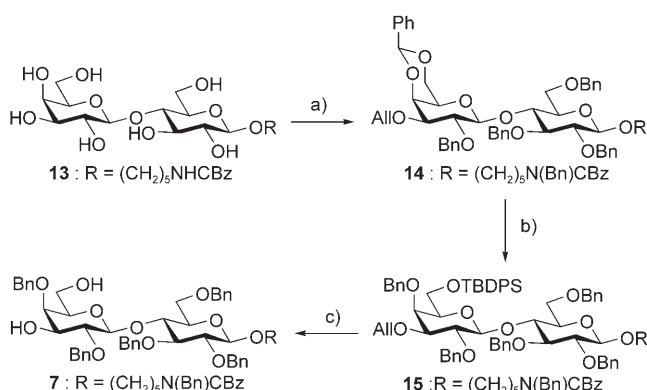


Scheme 4. Synthesis of lactosaminyl disaccharide building block **6**.

a) 1. NaOMe, MeOH/CH₂Cl₂; 2. PhCH(OMe)₂, cat. CSA, CH₃CN; 3. levulinic acid, DCC, DMAP, 76% over 3 steps; b) 1. TFA/CH₂Cl₂/H₂O = 4:10:1; 2. BzCl, pyridine, CH₂Cl₂, 73% in 2 steps; c) 1. **8**, BSP, Tf₂O, AW-300 molecular sieves, CH₂Cl₂, 54%; d) H₂NNH₂/xH₂O, pyridine, AcOH, 88%. CSA = camphorsulfonic acid, DCC = 1,3-dicyclohexylcarbodiimide, DMAP = *N,N*-dimethylaminopyridine, BSP = benzenesulfinyl piperidine, Tf₂O = trifluoromethanesulfonic anhydride, TFA = trifluoroacetic acid.

generated from D-glucosamine hydrochloride in three steps, underwent sequential deacetylation, benzylidenation, and levulinoylation at O3 to yield compound **11**. Hydrolysis of **11** gave the corresponding 4,6-diol intermediate, which upon subsequent selective O6-benzoylation with benzoyl chloride afforded the acceptor **9**. Coupling of the galactosyl thioglycoside **8** and **9**, with activation by benzenesulfinyl piperidine/trifluoromethanesulfonic anhydride (BSP/Tf₂O) gave the β -linked disaccharide **12** in 54% yield.^[15] The levulinoyl group was finally removed with hydrazine hydrate in an acetic acid/pyridine mixture and the disaccharide building block **6** was isolated in 88% yield.^[18]

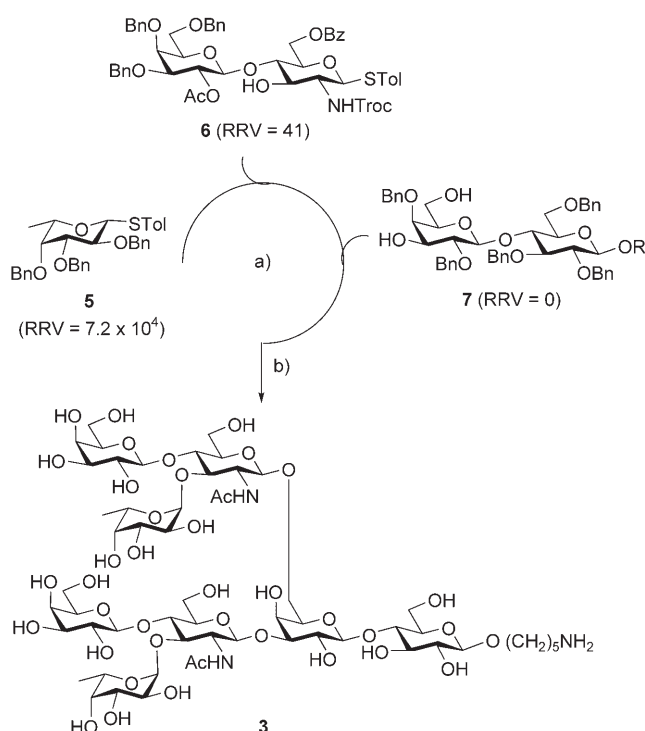
The lactosyl building block **7** was prepared from β -lactose octaacetate (Scheme 5), which was first transformed into the corresponding lactosyl bromide by using 33% hydrogen



Scheme 5. Synthesis of lactosyl building block **7**. a) 1. Bu₂SnO, C₆H₆; 2. AlBr, TBAI, C₆H₆; 3. PhCH(OMe)₂, cat. CSA, CH₃CN; 4. BnBr, 60% NaH, DMF, 54% over 4 steps; b) 1. TFA/CH₂Cl₂/H₂O = 4:10:1; 2. TBDPSCI, Et₃N, CH₂Cl₂; 3. BnBr, 60% NaH, DMF, 52% over 3 steps; c) 1. TBAF, THF; 2. Ph(PPh₃)₃Cl, DBU, EtOH/PhCH₃/H₂O = 8:3:1; 3. 1 M HCl_{aq}/acetone = 1:9, 69% over 3 steps. All = allyl, TBDPS = *tert*-butyldiphenylsilyl, TBAI = tetrabutylammonium iodide, TBAF = tetrabutylammonium fluoride, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

bromide in acetic acid and further coupled with *N*-(5-hydroxypentyl)carbamate in the presence of silver carbonate to provide an inseparable mixture of the expected β -lactoside and its orthoester by-product. The mixture was subjected to deacetylation to obtain the desired β -lactoside **13** in 46% yield over three steps. Regioselective allylation of compound **13** at O3' with dibutyltin oxide and allyl bromide afforded the allyl ether product,^[19] from which the fully protected lactoside **14** was obtained through 4,6-*O*-benzylidenation and perbenzylation (54% over 4 steps). The benzylidene acetal group of **14** was removed under acidic conditions and the primary hydroxy group of the generated diol was regioselectively silylated in excellent yield followed by benzylation at O4' to yield **15**. The TBDPS group was cleaved by treatment with TBAF and the C3' allyl ether was removed by using a two-step sequence. Isomerization of the terminal double bond with Wilkinson's catalyst ([RhCl(PPh₃)₃]) and DBU, with concomitant hydrolysis of the resulted enol ether using a mixture of aqueous HCl and acetone provided **7** in good yield.^[20]

With the desired building blocks **5–7** in hand, we proceeded to synthesize the antigen N3 minor octasaccharide **3** by a reactivity-based one-pot strategy, as outlined in Scheme 6. Coupling of fucosyl thioglycoside **5** with the less reactive lactosaminyl disaccharide unit **6** using BSP/Tf₂O as a promoter proceeded cleanly to give the Le^x trisaccharide fragment. After completion of this reaction, without workup, the lactosyl building block **7** was added in the same pot, followed by addition of NIS and TfOH as activators to introduce two trisaccharide moieties at the same time. The



Scheme 6. One-pot synthesis of antigen N3 minor **3**. a) 1. BSP, Tf₂O, AW-300 molecular sieves, CH₂Cl₂, –70 to –10°C; 2. NIS, TfOH, 0°C to RT; b) 1. Zn, AcOH, RT; 2. Ac₂O, DMAP, pyridine, 0°C to RT; 3. NaOMe, MeOH, RT; 4. Pd-black, H₂ (1 atm), MeOH with 5% formic acid, 11% from (a). NIS = *N*-iodosuccinimide.



B) Control experiment: N3 minor **3** was noncovalently deposited on the silylated amino surface **20**, and showed no signal by DIOS-MS after it had been washed.

reaction was stirred at room temperature to obtain the octasaccharide **4**, which has the skeleton of N3 minor. The mixture of the desired octasaccharide and the inseparable unknown by-product was isolated after the one-pot glycosylation reaction. The by-product was removed after deprotection and chromatography to give pure N3 minor antigen (see the Supporting Information). The two trichloroethyl carbamate (Troc) functional groups were removed with activated Zn nanoparticles in acetic acid, followed by acetylation of the free amino groups with acetic anhydride and pyridine in the presence of catalytic amounts of DMAP to afford the corresponding NHAc derivative. Cleavage of the *O*-acyl groups under Zemplén conditions and deprotection of the benzyl ether and benzyl carbamate groups were accomplished by hydrogenolysis with palladium black in 5% formic acid/methanol solution. The target antigen N3 minor **3** was finally obtained in 11% yield based on the one-pot glycosylation of building blocks **5–7**. The characterization and configuration of **3** were confirmed by NMR spectroscopic and HRMS analysis.

The preparation of the photocleavable sugar array on porous silicon is shown in Scheme 7. 5-Hydroxyl-2-nitrobenzaldehyde (**16**) was reduced with sodium borohydride (NaBH_4) to give compound **17** (97%), which was subsequently subjected to regioselective alkylation with methyl 4-bromobutyrate and TBAF to provide **18** in 88% yield.^[21] Hydrolysis of the methyl ester **18** using LiOH, followed by reaction with *N*-hydroxysuccinimide gave the succinic ester **19** (82% over 2 steps). Coupling of **19** with the silylated amino surface **20**^[10] furnished the photocleavable linker with a free hydroxy group, which was transformed into the carbamate ester **21** in the presence of *N,N'*-disuccinimidyl carbonate and triethylamine. Finally, the N3 minor **3** was printed directly on the plate of **21**. Extensive washing of the plate with MeOH and H_2O resulted in the formation of the photocleavable sugar array on the porous silicon (for detailed experimental procedures, see the Supporting Information). DIOS-MS analysis showed a strong signal at m/z 1473 ($[M + \text{Na}]^+$) and a signal at m/z 1327 (corresponding to the defucosylated molecule). In the control experiment, we printed the N3 minor **3** on the silylated amino surface **20**, which showed no signal after it had been washed. A mannose array was also prepared for the binding study with ConA, and the result showed that nonspecific lectin-surface interactions are insignificant.

In summary, we have successfully synthesized the tumor-associated antigen N3 minor octasaccharide by using the reactivity-based one-pot strategy. The strategy proved that complex molecules can be rapidly assembled using readily available thioglycosides with defined relative reactivity values as building blocks. In addition, we have developed a novel strategy for arraying synthetic carbohydrates on porous silicon surface containing a photocleavable linker for direct characterization by mass spectrometry. This strategy should be useful for preparation of a large glycan array for high-throughput analysis.

Keywords: carbohydrates · glycan array · glycosylation · mass spectrometry · synthesis design

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