Solid-Phase Random Glycosylation

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Two different approaches were employed to study solid phase random glycosylations to obtain oligosaccharide libraries. In approach I, Wang resin esters were attached to the acceptors structures. Following their glycosylation and resin cleavage, the peracetylated components of the oligosaccharide libraries were characterized. In approach II, polymer-linked donor components could be employed and processed correspondingly. Approach I proved to be superior regarding yield and versatility of products and also allowed the formation of higher oligomers.

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Introduction

Generation and biological screening of compound libraries is the fastest way to identify lead compounds for pharmaceutical purposes. The development of analytical separation techniques and screening methods in the past 20 years gave powerful tools facilitating the research to find new potential drug molecules. Peptides were the first naturally occurring oligomeric or polymeric biomolecules to be successfully generated as combinatorial libraries.[1] The preparation of the first oligonucleotide library followed about a decade later,[2] whereas the first oligosaccharide library was published in 1995 by Hindsgaul et al.[3] In that paper, the authors describe the solution phase synthesis of three different sublibraries of trisaccharides by random glycosylation. Contrary to conventional glycosylations, in which the acceptor is selectively protected having only the desired hydroxyl function available for reactions, the acceptor at random glycosylations contains more than one available hydroxyl function, enabling the synthesis of several products in a single glycosylation step. Following this line applying the "latent-active" strategy,[4] linear and branched trisaccharide libraries were prepared using a split mix method by Boons et al.[5,6] In 1998 the same group published the preparation of a trisaccharide library using a solid support methodology,[7] applying a thioglycoside donor loaded to Tentagel, to generate a library of twelve compounds. A significant milestone in the development of carbohydrate libraries was a 1296 member library made by a split-mix protocol by Kahne's group.[8] Seventy-two different di- and trisaccharides were prepared and subsequently derivatized to reach the final library. Since these report, there have been no additional publications concerned with random glycosylation either in solution or in solid phase. Although great advances have been made for the preparation of carbohydrate oligomers in both solution and solid phase, the synthesis of these biologically important biopolymers remains a challenging and time-consuming procedure. The presence of multiple functional groups possessing similar steric environments on the carbohydrate nucleus creates a significant challenge to the regiospecific functionalization of these molecules relative to the preparation of oligopeptides and oligonucleotides. We do believe that still a large number of fundamental studies have to be performed until a general routine for the synthesis of complex oligosaccharides is developed. The purpose of this study was to examine and evaluate different approaches resulting in oligosaccharide libraries.

Results and Discussion

Herein, we present random glycosylations on solid phase utilizing two different approaches. In approach I (Schemes 1−3), a glycosyl acceptor was immobilized on solid support, whereas in approach II (Scheme 4 and) the glycosyl donor was attached to the resin.

The acceptor bound approach I was used to load monosaccharides with two (**1**),[9] three (**6**),[10] or four unprotected hydroxyl groups (**11**) to Wang resin via a succinic acid linker. The resin bound monosaccharide acceptors obtained this way were glycosylated with thioglycoside donor **2**[11] affording resin bound di- or trisaccharides. These oligosaccharides were cleaved from the resin with sodium methoxide affording a crude mixture, which was subsequently acetylated and separated by column chromatography. The isolated fractions were analyzed by NMR methods to identify the structures of oligosaccharides in all fractions.

Purification of the random glycosylation mixture using polymer bound **1** as acceptor yielded two fractions (Scheme 1, Table 1, entry 1). The first fraction contained only the acetylated acceptor **3**, whereas the second fraction was a mixture of the two expected disaccharides **4** (β,1→4) and **5** (β,1→3) in a ration of 2:1. Because of the neighboring group participation of the ester in position 2 of the donor, no formation of an α-glycosidic linkage

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Scheme 1. Approach I: Solid-Phase Random Glycosylation with Resin-Bound Diol Acceptor

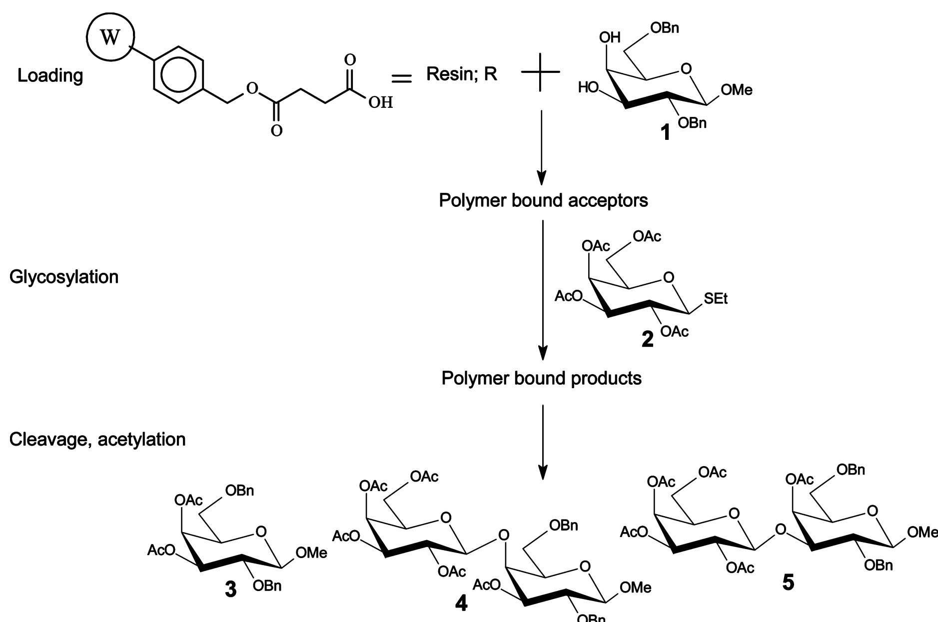

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Table 1. Summary of the Random Glycosylations, Isolated Fractions, and Yields

entry	donor	acceptor	fraction	products yields	approach
1	**2**	**1**	1	**3** (50%)	I
	2			**4** (30%)	
				5 (15%)	
2	**2**	**6**	1	**7** (40%)	I
	2			**8** (20%)	
				9 (20%)	
			3	**10** (15%)	
3	**2**	**11**	1	**12** (35%)	I
	2			**13** (20%)	
				14 (7%)	
			3	**15** (8%)	
				16 (2%)	
			4	**17** (20%)	
4	**18**	**1**	1	**19** (40%)	
				20 (40%)	II
5	**18**	**6**	1	**21** (5%)	II
				22(15%)	
	2			**23** (60%)	
6	**18**	11		no product obtained	II

could be observed in this or any of the following experiments. Considering the formed disaccharides the immobilization of the monosaccharide acceptor occurred via either of the two available hydroxyl functions, probably in favor of the more reactive 3−OH function. Under the chosen reaction conditions only 50% conversion for the acceptor was observed. It was possible to increase the conversion by iteration of the glycosylation step. Repeating glycosylation three times with the same ratio of donor, the percentage of unreacted acceptor decreased to 5% according to NMR data (data not shown). The iteration of the glycosylation step was not conducted for the other experiments.

Separation of the glycosylation mixture of triol **6** as an acceptor (Scheme 2, Table 1, Entry 2) afforded three fractions. Again, the first fraction was the unreacted monosaccharide acceptor **7**, whereas the second fraction contained a mixture of two disaccharides **8** and **9** in equal amounts. These were the β,1→3- and β,1→4-linked disaccharides, whereas the corresponding β, 1 → 6 disaccharide was not observed by NMR. The main product of the third fraction was the β,1→3-, β,1→4- linked branched trisaccharide **10**. Another trisaccharide was present in the fraction in traces (yield ≤2%), but the structure remained unclear after detailed NMR analysis. There was no evidence for the presence of a β,1→6- linked disaccharide in this fraction by NMR or MALDI-TOF spectra. The coupling of the acceptor **6** to the resin must have occurred predominantly via the 6-OH function.

Purification of the glycosylation mixture of acceptor **11** bound to the resin resulted in four main fractions (Scheme 3, Table 1, entry 3). The first fraction contained the nonglycosylated monosaccharide **12**, the second and the third fractions were mixtures of all possible disaccharides (**13**, **14**, **15**, and **16**). The second fraction consisted of a mixture of the β,1→2- and the β,1→6-linked disaccharides, and the third contained a mixture of the β,1→3- and β,1→4-linked ones. The main product in the fourth fraction was the β,1→2-, β,1→3-linked branched trisaccharide **17**, but traces of two other trisaccharides were present in this fraction. There were a few signals in the NMR spectra which suggested that one was probably the β,1→2-, β,1→4-linked branched trisaccharide, whereas the structure of the third trisaccharide remained unknown. Because of these uncertainties the compounds are not presented in Scheme or in Table. No tetrasaccharides were formed during the glycoslation according to MALDI-TOF analysis of the crude mixture.

The donor-bound approach II (Schemes 4 and 5) was used to attach the thioglycoside donor **18**[12] having one free hydroxyl function to the same resin used for the previous approach. The polymer-bound donor obtained was subsequently used to glycosylate monosaccharides having more than one free hydroxyl function. During these reactions formation of different disaccharides and some donor degradations were observed. In contrast to approach I, formation of tri- or tetrasaccharides was not possible.

Table 2. NMR Data of Compounds[a]

	3	**4**	**5**	**7**	**8**	**9**	**10**
A1	4.38 (7.6 Hz)	4.31 (7.5 Hz) 105.33	4.33 (7.6 Hz) 105.01	4.39 (7.6 Hz)	4.29 (7.9 Hz) 105.00	4.30 (7.6 Hz) 105.00	4.22 (7.6 Hz) 104.81
A2	3.48	3.61 80.26	3.50 77.79	3.60	3.61 79.89	3.51 77.39	3.48 79.38
A3	4.97	3.83 77.71	4.88	4.97	3.48 77.29	4.85 74.60	3.76 80.57
A4	5.42	5.40 69.91	4.07 75.94	5.36	5.36 69.45	4.07 75.36	4.15 74.84
A5	3.83	3.72 73.14	n.d.	3.87	n.d.	3.66 72.00	n.d.
A6	3.57, 3.59	3.67	3.75, 3.58	4.19, 4.11	4.10, 4.10 64.21	4.36, 4.26 64.21	n.d.
OMe	3.59	3.58	3.59	3.59	3.56 57.68	3.57 57.68	3.51 57.24
C1		4.85 (8.2 Hz) 101.04			4.89 (7.6 Hz) 101.23		4.87 (7.9 Hz) 101.81
C2		5.16 69.62			5.15 69.60		5.18 69.54
C3		4.94 71.32			4.94 71.13		5.14 71.17
C4		5.32 67.20			5.33 67.31		5.34 67.47
C5		nd			3.76 71.28		n.d.
C6		nd			4.10, 4.10 61.97		n.d.
D1			4.39 (7.9 Hz) 102.04			4.34 (7.9 Hz) 102.33	5.06 (7.6 Hz) 100.45
D2			5.22 69.51			5.21 69.40	5.19 67.69
D3			4.98 71.26			4.98 71.08	4.96 71.15
D4			5.33 67.21			5.34 67.31	5.34 67.47
D5			3.79 71.12			3.82 70.98	nd
D6			4.10, 3.98			nd	nd

	13	**14**	**15**	**16**	**17**	**19**	**20**	**21**	**22**	**23**
A1	4.40 (7.6 Hz) 103.40	4.38 (7.9 Hz) 102.55	4.29 (7.9 Hz) 102.18	4.36 (7.9 Hz) 101.83	4.40 (7.3 Hz) 102.87	4.26 (7.9 Hz) 104.84	4.29 (7.6 Hz) 104.93	4.25 (7.8 Hz)	4.27 (7.6 Hz) 104.77	4.33 (7.9 Hz) 105.03
A2	3.81 76.48	5.16	5.17 71.31	5.09	3.81 80.17	3.57 80.21	3.52 77.75	nd	3.53 77.57	3.55 76.99
A3	4.98 71.88	4.96	3.84 71.54	4.89	4.07 74.59	4.75 77.74	4.62 82.22	nd	4.79	4.92 72.94
A4	5.37 67.38	5.36	5.38 69.46	4.10	5.34 68.27	5.36 70.34	4.02 75.05	nd	3.99 75.19	5.29 68.60
A5	3.87	nd	nd	nd	nd	3.66 73.57	nd	3.68	n.d.	3.79 72.71
A6	4.23, 4.09 61.81	3.84, 3.75 67.48	4.17, 4.20 62.22	nd	nd	nd	nd	4.43, 4.05	4.31, 4.24	3.66, 3.66 66.68
OMe	3.58 57.84	3.53 57.56	3.48 56.93	3.48	3.56 57.42	3.52 57.17	3.56 57.70	3.55 57.64	3.52 57.12	3.54 57.68
B1	4.77 (7.9 Hz) 101.62				4.91 (8.2 Hz) 100.10					
B2	5.16 69.78				5.21					
B3	4.96 72.07				4.99 71.52					
B4	5.34 67.71				5.39 67.27					
B5	3.87				n.d.					
B6	4.23−09				n.d.					
C1			4.56 (7.9 Hz) 101.31		4.81 (7.9 Hz) 99.51	4.67 (7.9 Hz) 101.67		4.40 (8.2 Hz)		
C2			5.09 69.26		5.06 70.35	5.30 72.14		5.31		
C3			4.93 70.77		5.18 70.17	3.39 80.48		nd		
C4			5.35 67.17		5.34 68.25	3.93 73.28		5.30		
C5			nd		nd	nd		nd		
C6			nd		nd	nd		nd		
D1				4.45 (7.9 Hz)			4.24 (7.9 Hz) 102.43		4.20 (7.6 Hz) 102.49	
D2				5.25			5.30 72.14		5.31 71.97	
D3				5.00			3.47 80.41		3.46 80.48	
D4				5.35			3.91 73.08		3.93 73.00	
D5				nd			nd		nd	
D6				nd			nd		nd	
E1		4.53 (7.9 Hz) 101.17								4.39 (7.9 Hz) 101.11
E2		5.15								5.33 71.24
E3		4.96								3.46 80.73
E4		5.37 67.38								3.94 73.04
E5		nd								3.54 73.94
E6		nd								3.58, 68.13

[a] ^{1}H, $J_{1,2}$ in parentheses, and ^{13}C (nd, not determined).

The product mixture was cleaved from the resin and acetylated as previously described, followed by separation on column chromatography and analysis of the fractions obtained (Table 1, entries 4 and 5).

Glycosylation of diol acceptor **1** with the donor **18** bound to the resin, followed by cleavage and acetylation, resulted in the formation of both disaccharides **19** and **20** (Scheme 4, Table 1, entry 4) and 20% of other products (monosaccharides, degradation of the donor). Somewhat surprising, the disaccharides were formed in equal amounts, as judged by integration of NMR spectra.

Glycosylation of triol acceptor **6** with polymer bound donor **18** and subsequent cleavage and acetylation afforded all possible disaccharides **21**, **22** and **23** (Scheme 5, Table 1, Entry 5). The first fraction consisted of a mixture of β,1→3- and β,1→4- linked disaccharides and the second fraction contained as main product the 1→6-linked disaccharide in 60%. The ratio of the products did not fully correspond to the expectations considering the reactivities of the three free hydroxyl functions of the acceptor. As expected, the main product was the 1→6-linked, but the ratio of the other two disaccharides did not reflect the

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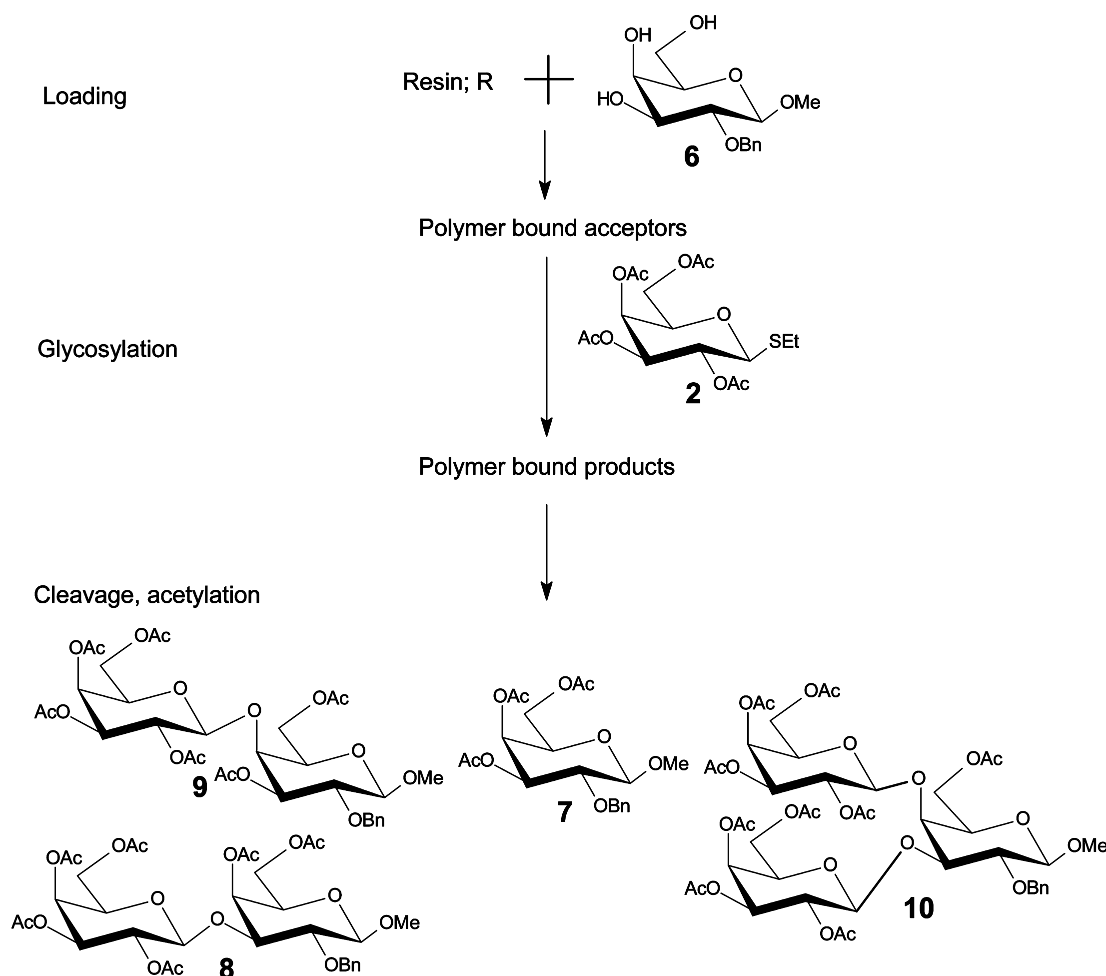
Scheme 2. Approach I: Solid-Phase Random Glycosylation with Resin-Bound Triol Acceptor



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usually higher reactivity of a hydroxyl group at position 3 compared to position 4.

Attempted glycosylation of tetraol acceptor **11** using polymer bound donor **18** did not afford any disaccharides (Table 1, entry 6). Only degradation products derived from donor **18** were observed after attempted cleavage of the products from the resin.Neat acetonitrile as solvent dissolved the acceptor; however glycosylation could not be achieved because of poor resin swelling in this solvent.

Conclusion

Random glycosylation on the solid phase has been performed using two approaches. When the acceptor was bound to the solid support, di- and trisaccharides were isolated and characterized. However, when the donor was linked to the solid phase disaccharides were isolated. To avoid formation of anomeric mixtures participating groups were present in position 2 of the glycosyl donors. In order to obtain high yields, the use of the acceptor bound approach is suggested, since it is very difficult to avoid degradation of glycosyl donors. For formation higher oligosaccharides this approach shows considerable limitations. The advantages of the donor-bound approach are easy removal of unreacted acceptor and much higher product diversity.

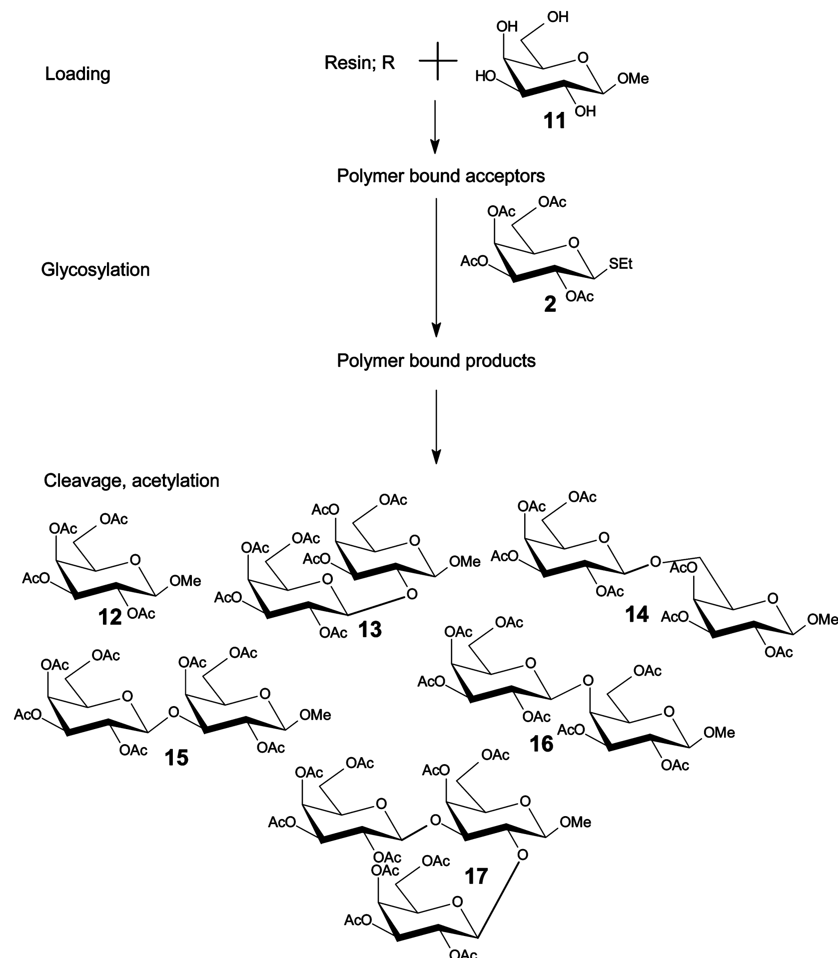
Experimental Section

General. Commercially available starting materials were used without further purification. Wang resin (substitution 1.0 mmol/g) was purchased from Novabiochem (Darmstadt, Germany). Solvents were dried according to standard methods. NMR spectra were recorded on a Bruker AMX-400 (100.62 MHz for ^{13}C) or DRX-500 (125.83 MHz for ^{13}C) spectrometer in $CDCl_3$ as a solvent. All chemical shifts are quoted in parts per million downfield from the characteristic signals of the used solvent (^{1}H 7.26 ppm; ^{13}C 77.00 ppm). Kieselgel 60 (E. Merck, Darmstadt, Germany) was used for column chromatography. MALDI-TOF measurements were carried out on a Bruker Biflex III mass spectrometer. 2,5- Dihydroxybenzoic acid was used as matrix, and 100−200 laser shots were applied for each spectrum. All estimation of the ratios of the compounds within a fraction based on the integration of the signals in the NMR spectra.

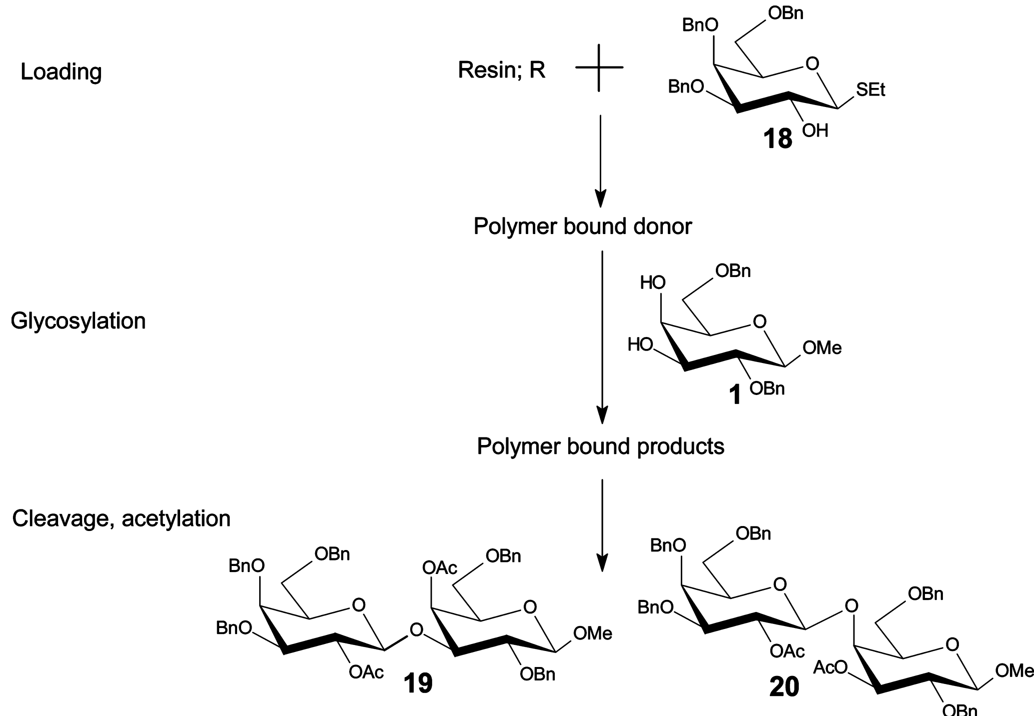
Functionalization of Wang Resin. A mixture of succinic anhydride (10 equiv) and DMAP (100 mg) in DCM (5 mL) and pyridine (2 mL) was added to Wang resin (1 g, preswelled in DCM). The mixture was shaken for 10 h; then the resin was drained and washed with DCM (5 × 5 mL), DMF (5 × 5 mL), and DCM (5 × 5 mL).

Loading. Monosaccharide derivative (0.2 mmol), DCC (100 mg), and DMAP (50 mg) were added in DCM (5 mL) to the functionalized resin (1 g) and the mixture was shaken for 10 h. Then the resin was drained and washed with DCM

Scheme 3. Approach I: Solid-Phase Random Glycosylation with Resin-Bound Tetraol Acceptor



Scheme 4. Approach II: Solid-Phase Random Glycosylation of a Diol with Resin-Bound Donor



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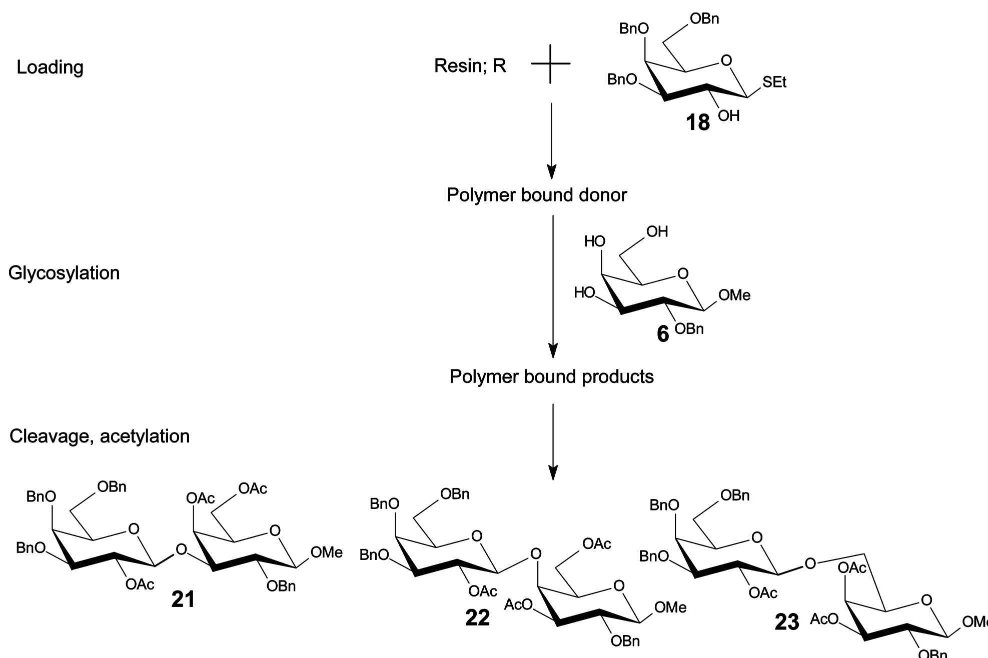
(2 × 5 mL), DMF (3 × 5 mL), DCM (5 × 5 mL), and MeOH (2 × 5 mL). The resin was dried overnight in high vacuum.

Determination of Loading. To the sample of resin (~50 mg, preswelled in DCM), NaOMe (10 mg) in DCM (2 mL) and MeOH (0.5 mL) was added and the mixture was

Scheme 5. Approach II: Solid-Phase Random Glycosylation of a Triol with Resin-Bound Donor



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shaken for 1 h. The resin was drained and washed with DCM (5 × 3 mL). The combined filtrates were washed with water (2 × 3 mL), dried, filtered, and concentrated. Column chromatography of the residue gave the monosaccharide derivative.

The loading was calculated using to the following equation, where x is the loading in mol/g, z is the mass of the sugar cleaved from the resin in mg, y is the mass of the loaded resin in mg, and M is the molecular weight of the sugar residue cleaved from the resin in g/mol:

$$x = \frac{z}{y \times M} \quad (1)$$

Obtained Loadings.

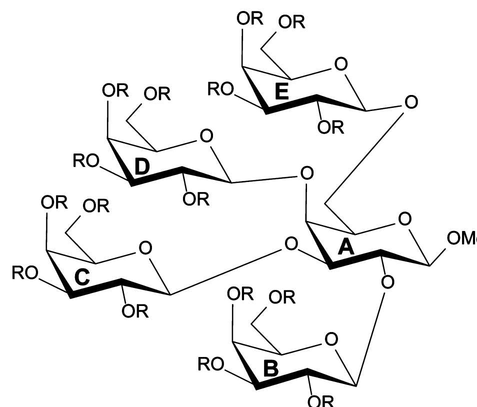
1: 0.60 mmol/g
6: 0.65 mmol/g
11: 0.52 mmol/g
18: 0.50 mmol/g

Coupling. The mixture of the sugar loaded resin (0.065 mmol), monosaccharide acceptor/donor (0.1 mmol), and 4 Å molecular sieves (50 mg) was stirred in DCM (3 mL) for 2 h. A mixture of NIS (1.3 eq for the donor) and AgOTf (0.1 eq for the donor) in acetonitrile (3 mL) was added at −40 °C. The mixture was allowed to warm to rt and was stirred for 10 h. The reaction was stopped by adding pyridine (100 μL); the resin was drained and washed with DCM (5 × 5 mL), DMF (5 × 5 mL), and DCM (5 × 5 mL).

Cleavage. NaOMe (50 mg) in DCM (5 mL) and MeOH (1 mL) was added to the resin and shaken for 30 min. The resin was drained and washed with DCM (5 × 5 mL). The combined organic phases were washed with water (2 × 10 mL), dried, filtered, and concentrated.

Acetylation. The crude cleaved material was dissolved in pyridine (10 mL) and cooled to 0 °C. Ac_2O (5 mL) was added and the mixture was stirred for 10 h. The mixture was concentrated and column chromatography of the residue gave the oligosaccharide derivatives.

General Scheme for the NMR Data.



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Supporting Information Available. 1D NMR, 2D NMR, and MALDI-TOF spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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