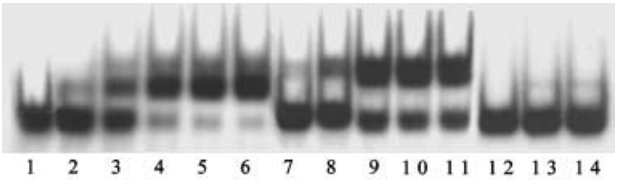

Figure 4. Autoradiograms showing the binding of hybrid **5** to ^{32}P-labeled DNAs. Lanes 1–6: T/CRE^{hs}, **5**: 0, 7.7, 19, 38, 58, 77 nM respectively; Lanes 7–11: T/CRE^{hs}*m*, **5**: 7.7, 19, 38, 58, 77 nM respectively; lanes 12–14: CRE^{hs}, **5**: 38, 77, 154 nM respectively. Binding reactions were performed over 10 min at 4 °C using < 1 nM labeled DNAs in a binding mixture (20 μL) containing 20 mM tris(hydroxymethyl)aminomethane (pH 7.5), 100 mM KCl, 2 mM $MgCl_2$, 2 mM ethylenediaminetetraacetate, 10% glycerol, 0.3 mg mL^{-1} *N,O*-bovine serum albumin (BSA), and 2% NP-40. The products were resolved by polyacrylamide gel electrophoresis using a 10% nondenaturing acrylamide gel and 0.5X TBE (25 mM tris borate and 0.5 mM EDTA) buffer.
CRE^{hs}: 5′-d(CGAC<u>GTCAT</u>CGGAGGTCCT)-3′
3′-d(GCTG<u>CAGTA</u>GCCTCCAGGA)-5′

makes non-specific electrostatic contacts to the phosphate groups.[18]

In conclusion, appropriate linking of a b-ZIP basic region to a minor groove binding tripyrrole allowed for specific binding to its cognate DNA site. The hybrid compound **5** shows considerably higher affinity for its designated target DNA sequence than that of its isolated components for their respective cognate subsites. Although further refinement of the design is necessary to obtain compounds with higher affinities and better specificities, the work described herein confirms the viability of this new type of major–minor groove DNA-binding molecules.

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Solid-Phase Capture–Release Strategy Applied to Oligosaccharide Synthesis on a Soluble Polymer Support**

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Advances in the techniques for oligosaccharide synthesis have lagged behind those for other classes of biological oligomers. For the preparation of oligopeptides and oligo-

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

nucleotides, the automated synthesizer has become a routine tool, in which polymer-support synthesis plays a key role.

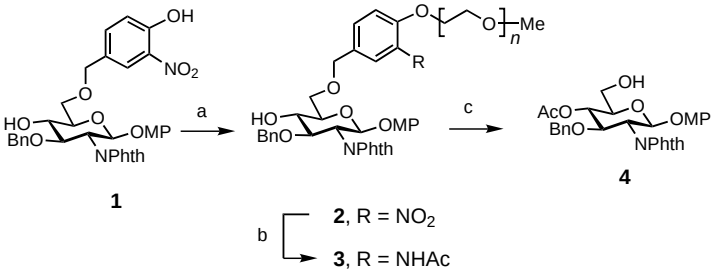
Considering the structural diversity of glycoconjugate-derived oligosaccharides,[1] the potential utility of oligosaccharide synthesis on a polymer support is obvious.[2] This technology would greatly facilitate glycoconjugate synthesis and could possibly reap the benefits of automation.[3] However, several problems must be solved before this goal can be reached: firstly, the difficulty in monitoring reaction progress; secondly, the reduced reactivity of substrates bound to the polymer support; and thirdly, the limitations in scaling up. Addressing these issues, we recently developed a methodology for the monitorable synthesis of oligosaccharides on a soluble polymer support based on the "tag–reporter" concept.[4] It exploited low molecular weight poly(ethylene)glycol (PEG),[5] which served both as a "reporter" in the monitoring of the reaction by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and as a "tag" in the separation of support-bound materials from the reaction mixture due to its high polarity.[6, 7]

Although syntheses on polymer supports can indeed be monitored,[4, 8] the success of this method assumes all reactions can be driven to near completion. This seems to be a difficult requirement, considering that typical O-glycosylations proceed in 50–80% yield.[9] Now, we wish to report a refined strategy for the synthesis of PEG-tagged oligosaccharides that circumvents this obstacle.

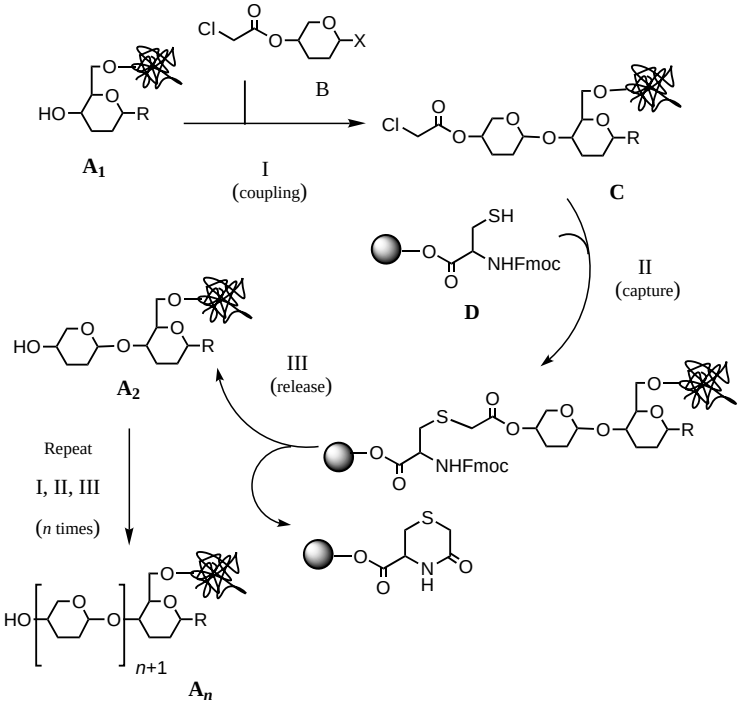
This new approach is based on the concept of resin-aided capture–release (Scheme 1).[10] It employs a glycosyl acceptor bound to a low molecular weight PEG support ($\mathbf{A_1}$) in combination with a chloroacetyl (CA) carrying donor (**B**), as described before.[4] The PEG-bound component can be recovered by filtration through a pad of silica gel. At this stage, excess donor and side product(s) can be removed. The coupled product (**C**), potentially contaminated with unreacted acceptor $\mathbf{A_1}$, is then captured onto solid phase by a chemoselective reaction between CA and a resin-bound thiol group (**D**). After removal of Fmoc, the exposed amine function should cyclize spontaneously to release a disaccharide ($\mathbf{A_2}$), which is now ready for the next coupling. Repetition of this cycle should provide facile access to correctly assembled oligomers $\mathbf{A}_n$.

To examine the adequacy of this strategy, we selected a lactosamine repetition sequence as the target. It is well recognized that polylactosamine [(Galβ1 →4GlcNAcβ1 →3)$_n$] is an important structural motif of Asn-linked[11] and Ser/Thr-linked[12] glycoproteins and of glycosphingolipids.[13]

PEG-supported monosaccharide **2**, which carries the nitro-modified Wang resin-type linker,[14] was prepared as depicted in Scheme 2. Previously reported **1**[4] was treated with PEG



Scheme 2. Preparation of PEG-supported monosaccharide **2**. a) PEG monomethyl ether, DEAD, Ph_3P, THF, RT, 18 h, 99%; b) acetylacetone, Zn/Cu, THF, RT, 16 h, then Ac_2O, Et_3N; c) DDQ, $ClCH_2CH_2Cl/H_2O$, RT, 4 h, 92% overall. DEAD = diethyl azodicarboxylate, DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone, MP = p-$MeOC_6H_4$, Phth = phthaloyl.



Scheme 1. Capture–release strategy for the synthesis of disaccharides $\mathbf{A_2}$ and oligosaccharides $\mathbf{A}_n$ on a polymer support. Fmoc = 9-fluorenylmethoxycarbonyl.

methyl ether (av $M_w = 550$) under Mitsunobu conditions. Nearly quantitative carbohydrate incorporation was supported by ^{1}H NMR spectroscopy and confirmed by the cleavage experiment performed under the conditions reported by Kusumoto et al. for the removal of the nitrobenzyl group.[15] Namely, the nitro group of **2** was first converted to the acetamido group in **3**, which was then treated with DDQ[16] to afford compound **4** in high overall yield.

Galactosyl donor **9**, which has a chloroacetyl group, was prepared from phenylsulfanylgalactoside **5**[17] via **6**–**8** (Scheme 3). The coupling of **2** and **9** was performed with dimethyl(methylsulfanyl)sulfonium triflate (DMTST)[18] in CH_2Cl_2 (Scheme 4). To evaluate the efficacy of the capture–release process only a substoichiometric amount (0.9 equiv) of **9** was used for this reaction. The reaction progress was monitored by MALDI-TOF mass spectrometry as described previously.[4, 19] The mixture was passed through a pad of silica gel that was first washed with AcOEt, and the PEG-bound component was eluted with AcOEt/MeOH 3:1. Analysis of this fraction by ^{1}H NMR spectroscopy revealed the presence of disaccharide **10** and unreacted acceptor **2** in a ratio of about 10:3 (Figure 1A). This indicates that the

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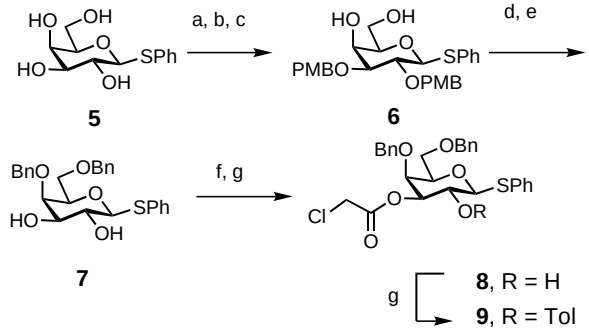

Scheme 3. Preparation of galactosyl donor **9**. a) $(MeO)_2CMe_2$, CSA, MeCN, RT, 10 min; b) *p*-methoxybenzyl chloride (PMBCl), NaH, DMF, RT, 15 h; c) 60% AcOH, RT, 13 h, 56% overall; d) benzyl bromide, NaH, DMF, RT, 5 h; e) DDQ, CH_2Cl_2/H_2O, RT, overnight, 79% from **6;** f) $(ClCH_2CO)_2O$, pyridine, CH_2Cl_2, $-40\,^{\circ}C$, 3 h, 33%; g) *p*-MeC_6H_4COCl (TolCl), pyridine, CH_2Cl_2, $0\,^{\circ}C \rightarrow RT$, 3 h, 85%. CSA = 10-camphorsulfonic acid, DMF = dimethylformamide.

glycosylation proceeded in a highly efficient manner (ca. 85% based on donor **9**) and that the entropic disadvantage inherent to reactions on a polymer support was kept to a minimum through the use of low molecular weight PEG. Additionally, potential contamination by moisture due to the hygroscopic nature of PEG was practically inconsequential.

The mixture of disaccharide **10** and unreacted **2** was then subjected to the capture–release cycle. According to our expectations, thiol-containing resin **12** generated from commercially available S*t*Bu-protected cysteine-loaded Wang resin **11** efficiently captured the coupled product. This step was monitored by chloroacetyl color test with *p*-nitrobenzylpyridine–piperidine.[4, 20] Subsequent release was effected by treatment with 4-(aminomethyl)piperidine[21] to afford **14** with excellent purity. As apparent from ^1H NMR analysis, unreacted acceptor **2** was completely removed (Figure 1B). At this stage, the coupled product was fully characterized as disaccharide **15**, which was obtained from **14** after cleavage from PEG (Scheme 4).

Further chain elongation was performed according to Scheme 5. Namely, the second glucosamine residue was incorporated by using trichloroacetimidate **16**. Crude trisaccharide **17** was then subjected to capture–release to give **18**. After final glycosylation with thioglycoside **9** and capture–release, tetrasaccharide **20** was cleaved from PEG to provide **21**. Complete deprotection was performed in a standard manner to give the free tetrasaccharide Galβ1 $\rightarrow$4GlcNAcβ1 $\rightarrow$3Galβ1 $\rightarrow$4GlcNAcβ1 $\rightarrow$ OMP.

We have developed a resin-aided capture–release strategy for oligosaccharide synthesis on a polymer support. As an initial demonstration of the strategy, we examined the construction of an oligosaccharide consisting solely of synthetically straightforward 1,2-*trans* (β) glycosidic linkages. More systematic studies should prove the generality of this strategy to a wide range of glycoconjugate-derived glycans.

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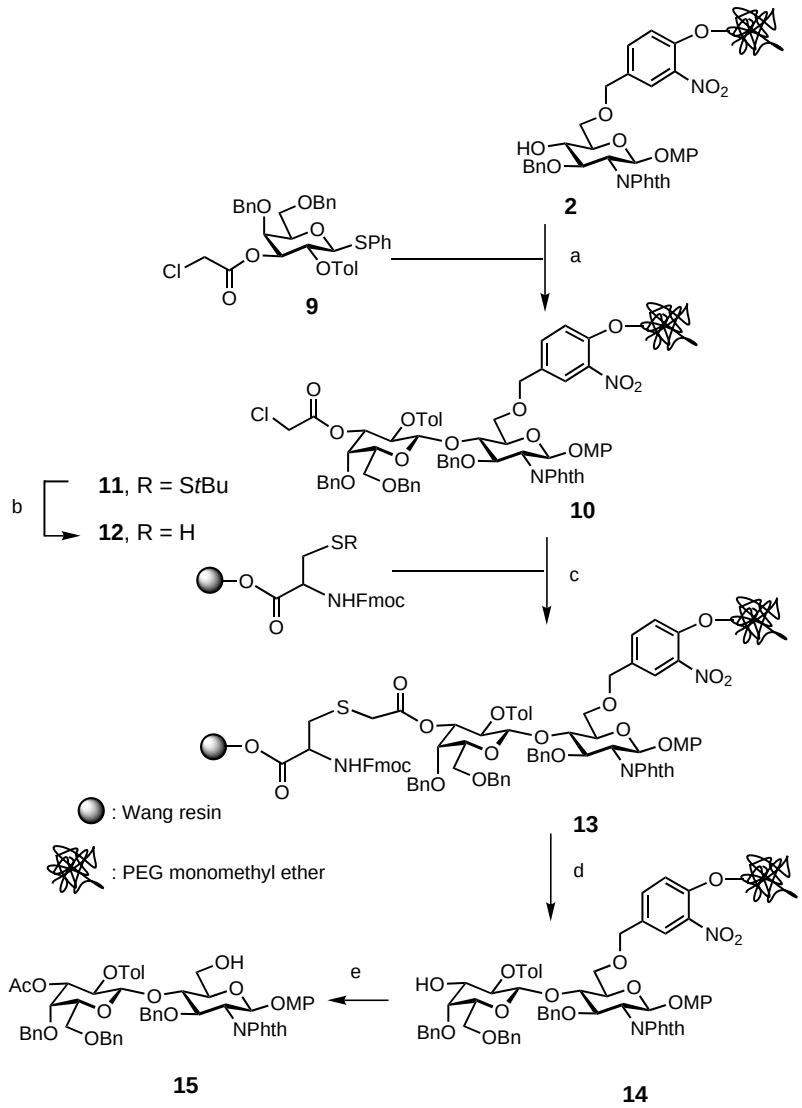

Scheme 4. Capture–release of disaccharide **10**. a) DMTST, MS 4 Å/CH_2Cl_2, $-20 \rightarrow 0\,^{\circ}C$, 14 h, 94%; b) nBu_3P, EtOH/CH_2Cl_2/H_2O (4:6:1), RT, 3 h, 98%; c) **12** (3 equiv), iPr_2EtN (3 equiv), CH_2Cl_2/MeCN (1:1), RT, 14 h; d) 4-(aminomethyl)pyridine (15 equiv), THF, RT, 12 h, then Amberlyst 15E, 82% from **10;** e) acetylacetone, Zn/Cu, THF, RT, 18 h, then Ac_2O, Et_3N; f) DDQ, $ClCH_2CH_2Cl/H_2O$, RT, 3 h, 86% from **14**.

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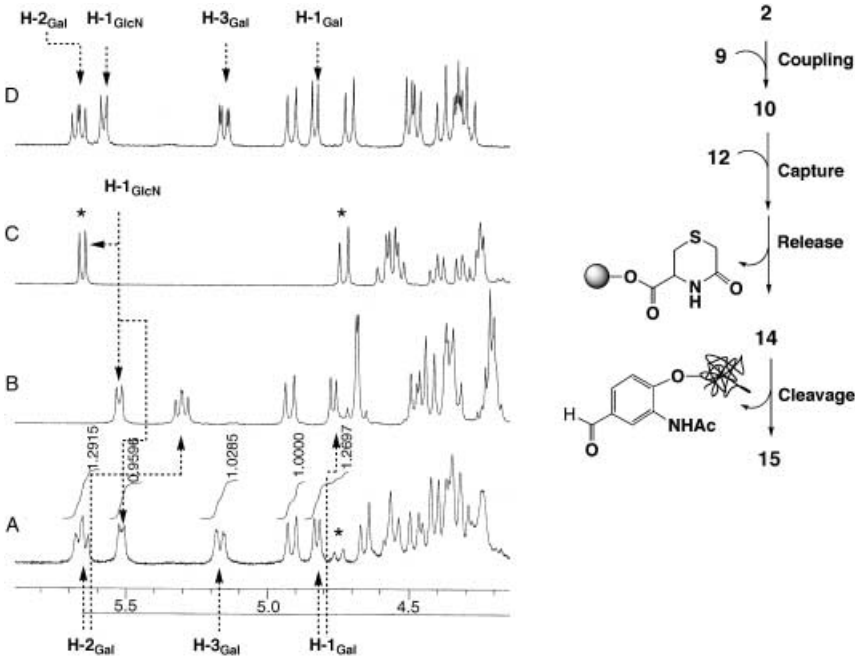
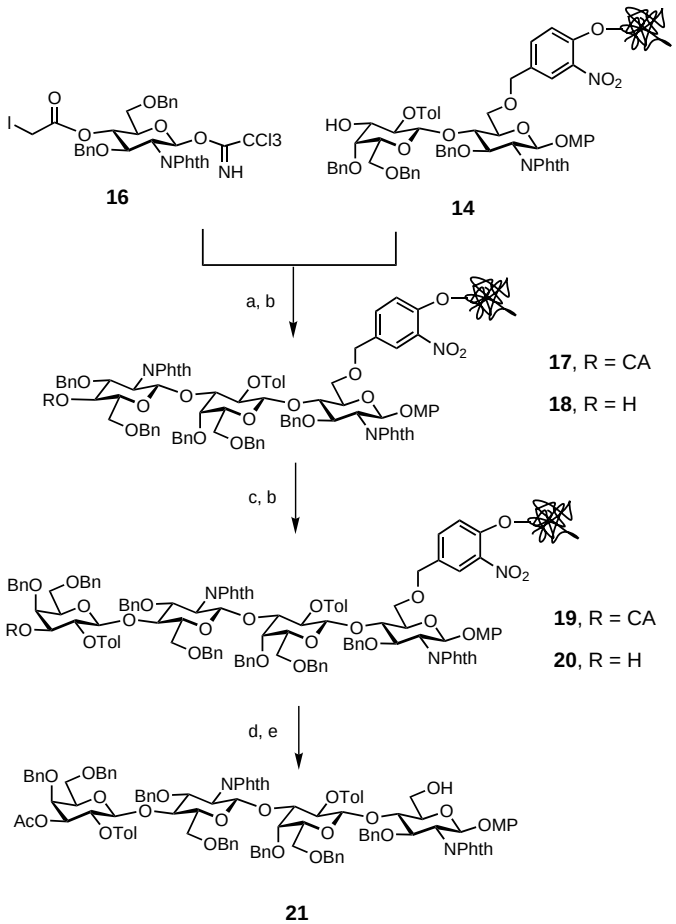


Figure 1. ^{1}H NMR spectra of crude **10** before capture (A), released disaccharide **14** (B), PEG-supported acceptor **2** (C), and disaccharide **15** after cleavage (D), and schematic reaction sequence. The starred signals belong to unreacted **2**.



Scheme 5. Synthesis of tetrasaccharide **21**. a) TMSOTf/CH_2Cl_2, $-20\,^\circ$C, 1 h, 95%; b) **12**, iPr$_2$EtN, MeCN/CH_2Cl_2, RT, then 4-(aminomethyl)pyridine, THF, RT, 79% (**18**), 80% (**20**); c) **9**, DMTST, MS 4 Å, CH_2Cl_2, $0\rightarrow10\,^\circ$C, 5 h, 88%; d) acetylacetone, Zn/Cu, THF, RT, 18 h, then Ac_2O, Et_3N; e) DDQ, $ClCH_2CH_2Cl/H_2O$, RT, 3 h, 72% overall. TMSOTf = trimethylsilyl triflate.

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