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The parallel synthesis of a disaccharide library using a solid phase, peptide-templated strategy

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A novel strategy for the synthesis of oligosaccharides, involving the use of a solid phase peptide template,[1] has been successfully applied to the construction of a twelve member disaccharide library.

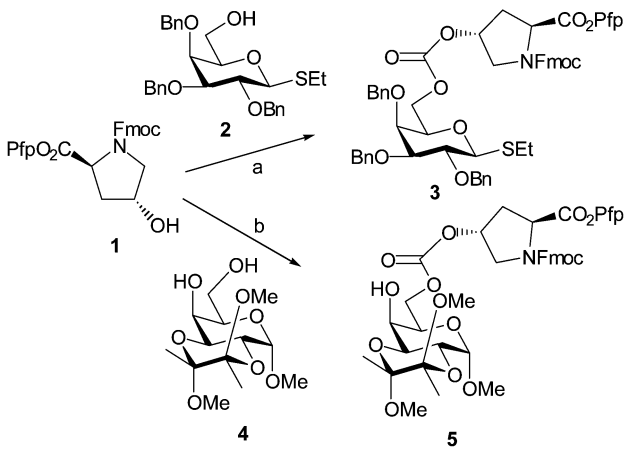
The solid phase synthesis of glycosides is highly desirable as supported synthesis is highly amenable to parallel and automated technologies.[1] We recently reported a solid supported approach to disaccharide synthesis in which monosaccharide building blocks are first attached to a peptide template.[2–4] Following preparation of a peptide decorated with glycosyl donors and acceptors, glycosidation is performed on a solid phase. Cleavage of base labile carbonate linkages then separates the target disaccharides from the support. Preliminary studies revealed highly efficient reactions and interesting selectivities when carbohydrates were prepared under these conditions. Peptide templated approaches to glycoside synthesis are easily transferred to the solid phase due to the highly optimised procedures for solid phase peptide synthesis. In order to demonstrate the applicability of this strategy to parallel synthesis and future automation we now report the application of this methodology to the synthesis of a twelve membered disaccharide library.

Tribenzylated thioglycosides of glucose, mannose and galactose (**2**) were prepared using standard protocols;[5] these were then coupled to protected hydroxyproline (Hyp) (**1**)[6] *via* a mixed carbonate link between the hydroxyl group of Hyp and the 6-OH of the sugars to form the glycosyl donor building blocks (*e.g.* **3**) in yields of 60–80%. Methyl glycosides of glucose, mannose and galactose were BDA protected[7] and then coupled to Hyp in an analogous fashion to give four glycosyl acceptor units (*e.g.* **5**). Typical examples are shown in Scheme 1.

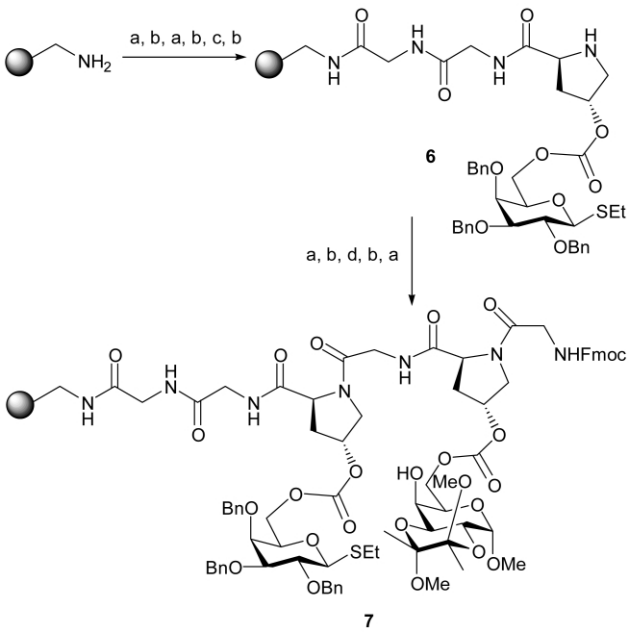
Peptide templates were then constructed in a parallel manner on Aminomethyl Novagel™ resin using standard Fmoc peptide coupling protocols.[8] Syntheses were performed in 3 ml fritted plastic tubes on a 16 port vacuum tank. Agitation was not required during peptide coupling. Two glycine residues were added prior to the first building block to avoid any clashes between the resin backbone and the sugar units. In the parallel synthesis each of the three glycosyl donors were added to four portions of resin *via* an extended coupling step. Another glycine residue was added as a spacer unit followed by the four glycosyl acceptor building blocks to each of the different glycosyl donor-bearing resin portions (*e.g.* **6**). A final glycine residue was added and its Fmoc group left intact. The preparation of the twelve peptide templates (*e.g.* **7**) took only 2 days to complete. The synthesis of one library member is shown in Scheme 2.

The resin-bound peptide templates were then exposed to the glycosidation conditions (NIS, cat. TMSOTf, 4 Å mol. sieves, THF : CH_2Cl_2 4 : 1, 20 h) and the final Fmoc group removed. Liberation of the target disaccharides from the template is performed under basic conditions, however, our original protocol required an aqueous work-up to isolate the target disaccharide.[2] Such manipulations are not desirable in parallel synthesis so the use of alternative cleavage conditions was investigated. It was found that when small quantities of NaOH were used (NaOH (1 mg) in methanol : THF (2 : 8, 2 ml, 5 h)) complete cleavage of the products was effected and work up could be avoided. The resin was agitated on a rotating wheel during glycosidation and cleavage steps.

Mass spectral analyses showed that the target disaccharides had all been prepared successfully. Preparative reverse phase HPLC was used to separate the α and β anomers of each of the disaccharides. All the disaccharides were successfully separated and each anomer fully characterised by ^1H and ^{13}C NMR spectroscopy. Structures, yields and anomeric ratios are shown in Table 1. Theoretical yields were calculated from the loading of the resin as quoted by the manufacturer. Quoted yields represent the



Scheme 1 Synthesis of the galactose donor and acceptor building blocks (Pfp = pentafluorophenyl): a) $COCl_2$, DCM, py, −78 °C to RT, then **2**, DCM, py; b) $COCl_2$, DCM, py, −78 °C to RT, then **4**, DCM, py.



Scheme 2 Synthesis of peptide template bearing galactose glycosyl donor and acceptor building blocks. a) Fmoc-GlyOH, HOBt, HBTU, DIPEA; b) 20% piperidine, DMF; c) **3**, HOBt; d) **5**, HOBt.

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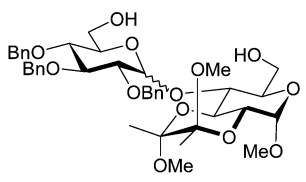
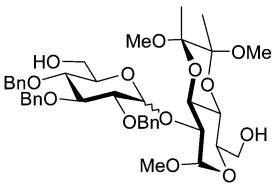
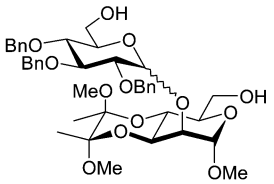
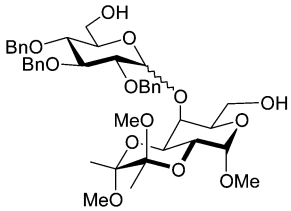
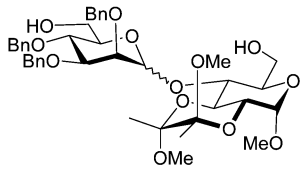
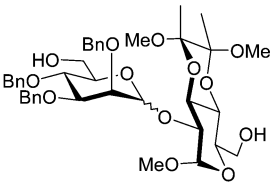
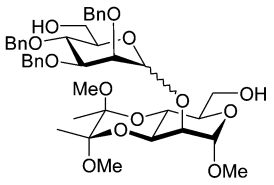
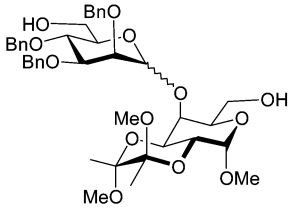
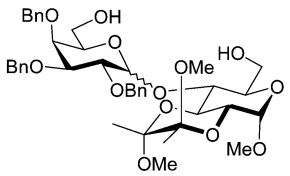
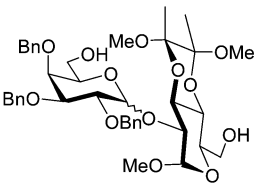
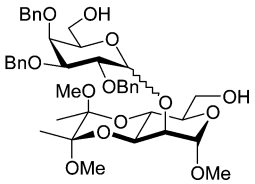
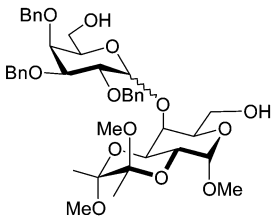
Table 1 Disaccharide library prepared using peptide templated methodology

			
Glc(1,4)Glc 78:22[a] 40%[b]	Glc(1,2)Glc 72:28[a] 57%[b]	Glc(1,2)Man 71:29[a] 42%[b]	Glc(1,4)Gal 86:14[a] 56%[b]
			
Man(1,4)Glc 82:18[a,c] 30%[b]	Man(1,2)Glc 57:43[a,c] 54%[b]	Man(1,2)Man 72:28[a,c] 50%[b]	Man(1,4)Gal 81:19[a,c] 40%[b]
			
Gal(1,4)Glc 80:20[a] 41%[b]	Gal(1,2)Glc 65:35[a] 28%[b]	Gal(1,2)Man 70:30[a] 63%[b]	Gal(1,4)Gal 68:32[a] 44%[b]

[a] Ratio α : β determined by NMR and HPLC. [b] Overall yield for synthesis, including peptide assembly. [c] The configuration of the major isomer was determined from the $^1J_{C1–H1}$ value.

cumulative yield for the peptide assembly and the subsequent glycosidation, cleavage and HPLC steps, demonstrating the highly efficient nature of the reaction sequence. Typically, syntheses performed on 50 mg of resin yielded 10 mg of the major disaccharide. It is notable that, apart from being mixtures of anomers, the products are very pure directly following the synthesis. Anomeric selectivities follow the trends observed in preliminary studies with the α anomer dominating with ratios of typically 3 : 1. It is interesting to note that significantly more β mannoside is produced using this methodology than observed using classical approaches. The template or resin must be increasing the propensity for β glycoside formation. Our preliminary results showed higher α selectivity in the synthesis of the Glc(1,4)Glc disaccharide on a slightly different template.[2] Experiments to understand the origins of these selectivities are in progress.

In summary, we have successfully exploited the efficiency of peptide templated glycoside synthesis to prepare a 12 member library of disaccharides. Our method differs from existing approaches to parallel saccharide synthesis in that it uses a peptide coupling, rather than a glycosidation, as the diversity introducing step.[9,10] As a result simple procedures can be followed and, even without complex automation, the simplicity of the protocols enabled the library to be prepared in only 4 days.

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Notes and references

1 H. M. I. Osborn and T. H. Khan, *Tetrahedron*, 1999, **55**, 1807; P. H. Seeberger, *Chem. Commun.*, 2003, 1115.

2 D. R. Greenwell, A. F. Ibnouzaki and S. L. Warriner, *Angew. Chem., Int. Ed.*, 2002, **41**, 1215.

3 For an alternative approach to peptide templated saccharide synthesis see: R. J. Tennant-Eyles, B. G. Davis and A. J. Fairbanks, *Chem. Commun.*, 1999, 1037; R. J. Tennant-Eyles, B. G. Davis and A. J. Fairbanks, *Tetrahedron: Asymmetry*, 2000, **11**, 231; R. J. Tennant-Eyles, B. G. Davis and A. J. Fairbanks, *Tetrahedron: Asymmetry*, 2000, **11**, 231; R. J. Tennant-Eyles, B. G. Davis and A. J. Fairbanks, *Tetrahedron: Asymmetry*, 2003, **14**, 1201.

4 Non-peptide tethers have been used by several groups to control the regio- and stereoselectivity of glycosidation reactions. For example: F. Barresi and O. Hindsgaul, *J. Am. Chem. Soc.*, 1991, **113**, 9376; M. Bols, *J. Chem. Soc., Chem. Commun.*, 1992, 913; S. Valverde, A. M. Gomez, A. Hernandez, B. Herradon and J. C. Lopez, *J. Chem. Soc., Chem. Commun.*, 1995, 2005; T. Ziegler, G. Lemanski and J. Hurttlen, *Tetrahedron Lett.*, 2001, **42**, 569; M. Müller U. Huchel, A. Geyer and R. R. Schmidt, *J. Org. Chem.*, 1999, **64**, 6190.

5 A. K. Ray and N. Roy, *Carbohydr. Res.*, 1990, **196**, 95.

6 H. Franzyk, M. Meldal, H. Paulson and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2883.

7 S. V. Ley, D. K. Baeschlin, D. J. Dixon, A. C. Foster, S. J. Ince, H. W. M. Priepke and D. J. Reynolds, *Chem. Rev.*, 2001, **101**, 53; J. L. Montchamp, F. Tian, M. E. Hart and J. W. Frost, *J. Org. Chem.*, 1996, **61**, 3897; U. Berens, D. Leckel and S. C. Oepen, *J. Org. Chem.*, 1995, **60**, 8204.

8 *Fmoc Solid Phase Peptide Synthesis, A Practical Approach*, ed. W. C. Chan and P. White, Oxford Univeristy Press, Oxford, New York, 2000.

9 R. Liang, L. Yan, J. Loebach, M. Ge, Y. Uozumi, K. Sekanina, N. Horan, J. Gildersleeve, C. Thompson, A. Smith, K. Biswas, W. C. Still and D. Khane, *Science*, 1996, **274**, 3053; T. Zhu and G-J, Boons, *Angew. Chem., Int. Ed.*, 1998, **37**, 1898.

10 *Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries*, ed. P. H. Seeberger, Wiley, New York, 2001.