

## Combinatorial Syntheses of Trisaccharide Libraries on a Soluble Polymeric Support

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**Abstract:** A new synthetic strategy for demixing libraries of compounds has been developed using a multi-linker approach. The linker-tagged building blocks are immobilized on a soluble polymeric support. Key to the success of this approach is the use of a temporary protecting group which is compatible with the linker system and stable under glycosylation conditions.

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### INTRODUCTION

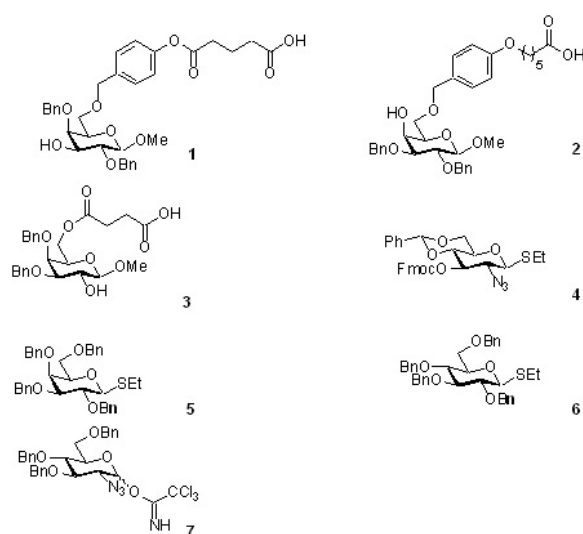
The chemical synthesis of structurally defined oligosaccharides would be highly desirable to speed up the discovery and development of new pharmaceutical agents. Therefore, laboratories are applying the techniques of combinatorial chemistry to synthesize small molecule libraries to probe for lead compounds using high-throughput screening assays. Since chemistry is currently the limiting step in the drug discovery process, (Gravert *et al.*, 1996) improved methods for small molecule library synthesis are required. Solid-phase combinatorial methods have been pursued to facilitate product purification, the resin-bound product can simply be filtered and excess reagents and impurities rinsed away. However, these insoluble supports create heterogeneous reaction conditions that lead to nonlinear kinetic behavior, unequal distribution and/or access to the chemical reaction, solvation problems, and pure synthetic problems traditionally associated with solid-phase synthesis besides the difficulty of monitoring the progress of the reaction. A decade ago, liquid polymer-, dendrimer-, and fluorosupported syntheses emerged as attractive alternatives for solid-phase organic chemistry. (Toy *et al.*, 2000). In general, these liquid polymer supported methods have similar reaction kinetics compared to traditional solution-phase chemistry. In particular, polyethylene glycol  $\omega$ -monomethyl ether [HOCH<sub>2</sub>-CH<sub>2</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>n</sub>OMe (MPEG)] introduced by Krepinski and co-workers. (Douglas *et al.*, 1997; Krepinsky *et al.*, 2001). has been recognized as an inexpensive and convenient soluble polymer (Dickerson *et al.*, 2002; Oikawa *et al.*, 2005; Hong *et al.*, 2006; Seeberger *et al.*, 2008) While insoluble in simple dialkyl ether, MPEG dissolves readily in halogenated solvents allowing it to react under homogenous conditions and its reactions to be monitored by NMR spectroscopy. Despite these attractive features, liquid polymer supported methods have rarely been used in combinatorial chemistry. (Han *et al.*, 1995).

From our earlier work, (Elsayed *et al.*, 2002) a library of 18 disaccharides was synthesized using glycosyl acceptors **1-3** that are tagged by a series of selective cleavable linkers. Those linkers which contain a carboxylic moiety were utilized for the attachment to amino functionalized MPEG. Glycosylation with the donors **5-7** followed by an orchestral release from the supporting system by selective cleavage of different linkers afforded a library of 18 disaccharides. Herein, a further step was taken to synthesize trisaccharide libraries including a number of biologically important amino sugars (Berdy *et al.*, 1980). Key to the success of this approach is the use of the Fmoc protected thioglycoside **4**. (Fmoc) 9-fluorenyl-methoxy carbonyl will serve as a temporary protecting group that will be removed following each coupling. Also, Fmoc was chosen since it is expected to be cleaved from the resin-bound disaccharides without affecting the linker system.

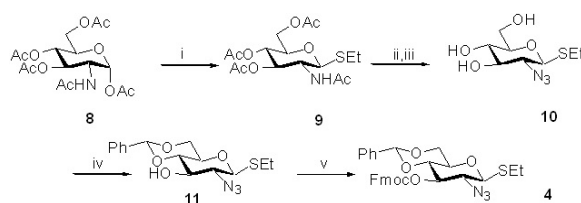
With ample quantities of the spacer modified (**1,2**, and **3**) in hand which were synthesized from known galactosides (Maddali *et al.*, 1990). (Fig. 1), attention was focused on the synthesis of the Fmoc-containing glycosyl donor **4**. 1,3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy  $\alpha$ -D-glucopyranoside **8** was treated with ethylthiotrimethylsilane in the presence of zinc iodide (Hanessian *et al.*, 1993) to give exclusively the *B*-anomer of the thioethylglucoside **9** (Scheme 1). Compound **9** underwent simultaneous *N* and *O* deacetylation when treated with 1M NaOH to give the corresponding triol (Buskas, 1994). followed by diazo transfer by treatment with trifluoromethylsulfonyl azide and *N,N*-dimethylaminopyridine (DMAP) in dichloromethane to yield the azide **10**. The 4,6 diol of **10** was protected as benzylidene acetal by treatment with benzaldehyde dimethyl acetal using *p*-TsOH as a catalyst in DMF (Garegg, 1997). to give **11**. Finally, the C-3 hydroxyl group was functionalized into Fmoc (Gioeli *et al.*, 1982) by treating compound **11** with 9-fluoenylmethoxycarbonyl chloride in the presence of pyridine to give the target glycosyl donor **4**.

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**Fig. 1:** Building blocks for library synthesis

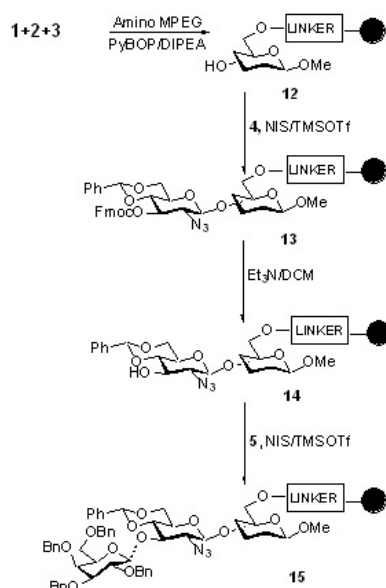


**Scheme. 1:** Reagents and conditions: (i)  $\text{EtSSiMe}_3$ ,  $\text{ZnI}_2$ ,  $\text{ClCH}_2\text{CH}_2\text{Cl}$ , 8h,  $50^\circ\text{C}$  (86%); (ii)  $\text{NaOH}$ , 1M, 15h, reflux (85%); (iii)  $\text{N}_3\text{OTf}$ , DCM, DMAP, 12h; (iv)  $\text{PhCH}(\text{Ome})_2$ , *P*- $\text{TsOH}$  (83%); (v)  $\text{FmocCl}$ , Py. (70%).

Mixing of compounds **1-3** followed by attachment to amino-modified MPEG (Mw 5000) by standard amide bond formation using PyBOP/DIPEA afforded library **12** (Scheme 2). After purification by precipitation with diethyl ether, the crude product was analyzed by NMR and Maldi-Tof MS. The analysis indicated the presence of the three monosaccharides in approximately equal molar amounts. The monosaccharide library **12** was glycosylated with the glycosyl donor **4** using NIS/TMSOTf (Veeneman *et al.*, 1990). as the activator and dichloromethane/ diethyl ether as the solvent mixture (scheme 2). The resulting MPEG-bound disaccharides **13** were easily purified by selective precipitation, filtration and washing. Detailed NMR analysis of **13** showed that the first glycosidic linkages was formed as a mixture of anomers ( $\alpha/\beta=9:1$ ). The next stage of the synthesis entailed removal of Fmoc group of the disaccharide library **13** using  $\text{Et}_3\text{N}$  in  $\text{CH}_2\text{Cl}_2$  (Nicolau *et al.*, 1997; Nicolau *et al.*, 1998). to afford the disaccharide glycosyl acceptor libraries **14**. NMR analysis showed that the deprotection condition of Fmoc did not affect other protecting groups or the linker system. The resulting MPEG-bound disaccharide acceptors **20** were precipitated by addition of diethyl ether and collected by filtration. After concentration in *vacuo*, the crude product was analyzed by NMR and Maldi-Tof MS. The analysis showed the presence of the disaccharide libraries **14** and the absence of the Fmoc group. After selective precipitation, filtration, and washing, the resin-bound disaccharide library **14** was split into three pools and the first pool was glycosylated with 5 equivalents of the fully thiogalactosyl donor **5** in the presence of the activating mixture NIS/TMSOTf and dichloromethane as the solvent to yield a library of MPEG-bound trisaccharides **22**. Working up of the reaction started with filtration and then extraction with 5% aqueous solution of sodium thiosulfate followed by concentration of the dichloromethane layer. The resin bound trisaccharide was precipitated from cold diethyl ether followed by recrystallization from warm ethanol.

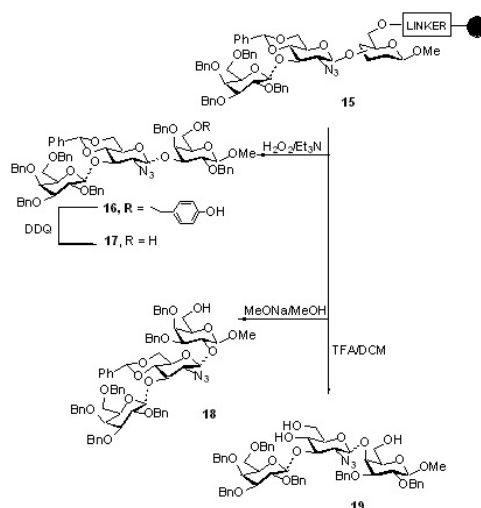
In the next stage of the synthesis, the library **15** was dissolved in ethanol and treated with hydrogen peroxide/ $\text{Et}_3\text{N}$  (Zhu and Boons, 2000; Zhu and Boons, 2001). to cleave the phenolic ester linkage releasing the trisaccharide **16** (Scheme 3). The filtrate containing the released trisaccharide was concentrated under *vacuo* and the residue was purified by silica gel column chromatography to give **16** in an overall yield of 55%. NMR analysis showed the presence of trisaccharide **16** and the absence any of the other trisaccharides and intact

disaccharide library indicating that the cleavage was indeed selective and the glycosylation had proceeded to completion. The released trisaccharide was oxidized by using DDQ (Jobron and Hingsaul, 1999). to remove the *P*-alkoxy group to give compound **17**.



**Scheme. 2:**

The precipitated MPEG-bound trisaccharide mixture which contained the other remaining trisaccharide libraries was dissolved in methanol and treated with catalytic amount of freshly prepared MeONa to cleave the succinic linker in order to release the trisaccharide **18**. After work-up steps which include precipitation, filtration, and recrystallization, the released trisaccharide **18** was concentrated and purified by silica gel chromatography (60% yield). The remaining recrystallized resin-bound trisaccharide was dissolved in dichloromethane and then treated with TFA to release the third protected trisaccharide **19** from the resin (55% yield).



**Scheme. 3:**

In two separate glycosylations, library **14** was coupled with glycosyl donors **6** (Dasgupta and Garegg 1989). and **7** (Kinzy and Schmidt 1985). using NIS/TMSOTf (Veeneman *et al.*, 1990) or TMSOTf, (Schmidt, 1986). respectively, as the activators. The resulting libraries of trisaccharides were purified by precipitation followed by filtration and washing and then demixed by selective cleavage of the linkers. All the trisaccharides were deprotected using the standard procedures to give a library of thirty-six trisaccharides. All of the obtained

trisaccharide libraries were mixtures of anomers since we did not have any neighboring group participation protecting groups on C-2 on any of the glycosyl donors or acceptors.

In conclusion, it has been demonstrated that combinatorial syntheses of trisaccharide libraries that are attached to a soluble polymeric support using selective cleavable linkers is a reliable methodology. Major attractions of the methodology are that libraries of linker-tagged disaccharides can repeatedly be used in glycosylation with different glycosyl donors to give a larger number of oligosaccharide libraries. Currently, we are expanding this methodology by developing several other selective cleavable linkers with other types of liquid supported synthesis. It is to be expected that the new methodology can be applied to other types of liquid supported synthesis and in particular the combination of selective cleavable linkers with fluoruous tags will be attractive to demix a relatively larger number of oligosaccharides. Also, other temporary protecting groups which are compatible with the linkers and glycosylation conditions are under investigation.

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Selected <sup>1</sup>H-NMR data (CDCl<sub>3</sub>, 300MHz). Methyl *O*- (α/β-D-galactopyranosyl)-(1→3)-*O*- (β-2-amino-2-deoxy-D-glucopyranosyl)- (1→3)- β-D-glucopyranoside: δ 5.3 (d, *J*=3.5Hz), 4.33 (d, *J*=7.2Hz), 4.72 ((d, *J*=3.6Hz); Methyl *O*- (α/β-D-galactopyranosyl)-(1→3)-*O*- (β-2-amino-2-deoxy-D-glucopyranosyl)- (1→2)- β-D-glucopyranoside: δ 5.3 (d, *J*=3.5Hz), 4.33 (d, *J*=7.2Hz), 4.72 ((d, *J*=3.6Hz); Methyl *O*- (α/β-D-galactopyranosyl)-(1→3)-*O*- (β-2-amino-2-deoxy-D-glucopyranosyl)- (1→4)- β-D-glucopyranoside: δ 5.3 (d, *J*=3.5Hz), 4.33 (d, *J*=7.2Hz), 4.72 ((d, *J*=3.6Hz); Methyl *O*- (α/β-D-galactopyranosyl)-(1→3)-*O*- (α-2-amino-2-deoxy-D-glucopyranosyl)- (1→3)-β-D-glucopyranoside: δ 5.1 (d, *J*=3.8Hz), 4.62 (d, *J*=7.6Hz), 4.70 ((d, *J*=3.3Hz); Methyl *O*- (α/β-D-glucopyranosyl)-(1→3)-*O*- (β-2-amino-2-deoxy-D-glucopyranosyl)- (1→3)- β-D-glucopyranoside: δ 5.5 (d, *J*=3.5Hz), 4.33 (d, *J*=7.2Hz), 4.72 ((d, *J*=3.6Hz); 4.73 (d, *J*=4.03Hz); Methyl *O*- (α/β-D-glucopyranosyl)-(1→3)-*O*- (α-2-amino-2-deoxy-D-glucopyranosyl)- (1→3)- β-D-glucopyranoside: δ 5.1 (d, *J*=3.5Hz), 4.33 (d, *J*=7.2Hz), 4.72 ((d, *J*=3.6Hz); 4.71 (d, *J*=3.2Hz); Methyl *O*- (α/β-2-amino-2-deoxy-D-glucopyranosyl)-(1→3)-*O*- (α-2-amino-2-deoxy-D-glucopyranosyl)- (1→3)- β-D-glucopyranoside: δ 5.1 (d, *J*=3.4Hz), 4.28 (d, *J*=7.6Hz), 4.72 ((d, *J*=3.6Hz); 4.73 (d, *J*=4.03Hz); Methyl *O*- (α/β-2-amino-2-deoxy-D-glucopyranosyl)-(1→3)-*O*- (β-2-amino-2-deoxy-D-glucopyranosyl)- (1→3)- β-D-glucopyranoside: δ 5.38 (d, *J*=3.0Hz), 4.33 (d, *J*=7.9Hz), 4.94 ((d, *J*=3.3Hz); 4.88 (d, *J*=3.0Hz);