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Demixing libraries of saccharides using a multi-linker approach in combination with a soluble polymeric support

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Abstract—A new method for demixing libraries of compounds that are attached to a soluble polymeric support by tagging starting materials with selective cleavable linkers is described. © 2002 Elsevier Science Ltd. All rights reserved.

Liquid polymer-, dendrimer- and fluorous supported syntheses are emerging as attractive alternatives for solid-phase organic chemistry.[1] These synthetic approaches have the favorable properties of traditional solution phase chemistry, while the macromolecular or fluorous properties of the liquid supporting system facilitate product purification. For example, compounds that are linked to polyethylene glycol monomethyl ether (MPEG)[2] are soluble in many organic solvents; however, due to the helical structure of MPEG, it has a high propensity to crystallize in diethyl ether and ethanol and, thus, product purification by crystallization can be accomplished at each stage of the synthesis. Dendrimer supported synthesis exploits that compounds can be easily separated from excess reagents by size exclusion chromatography,[3] whereas fluorous supported synthesis relies on selective extraction in a fluorocarbon solvent.[4] In general, these liquid polymer supported methods have similar reaction kinetics compared to traditional solution-phase chemistry; they do not require the use of a large excess of reagents, and they allow supported intermediates and final products to be easily characterized. Despite these attractive features, liquid polymer supported methods have rarely been used in combinatorial chemistry[5] and one of their main shortcomings is that there are no general methods for synthesizing mixtures of compounds that at the end of a sequence of reactions can easily be separated to give individual compounds.[6,7] A notable exception is a method developed by Curran and co-workers[8] whereby four different compounds can be linked to four different fluorous tags of increasing fluorine content. The tagged compounds can then be mixed and after multiple reactions, demixed by fluorous chromatography to provide individual compounds.

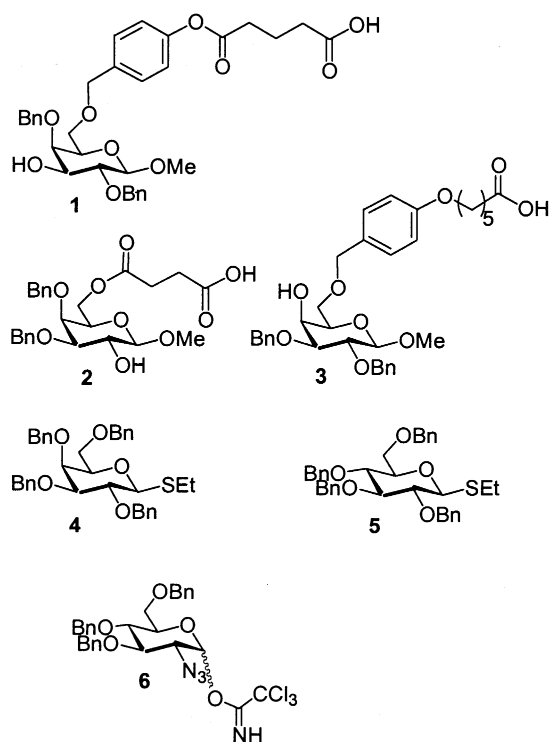

Figure 1. Building blocks for library synthesis.

Herein we report a novel and general method for demixing liquid phase supported compounds by an orchestrated release from the supporting system by selective cleavage of different linkers. Thus, in the proposed strategy, a series of substrates will be tagged

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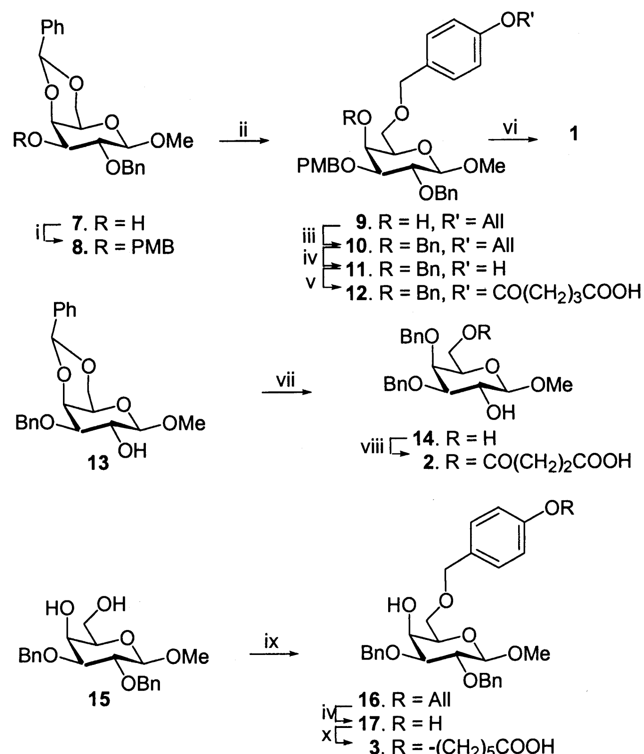
with a series of selective cleavable linkers. These linker-tagged compounds can then be mixed, attached to a soluble support and used in a series of combinatorial reactions. At the end of the reaction sequence, individual compounds can be obtained by selective cleavage of the linkers. The new method was applied to the preparation of a library of 18 disaccharides[9–13] using glycosyl acceptors **1**–**3** that are tagged by unique linkers, glycosyl donors **4**–**6** and MPEG as the liquid supporting system.[14]

Compound **1** has a free C-3 hydroxyl and is functionalized with a phenolic ester linker (Fig. 1), which is stable towards Lewis acid conditions used in glycosylations but can be cleaved within minutes by treatment with hydrogen peroxide/Et_3N.[15,16] After detachment, a *p*-hydroxyl benzyl ether will be obtained which can easily be removed by oxidation with DDQ.[17] Compound **2** has a free C-2 hydroxyl and is derivatized with a succinic ester linker.[18] This ester is substantially more stable than the phenolic ester linkage of **1** and requires treatment with sodium methoxide for cleavage. Glycosyl acceptor **3** has a free C-4 hydroxyl and is derivatized with an acid sensitive *p*-alkoxybenzyl linker,[19] which is orthogonal with the linkers of **1** and **2**. The linkers of **1**–**3** contain a carboxylic acid moiety, which will be utilized for the attachment to amino-functionalized MPEG.

Spacer modified **1** was synthesized from known galactoside **7**[20] by first installation of a *p*-hydroxybenzyl group at C-6 (**11**), which was acylated with glutaric anhydride (Scheme 1). Thus, the C-3 hydroxyl of **7** was protected as a temporary *p*-methoxybenzyl ether (PMB) by reaction with PMBCl and NaH in DMF to give fully protected **8** in a yield of 95%. The benzylidene acetal of **8** was removed using aqueous acetic acid and the C-6 hydroxyl of the resulting diol was functionalized as a *p*-allyloxybenzyl ether by first formation of an intermediate 4,6-*O*-stannylene acetal,[21] which was regioselectively reacted with *p*-allyloxybenzyl chloride in the presence of *t*-butylammonium bromide to give **9**. Next, the remaining C-4 hydroxyl of **9** was benzylated under standard conditions to give **10**, which was treated with $Pd(PPh_3)_4$ in refluxing ethanol to remove the phenolic allyl ether to afford **11**. Reaction of **11** with glutaric anhydride in the presence of DMAP in pyridine gave linker modified **12** in a yield of 61% and finally, the target compound **1** was obtained by removal of the PMB ether by treatment with TFA in dichloromethane.

Target compound **2** was easily obtained by regioselective opening of the benzylidene acetal of **13**[22] with $BH_3{\cdot}Me_3N$ complex in the presence of $AlCl_3$ followed by selective acylation of the primary alcohol of the resulting **14** with succinic anhydride. The latter reaction only proceeded with high regioselectivity when first an intermediate tri-*n*-butylstannyl ether was formed by reaction with $(Bu_3Sn)_2O$.

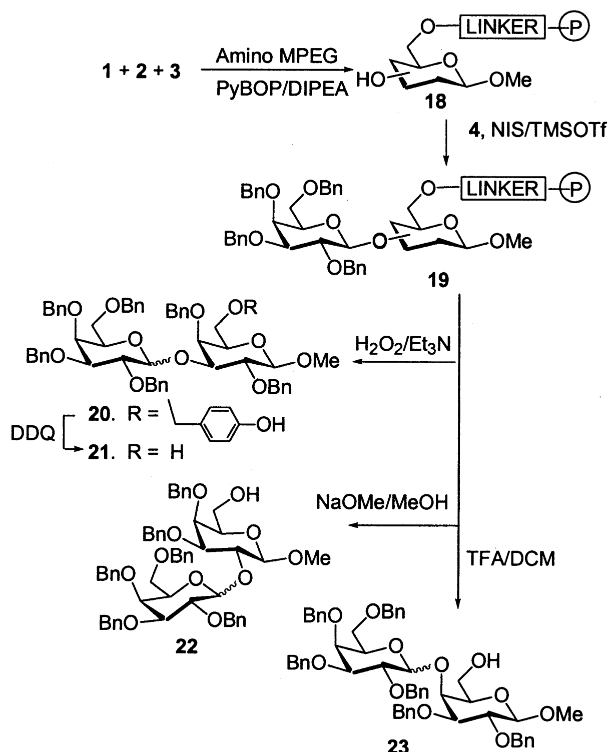
Spacer modified **3** was also prepared from an intermediate *p*-hydroxybenzyl ether (**17**), which in this case, was alkylated with 6-bromohexanoate. Thus, the C-6



Scheme 1. *Reagents and conditions*: (i) PMBCl, NaH, DMF (95%); (ii) aq. AcOH then $Bu_2Sn(OMe)_2$, *p*-allyloxybenzyl chloride, Bu_4NI (62%); (iii) BnBr, NaH, DMF (82%); (iv) $Pd(PPh_3)_4$, EtOH (63%); (v) glutaric anhydride, Py, DMAP (79%); (vi) TFA, DCM (30%); (vii) $BH_3{\cdot}Me_3N$, $AlCl_3$ (68%); (viii) $(Bu_3Sn)_2O$, succinic anhydride (60%); (ix) $Bu_2Sn(OMe)_2$, *p*-allyloxybenzyl chloride, Bu_4NI (80%); (x) $Br(CH_2)_5CO_2Et$, Cs_2CO_3, DMF; then MeOH, NaOH (86%).

hydroxyl of diol of **11**[23] was selectively functionalized as a *p*-allyloxybenzyl ether to give **16** by reaction of an intermediate stannylene acetal[21] with *p*-allyloxybenzyl chloride in the presence of n-Bu_4NI in toluene. Removal of the allyl ether of **16** by treatment with $Pd(PPh_3)_4$ in refluxing ethanol afforded **17** in a high yield. The target compound **3** was obtained by alkylation of the *p*-hydroxybenzyl ether of **17** with ethyl 6-bromohexanoate in the presence of Cs_2CO_3 followed by hydrolysis of the ethyl ester using aqueous sodium hydroxide in methanol.

With ample quantities of linker-modified acceptors in hand, attention was focused on the combinatorial synthesis of a library of eighteen disaccharides (Scheme 2). Mixing of compounds **1**–**3** followed by attachment to amino-modified MPEG (Mw 5000) by standard amide bond formation afforded library **18**. The mixture of immobilized acceptors (**18**) could be easily purified by precipitation with diethyl ether and inspection of the NMR spectrum of the resulting material indicated that the three monosaccharides were present in approximately equal molar quantities. The monosaccharide library **18** was split into three pools and the first pool was glycosylated with thiogalactosyl donor **4**[24] using NIS/TMSOTf[25] as the activator and dichloromethane/diethyl ether as the solvent mixture. The resulting



Scheme 2. Combinatorial synthesis and demixing disaccharide library.

MPEG-bound disaccharides (**19**) were easily purified by selective precipitation, filtration and washing.

The next stage of the synthesis entailed demixing of library **19** by selective cleavage of the linkers followed by precipitation of the MPEG-bound compounds (Scheme 2) to give the individual disaccharides **20**, **22** and **23**. Thus, library **19** was first treated with H_2O_2 and Et_3N in dichloromethane for 10 min after which the MPEG-bound compounds were precipitated by addition of diethyl ether and collected by filtration. The filtrate, which contained the released disaccharide **20**, was concentrated and the crude reaction product analyzed by NMR and Maldi-Tof MS. The analysis showed only the presence of disaccharide **20** and the absence of any of the other disaccharides and intact acceptor indicating that the cleavage was indeed selective and that the glycosylation had proceeded to completion. The crude reaction mixture was purified by silica gel column chromatography to give **20** in an overall yield of 65% as a mainly the α anomers (α/β = 9/1). The precipitated MPEG, which contained the two remaining disaccharides, was dissolved in methanol and treated with NaOMe to cleave the succinic ester linkage to release **22**. This compound was isolated in a 61% yield as a mixture of anomers (α/β = 4/1) by the same procedure as described for **20**. Finally, disaccharide **23** was obtained in an overall yield of 45% (α/β = 3/1) by treatment of the polymer with TFA in dichloromethane followed by a standard isolation procedure.

In two separate glycosylations, library **18** was coupled with glycosyl donors **5**[26] and **6**[27] using NIS/TMSOTf[25] or TMSOTf,[28] respectively, as the activators. The resulting libraries of disaccharides were purified by precipitation followed by filtration and washing and then demixed by selective cleavage of the linkers. All disaccharides were deprotected using standard procedures to give the following disaccharides: α/β-D-Gal*p*(1-2)-β-D-Gal*p*-OMe (α/β = 4:1, 61%); α/β-D-Gal*p*(1-3)-β-D-Gal*p*-OMe (α/β = 9:1, 65%); α/β-D-Gal*p*(1-4)-β-D-Gal*p*-OMe (α/β = 3/1, 45%); α/β-D-Glc*p*(1-2)-β-D-Gal*p*-OMe (α/β = 3/1, 55%); α/β-D-Glc*p*(1-3)-β-D-Gal*p*-OMe (α/β = 1/1, 62%), α/β-D-Glc*p*(1-4)-β-D-Gal*p*-OMe (α/β = 3/2, 50%); α/β-D-GlcNH$_2$*p*(1-2)-β-D-Gal*p*-OMe (α/β = 3/1, 55%); α/β-D-GlcNH$_2$*p*(1-3)-β-D-Gal*p*-OMe (α/β = 5/1, 60%); α/β-D-GlcNH$_2$*p*(1-4)-β-D-Gal*p*-OMe (α/β = 9/1, 50%).[29]

In conclusion, we have developed a new method for demixing libraries of compounds that are attached to a soluble polymeric support by tagging starting materials with selective cleavable linkers. Major attractions of the methodology are that libraries of linker-tagged monosaccharides can repeatedly be used in glycosylations with different glycosyl donors to give a large number of oligosaccharide libraries. Each of these libraries can then be demixed by simple chemical manipulations to give well-defined products. Unlike deconvolution procedures based on tagging of beads, the method described here provides *preparative* quantities of material that can be characterized by conventional methods. This is important because oligosaccharide synthesis is prone to side-product formation and there are also no reliable strategies for deblocking oligosaccharides attached to the polymeric support. 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 fluorous tags will be attractive to demix a relatively large number of compounds. Currently, we are expanding the new methodology by developing several other linkers that will be compatible with the existing linkers and by employing temporary protecting groups to prepare larger oligosaccharides.

Acknowledgements

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