Reagent switchable stereoselective β(1,2) mannoside mannosylation: OH-2 of mannose is a privileged acceptor†‡

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The discovery of novel conditions for highly β-stereoselective (>9 : 1) mannosylation of OH-2 of mannosides using a straightforward perbenzylthioglycoside donor has allowed ready assembly of β-mannosyl oligosaccharides including the repeating trisaccharide motif of the O5 antigen of pathogen *Klebsiella pneumoniae.*

The stereoselective formation of *cis*-glycosidic linkages is still a major synthetic challenge in the synthesis of complex oligosaccharides.[1–3] Although significant advances have been made in methodology, these methods are not fully understood and generalities are often hard to find.[2]

Common methods for the synthesis of β-mannosides (Fig. 1) involve the synthesis of a β-glucoside, followed by inversion of C-2 configuration either by a nucleophilic displacement[4,5] or an oxidation–reduction procedure.[6,7] Intramolecular aglycon delivery has also been developed by several groups as a method for the synthesis of 1,2-*cis* glycosides and has been applied to the synthesis of β-mannosides.[8–14]

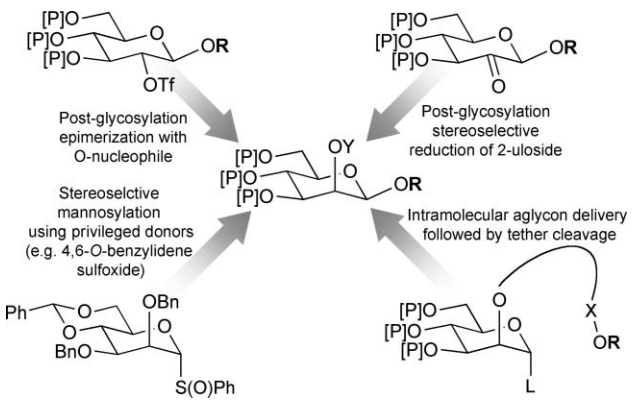

Fig. 1 Selected, illustrative methods for the synthesis of β-mannosides.

The use of sulfoxide glycosyl donors, first activated with triflic anhydride before addition of the acceptor, has also proved successful.[15,16] In such systems, the use of 4,6-*O*-benzylidene-type protection with non-participating groups on *O*-2 and *O*-3 of the donor is important in gaining high β-selectivity. The mechanism proposed involves the formation of an α-triflate, which then undergoes an S_N2-like displacement giving β-mannoside product. Crich *et al*. have used kinetic isotope effect experiments to explore this mechanism and suggest that the 4,6-*O*-benzylidene acts to prevent rehybridisation at the anomeric carbon thus favouring the triflate intermediate.[17] This methodology has been widely used in the synthesis of biologically significant oligosaccharides containing *cis*-glycosidic linkages,[2,18,19] although some linkages still remain as difficult targets.[3,20]

Our laboratory has been particularly interested in the synthesis of fragments of D-mannosyl-containing sections of pathogen coats such as the high-mannose oligosaccharide **1** found on the surface of the HIV-1 envelope glycoprotein gp120,[21] which contains predominantly alpha mannosides, and the O5 antigen of the opportunistic gram-negative pathogen *Klebsiella pneumoniae* serotype O5 **2** (Fig. 2). This paper describes the development of mannosylation methodology and its application to the synthesis of key di-, tri- and tetra-saccharide fragments of both of these structures.

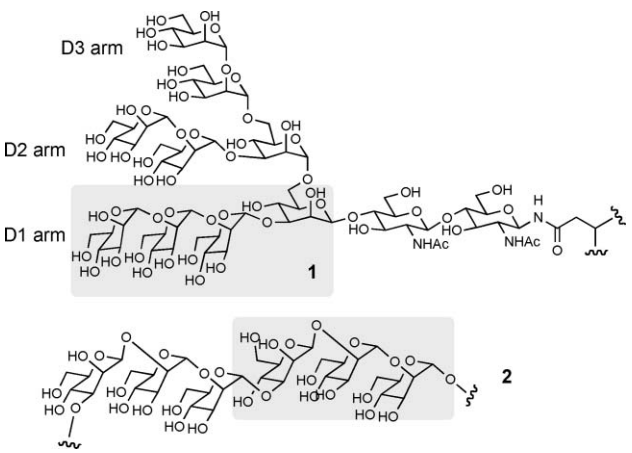

Fig. 2 D-Mannosyl targets: high mannose oligosaccharide of gp120 **1** and O5 antigen from gram-negative pathogen *Klebsiella pneumoniae* serotype O5. Target fragments highlighted in grey.

Glycan **1** contains a number of key α1,2 and α1,3 mannosyl linkages. The alpha-mannosyl linkage configuration is, as a *trans* linkage, one of the most ready to form in a stereoselective manner being favoured both by participating groups, sterics and the anomeric effect.[22,23] Access to the *cis* beta-mannosyl linkage therefore remains a key challenge in organic chemistry (*vide supra*).[2]

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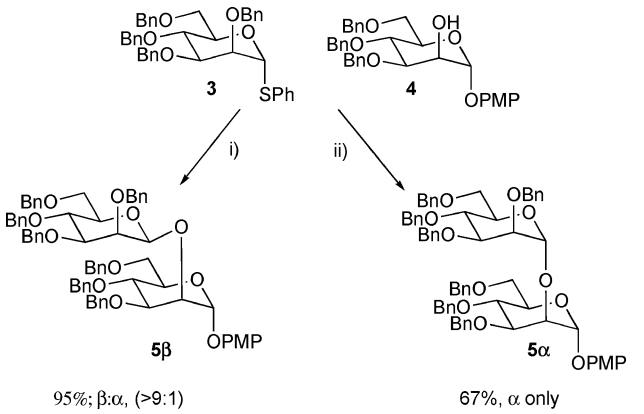
We were therefore particularly surprised and pleased to find that using the recently reported dimethyl disulfide-triflic anhydride[24] conditions (conditions A) for activation of thioglycosides, we observed the formation of Man-β1,2-Man product in very good yield and good selectivity (β : α > 9 : 1) (Scheme 1). We reasoned that this unexpected selectivity might be applied to the synthesis of the repeating trisaccharide unit of **2**, which contains a key β1,2-mannosyl linkage.



Scheme 1 (i) *Conditions A:* Tf_2O, Me_2S_2, TTBP, 4 Å mol sieves, −78 °C, (ii) *conditions B:* DMTST, TTBP, 4 Å mol sieves, −78 °C→RT.

Reaction of fully benzylated thiophenyl mannosyl donor **3** with acceptor **4** gave the β-product **5**β in a >9 : 1 ratio in 95% yield (Scheme 1).[25] However, in striking contrast, when the same glycosylation was performed using the powerful and long used alkylsulfenylating agent dimethyl(methylthio) sulfonium triflate (DMTST, conditions B)[26] the α-product **5**α only was obtained in 67% yield. The identity and configuration of the products were unambiguously identified (as for all mannosides) by a range of methods including, critically, ^1H-^{13}C ^1J coupling constants,[27] which in all cases were in the range 150–156 Hz for β-products and 170–172 Hz for all α-products. The reagent switchable α↔β selectivity observed here for the β-1,2-mannosylations is to the best of our knowledge unprecedented. The β-selectivity obtained with conditions A was of particular additional significance as donor **3** does not possess the 4,6-*O*-benzylidene protection previously used in other systems such as the Crich sulfoxides.[16]

These intriguing results led us to investigate the scope of these reaction conditions and the resulting stereoselectivity in the formation of other mannosides (Table 1). First, mannosylation under conditions A was performed using the same acceptor **3** with the alternative peracetylated donor **5** that bears a participating *O*-2 protecting group: unsurprisingly, participation dominates selectivity and only the α-product **6** was obtained in a yield of 55% (entry 3, table 1). Yet, the strong β-selectivity observed for OH-2 of D-mannose was again observed, under the dimethyl disulfide-triflic anhydride conditions (conditions A), during the installation of a terminal mannosyl residue to the truncated D1-arm acceptor trisaccharide **19**: only the β-linked product **20**β was observed in 47% yield (entry 9). Consistent with the reagent control shown in the reaction of **3** with **4**, application of conditions B to the same reactions gave primarily α-linked product **20**α (entry 10). Yields for both of these mannosylations of **19** to yield tetrasaccahrides are more modest, consistent with the more hindered acceptor. These results and their switchability are summarized in Table 2.

A range of representative sugar alcohol acceptors other than OH-2 mannosides were then tested (primary hydroxyl **8**, secondary axial hydroxyl **10**, secondary equatorial hydroxyl **12**, **14**, **17**). Together the results suggested a unique privileged reactivity of the 2-OH of mannosides. Glycosylation of less hindered primary acceptor **8** using donor **3** gave a poor β-selectivity (**9** β : α = 1 : 3) in 53% yield (entry 4). Glycosylation of the hindered axial 4-OH in the galactose acceptor **10** gave **11** exclusively with α-selectivity in 75% yield (entry 5). Next the GlcNAc acceptor **12** was used, which in principle would allow access to one of the key linkages (Manβ1,4GlcNAc) of the core pentasaccharide found in *N*-linked glycoproteins; under glycosylating conditions A, only the α-product **13** was observed in 78% yield (entry 6). Glycosylation with the glucoside acceptor **14** also only gave the α anomer in 64% yield (entry 7). Even use of disaccharide thioglycoside donor **16** gave only the α-product **18** from secondary equatorial hydroxyl acceptor **17** (entry 8).

These striking results suggested that this strongly selective transformation is only applicable to the synthesis of β-(1→2)-mannosylmannoside linkages and that this reactivity is switchable only for this linkage (Table 2).

This highly selective yet narrowly applicable methodology was then tested in the target synthesis of the O5 antigen of the opportunistic gram-negative pathogen *Klebsiella pneumoniae* serotype O5. This organism's cell wall contains a repeating trimannoside motif found in **22** (Fig. 2 and Scheme 2), which critically contains the target Manβ(1,2)Man linkage that is addressable by our method.[28,29] Thus, disaccharide acceptor **21** was reacted with donor **3** using the triflic anhydride and dimethyldisulfide activating conditions (conditions A). As expected, this successfully installed the β-(1→2)-mannoside linkage in 84% yield and with high β-selectivity (α : β ratio of 1 : 11); the two anomers were found to be easily separable by column chromatography. Again, use of conditions B did not yield target **22** but instead gave primarily alpha linked product (Table 2).

In conclusion, we have shown that donor **3** when activated using triflic anhydride and dimethyldisulfide is an efficient reagent for the synthesis of β-(1→2)-mannoside linkages. The selectivity of this reaction dramatically decreased during the synthesis of other glycosidic linkages. This method is therefore narrow in the linkage type to which it might be applied but was successfully applied here to the synthesis of the naturally-occurring pathogen trisaccharide motif **22**. This reaction is of particular note as the donor has not been conformationally constrained by the presence of a 4,6-*O*-benzylidene protecting group and is reagent switchable. We are currently exploring the mechanistic origin of this startling selectivity but some mechanistic rationale for the observed outcomes may be suggested with the caveat that detailed studies are ongoing.

Given the simplicity of the donor system, which contrasts with the designed use of, for example, 4,6-*O*-benzylidenes and other 'torsionally disarmed' donors,[16,30] the mechanistic origins of the reagent-switchable selectivity in this more conformationally flexible system are intriguing. Electronic effects in both 4,6-*O*-benzylidene mannosyl donors[31] and related uronic acids[32] are also suggested to influence stereoselectivity; again these are features that per-benzylated donor **3** lacks. This suggests that other factors

Table 1 Representative mannosylations with triflic anhydride (conditions A) or dimethyl disulfide (conditions B)

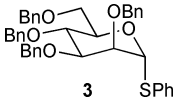
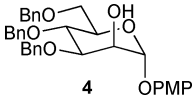
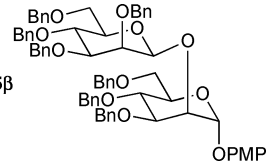
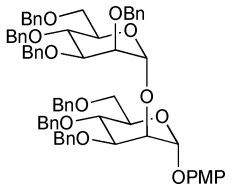
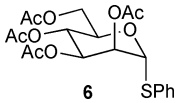
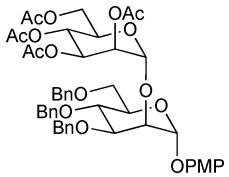
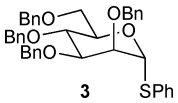
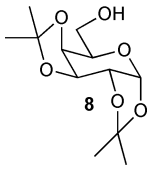
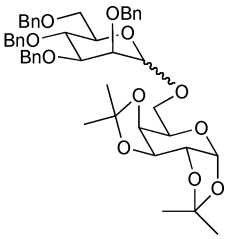
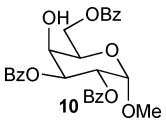
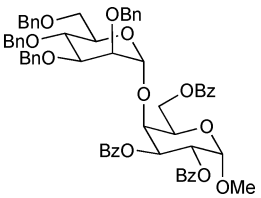
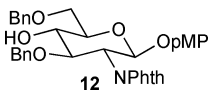
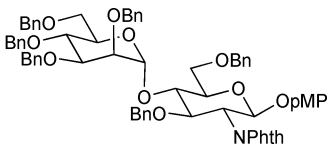
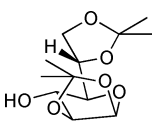
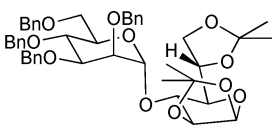
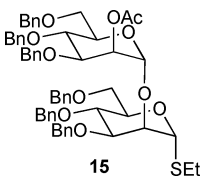
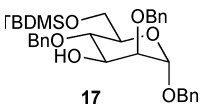
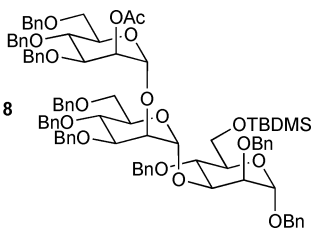
Entry	Donor	Acceptor	Product	Conditions	Yield (β/α)
1	 **3**	 **4**	**5β** 	A	95% (>9 : 1)
2	**3**	**4**	**5α** 	B	67% (α only)
3	 **6**	**4**	**7** 	A	55% (α only)
4	 **3**	 **8**	**9** 	A	53% (1 : 3)
5	**3**	 **10**	**11** 	A	75% (α only)
6	**3**	 **12**	**13** 	A	78% (α only)
7	**3**	 **14**	**15** 	A	64% (α only)
8	 **15**	 **17**	**18** 	A	68% (α only)

Table 1 *(Conti.)*

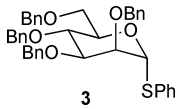
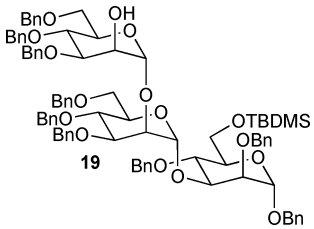
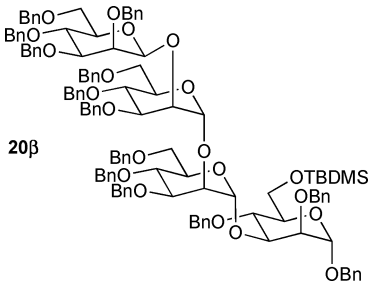
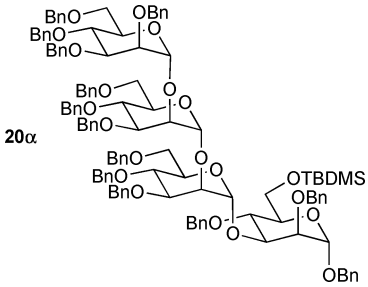
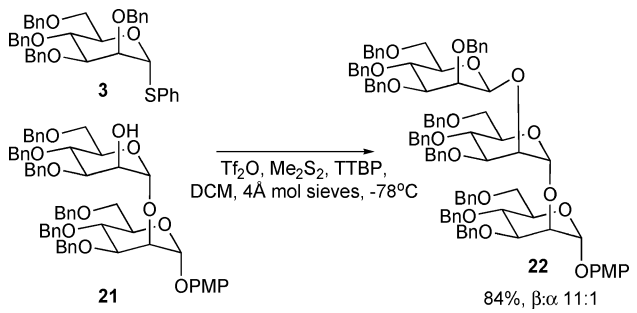
Entry	Donor	Acceptor	Product	Conditions	Yield (β/α)
9				A	47% (β only)
10	**3**	**19**		B	45% (1 : 2.5)

Table 2 Summary of β(1,2)-mannosylations

Entry	Donor	Acceptor	Conditions	β	α	Yield (%)
1	**3**	**4**	A	>9	1	95
2	**3**	**4**	B	0	1	67
3	**3**	**19**	A	1	0	47
4	**3**	**19**	B	1	2.5	41
5	**3**	**21**	A	11	1	84
6	**3**	**21**	B	1	3	75



Scheme 2 Preparation of the repeating trisaccharide motif of the O5 antigen of pathogen *Klebsiella pneumoniae.*

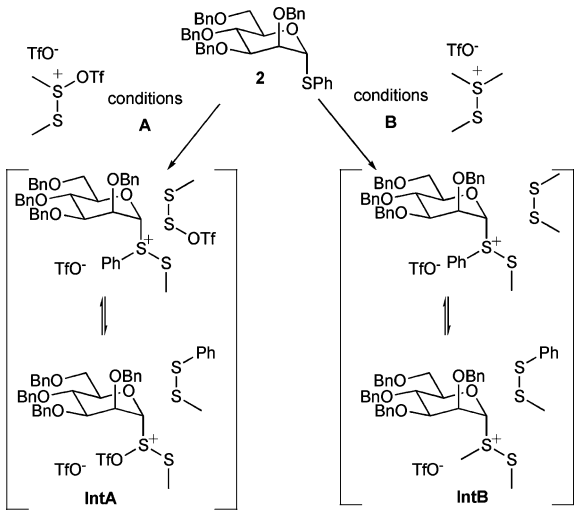


Fig. 3 Putative differential activation of **2** leading to potential equilibration of intermediates (selected examples of alpha sulfeniums shown, although, beta intermediates and triflates cannot be discounted).

dominate. The donors used here are exclusively alpha thiomannosides with an axial leaving group. Were clean S_N2-chemistry to dominate, as has been suggested in a number of systems,[17] then beta products would be expected; S_N2 chemistry has been invoked to explain the beta selectivity obtained from intermediate mannosyl alpha-triflates.[17] Deuterium secondary kinetic isotope effect experiments[17] are currently underway to address this issue.

In this context, the switch in activation conditions (A to B) differs essentially only in the nature of the leaving group for the sulfenium reagent that is generated *in situ* (see Fig. 3). Putative post-activation equilibration might give rise to a number of intermediates with varied anomeric leaving groups (or corresponding glycosyl cation·anion pairs). The different conditions (A and B) may differ in their outcome as a result of cleaner S_N2-substitution on an intermediate such a **IntA**. Initial experiments intended to observe by NMR the intermediates formed from **2** upon activation by either conditions were inconclusive. Of course, under conditions of rapid equilibration, the differing rates of intermediate substitution will critically determine reaction product distribution and hence observation of the equilibrium position of these intermediates would add little to illuminating the origin of selectivity.

The restriction of this beta selectivity to only the OH-2 mannoside acceptors indicates a strong role for the nucleophile/acceptor in these reactions. The axial OH-4 hydroxyl of acceptor **9** gives rise to exclusive alpha mannosylation products, indicating that this substrate-controlled aspect of stereoselectivity extends beyond the relative conformational position of the OH-2 nucleophile of the alpha-mannoside acceptors that we have shown here are privileged acceptors. The possibility therefore remains that the unique O1ax/O2ax/O3eq geometry plays a key role in determining selectivity and other acceptors containing this relative configuration are being now being explored.

Acknowledgements

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Notes and references

1 H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 823–839.
2 J. J. Gridley and H. M. I. Osborn, *J. Chem. Soc., Perkin Trans. 1*, 2000, **10**, 1471–1491.
3 A. J. Fairbanks, *Synlett*, 2003, 1945–1958.
4 S. David, A. Malleron and C. Dini, *Carbohydr. Res.*, 1989, **188**, 193–200.
5 J. Alais and S. David, *Carbohydr. Res.*, 1990, **201**, 69–77.
6 M. A. Shaban and R. W. Jeanloz, *Carbohydr. Res.*, 1976, **52**, 103–114.
7 N. K. Kochetkov, B. A. Dmitriev, N. N. Malysheva, A. Y. Chernyak, E. M. Klimov, N. E. Bayramova and V. I. Torgov, *Carbohydr. Res.*, 1975, **45**, 283–290.
8 F. Barresi and O. Hindsgaul, *J. Am. Chem. Soc.*, 1991, **113**, 9376–9377.
9 F. Barresi and O. Hindsgaul, *Can. J. Chem.*, 1994, **72**, 1447–1465.
10 Y. Ito, Y. Ohnishi, T. Ogawa and Y. Nakahara, *Synlett*, 1998, 1102–1104.
11 I. Cumpstey, A. J. Fairbanks and A. J. Redgrave, *Org. Lett.*, 2001, **3**, 2371–2374.
12 C. M. P. Seward, I. Cumpstey, M. Aloui, S. C. Ennis, A. J. Redgrave and A. J. Fairbanks, *Chem. Commun.*, 2000, 1409–1410.
13 Y. Ito and T. Ogawa, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1765–1767.
14 A. Dan, Y. Ito and T. Ogawa, *J. Org. Chem.*, 1995, **60**, 4680–4681.
15 D. Crich and S. X. Sun, *J. Org. Chem.*, 1997, **62**, 1198–1199.
16 D. Crich and S. X. Sun, *Tetrahedron*, 1998, **54**, 8321–8348.
17 D. Crich and N. S. Chandrasekera, *Angew. Chem., Int. Ed.*, 2004, **43**, 5386–5389.
18 D. Crich, W. J. Li and H. M. Li, *J. Am. Chem. Soc.*, 2004, **126**, 15081–15086.
19 D. Crich, A. Banerjee and Q. J. Yao, *J. Am. Chem. Soc.*, 2004, **126**, 14930–14934.
20 A. V. Demchenko, *Curr. Org. Chem.*, 2003, **7**, 35–79.
21 D. A. Calarese, C. N. Scanlan, M. B. Zwick, S. Deechongkit, Y. Mimura, R. Kunert, P. Zhu, M. R. Wormald, R. L. Stanfield, K. H. Roux, J. W. Kelly, P. M. Rudd, R. A. Dwek, H. Katinger, D. R. Burton and I. A. Wilson, *Science*, 2003, **300**, 2065–2071.
22 G. J. Boons, *Tetrahedron*, 1996, **52**, 1095–1121.
23 B. G. Davis, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2137–2160.
24 J. Tatai and P. Fugedi, *Org. Lett.*, 2007, **9**, 4647–4650.
25 *Conditions A:* acceptor (1 eq), donor (1.1 eq) and 2,6-di-*tert*-butyl-4-methylpyridine (5 eq) were dried in a desiccator overnight. The reagents were dissolved in DCM (2 mL) and transferred using a cannula to a flame dried flask containing 4 Å molecular sieves. The mixture was stirred for 1 h and cooled to −78 °C. DCM (2 mL) was added to a flame dried flask containing 4 Å molecular sieves and stirred for 1 h then cooled to 0 °C. To this flask was added dimethyldisulfide (4 eq) and trifluoromethylsulfonic anhydride (4 eq). After 2 min, the solution was transferred to the flask containing the sugar reagents at −78 °C. The mixture was stirred at −78 °C under an atmosphere of argon. After 1 h, the reaction mixture was quenched with triethylamine (1 mL) and filtered through celite®. The filtrate was concentrated *in vacuo* and the residue purified by flash column chromatography.
26 P. Fugedi and P. J. Garegg, *Carbohydr. Res.*, 1986, **149**, C9–C12.
27 K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, 1974, 293–299.
28 P.-E. Jansson, J. Lonngren, G. Widmalm, K. Leontein, K. Slettengren, S. B. Svenson, G. Wrangsell, A. Dell and P. R. Tiller, *Carbohydr. Res.*, 1985, **145**, 59–66.
29 J. D. C. Codee, L. H. Hossain and P. H. Seeberger, *Org. Lett.*, 2005, **7**, 3251–3254.
30 T. Nukada, A. Berces and D. M. Whitfield, *Carbohydr. Res.*, 2002, **337**, 765–774.
31 H. H. Jensen, L. U. Nordstrom and M. Bols, *J. Am. Chem. Soc.*, 2004, **126**, 9205–9213.
32 L. J. Van Den Bos, J. Dinkelaar, H. S. Overkleeft and G. A. Van Der Marel, *J. Am. Chem. Soc.*, 2006, **128**, 13066–13067.

