A Two-Directional Approach for the Solid-Phase Synthesis of Trisaccharide Libraries

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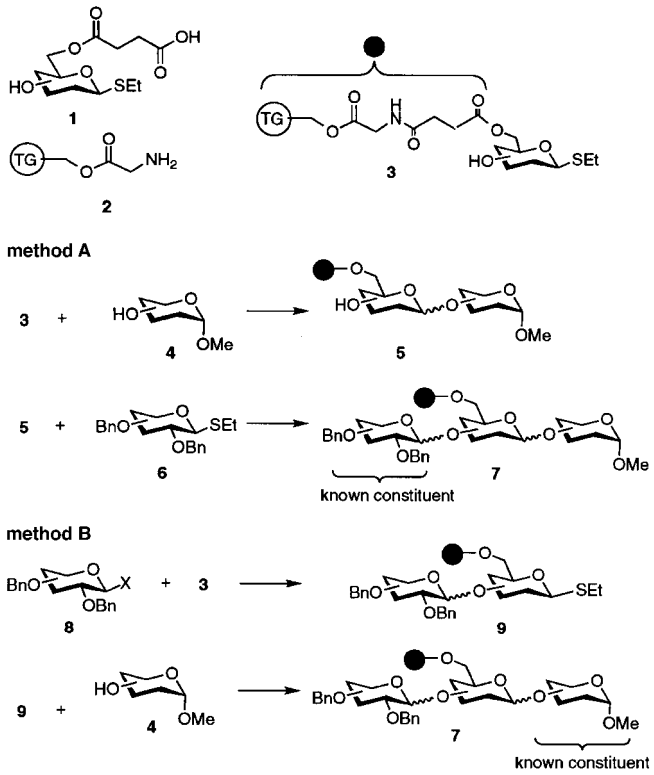
In recent years the search for novel pharmacological agents has focused on the preparation of "chemical libraries" as potential sources of new lead compounds for drug discovery.[1] Combinatorial chemistry has been developed mainly for the preparation of peptide and nucleic acid libraries and some small molecules, but only very few reports deal with combinatorial carbohydrate chemistry.[2] We report here the synthesis of a combinatorial saccharide library on a solid support whereby all glycosidic linkages are intentionally synthesized as mixtures of anomers. This approach addresses problems associated with classical oligosaccharide solid-phase synthesis.[3] In addition, a novel two-directional approach[4] for the assembly of the oligosaccharides on the solid support is used in which each immobilized saccharides can act as a glycosyl donor as well as an acceptor.

The strategic considerations for the preparation of trisaccharide libraries are summarized in Scheme 1. The thioglycosyl building blocks **1** are immobilized onto a solid support through the formation of an amide linkage between the carboxylic acid of a succinic acid moiety and the amine group of a glycine-derivatized polymer. This amide bond formation should offer a very reliable approach for immobilization. However, a product can simply be removed from the solid support by base-mediated cleavage of the ester linkage between the carbohydrate and the succinimide residue. Glycine-derivatized TentaGel hydroxyl resin (**2**; TG = TentaGel)was selected as the solid support so that the high flexibility and mobility of its polyethylene glycol moieties could be utilized, thus ensuring high reactivities of the immobilized compounds.[5] A resin-bound saccharide **3** can first act as a glycosyl donor, and on reaction with a glycosyl acceptor **4** in solution will give a disaccharide **5**. It is hoped that oligomerization will be prevented by site isolation of the immobilized substrates. The resulting disaccharide can immediately be used in the next step as a glycosyl acceptor. When coupled with a thioglycoside **6** that is in solution, **5** will give a resin-bound trisaccharide **7**. A library of trisaccharides can be prepared by a mix-and-split approach. Thus, **3** can be coupled in individual glycosylation reactions to a range of acceptors, and the products can be mixed and split before each pool of resin-bound acceptors are glycosylated with a range of donors. After cleavage of the resin and deprotection, a library with a known residue at the nonreducing end is generated (method A). Alternatively, compound **3** can act as an acceptor and on coupling with donor **8** (X = F, SOPh, or $OCNHCCl_3$) will give a resin-bound glycosyl donor **9**, which in a subsequent step can be coupled with an acceptor **4** to give a trisaccharide. A mix-and-split approach will thus give a trisaccharide library with a known residue at the reducing end of the saccharides (method B). A key principle will be that each glycosylation will be performed under conditions that give a mixture of anomers. Recently, we established[6] that glycosylations[7] of thioglycosides mediated by *N*-iodosuccinimide/trimethylsilyl trifluoromethanesulfonate (NIS/TMSOTf) in dichloromethane at room temperature consistently give mixtures of anomers.



Scheme 1. A two-directional strategy for the syntheses of trisaccharide libraries.

Treatment of the dibutyltin acetal of diol **11** with succinic anhydride afforded **12** in an excellent yield of 85% (Scheme 2).[8] Attachment of a Fmoc-protected glycine (Fmoc = fluorenyloxycarbonyl) to the TentaGel hydroxyl resin **13** (0.37 mmol g^{-1} resin) under standard conditions[9a] and subsequent removal of the Fmoc group by treatment with piperidine gave polymer **2**. Compound **12** was immobilized by amide bond formation with **2** in the presence of benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) to give the polymer bound monosaccharide **15**(Scheme 2).[9b] Compound **15** was coupled with **16** in the presence of NIS/TMSOTf to give the desired disaccharide **17**, which after washing was used as an acceptor. Glycosylation with the perbenzylated thioglycosyl donor **18** gave the trisaccharide **19** (Scheme 3). This latter coupling was repeated due to an incomplete reaction (the 4′-OH group of **17** has a low reactivity). Completion of each reaction step was ascertained by thin-layer chromatography and matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of the crude product after cleavage of a small amount of beads (3–5 mg) by treatment with base. Finally, treatment of **19** with MeONa/MeOH in 1,4-dioxane gave trisaccharide **20** (≈ 65% overall yield based on the resin

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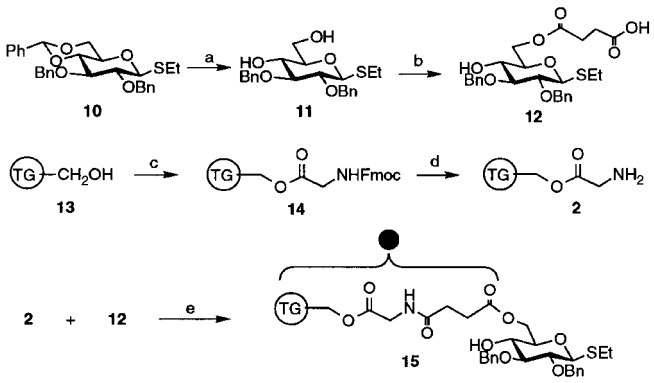
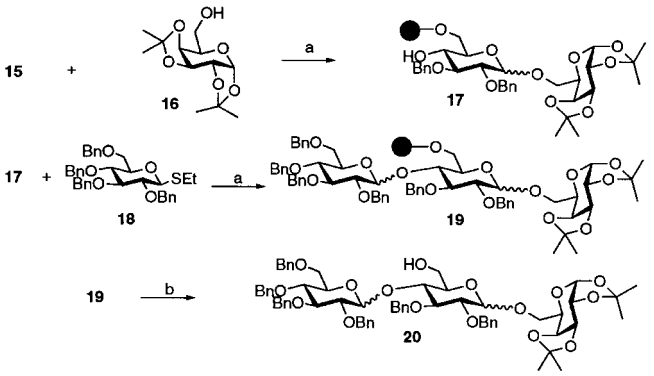


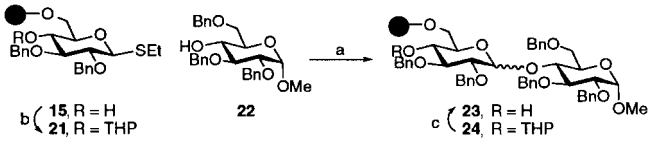
Scheme 2. Synthesis of the polymer-bound thioglycoside **15**. a) Trifluoroacetic acid (TFA), CH_2Cl_2, 95%; b) $Bu_2Sn(OMe)_2$, benzene, Dean–Stark conditions then succinic anhydride, pyridine, 85%; c) *N,N′*-diisopropylcarbodiimide (DIC), 4-dimethylaminopyridine (DMAP), Fmoc-GlyOH, DMF; d) piperidine (20% in DMF), >90% overall yield; e) PyBOP, diisopropylethylamine (DIPEA), DMF.



Scheme 3. Synthesis of trisaccharide **20** (method A). a) NIS/TMSOTf, CH_2Cl_2, 4-Å molecular sieves; b) MeONa, MeOH, 1,4-dioxane then DOWEX50 (H^+) ion-exchange resin.

loading; Scheme 3). Analysis by NMR spectroscopy and mass spectrometry showed that no oligomerization had occurred and that all glycosidic linkages were formed as mixtures of anomers (Glc(1-4)Glc: $\alpha/\beta \approx 2/1$ and Glc(1-6)Gal: $\alpha/\beta \approx 1/1$). Thus, the NIS/TMSOTf-mediated glycosylation of thioglycosides in dichloromethane is highly compatible with the solid support and linker system employed. It is noteworthy that the glycosylation rates are significantly decreased (4 h) compared to those for similar reactions in solution (5–10 min). However, this feature does not affect the reliability of the glycosylation approach.

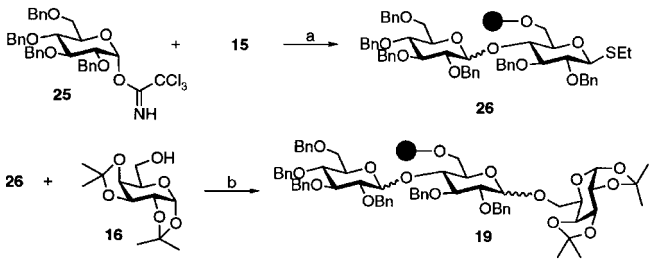
Encouraged by these results, we turned to the glycosyl acceptor **22** that has an unreactive 4-hydroxyl group. Unfortunately, coupling of **15** with **22** in the presence of NIS/TMSOTf gave a small amount of oligomeric products (5–10%) in addition to the required product **23** (Scheme 4). A



Scheme 4. Synthesis of polymer-bound disaccharide **23**. a) NIS/TMSOTf, CH_2Cl_2, 4-Å molecular sieves; b) DHP, PPTS; c) AcOH, THF, H_2O.

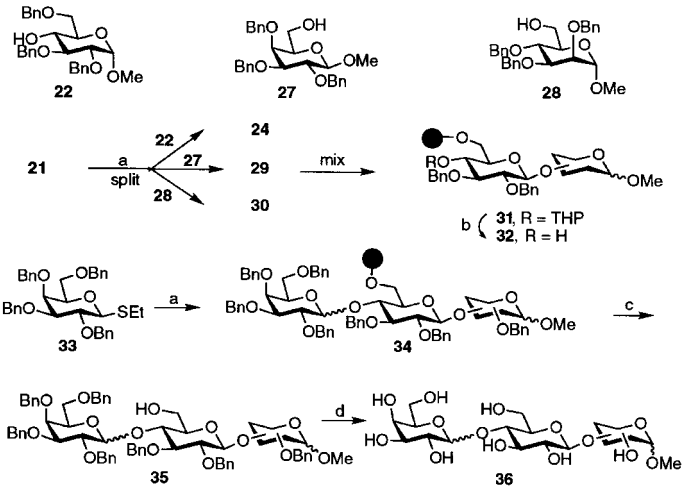
similar result was obtained when the more rigid Merrifield resin (1% cross-linked) was employed as the solid support. The hydroxyl group of **15** was therefore protected as a tetrahydropyranyl (THP) ether to avoid oligomerization. Thus, treatment of **15** with 3,4-dihydro-2-pyran (DHP) in the presence of pyridinium *p*-toluenesulfonate (PPTS)[10] gave **21** in virtually quantitative yield. This product was successfully coupled with **22** to give the disaccharide **24**. The THP group of **24** was easily removed by treatment with acetic acid/water to yield **23**.

Immobilized **12** also proved to be a good glycosyl acceptor when coupled with a perbenzylated trichloroacetimidate donor. Thus, the TMSOTf-mediated coupling of **15** with an excess of **25** gave resin-bound **26.** This product was further coupled with acceptor **16** to give trisaccharide **19** (60% overall yield based on the resin loading) (Scheme 5).



Scheme 5. Synthesis of polymer-bound trisaccharide **19** (method B). a) TMSOTf, CH_2Cl_2, 4-Å molecular sieves; b) NIS/TMSOTf, CH_2Cl_2, 4-Å molecular sieves.

To verify the proposed strategy, a relatively small library of 12 trisaccharides was prepared (Scheme 6). Three different glycosyl acceptors **22**, **27**, and **28** were coupled in individual



Scheme 6. Synthesis of the trisaccharide library **36**. a) NIS/TMSOTf, CH_2Cl_2, 4-Å molecular sieves; b) HOAc/THF/H_2O, 4/2/1, 50 °C; c) MeONa, MeOH, 1,4-dioxane then DOWEX50 (H^+) ion-exchange resin; d) $Pd(OAc)_2$, H_2, EtOH.

reactions with **21**, with NIS/TMSOTf as the promoter, to give three different disaccharides (**24**, **29**, and **30**, respectively) as mixtures of anomers. The beads were combined, the THP group removed by treatment with HOAc/THF/H_2O (4/2/1),[11]

and the mixture of disaccharide acceptors coupled with the glycosyl donor **33,** again with NIS/TMSOTf as the promoter. The trisaccharide library was released from the polymer after thin-layer chromatography and MALDI-TOF MS had shown that all the disaccharides had been consumed. The library was purified by size-exclusion column chromatography (LH-20; CH_2Cl_2/MeOH, 1/1), and the benzyl groups removed by hydrogenation over $Pd(OAc)_2$ to give the deprotected library **36** (55 % overall yield based on the resin loading).[12]

To further examine the quality of this library, a monosaccharide compositional analysis was performed. A portion of the trisaccharide library was treated with aqueous trifluroacetic acid (2 M) at 100 °C for 4 h. Analysis of the resulting mixture of monosaccharides by HPLC (Dionex with a PA1 column and pulsed amperometric detection) showed that galactose, glucose, and mannose were present in approximately the required ratio.[13]

In conclusion, a highly efficient approach for the synthesis of oligosaccharide and saccharide libraries on a solid-support has been described. Saccharides can be reliably immobilized by amide bond formation between a succinimide linker and a glycine-derivatized TentaGel resin. The products can be analyzed after each reaction step by MALDI-TOF MS and thin-layer chromatography by cleavage of the base-labile ester linker from a small amount of resin. The glycosylation strategy is two-directional: The immobilized thioglycoside acts first as a donor, and the product bearing a free hydroxyl group is used in subsequent glycosylation as an acceptor and glycosylated with a thioglycosyl donor. A mix-and-split approach gave a library with a known monosaccharide residue at the nonreducing end. Immobilized **12** can also act first as a glycosyl acceptor and the resulting disaccharides can be used as a glycosyl donor.

Experimental Section

General procedure for NIS/TMSOTf-mediated glycosylation on a solid support: The polymer-bound thioglycosyl donor (0.1 mmol) was placed in a round-bottom flask just covered with dichloromethane and allowed to swell for 15 min. Glycosyl acceptor (0.5 mmol) in dichloromethane (4 mL) and 4-Å molecular sieves (200 mg, beads) were added, and the suspension was shaken at room temperature for 15 min. NIS (0.5 mmol) and TMSOTf (0.05 mmol) were then added, and the mixture was shaken at room temperature for 2 – 4 h. After removal of the molecular sieves by decanting, the resin was washed successively with DMF (3 × 20 mL), dichloromethane (3 × 20 mL), and methanol (3 × 20 mL), and dried in vacuo over P_2O_5 for 24 hours.

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[9] a) The degree of glycine attachment was estimated photometrically from the amount of Fmoc chromophore released upon treatment of **15** with piperidine/DMF. b) The progress of the coupling reactions was monitored by the Kaiser test; for details, see *Novabiochem* Catalog & Peptide Synthesis Handbook **97/98**.

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[12] All saccharides in the library were prepared as individual compounds, and each trisaccharide was formed as a mixture of anomers in a range of $\alpha/\beta = 1/1 - 2/1$.

[13] Expected product ratios: Gal/Glc/Man: 1/1/0.25, found: 1/0.9/0.25.

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