Peptide templated glycosidic bond formation: a new strategy for oligosaccharide synthesis†

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Glycosidation reactions performed between glycosyl donors and acceptors covalently linked to a peptide template produced increased regio- and stereo-selectivities, which were dependent on the nature of the peptide, and which may be rationalised by simple molecular modelling.

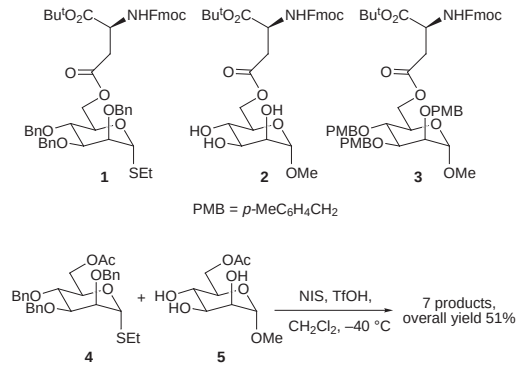
Despite an enormous effort over the course of the last hundred years, the stereo- and regio-selective construction of glycosidic bonds remains an unsolved problem. Extremely significant advances have been made since Koenigs and Knorr disclosed their classic glycosidation methodology back at the beginning of the twentieth century,[1] and numerous excellent reviews on some of the most significant methodological developments exist.[2] However the ultimate goal for the synthetic chemist is the reduction of the synthesis of oligosaccharides to an automatable solid phase process, a feat which revolutionalised the synthesis of oligonucleotides and oligopeptides. We would therefore like to disclose our preliminary investigations into a completely novel approach to the construction of glycosidic linkages. Herein our strategy is to abandon the classical philosophy of the search for a 'general glycosidation reaction', and to attempt to use combinatorial techniques to search for separate synthetic solutions to the construction of each individual glycosidic bond.

The technique of molecular tethering, first introduced to glycosidation chemistry by Stork,[3] and also used by Hindsgaul[4] and Ogawa[5] amongst others, has proven to be an excellent approach for the synthesis of β-mannoside bonds. More recently, several groups have disclosed the use of more extended tethers for the stereo- and even regio-selective construction of glycosidic linkages.[6] As a solution to the problems of glycosidic bond formation we have combined the use of tethering, which displays great promise for the construction of particular glycosidic linkages, with a combinatorial approach. In theory this will allow us to search rapidly through libraries of tethers, screening their potential to promote the formation of particular glycosidic linkages in stereo- and regio-selective fashions.

Since we required tethers in which structural diversity can be readily introduced by solid phase synthesis, our initial investigations have focussed on peptides. Ultimately this may allow us to use existing automatable methodology for solid phase peptide synthesis for oligosaccharide construction. The question therefore is: can a peptide sequence, itself readily synthesizable quantitatively in an automated manner on the solid phase, be effectively translated into an oligosaccharide sequence?

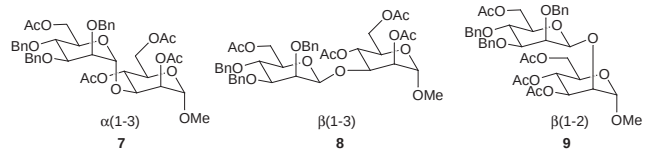
We recently disclosed[7] the synthesis of the novel glycosyl amino acids **1**, **2** and **3**, wherein the sugar moieties are linked through the free OH-6 hydroxy to the β-carboxy of a suitably protected aspartic acid residue. We now disclose the results of several glycosidation reactions undertaken after linking glycosyl donor **1** and glycosyl acceptor **2** together *via* short peptide chains.

† Detailed NMR data are available for selected products, see http://www.rsc.org/suppdata/cc/1999/1037/



Scheme 1

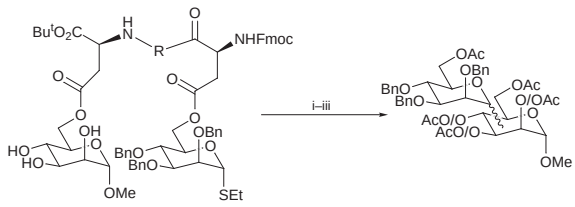
Firstly, a control reaction of the protected thioglycosyl donor **4** with the triol **5** under standard conditions was investigated, and found to proceed in moderate yield but with extremely poor selectivity (Scheme 1). Of the seven products only two were found to be disaccharides, which were identified as the α(1-3) and β(1-3) linked materials **7** and **8** respectively. Four of the



other products were found to be undesired trisaccharides, and the final material was simply the product of hydrolysis. Armed with the knowledge that the simple intermolecular reaction was particularly unselective we then undertook an investigation of several intramolecular reactions between the same acceptor and donor, when they were instead covalently linked to a peptide template.

Aspartate linked glycosyl donor **1** and acceptor **2** were elaborated to the oligopeptides **6a**–**g** using standard protecting group manipulations and EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) mediated peptide coupling. Intramolecular glycosidation mediated by *N*-iodosuccinimide (NIS) and TfOH was then undertaken (Scheme 2). The resulting disaccharides were released from the peptide template by treatment with K_2CO_3 in MeOH, before complete acetylation and structural assignment. The results of this series of glycosidation reactions are summarised in Table 1.

Several points are worthy of comment. Firstly, the overall yields for the peptide templated glycosidation reactions are in all cases similar to those observed for the control reaction. Secondly, the absence of any trisaccharide products, in contrast to the control, is a clear indicator that all glycosidation is effected intramolecularly. Thirdly we were delighted to find that moderate levels of regio- and stereo-selectivity were achieved, and that the product distribution was dependent on the nature of the peptide template. It is clear that the α(1-3) **7** and



6a R = —
b R = Gly
c R = Ala
d R = Phe
e R = Asn
f R = Pro
g R = GlyGly

Scheme 2 *Reagents and conditions*: i, NIS, TfOH, −40 °C, CH_2Cl_2; ii, K_2CO_3, MeOH; iii, Ac_2O, pyridine.

Table 1 Results of peptide templated glycosidation reactions

Peptide[a]	Total yield[b] (%)	Major products and yields[b] (%)	Yields of acetylated disaccharides[c] (%)
Bu^{t}AspAspFmoc **6a**	59	α(1-3) 11	α(1-3) 60
		β(1-3) 23	β(1-3) 78
		β(1-2) 13	β(1-2) 54
Bu^{t}AspGlyAspFmoc **6b**	41	α(1-3) 21	α(1-3) 58
		β(1-3) 20	β(1-3) 77
Bu^{t}AspAlaAspFmoc **6c**	43	α(1-3) 20	α(1-3) 56
		β(1-3) 23	β(1-3) 59
Bu^{t}AspPheAspFmoc **6d**	44	α(1-3) 13	α(1-3) 58
		β(1-3) 18	β(1-3) 71
Bu^{t}AspAsnAspFmoc **6e**	43	β(1-3) 20	β(1-3) 63
Bu^{t}AspProAspFmoc **6f**	49	β(1-2) 16	β(1-2) 73
		β(1-3) 19	β(1-3) 62
		α(1-2) 14	α(1-2) 79
Bu^{t}AspGlyGlyAspFmoc **6g**	56	α(1-3) +	α(1-3) 30
		β(1-3) 56	β(1-3) 42

[a] Glycosyl donor linked *via* β ester to N-terminal aspartate, glycosyl acceptor linked *via* β ester to C-terminal asparate. [b] Minor or inseparable reaction products not identified. [c] Overall yield for cleavage from peptide backbone and subsequent acetylation.

β(1-3) **8** products dominate throughout, though the relative amount of these two isomers is variable. It would also seem that simple variation of the intermediate amino acid in the tripeptides from glycine (Gly) **6b** to alanine (Ala) **6c**, to phenylalanine (Phe) **6d** actually influences the outcome of the glycosidation reaction only slightly. However, with the use of proline (Pro) as the linking residue (**6f**) then a much higher degree of β selectivity is observed; β(1-2) linked disaccharide **9** now becomes a major product, together with the previously observed β(1-3) disaccharide **8**, and now none of the α(1-3) linked product **7** is formed. Moreover, increasing the length of the peptide by the addition of another Gly residue did not greatly alter the outcome (**6g**).

In order to begin to understand the dependence of product distribution upon peptide sequence we undertook some simple molecular modelling. Thus, a number of potential energy minima locations for a selection of these glycopeptides were probed by variation of the starting point through varying the torsional angles in the linker. This allowed us to probe a number of representative points at different relative orientations of the glycosyl acceptor ranging from the β face to the α face of the glycosyl donor. It was found that all of the modelled peptides AspGlyAsp, AspAlaAsp and AspPheAsp have minima in positions above both and α and β faces, *i.e.* there is little facial distinction, although the α face minima are typically slightly lower in energy. For example, Fig. 1(*a*) shows (hydrogen atoms omitted for clairty) the Bu^{t}AspGlyAspFmoc peptide system **6b** in which the glycosyl acceptor occupies a minimum above the α face of the glycosyl acceptor. In contrast the half chair conformation of the proline residue shown in Fig. 1(*b*) biases the AspProAsp model towards a β face energy minimum. This therefore suggests that the increased β selectivity observed for the AspProAsp peptide is a consequence of the conformation of this tether. In addition, changing identity of the intermediate amino acid from Gly to Ala or Phe changed the conformations of these minima only very slightly. This is consistent with the similar product distributions that were observed for these three peptide linkers. Finally, these simple models also provided a plausible explanation for the preponderance of (1-3)-linked disaccharide products; in all of the minimised models the 3-hydroxy of the acceptor is readily disposed to react with the anomeric centre of the donor.

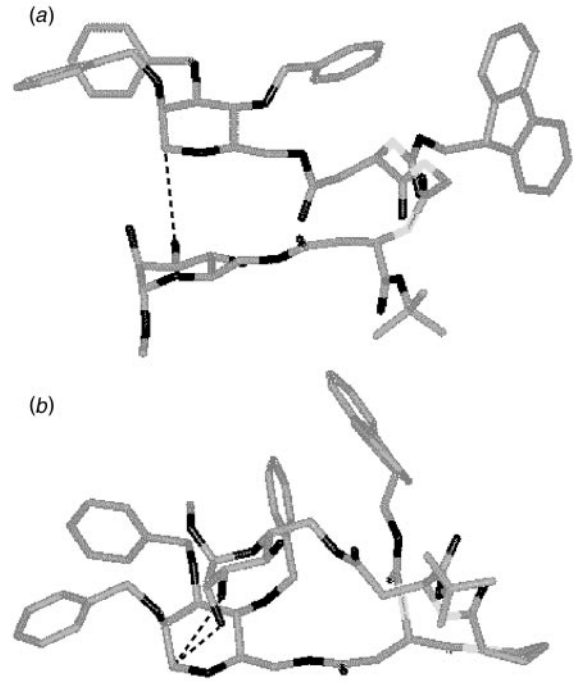


Fig. 1 Minimized structures of tripeptide-tethered systems illustrating the positions of O-2 and O-3 of the glycosyl acceptor relative to the anomeric centre of the glycosyl donor (hydrogens omitted for clarity): (*a*) Bu^{t}AspGlyAspFmoc **6b** and (*b*) Bu^{t}AspProAspFmoc **6f**.

In summary, we have successfully demonstrated for the first time that glycosidation reactions may be achieved between glycosyl donors and acceptors which are bound to a peptide template. In addition these reactions show enhanced, though at present modest, regio- and stereo-selectivities, the product distribution of which can be rationalised by simple molecular modelling. Further investigations in these areas are currently in progress and results will be published in due course.

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Notes and references

1 W. Koenigs and E. Knorr, *Chem. Ber.*, 1901, **34**, 957.
2 For some recent reviews and books see: K. Toshima and K. Tatsuta, *Chem. Rev.*, 1993, **93**, 1503; G.-J. Boons, *Tetrahedron*, 1996, **52**, 1095; *Modern Methods in Carbohydrate Synthesis*, ed. S. H. Khan and R. A. O'Neill, Harwood Academic, Amsterdam, 1996; *Preparative Carbohydrate Chemistry*, ed. S. Hanessian, Marcel Dekker, New York, 1997.
3 G. Stork and G. Kim, *J. Am. Chem. Soc.*, 1992, **114**, 1087.
4 F. Barresi and O. Hindsgaul, *Can. J. Chem.*, 1994, **72**, 1447; F. Barresi and O. Hindsgaul, *Synlett*, 1992, 759; F. Barresi and O. Hindsgaul, *J. Am. Chem. Soc.*, 1991, **113**, 9376.
5 Y. Ito, Y. Ohnishi, T. Ogawa and Y. Nakahara, *Synlett*, 1998, 1102; A. Dan, Y. Ito and T. Ogawa, *J. Org. Chem.*, 1995, **60**, 4680; Y. Ito and T. Ogawa, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1765.
6 T. Zeigler, G. Lemanski and A. Rakoczy, *Tetrahedron Lett.*, 1995, **36**, 8973; T. Zeigler and R. Lau, *Tetrahedron Lett.*, 1995, **36**, 1417; R. Lau, G. Schülle, U. Schwaneberg and T. Zeigler, *Liebigs Ann.*, 1995, 1475; H. Yamada, K. Imamura and T. Takahashi, *Tetrahedron Lett.*, 1997, **36**, 391; S. Valverde, A. M. Gómez, A. Hernández, B. Herradón and J. C. López, *J. Chem. Soc., Chem. Commun.*, 1995, 2005.
7 R. J. Tennant-Eyles and A. J. Fairbanks, *Tetrahedron : Asymmetry*, 1999, **10**, 391.

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