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Solid-phase carbohydrate synthesis via on-bead protecting group chemistry

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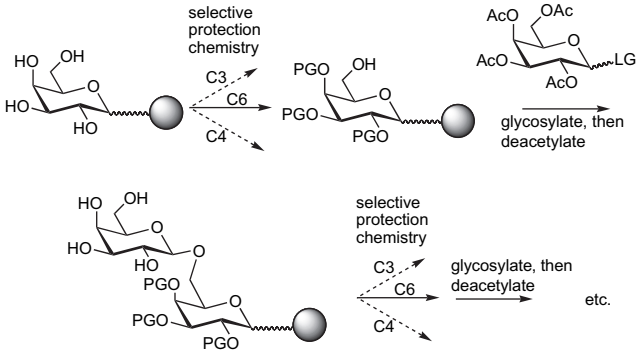
Abstract—Di- and tri-saccharides were synthesized on a solid phase. The procedure started with a non-protected sugar linked via either cysteine or glutamine to a polystyrene resin. Selective dimethoxytritylation chemistry and subsequent steps yielded a resin-bound acceptor that could be glycosylated to yield β1,6-linked disaccharides. Reiteration of the procedure produced the trisaccharide.

1. Introduction

The difficulties in synthesizing complex synthetic carbohydrates limit our understanding of their roles in biological systems and also limit carbohydrate-based drug development. Carbohydrates are nevertheless emerging as a class of compounds, which play crucial roles in major diseases such as (avian) flu,[1] AIDS,[2] and cancer.[3] The shift of the synthesis of oligomeric nucleotides and peptides to the solid phase, and subsequent automation, has led to major steps forward in their ease of preparation and application in biology. For carbohydrates, a universal solid-phase synthetic method is more difficult but would have a great scientific impact. Solid-phase synthesis of carbohydrates was first reported in 1971[4] and since then much progress was made.[5] The recent automation of the process represents a milestone.[6] All published strategies in common are based on a two-step protocol, which includes an *on-bead* glycosylation step and an *on-bead* deprotection step. This means that strategically protected carbohydrate donors are used for Glycosylation, which, after coupling, can selectively be deprotected at the desired position to afford a new glycosyl acceptor for further elaboration. Although connecting the building blocks one by one is a rapid procedure, the synthesis of all the desired building blocks is a major effort to be customized for each desired target carbohydrate. In response to this, we opted to explore the use of resin-bound unprotected sugars to be selectively converted to acceptors by on-bead selective chemistry commonly used in solution (Scheme 1). In principle, a single carbohydrate can thus be elaborated into several disaccharides depending on the protecting chemistry used. Such selective chemistry would have to be sequence independent, and based on an excess of reagent to push reactions to completion. If these procedures prove to be simple and compatible with automation, many complex sugars, including branched ones, can be made from a few building blocks by reiteration of the steps. To take the first step toward this distant goal, we started with the well-studied selective C-6 chemistry, based on the bulky 4,4-dimethoxytrityl (DMT) protecting group that selectively reacts with primary hydroxyl groups even when applied in excess. The DMT group was chosen for the mild cleavage conditions (1% dichloro acetic acid in CH_2Cl_2) and the visible color release by the DMT-cation that can be used for verification.



Scheme 1. General solid-phase synthesis of carbohydrates including a selective on-bead protection step followed by glycosylation and deprotection and reiteration. A selective C6 protection step is exemplarily shown as used in this paper.

As a linker, the trifunctional amino acid cysteine (**1**) was chosen. Its sulfur atom can be linked to the sugar anomeric center.[7] After all synthetic procedures, this thioglycosidic

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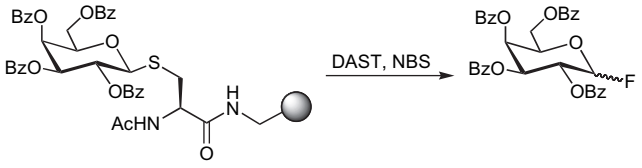
linkage can selectively be cleaved with *N*-bromosuccinimide (NBS) and a variety of nucleophiles.[8] The cleavage conditions we used involved DAST (diethylaminosulfur trifluoride), which yielded the anomeric fluorides that can be further functionalized (Scheme 2). Another attractive feature of the cysteine linker is its amino group that was Fmoc protected, and thus a useful tool for determination of the loading upon deprotection. Furthermore, the carboxylic acid function of cysteine was used for coupling the construct to an amine functionalized polystyrene resin.



Scheme 2. Cleavage conditions used for the cysteine bound carbohydrates from the resin by DAST and NBS.

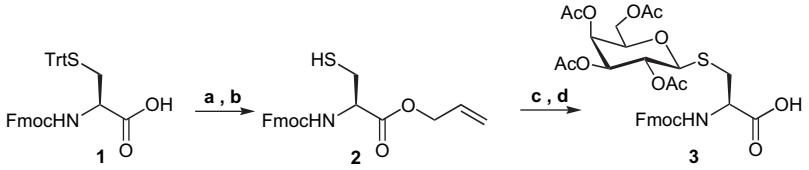
2. Results and discussion

2.1. S-Linked galactoside acceptor route

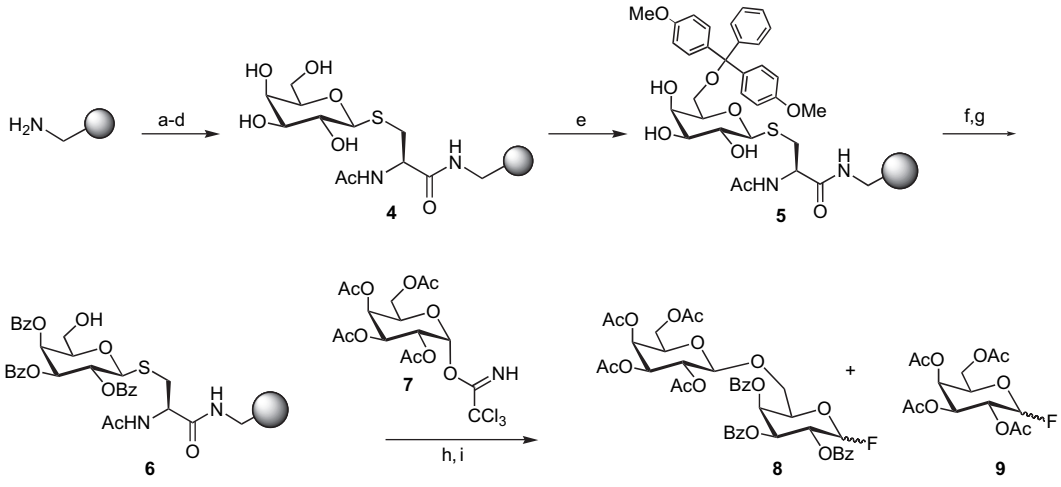
The S-linked amino acid **3** was synthesized starting from commercially available Fmoc-Cys[*S*-trityl]-OH **1** (Scheme 3). Its carboxylic acid group was first protected as the allyl ester by allyl bromide and K_2CO_3 as the base. The trityl group was removed under acidic conditions and the acetylated carbohydrate was introduced by the action of $SnCl_4$ as described.[9] The allyl ester was removed by $Pd(PPh_3)_4$ using anilinium *p*-toluenesulfinate as a scavenger yielding **3**.

The amino acid **3** was coupled to an aminomethylated polystyrene resin using the HATU coupling reagent and DIPEA (Scheme 4). By Fmoc deprotection analysis, a resin loading of 0.70 mmol/g was determined. The Fmoc group was cleaved by 20% piperidine in NMP and the free amine was subsequently acetylated with acetic anhydride. The carbohydrate was deacetylated using 5% N_2H_4 in DMF to yield resin **4**. To verify its identity and evaluate the DAST/NBS cleavage method, the carbohydrate was fully benzoylated with Bz-Cl in pyridine. Subsequent treatment of the resin with NBS (5 equiv) and DAST (5 equiv) for 10 min afforded the desired anomeric fluoride (Scheme 2).

Introduction of the DMT group was achieved by treating resin **4** with 10 equiv of DMT-Cl in pyridine. The remaining hydroxyl functions of resin **5** were capped with Bz-Cl in pyridine followed by cleavage of the DMT group with 1% DCA in CH_2Cl_2 to yield resin **6**. To verify the identity of this resin, part of it resin was acetylated (Ac_2O) and cleaved (DAST/NBS) to afford the desired monoacetylated glycosyl fluoride. This confirmation set the stage for the glycosylation reaction of **6** for which galactose donor **7** (5 equiv) was used in the presence of TMSOTf (0.3 equiv) as a promoter. The procedure was run twice and was followed by NBS/DAST cleavage of the products from the resin. Product analysis revealed that indeed the desired disaccharide **8** was obtained, but also the peracetylated glycosyl fluoride **9**. The reaction was run several times and the ratio of **8**:**9** varied widely between 1/1 to 9/1. The formation of **9** can be explained by an unwanted aglycon transfer mechanism, as has been noticed



Scheme 3. Synthesis of amino acid **5**; reagents and conditions: (a) allyl bromide, K_2CO_3, DMF, 2 h, rt; (b) TFA, TES, CH_2Cl_2, 2 h, rt, 64% over two steps; (c) galactose penta-acetate, $SnCl_4$, CH_2Cl_2; and (d) $Pd(PPh_3)_4$, anilinium *p*-toluenesulfinate, quantitative over two steps.



Scheme 4. Reaction conditions: (a) **3**, HATU, DIPEA; (b) piperidine, NMP; (c) Ac_2O, pyridine; (d) N_2H_4, DMF; (e) DMT-Cl, pyridine; (f) Bz-Cl, pyridine; (g) 1% dichloro acetic acid, CH_2Cl_2; (h) **7**, TMSOTf, CH_2Cl_2; and (i) DAST, NBS.

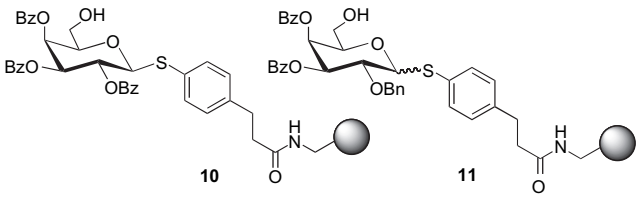


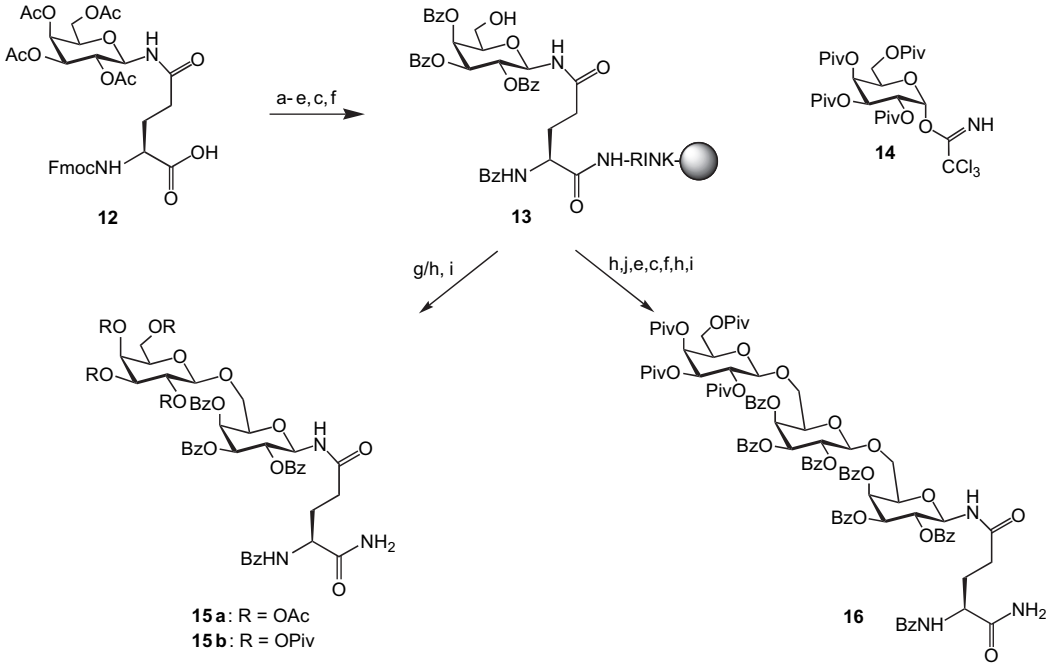
Figure 1. Modified acceptors containing an aryl thioglycosidic linkage to the solid phase.

before for reactions in solution.[10] It is believed to result from the relatively high nucleophilicity of the sulfur atom that can to some extent outcompete the free OH of the acceptor. Modifications of the glycosylation conditions could not prevent the formation of **9**. Furthermore the use of the analogous perbenzylated donor did not yield any product.

In solution, the unwanted aglycon transfer was suppressed by changing from an anomeric thioalkyl group to a thioaryl group.[10a] Furthermore, changing the protecting group pattern was also shown to suppress the transfer.[10b] To this end, two new resin-bound acceptors **10** and **11** were prepared as shown in Figure 1. The first modification was the introduction of an arylthio linkage as in **10**. In **11**, an additional modification was made. Replacing the participating benzoyl group for a non-participating benzyl group was expected to disfavor the aglycon transfer reaction. Both resins were prepared from the commercially available 3-[4-(trityl-mercapto)phenyl]propionyl aminomethyl functionalized polystyrene resin, starting with removal of its trityl group. Subsequent glycosylation with the appropriate glycosyl donors and deacetylation followed by the C-6 chemistry protocol of Scheme 4, yielded the two acceptor resins **10** and **11**. The glycosylation of these resins with **7** as before, followed by NBS/DAST cleavage yielded the disaccharide **8** but unfortunately also the aglycon transfer product **9**. In both cases **9** was obtained in the range of 40–80%.

2.2. N-Linked galactoside acceptor route

In order to avoid the aglycon transfer, a more stable glycosidic linkage was used based on an anomeric glutamine linked to the resin. While this modification no longer allows anomeric manipulation/conjugation of the synthesized carbohydrate, the glycopeptides that result from this pathway are highly versatile and useful building blocks. Firstly, building block **12**[11] was coupled to a Rink-amide linker of a polystyrene resin (Scheme 5). The attached glyco-amino acid is cleavable at the glutamine carboxy function by TFA. The coupling of **12** to the resin was performed by HATU and DIPEA. The subsequent reaction was a piperidine-mediated Fmoc cleavage. A loading of 0.48 mmol/g was determined at this stage. The liberated amine was capped with benzoyl chloride and the acetates were removed by 5% N_2H_4 in DMF. Selective tritylation of the primary hydroxyls was found to work best using 5 equiv of DMT-Cl in pyridine as a solvent. As before, the remaining hydroxyl functions were benzoylated, and the primary hydroxyl was liberated to yield resin **13**. Glycosylation of this acceptor with donor **7** (5 equiv) and TMSOTf (0.3 equiv) as a promoter yielded the expected disaccharide **15a**, which was isolated by silica chromatography. The product was obtained in ca. 25% yield (i.e., 86% per reaction step). Since ortho ester formation is a possible side reaction, the glycosylation was repeated with pivaloyl protected donor **14**, which is less prone to ortho ester formation. Again the desired disaccharide (**15b**) was obtained after HPLC purification in a slightly higher overall yield of 30% (i.e., 88% per reaction step). Using this strategy the procedure was reiterated for the synthesis of a trisaccharide. The resin-bound disaccharide was subjected to NaOMe deacylation followed by the selective C-6 chemistry reaction



Scheme 5. Reaction conditions: (a) Rink-polystyrene resin, HATU, DIPEA, DMF; (b) piperidine, NMP; (c) Bz-Cl, pyridine; (d) N_2H_2, DMF; (e) DMT-Cl, pyridine; (f) 1% dichloro acetic acid, CH_2Cl_2; (g) **7**, TMSOTf, CH_2Cl_2, 0 °C; (h) **14**, TMSOTf, CH_2Cl_2, 0 °C; (i) TFA, H_2O (95/5); and (j) NaOMe, MeOH.

sequence and, after glycosylation with **14** the desired trisaccharide **16**, was isolated after cleavage from the resin and straightforward HPLC purification.

3. Conclusions

The solid-phase synthesis of β1,6-linked galactosides proved possible with a resin-bound unprotected sugar as the starting point. Selective resin compatible protection chemistry involving the C6 primary hydroxyl yielded acceptors that could be glycosylated. The synthesis greatly benefited in the practical sense, from being performed on-resin, as it requires only washing steps. Glycosylation thus led to disaccharides, and finally a trisaccharide preparation demonstrated that reiteration of the procedure was possible. The synthesis with cysteine as the linking moiety connecting the sugar to the resin suffered from unwanted aglycon transfer, even when remedies that had been effective in solution were not able to fully prevent the transfer. Recently reported insights suggest that the use of two methyl groups flanking the sulfur on the aromatic ring would be sufficient to prevent the transfer.[10c] When glutamic acid was used to link the sugar, stable glycopeptidic products were obtained from the applied reaction sequence that could be cleaved from the resin via the amino acid's carboxyl group. The work indicates that protecting group chemistry can be performed on-resin and has the potential for the synthesis of a wide variety of oligosaccharides from a limited set of glycosyl donor molecules. To make this chemistry applicable it needs to be extended to selectively produce other acceptors on-bead. As such the versatile 4,6-*O*-benzylidene group was successfully generated on-bead using an excess of reagents (not shown), although reproducibility needs to be improved. In conclusion, the presented work is an initial step in the exploration of on-bead carbohydrate synthesis that goes beyond glycosylation and deprotection steps, and may ultimately reduce the labor involved in the building block synthesis.

4. Experimental section

4.1. General remarks

Unless stated otherwise, chemicals were obtained from commercial sources and used without further purification. All solvents were purchased from Biosolve (Valkenswaard, The Netherlands) and were stored over molecular sieves (4 Å). Pyridine was purchased from Acros (Geel, Belgium) and stored over molecular sieves (4 Å). Both aminomethyl polystyrene resin and Rink-amide functionalized aminomethyl polystyrene resin were obtained from Rapp Polymere (Tübingen, Germany). 3-[4-(Tritylmercapto)phenyl]propionyl aminomethyl functionalized polystyrene resin was obtained from Novabiochem (Läufelfingen, Switzerland). A solution of Ac_2O (4.72 mL), DIPEA (2.18 mL), and HOBT (230 mg) in NMP (100 mL) was used as the capping mixture. TLC was performed on Merck precoated Silica 60 plates. Spots were visualized by UV light and by 10% H_2SO_4 in MeOH. Analytical HPLC runs were performed on a Shimadzu automated HPLC system equipped with an evaporative light scattering detector (PL-ELS 1000, Polymer Laboratories) and a UV/VIS detector operating at 220 and 254 nm. Preparative HPLC runs were performed on a Gilson HPLC workstation. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were performed on a Varian G-300 spectrometer and ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded on a Varian INOVA-500 spectrometer. Chemical shifts are given in parts per million relative to TMS (^{1}H, 0.00 ppm) and $CDCl_3$ (^{13}C, 77.0 ppm). Assignments of the NMR spectra of **15** and **16** were made on the basis of their HSQC, HMBC, and TOCSY spectra.

4.1.1. Fmoc-Cys-OAllyl (2). To a solution of Fmoc-Cys[Trt]-OH (5.86 g, 10 mmol) and K_2CO_3 (1.38 g, 10 mmol) in dry DMF (50 mL) was added allyl bromide (1.74 mL, 20 mmol). TLC showed complete conversion after 2 h. The mixture was concentrated in vacuo and then taken up in EtOAc (100 mL). The organic solution was washed with 1 M $KHSO_4$ (50 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica gel (hex/EtOAc 7/1→2/1) yielded Fmoc-Cys[Trt]-OAllyl as a white foam. ^{1}H NMR spectrum is consistent with Ref. 12: (300 MHz, $CDCl_3$): 7.76–7.19 (23H, m, CH_{arom}), 5.92–5.81 (1H, m, OCH_2CHCH_2), 5.33 (1H, d, Fmoc NH, J=0.9 Hz), 5.27–5.23 (2H, m, OCH_2CHCH_2), 4.62 (2H, d, OCH_2CHCH_2), 4.42–4.35 (3H, m, Fmoc CH_2, CHα), 4.23 (1H, t, Fmoc CH, J=6.9 Hz), and 2.66 (2H, m, CH_2β). To a solution of Fmoc-Cys[Trt]-OAllyl (≈9 mmol) and TES (7.26 mL, 45 mmol) in CH_2Cl_2 (100 mL) was added TFA (3.35 mL, 45 mmol) dropwise over 5 min and the mixture was stirred for additional 2 h. TLC (hex/EtOAc 3/1) showed completion of the reaction. The mixture was concentrated in vacuo and co-evaporation with toluene, ethanol, and CH_2Cl_2 yielded crude **2**. Silica gel chromatography (CH_2Cl_2/hex 4/1→9/1) yielded the product as a white amorphous solid (2.45 g, 64% over two steps). ^{1}H NMR spectrum was consistent with Ref. 13: (300 MHz, $CDCl_3$): 7.77–7.32 (8H, m, Ph), 5.97–5.88 (1H, m, OCH_2CHCH_2), 5.71 (1H, br d, Fmoc NH, J=7.4 Hz), 5.39–5.27 (2H, m, OCH_2CHCH_2), 4.71–4.63 (3H, m, OCH_2CHCH_2 and CHα), 4.44 (2H, d, Fmoc CH_2, J=1.1 Hz), 4.23 (1H, t, Fmoc CH, J=6.9 Hz), 3.05–2.99 (2H, m, CH_2β), and 1.37 (1H, t, SH, J=8.8 Hz). ^{13}C NMR (75.5 MHz, $CDCl_3$): 141.3, 127.8, 127.1, 125.1, and 120.0 (Ph), 131.2 (OCH_2CHCH_2), 119.4 (OCH_2CHCH_2), 67.1 (OCH_2CHCH_2), 66.5 (Fmoc CH_2), 55.2 (Cα), 47.1 (Fmoc CH), and 27.2 (CH_2β).

4.1.2. Fmoc-Cys[Gal(OAc)$_4$]-OH (3). To a solution of **2** (950 mg, 2.48 mmol) and β-D-galactose penta-acetate (1.93 g, 4.95 mmol) in dry CH_2Cl_2 (40 mL) was added dropwise $SnCl_4$ (0.42 mL, 3.72 mmol). Immediately a yellow colour appeared. The mixture was stirred for 4 h. TLC (hex/EtOAc 1/1) indicated the complete conversion of **2** to a single product. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with 5% $NaHCO_3$ solution (75 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography on silica gel was used to purify Fmoc-Cys[Gal(OAc)$_4$]-OAllyl but the excess of galactose penta-acetate could not completely be removed. ^{1}H NMR spectrum was consistent with Ref. 14 (300 MHz, $CDCl_3$): 7.76 –7.27 (8H, m, Ph), 5.99 (1H, br d, Fmoc NH, J=7.8 Hz), 5.95–5.84 (1H, m, OCH_2CHCH_2), 5.37 (1H, d, H-4, $J_{3,4}$=3.9 Hz), 5.34–5.18 (3H, m, OCH_2CHCH_2 and H-2), 5.01 (1H, dd, H-3, $J_{2,3}$=10.2 Hz,

$J_{3,4}$=3.3 Hz), 4.66 (2H, d, OCH_2CHCH$_2$), 4.60–4.54 (1H, m, CHα), 4.45 (1H, d, H-1, $J_{1,2}$=9.9 Hz), 4.39–4.34 (2H, m, Fmoc CH_2), 4.25 (1H, t, Fmoc CH, J=6.6 Hz), 4.05 (2H, d, 2H-6, $J_{5,6}$=6.3 Hz), 3.76 (1H, t, H-5, $J_{5,6}$=6.3 Hz), 3.29 (1H, dd, C*H*Hβ, J=3.9 Hz, J=14.7 Hz), 3.06 (1H, dd, CH*H*β, J=6.9 Hz, J=14.1 Hz), 2.12, 2.06, 2.00, and 1.93 (4×3H, 4×s, OC(O)CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): 170.3, 169.9, and 169.7 (O*C*(O)CH_3), 155.9 (OC(O)NH), 141.3, 127.7, 127.1, 125.0, and 120.0 (Ph), 131.3 (OCH$_2$*C*HCH$_2$), 118.9 (OCH$_2$CH*C*H$_2$), 83.5 (*C*-1), 71.5, 66.8, 66.7, and 66.3 (C-2, C-3, C-4, and C-5), 67.0 (O*C*H$_2$CHCH$_2$), 66.3 (Fmoc *C*H$_2$), 61.5 (*C*-6), 55.2 (CHα), 47.0 (Fmoc CH), 31.8 (CH_2β), and 20.5 (C(O)*C*H$_3$). A solution of the crude Fmoc-Cys[Gal(OAc)$_4$]-OAllyl (≈2.48 mmol) and anilinium *p*-toluenesulfinate (0.68 g, 2.73 mmol) was stirred in dry THF (40 mL) under an argon atmosphere. Pd(PPh$_3$)$_4$ (80 mg, 0.07 mmol) was added under argon. After stirring for 2 h, TLC indicated 100% conversion. The bright yellow mixture was concentrated in vacuo, dissolved in CH_2Cl_2 (100 mL), and washed twice with 1 M HCl (50 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was isolated by column chromatography on silica gel (hex/EtOAc 1/1→EtOAc/MeOH 1/1) as a yellow foam (1.656 g, 99% over two steps). The NMR spectrum of the product was consistent with Ref. 9: (300 MHz, $CDCl_3$): 7.75–7.35 (8H, m, Ph), 6.11 (1H, br d, Fmoc NH, J=7.50 Hz), 5.38 (1H, d, H-4, $J_{3,4}$=2.7 Hz), 5.20 (1H, t, H-2, J=9.9 Hz), 5.02 (1H, dd, H-3, $J_{2,3}$=9.9 Hz, $J_{3,4}$=3.3 Hz), 4.56–4.47 (3H, m, Fmoc CH_2 and H-1), 4.36 (1H, m, CHα), 4.23 (1H, t, Fmoc CH, J=6.30 Hz), 4.12 (1H, dd, H-6a, J=7.2 Hz, J=11.4 Hz), 4.02 (1H, dd, H-6b, J=6.3 Hz, J=11.4 Hz), 3.29 (1H, dd, C*H*Hβ, J=4.2 Hz, J=14.7 Hz), 3.10 (1H, dd, CH*H*β, J=6.0 Hz, J=14.1 Hz), 2.12, 2.04, 1.99 and 1.93 (4×3H, 4×s, C(O)CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): 170.8, 170.2 170.0, and 169.8 (*C*(O)CH_3), 141.2, 127.7, 127.1, 124.8, and 119.9 (Ph), 84.3 (C-1), 74.3, 71.5, 67.1, and 66.9 (C-2, C-3, C-4, and C-5), 66.8 (Fmoc CH_2), 61.5 (*C*-6), 53.9 (CHα), 46.9 (Fmoc CH), 31.8 (CH_2β), and 20.7 and 20.5 (OC(O)*C*H$_3$).

4.2. General procedure for solid-phase sugar deacetylation (procedure A)

The resin was treated with 5% $N_2H_4\cdot H_2O$ in DMF (4×30 min) and subsequently washed with DMF and CH_2Cl_2 (each 4×2 min).

4.3. General protocol for solid-phase C6-OH acceptor synthesis (procedure B)

The resin containing an unprotected galactose moiety was washed with an appropriate volume of pyridine (3×2 min). A solution of 4,4-dimethoxytrityl chloride (5–10 equiv) in dry pyridine was added to the resin and N_2 was bubbled through the mixture for 4 h. Then the resin was washed with CH_2Cl_2 (6×2 min). The resin was swollen again in pyridine and benzoyl chloride (10 equiv per OH) was added slowly. N_2 was bubbled through the mixture for 1 h followed by a washing step with CH_2Cl_2 (4×2 min). The benzoylation step was repeated. The resin was treated several times with a 1% dichloro acetic acid solution in CH_2Cl_2 (2 min each) until no color release was observed. This was followed by washing with CH_2Cl_2 (6×2 min) and Et_2O (2×2 min). The resin was dried under high vacuum.

4.4. General procedure for solid-phase glycosylations (procedure C)

In a flask the resin (100–300 mg) was combined with the glycosyl donor (5 equiv). An appropriate volume of dry CH_2Cl_2 was added and the mixture was kept under an N_2 atmosphere and cooled to 0 °C. After 30 min TMSOTf (0.3 equiv) was added. The mixture was slowly stirred at 0 °C for 1 h followed by 1 h at rt. A drop of Et_3N was added to neutralize the mixture after which the resin was transferred to a solid-phase tube and washed five times with CH_2Cl_2. The glycosylation procedure was subsequently repeated.

4.5. General procedure for cleavage of the thioglycosides from the resin by DAST/NBS (procedure D)

The resin was swollen in an appropriate amount of CH_2Cl_2 and DAST (5 equiv) was added followed by NBS (5 equiv). N_2 was bubbled through the mixture and after 30 min the mixture was filtered and the resin was washed with CH_2Cl_2 (4×2 min). The filtrate was concentrated to afford the crude anomeric fluorides, which were purified by silica gel column chromatography.

4.6. General procedure for cleavage of the Rink linker (procedure E)

The resin was shaken in a mixture of 95% TFA and 5% H_2O for 2 h. The resin was filtered off and washed with CH_2Cl_2 (5×). The filtrate was concentrated to obtain the crude products, which were purified by silica gel column chromatography followed by HPLC.

4.6.1. Resin (4). A mixture of **3** (775 mg, 1.15 mmol), HATU (437 mg, 1.15 mmol), and DIPEA (0.38 mL, 2.30 mmol) in dry NMP (10 mL) was added to aminomethylated polystyrene resin (500 mg, 0.58 mmol NH_2). The mixture was shaken for 20 h and the resin was filtered, washed with NMP and CH_2Cl_2 (3×10 mL, 2 min each). The Kaiser test indicated the reaction to be complete. The remaining amines were capped with capping reagent (10 mL) for 1 h. After a washing procedure with NMP and CH_2Cl_2 (both 3×10 mL, 2 min each, i.e., general washing procedure) the Fmoc group was cleaved by treatment with 20% piperidine in NMP (3×10 mL, each 20 min). After a general NMP/CH_2Cl_2 washing a Kaiser test showed the presence of free amines, which were acetylated twice (1.5 h each) by a mixture of pyridine (7.5 mL) and Ac_2O (5 mL). After washing no free amines were present as shown by the Kaiser test. Hydroxyl deprotection was performed according to procedure A.

4.6.2. Resin (6). Resin **4** was treated according to the procedure B to yield resin **6**.

4.6.3. Resin (10). In a solid-phase reaction tube commercially available 3-[4-(tritylmercapto)phenyl]propionyl aminomethyl functionalized polystyrene resin (1.00 g, 0.80 mmol) was treated several times (5 min each) with a 1/1 mixture of CH_2Cl_2 and TFA until the release of the yellow

trityl cation had ceased. The resin was washed with CH_2Cl_2 (6×) and Et_2O (2×) and dried for 2 h. In a flask the resin was combined with peracetylated galactopyranose (1.72 g, 4.40 mmol), CH_2Cl_2 (5 mL) was added followed by $BF_3 \cdot Et_2O$ (0.56 mL, 4.40 mmol). The reaction mixture was stirred for 20 h. Then the resin was filtered off and washed with CH_2Cl_2 (4×2 min) and Et_2O (2×2 min). The glycosylation procedure was repeated to guarantee a full loading of the resin. The resin was subjected to the deacetylation procedure A. A 50-mg sample was benzoylated and cleaved from the resin to indicate a loading of 0.8 mmol/g. Finally procedure B was followed.

4.6.4. Resin (11). Detritylation was performed as described for the synthesis of resin **10**. The 3-[4-(tritylmercapto)phenyl]propionyl aminomethyl functionalized polystyrene resin (0.44 mmol) was combined with donor 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β-D-galactopyranosyl trichloroacetimidate (476 mg, 0.88 mmol, for synthesis see Supplementary data). To this CH_2Cl_2 (15 mL) was added and the reaction mixture was cooled to 0 °C under an N_2 atmosphere. After 30 min TMSOTf (17 μL, 0.09 mmol) was added and the mixture was stirred slowly for 3 h. Then the mixture was warmed to rt and stirred additionally for 20 h. The resin was transferred to a solid-phase reaction vessel and washed with CH_2Cl_2 (4×) and Et_2O (2×). The glycosylation procedure was repeated to guarantee a full loading of the resin. The resin was subjected to the deacetylation procedure A. A 50-mg sample was benzoylated and cleaved from the resin to indicate a loading of 0.5 mmol/g. Finally procedure B was followed.

4.6.5. Fluoro *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α/β-D-galactopyranoside (8). Either resin **6**, or **10** or **11** (100–300 mg) was glycosylated according to procedure C and the product was cleaved from the resin according to procedure D, to yield monosaccharide **9** (clear glass) and the disaccharide **8** (white foam). Compound **8** (α/β=1/1): ^{1}H NMR (500 MHz, $CDCl_3$): 8.08–7.27 (15H, m, Ph), 6.07 (0.5H, d, H-1α, $J_{H,F}$=53 Hz), 5.98–5.84 (2H, m, H-2 and H-4), 5.70–5.58 (1H, 2×m, H-3), 5.63 and 5.53 (0.5H, 2×d, H-1β, $J_{H,F}$=51.8 Hz, $J_{1,2}$=6.4 Hz), 5.36 (1H, s, H-4′), 5.20 (1H, t, H-2′, $J_{2',3'}$=9.8 Hz), 5.02 (1H, d, H-3′, $J_{2',3'}$=10.3 Hz), 4.58–4.52 (1H, m, H-1′), 4.65 and 4.30 (1H, 2×br s, H-5), 4.09–3.83 (5H, m, H-5′, 2×H-6, and 2×H-6′), 2.15, 2.07, 2.01, and 1.99 (4×3H, 4×s, C(O)CH_3). ^{13}C NMR (75.0 MHz, $CDCl_3$): 170.2, 170.1, and 169.6 (*C*(O)CH_3), 165.4, 165.2, and 165.1 (*C*(O)Ph), 133.8–128.3 (Ph), 101.5 (C-1′), 73.4, 73.3, 70.7, 69.9, 69.6, 68.5, 67.6, 66.9, 66.8, and 61.1 (C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6, and C-6′), and 20.6 (*C*(O)CH_3).

4.6.6. Fluoro 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (9). Compound **9** (only β isomer): ^{1}H NMR spectrum is consistent with Ref. 15: (300 MHz, $CDCl_3$): 5.42 (1H, s, H-4), 5.36–5.29 (1H, m, H-2), 5.25 (1H, dd, $J_{1,2}$=7.2 Hz, $J_{H,F}$=49.2 Hz), 5.05 (1H, d, H-3, *J*=4.5 Hz), 4.21 (2H, d, 2×H-6, *J*=3.9 Hz), 4.06 (1H, t, H-5, *J*=3.6 Hz), 2.18, 2.11, 2.07, and 2.01 (4×3H, 4×s, C(O)CH_3).

4.6.7. Resin (13). Fmoc loaded Rink aminomethyl polystyrene resin (1.00 g, 0.78 mmol NHFmoc) was treated with 20% piperidine in NMP (3×10 mL, 15 min each). The resin was washed with NMP (4×10 mL, 2 min each) and CH_2Cl_2 (4×10 mL, 2 min each). Kaiser test indicated the reaction to be complete. To the resin was added a mixture of **12** (1.09 g, 1.56 mmol), HATU (593 mg, 1.556 mmol), and DIPEA (515 μL, 3.12 mmol) in dry NMP (10 mL). The resin was shaken for 20 h and the resin was filtered and washed with NMP (4×10 mL, 2 min each), CH_2Cl_2 (4×10 mL, 2 min each), and Et_2O (2×10 mL, 2 min each). Standard Fmoc analysis revealed a loading of 0.48 mmol/g. Although the Kaiser test revealed a complete coupling, a capping reaction (10 mL capping solution) was performed for 1 h. The Fmoc group was cleaved by piperidine as described above. The resin was swollen in dry pyridine (10 mL) and benzoyl chloride (0.91 mL, 7.8 mmol) was added. N_2 was bubbled through the mixture for 1 h followed by washing with CH_2Cl_2 (6×10 mL, 2 min each) and Et_2O (2×10 mL, 2 min each). Hydroxyl deprotection was performed according to procedure A, selective protecting group chemistry was performed according to procedure B.

4.6.8. *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-*N*-(carboxamido *N*-benzoyl-L-glutam-5-oyl)-β-D-galactopyranosylamine) (15a). Glycosylation of resin **13** with **7** according to procedure C, followed by cleavage from the resin by procedure E yielded **15a** (white solid) (16.4 mg, 25%): ν_{max} (KBr)=3500–3100 (br), 2924, 1734, 1262, 1070, and 711 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 8.10–7.23 (22H, m, Ph, NH_2), 6.74 (1H, br t, C(O)NHC-1), 5.92 (1H, dd, H-4, $J_{3,4}$=3.0 Hz), 5.73 (1H, dd, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=3.5 Hz), 5.67 (1H, t, H-2, $J_{1,2}$=9.0 Hz), 5.61 (1H, t, H-1, $J_{1,2}$=8.5 Hz), 5.36 (1H, s, NHBz), 5.32 (1H, d, H-4′, $J_{3,4}$=3.0 Hz), 5.17 (1H, dd, H-2′, $J_{2',3'}$=10.5 Hz, $J_{1',2'}$=8.0 Hz), 4.99 (1H, dd, H-3′, $J_{2',3'}$=10.0 Hz, $J_{3',4'}$=3.0 Hz), 4.58 (1H, d, H-1′, $J_{1',2'}$=8.0 Hz), 4.65–4.61 (1H, m, CHα), 4.26 (1H, t, H-5, $J_{5,6}$=6.0 Hz), 4.08–3.83 (4H, m, H-5′, 2×H-6′ and 2×H-6), 2.59–2.51 and 2.42–2.27 (2×1H, 2×m, CH_2γ), 2.10–1.94 (2H, m, CH_2β), 2.13, 2.06, 1.98, and 1.97 (4×3H, 4×s, C(O)CH_3). ^{13}C NMR (125.5 MHz, $CDCl_3$): 170.7, 170.5, 170.3, and 169.3 (*C*(O)CH_3), (*C*(O)NHC-1), 133.3–128.3 (Ph), 100.9 (C-1′), 78.9 (*C*-1), 75.5 (C-5), 71.5 (C-3), 70.8 (C-2), 70.8 (C-3′), 70.6 (C-5′), 68.0 (C-4), 68.2 (C-2′), 66.3 (H-6′), 66.7 (C-4′), 60.7 (H-6), 52.7 (Cα), 32.7 (CH_2γ), 21.4, 20.5, and 20.3 (C(O)*C*H_3). HRMS for $C_{53}H_{55}N_3O_{20}$ (M, 1053,338) M+H found 1054.295, calcd 1054.347.

4.6.9. *O*-(2,3,4,6-Tetra-*O*-pivaloyl-β-D-galactopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-*N*-(carboxamido *N*-benzoyl-L-glutam-5-oyl)-β-D-galactopyranosylamine) (15b). Glycosylation of resin **13** with **14** according to procedure C, followed by cleavage from the resin by procedure E yielded **15b** (white solid) (21.8 mg, 30%): ν_{max} (KBr)=3500–2800 (br), 1730, 1684, 1533, 1451, 1267, 1107, and 709 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 8.10–7.22 (22H, m, Ph, NH_2), 6.85 (1H, br t, (CO)NHC-1), 5.90 (1H, dd, H-4, $J_{3,4}$=3.5 Hz, $J_{4,5}$=1.0 Hz), 5.72 (1H, dd, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=3.5 Hz), 5.67 (1H, dd, H-2, $J_{1,2}$=8.5 Hz, $J_{2,3}$=10.0 Hz), 5.64 (1H, s, NHBz), 5.61 (1H, t, H-1, $J_{1,2}$=8.5 Hz), 5.35 (1H, d, H-4′, $J_{3',4'}$=3.5 Hz), 5.19 (1H, dd, H-2′, $J_{1',2'}$=8.0 Hz, $J_{2',3'}$= 10.5 Hz), 5.09 (1H, dd, H-3′, $J_{2',3'}$=10.5 Hz, $J_{3',4'}$=3.5 Hz), 4.62 (1H, d, H-1′, $J_{1',2'}$=8.0 Hz), 4.61 (1H, m, CHα), 4.26–4.23 (1H, m, H-5), 4.01–3.89 (4H, m, H-5′, H-6a, and 2×H-6′), 3.76 (1H, dd, H-6b, *J*=7.0 Hz, *J*=11.0 Hz),

2.56–2.50 and 2.42–2.37 (2×1H, 2×m, $CH_2\gamma$), 2.12–2.05 and 2.00–1.94 (2×1H, 2×m, $CH_2\beta$), 1.24, 1.16, 1.10, and 1.10 (4×9H, 4×s, C(O)C(CH_3)$_3$). ^{13}C NMR (125.5 MHz, $CDCl_3$): 177.9, 177.5, 177.0, and 176.8 (*C*(O)C(CH_3)$_3$), 167.9, 165.4, 165.3, 165.2, and 165.1 (*C*(O)Ph), 165.2 (*C*(O)NHC-1),130.0–128.3 (Ph), 100.9 (C-1′), 79.0 (*C*-1), 75.2 (C-5), 71.0 (C-3′), 71.0 (C-5′), 70.8 (C-3), 70.8 (C-2), 68.7 (C-4), 68.6 (C-2′), 67.6 (C-6′), 66.8 (C-4′), 61.0 (C-6), 52.5 (Cα), 38.7, 38.8, and 39.1 (C(O)*C*(CH_3)$_3$), 32.8 ($CH_2\gamma$), 27.1 ($CH_2\beta$), 27.2, 27.1, and 27.1 (C(O)C(*C*H_3)$_3$). HRMS for $C_{65}H_{79}N_3O_{20}$ (M, 1221.526) M+H found 1222.538, calcd 1222.534.

4.6.10. *O*-(2,3,4,6-Tetra-*O*-pivaloyl-β-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-*N*-(carbamoyl *N*-benzoyl-L-glutam-5-oyl)-β-D-galactopyranosylamine) (16). Glycosylation of resin **13** with **14** according to procedure C. The resin (208 mg) was subsequently treated with a 1% NaOMe solution in 20% MeOH/dioxane (6×5 mL, 20 min each). The resin was then washed with CH_2Cl_2 (6×5 mL, 2 min each) and Et_2O (2×5 mL, 2 min each). Selective protecting group chemistry was performed according to procedure B followed by glycosylation with **14** according to procedure C. Finally, cleavage from the resin was performed by procedure E and purification by HPLC to yield **16** (white solid) (1.9 mg, 2%, non-optimized): ν_{max} (KBr)= 3500–3100 (br), 2965, 2929, 1734, 1281, 1107, and 710 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 8.08–7.22 (37H, m, Ph, NH_2), 6.81 (1H, s, C(O)NH), 5.94 (1H, dd, H-4, $J_{3,4}$=3.0 Hz, $J_{4,5}$=1.0 Hz), 5.82 (1H, dd, H-4′, $J_{3',4'}$=3.0 Hz, $J_{4',5'}$=0.5 Hz), 5.67 (1H, dd, H-2′, $J_{1',2'}$=8.0 Hz, $J_{2',3'}$= 10.5 Hz), 5.66 (1H, dd, H-3′, $J_{2',3'}$=11.00 Hz, $J_{3',4'}$=3.5 Hz), 5.61 (1H, t, H-2, $J_{1,2}$=8.5 Hz), 5.56–5.53 (3H, m, H-1, NH, H-3), 5.30 (1H, d, H-4″, $J_{3'',4''}$=3.5 Hz), 5.11 (1H, dd, H-2″, $J_{1'',2''}$=8.0 Hz, $J_{2'',3''}$=10.5 Hz), 5.02 (1H, dd, H-3″, $J_{2'',3''}$= 10.5 Hz, $J_{3'',4''}$=3.5 Hz), 4.90 (1H, d, H-1′, $J_{1',2'}$=7.5 Hz), 4.61–4.57 (1H, m, CHα), 4.28 (1H, d, H-1″, $J_{1'',2''}$=7.5 Hz), 4.19 (1H, t, H-5, $J_{5,6}$=6.0 Hz), 4.04–4.00 (3H, m, H-5″, H-6a, and H-6a′), 3.89–3.77 (5H, m, H-5′, H-6b, H-6b′, and 2×H-6″), 2.56–2.50 and 2.39–2.34 (2×1H, 2×m, $CH_2\gamma$), 2.17–2.10 and 2.05–1.97 (2×1H, 2×m, $CH_2\beta$), 1.22, 1.11, 1.09, and 1.08 (4×9H, 4×s, C(O)C(CH_3)$_3$). ^{13}C NMR (125.5 MHz, $CDCl_3$): 177.8, 177.3, 176.9, and 176.6 (*C*(O)C(CH_3)$_3$), 167.7, 165.3, and 165.1 (*C*(O)Ph), 133.8–127.3 (Ph), 101.0 (C-1″), 100.5 (C-1′), 79.1 (C-1), 75.0 (C-5), 72.9 (C-5′), 71.7 (C-3), 71.6 (C-3′), 70.9 (C-3″), 70.8 (C-5″), 70.5 (C-2), 69.6 (C-2′), 68.7 (C-2″), 68.4 (C-4), 68.3 (C-4′), 67.4 (C-6), 66.6 (C-4″), 66.4 (C-6′), 60.7 (C-6″), 52.6 (Cα), 38.7, 38.8, and 39.1 (C(O)*C*(CH_3)$_3$), 32.7 ($CH_2\gamma$), 27.1 ($CH_2\beta$), 27.1, and 27.0 (C(O)C(*C*H_3)$_3$). HRMS for $C_{92}H_{101}N_3O_{28}$ (M, 1695.657) M+Na found 1718.638, calcd 1718.647.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.03.051.

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