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Synthesis and acid catalyzed hydrolysis of $B_{2,5}$ type conformationally constrained glucopyranosides: incorporation into a cellobiose analogue

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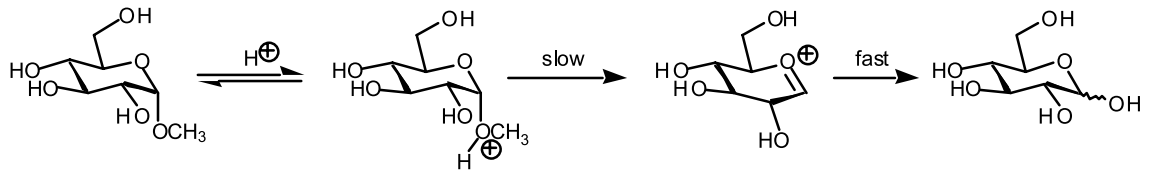
Abstract—Isopropyl and *p*-nitrophenyl α- and β-D-glucopyranosides, restrained in a conformation close to $B_{2,5}$ via an oxymethylene bridge have been synthesized. These four glucopyranosides were found to be hydrolyzed at similar rates, close to those observed for the parent unconstrained glucosides. In such derivatives, either α or β, the exocyclic cleaved bond is synperiplanar to an endocyclic oxygen lone pair. This conformationally locked glucopyranosyl moiety was also incorporated into a disaccharide, affording a conformationally restrained cellobiose analogue which was assayed against various glycosidases.

1. Introduction

Oligo- and polysaccharides, the most abundant biomolecules on earth, are made of monosaccharide monomers connected together through a glycosidic linkage. This bond, which is formed or cleaved during bioprocesses is thus inherently critical for the emergence of the numerous biological properties of this class of compounds. As a consequence, an in-depth understanding of the mechanism of glycosidic bond cleavage is essential.

An increasing number of articles dissecting the enzymatic cleavage of the glycosidic bond by glycosidases have appeared recently, taking advantage of kinetic studies and protein crystallography,[1] but few recent papers are indeed dealing with the non-enzymatic hydrolysis of glycosides:[2] the same set of pioneering articles is usually cited. The chemical hydrolysis of glycopyranosides is indeed a much-studied reaction with a well-established mechanism involving a specific acid catalysis, the rate determining step being the formation of a cyclic alkoxycarbenium ion intermediate (Scheme 1).[3]

Nevertheless, some features of this reaction have yet to be fully explored to quantify the effects, which control reactivity in glycosyl transfer. Bols and co-workers have demonstrated that steric effects are not the cause of the rate difference observed during hydrolysis of stereoisomeric glycopyranosides[4] ruling out long-standing Edward's proposal.[5] They rather suggested that the rate difference can be attributed to the different electron-withdrawing effects of axially and equatorially oriented hydroxyl groups involved in the destabilization of the transient cyclic alkoxycarbenium ion. These findings are in agreement with Withers results invoking a Kirkwood–Westheimer model of field effects to explain the opposite effect on the



Scheme 1. A mechanism for the chemical hydrolysis of alkyl glycopyranosides, involving the protonation of the exocyclic oxygen atom.

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hydrolysis rates of glycopyranosides of the deoxygenation of hydroxyl groups and their replacement by fluorine.[6] Furthermore, the contribution of torsional effects on reactivity in glycosyl transfer has a significant albeit not large effect on the rate of hydrolysis of glycopyranosides.[7]

The conformational aspects of the chemical hydrolysis of glycopyranosides have been less explored due to the short lifetime of the transient cyclic alkoxycarbenium and the difficulty of probing the conformation of this intermediate.[8] Bennet and Sinnott, using kinetic isotopic effects, have studied the acid-catalyzed hydrolysis of methyl D-glucopyranosides,[9] and concluded that methyl α-D-glucopyranoside was hydrolyzed via a skew or boat conformation whereas the β-anomer reacted through a chair-like conformation. Similar conclusions were drawn with xylopyranosides.[10] We disclose here another approach to generating the conformation adopted by the cyclic alkoxycarbenium and/or the glucopyranoside at the point of hydrolysis: locking the structure of the glycopyranoside in a defined conformation and a subsequent analysis of its behavior towards acid-catalyzed hydrolysis should tell us if this conformation can be operative during oxycarbenium formation.[11] A careful study is necessary because conformational restraints have been shown to have significant effects on the reactivity of tetrahydropyranyl acetals.[12]

Whatever the exact mechanism of glycoside hydrolysis, nucleophilic substitution at the glycosidic bond involves the sugar becoming either an oxycarbenium ion intermediate or passing through a transition state that is highly oxycarbenium ion-like.

Considering the complete map of pyranoid ring interconversions (Fig. 1), including the boat/skew-boat pseudorotational itinerary of the pyranoid ring,[13] and as suggested previously,[14] not only $^{4}H_{3}$ and $^{3}H_{4}$, but also $B_{2,5}$ and $^{2,5}B$

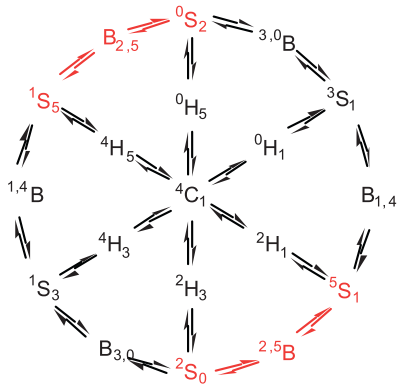


Figure 1. Partial map of pyranoside ring interconversions (adapted from Stoddart).[13]

conformations (Fig. 2) are possible candidates for the conformation of the glycopyranosyl oxycarbenium ion, wherein the four atoms C-5, O-5, C-1 and C-2 become coplanar as the anomeric center is rehybridized from sp^3 to sp^2. An increasing number of these boat type conformations have recently been convincingly observed in nature for some glycosidases that distort the substrate away from the ground state $^{4}C_{1}$ conformation before enzymatic hydrolysis.[15]

If the conformation adopted by the sugar prior to oxygen–carbon bond cleavage is indeed $B_{2,5}$ (or $^{2,5}B$), this implies that the antiperiplanar lone pair effect (ALPE)[16] is not operating in the hydrolytic process. Such a cleavage would rather be compatible with synperiplanar assistance, known to be effective in the hydrolysis of constrained tetrahydropyranyl acetals,[17] and consistent with the relevant least nuclear motion effect.[18]

2. Results

In this context, we report the synthesis of *p*-nitrophenyl and isopropyl α- and β-D-glucopyranosides locked in a $B_{2,5}$ conformation and on their rates of hydrolysis under acidic conditions. The selected constrained targets are **1**–**4**, carbon atoms 2 and 5 linked via an oxymethylene bridge.[19] We also envisioned an oxycarbonyl linkage to lock the conformation as in compound **5** (Fig. 3). The choice of both isopropyl and *p*-nitrophenyl glycosides is designed to detect the possibility of endocyclic cleavage, which is always a potential complication, particularly in the acid-catalysed hydrolysis of conformationally restricted glycosides. Comparison of kinetics for these compounds will enable us to tell whether this mechanism is operative because the endocyclic pathway for the hydrolysis of nitrophenyl glycosides would be orders of magnitude slower than for isopropyl derivatives.

Compounds **1**–**5** are confined for stringent geometrical reasons to the following conformational domain of the boat/skew boat itinerary close to $B_{2,5}$:

$$(^{1}S_{5} \rightleftharpoons B_{2,5} \rightleftharpoons {}^{0}S_{2})$$

3. Results and discussion

3.1. Chemical synthesis

The strategy used to synthesize compounds **1**–**5** starts from the known vinyl derivative **6**.[20] It was first converted

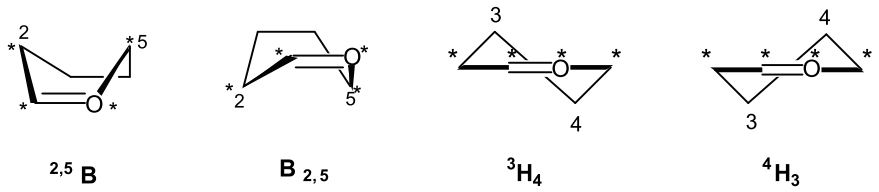


Figure 2. Possible conformations for a glycopyranosyl oxycarbenium ion. Hydroxyl groups have been omitted for the sake of clarity. Coplanar atoms are indicated with asterisks (adapted from Berti and Tanaka).[14]

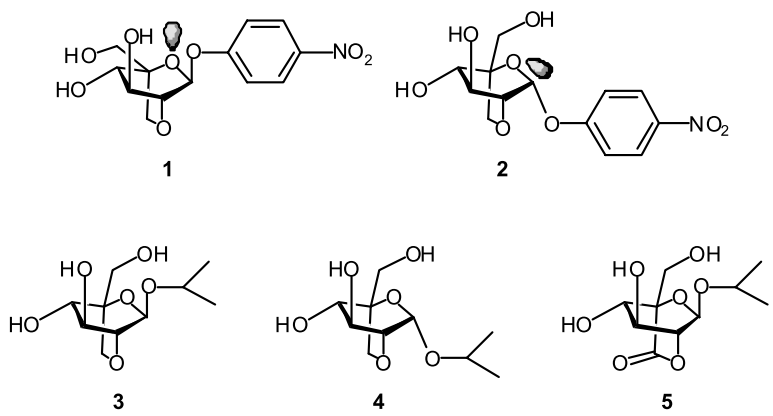
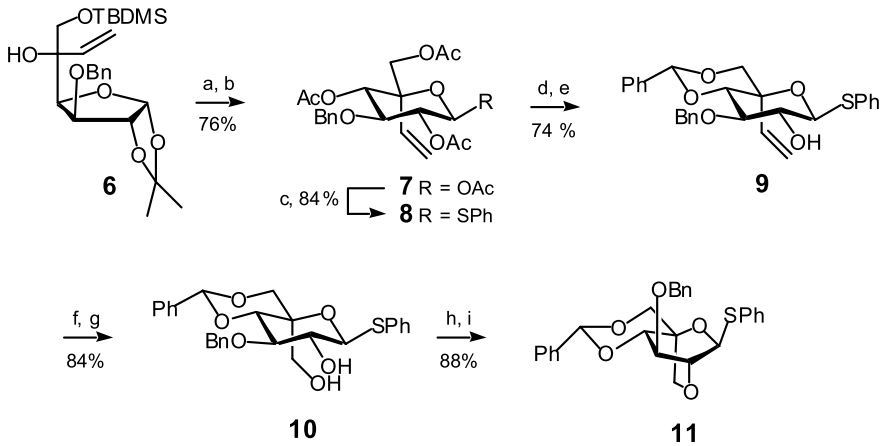


Figure 3. Structure of the conformationally constrained α- and β-D-glucopyranosides **1**–**5**, showing the *syn*-periplanarity of one pyranoside orbital of the intracyclic oxygen and of the glycosidic bond.



Scheme 2. Synthesis of the glycosyl donor **11**. Reagents and conditions: (a) IR-120 H^+ resin, dioxan, H_2O, 90 °C; (b) Ac_2O, DMAP, pyridine, rt; (c) PhSH, $BF_3{\cdot}OEt_2$, anhydrous CH_2Cl_2, rt; (d) CH_3ONa, CH_3OH, rt; (e) $PhCH(OMe)_2$, CSA, DMF, rt; (f) O_3, CH_2Cl_2, −78 °C; (g) $NaBH_4$, EtOH, rt; (h) TsCl, DMAP, pyridine, rt; (i) NaH, DMF, rt.

into the conformationally restrained glycosyl donor **11** (Scheme 2).

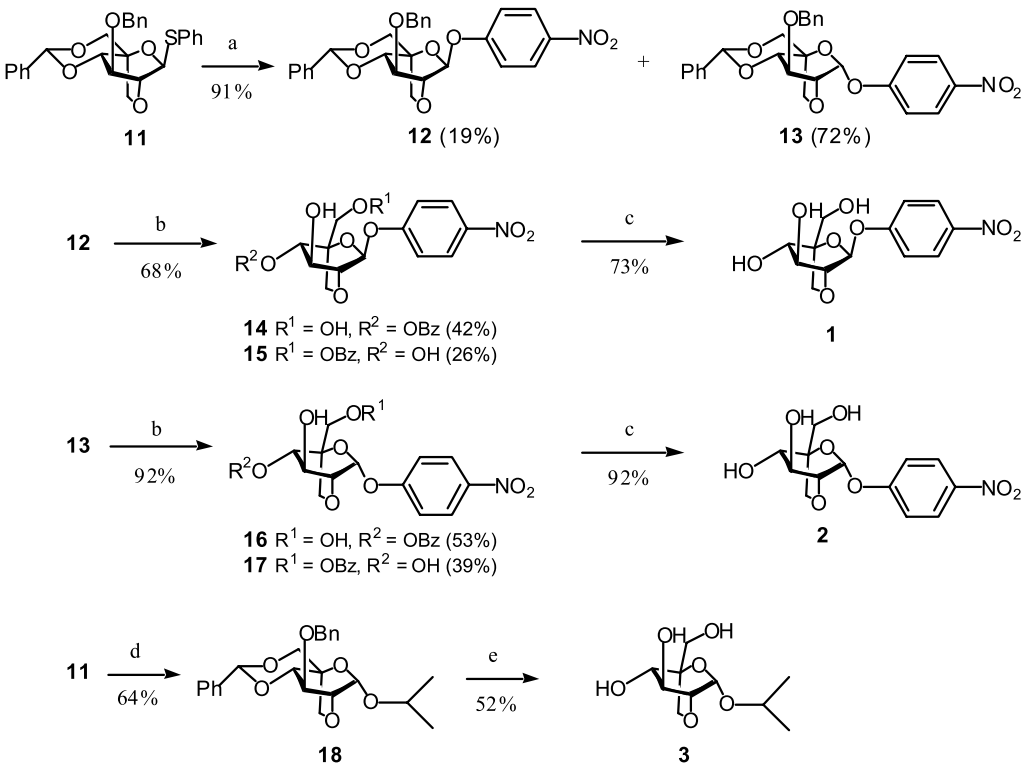
Treatment of compound **6** under acidic conditions afforded the corresponding glucopyranose derivative, which was isolated in 76% yield as the peracetylated derivative **7**. Reaction of **7** with PhSH and $BF_3{\cdot}OEt_2$ in dry dichloromethane gave the thiophenyl derivative **8** in 84% yield. Deacetylation of **8** was followed by the easy formation of alcohol **9**. Careful ozonolysis of compound **9**, in order to avoid sulfur oxidation, led to the corresponding aldehyde which was not isolated and directly reduced to the alcohol using sodium borohydride in ethanol to give diol **10** in 62% yield from the thiophenyl glycoside **8**. Subsequent tosylation of the 'neopentylic' alcohol of diol **10**, followed by treatment with NaH in DMF led to cyclisation, affording the glycopyranosyl donor **11** in 88% yield. Compound **11** constitutes a novel conformationally locked glucopyranosyl donor, which was now used to obtain the conformationally locked glycosides **1**, **2** and **3** (Scheme 3).

When compound **11** was treated with *para*-nitrophenol in the presence of *N*-iodosuccinimide (NIS) and triflic acid in dichloromethane, the *para*-nitrophenyl β-D-glucopyranoside **12** and α-D-glucopyranoside **13** were obtained in high yield (91%) and in a 1:4 ratio. Treatment of glucoside **12** with sodium bromate and sodium dithionite in ethyl acetate/water[21] simultaneously performed the opening of the benzylidene acetal and the cleavage of the benzyl ether to afford a mixture of the 4-*O*-benzoyl derivative **14** and the 6-*O*-benzoyl derivative **15** in 68% yield. Deprotection of the benzoate **14** and **15** with sodium methoxide in methanol afforded the *p*-nitrophenyl β-D-glucoside **1** in 73% yield.

The same sequence was uneventfully applied to compound **13** to afford the *p*-nitrophenyl α-D-glucoside **2** in 84% overall yield.

Compound **3** was also obtained from glycosyl donor **11**. Its treatment with dry isopropanol in the presence of *N*-chlorosuccinimide (NCS) gave the protected isopropyl α-D-glucoside **18** along with traces of the corresponding protected β-D-glucoside. Surprisingly, hydrogenolysis of **18** under various conditions only led to decomposition products. Fortunately, reduction with Na-liq. NH_3 afforded the pure α-D-glucoside **3** after careful column chromatography.

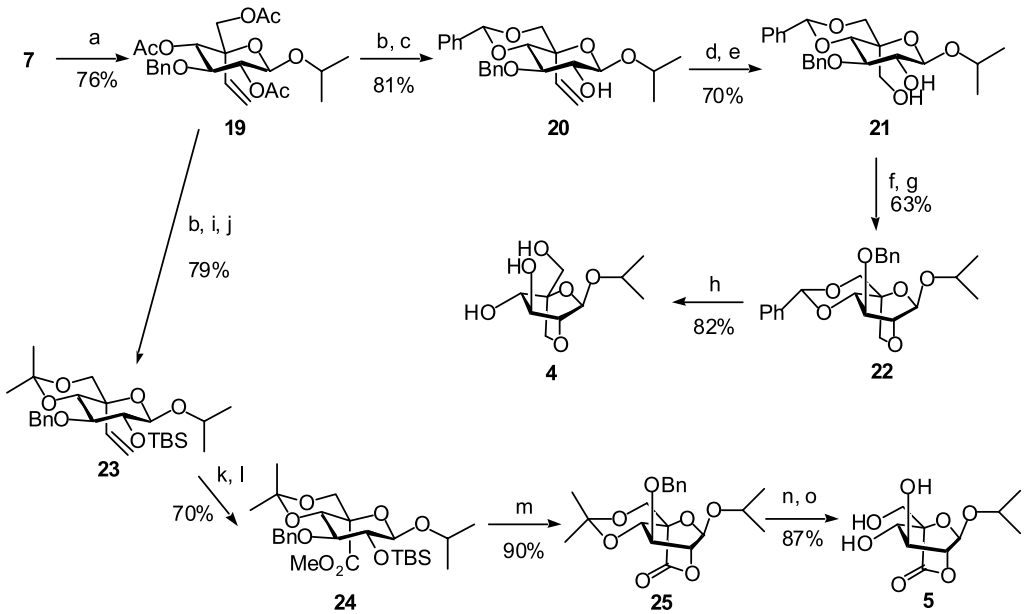
Compounds **4** and **5** were now synthesized from the isopropyl β-D-glucopyranoside **19**, obtained by glycosylation



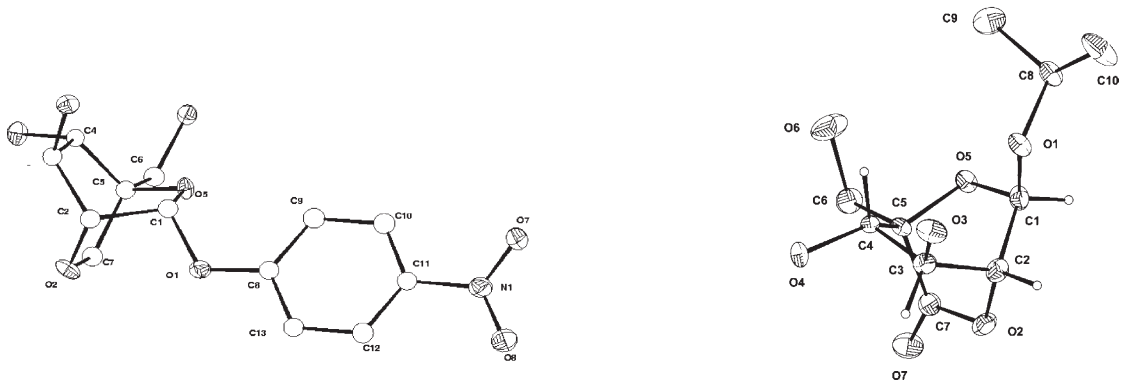
Scheme 3. Synthesis of compounds **1**, **2** and **3**. Reagents and conditions: (a) *para*-nitrophenol, NIS, TfOH, CH_2Cl_2, −30 °C; (b) $NaBrO_3$, $Na_2S_2O_4$, EtOAc, H_2O, rt; (c) CH_3ONa, CH_3OH, rt; (d) NCS, dry isopropanol, rt; (e) Na, liq. NH_3.

in 76% yield from the acetate **7** using anhydrous isopropanol and TMSOTf (Scheme 4). An unevent series of reactions similar to that previously described led to the target **4**.

The lactone **5** was obtained in eight steps from isopropyl β-D-glucopyranoside **19** which was first deacetylated and then fully protected using isopropylidene acetal and *tert*-butyldimethylsilyl protecting groups to give compound **23**. Prolonged ozonolysis of the C=C bond yielded the corresponding carboxylic acid which was not isolated and directly converted to its methyl ester **24** using iodomethane and $KHCO_3$ in DMF. Selective removal of the silyl protection group with TBAF gave the corresponding



Scheme 4. Synthesis of compounds **4** and **5**. Reagents and conditions: (a) TMSOTf, dry isopropanol, rt; (b) CH_3ONa, CH_3OH, rt; (c) $PhCH(OMe)_2$, CSA, DMF, rt; (d) O_3, CH_2Cl_2, −78 °C; (e) $NaBH_4$, EtOH, rt; (f) TsCl, DMAP, pyridine, rt; (g) NaH, DMF, rt; (h) H_2, Pd/C, CH_3OH, rt; (i) $Me_2CH(OMe)_2$, CSA, DMF, rt; (j) TBDMSCl, imidazole, DMF, 60 °C; (k) O_3, CH_2Cl_2, −78 °C; (l) MeI, $KHCO_3$, DMF, rt; (m) TBAF, THF, rt; (n) 60% aq. AcOH, 60 °C; (e) H_2, Pd/C, CH_3OH, rt.



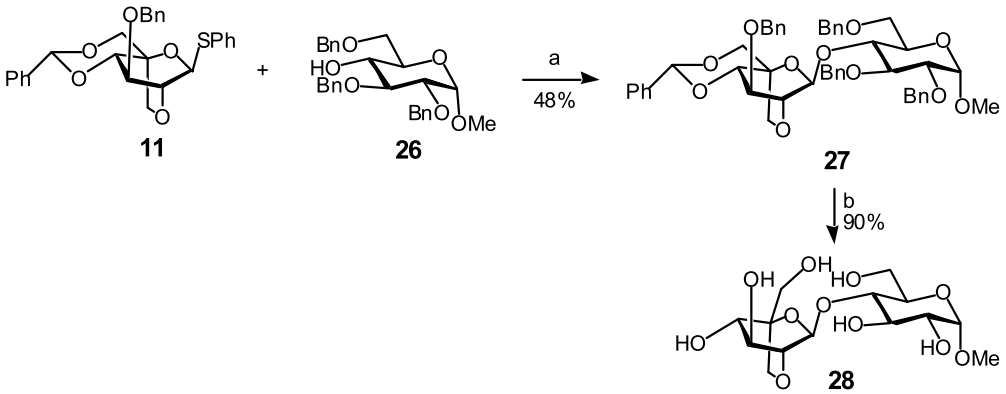
Compound **2** Compound **5**

Figure 4. Crystallographic structure of compounds **2** and **5**.

alcoholate with spontaneously lactonized, affording the fully protected δ-lactone **25**. Final deprotection using aq. AcOH followed by hydrogenolysis furnished compound **5** as a crystalline compound. Compound **5** is interesting regarding human α-L-iduronidase, a family 39 glycosidase supposed to distort its substrate in a ^{2,5}B conformation with a possible neighboring group participation of the carboxylate.[22] Lactone **5** could thus mimick the conformationally locked intermediate suggested by Withers.

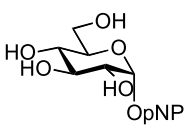
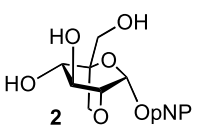
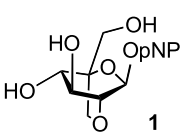
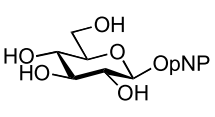
^{1}H NMR of compounds **1**–**5** indicated that they adopted in solution a conformation close to $B_{2,5}$.[23] This boat conformation was also observed in the solid state for compounds **2** and **5**, which were crystalline, and for which X-ray structures have been solved (Fig. 4).[11,24]

Finally, the conformationally restrained glycosyl donor **11** was used to obtain a cellobiose analogue containing a monosaccharide unit locked in $B_{2,5}$ conformation. Reaction of thiophenyl glucoside **11** with the known methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[25] **26** in the presence of NIS, triflic acid and 4 Å molecular sieves in dry dichloromethane afforded exclusively the β linked fully protected disaccharide **27**. This selectivity can be explained by the steric hindrance created by the oxymethylene bridge vis à vis the bulky glycosyl acceptor. Hydrogenolysis of the



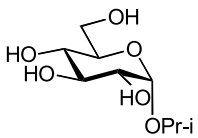
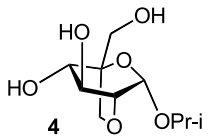
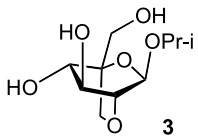
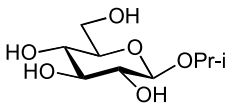
Scheme 5. Synthesis of disaccharide **28**. Reagents and conditions: (a) NIS, 4 Å molecular sieves, dry dichloromethane, −40 °C; (b) H_2, Pd/C, CH_3OH, rt.

Table 1. Hydrolysis of *p*-nitrophenyl compounds[a]

		 **2**	 **1**	
$10^4 k_{obs}/s^{-1}$	2.6±0.02	3.20±0.6	2.27±0.2	0.35±0.01[b]
k_{rel} (70 °C)	1.0	1.23	0.87	0.13[5]
$\Delta H^{\ddagger}$/kJ mol^{-1}	116±8	108±3	96.2±1.4	110±12
$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	24±23	2.4±10	−35±42	−8±34

[a] Notes. Data collected at three temperatures in the ranges 60–70 °C, in 1 M aqueous HCl. Rate constants quoted are for 70 °C (full data appear in Table 3 below). Rates and thermodynamic parameters for the parent glucosides are consistent with previous measurements.
[b] Data collected at three temperatures in the ranges 70–80 °C, in 1 M aqueous HCl.

Table 2. Hydrolysis of isopropyl compounds[a]

				
		4	**3**	
$10^4 k_{obs}/s^{-1}$	6.9±0.4	4.2±1.1	2.3±0.5	1.6±0.1
k_{rel} (80 °C)	1.0	0.61	0.33	0.23
No. signals followed[b]	3 (1)	4 (2)	5 (2)	2 (1)

[a] Notes. Reactions followed by ^{1}H NMR at 80 °C, in 1 M aqueous DCl/D_2O. For solubility reasons the solvent D_2O contained 10% of dioxan-d_8 for the reactions of the constrained compounds (**3** and **4**). Rate constants for the parent glucosides are consistent with previous measurements.
[b] Number of readily discernable peaks followed to stable end point (see the text).

benzyl groups yielded the cellobiose analogue **28** (Scheme 5).

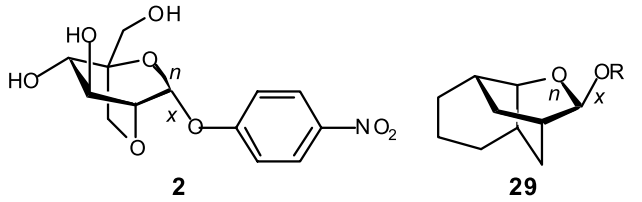
3.2. Reactivity of the constrained glycosides

We report rates of acid-catalyzed hydrolysis for the four conformationally constrained compounds **1**–**4** under standard conditions (1 M HCl, ionic strength 1.0 M). For comparison we have measured also the rates of hydrolysis of the corresponding unconstrained glucosides, *p*-nitrophenyl and isopropyl α- and β-glucopyranosides,[26] under the same conditions. Results appear in Tables 1 and 2.[27] The reactions of the *p*-nitrophenyl derivatives were studied over a range of temperatures and HCl concentrations, providing second order rate constants for acid catalysis and thermodynamic parameters for the hydrolysis reactions (see Section 5, Table 3). Table 1 compares first order rate constants in 1 M HCl (identical to the second order rate constants) at 70 °C for the *p*-nitrophenyl compounds. The hydrolyses of the isopropyl compounds were followed by ^{1}H NMR, in 10% dioxan-d_8—D_2O in 1 M DCl at 80 °C. Thus internal comparisons, for the *p*-nitrophenyl compounds (Table 1) and the isopropyl derivatives (Table 2) are for identical conditions, but comparisons between the tables are not. However, the differences are expected to be small: the solvent deuterium isotope effect, of the order of 2 for the acid catalyzed hydrolysis of alkyl glycosides, slows the reactions of the isopropyl compounds by this factor, partly compensating for the higher temperature (a factor of the order of 3 for 10 °C, according to the thermodynamic parameters of Table 1). The solvent effect of 10% dioxan is considered negligible.

Table 3. Second order rate constants for the acid-catalysed hydrolysis of *p*-nitrophenyl glycosides, in 0.8–1.0 M HCl and ionic strength 1.0

Compound	$k_{H+}\times10^5$/dm^3 mol^{-1} s^{-1} at temperature (in °C) given below				
	60	65	70	75	80
α-pNP-Glu	6.7±0.4	13.7±1.0	26.0±2.0		
2 α-constr	10.0±0.9	18.6±1.5	32.0±3.6		
1 β-constr	8.0±0.5	11.9±3.2	22.7±2.9		
β-pNP-Glu			3.5±0.2	5.6±0.4	10.9±0.7

The clear conclusion from the results summarized in Tables 1 and 2 is that compounds **1**–**4** are hydrolyzed at rates similar to those of the corresponding unconstrained α-glucosides. The severe conformational constraint imposed by the [2,2,2] bicyclic system, which excludes a significant antiperiplanar n_O-σ^*_{C-OR} interaction in the reactant, reduces reactivity scarcely at all for either *p*-nitrophenyl system **1** or **2**: and by factors of only 2 and 3 for the isopropyl derivatives **3** and **4**. In each case the differences are smaller than the well-known differences in reactivity between the anomers of the parent unconstrained glucosides. Apparently the synperiplanar lone pairs of these constrained compounds stabilize the developing oxocarbenium ion character in the transition state for acid catalyzed cleavage about as effectively as the antiperiplanar lone pairs of the unconstrained α-glucosides. In the case of the tetrahydropyranyl system **29** (R=Ar) (Fig. 5) it was concluded, on the basis of a crystal structure correlation (see below), that the synperiplanar n_O-σ^*_{C-OR} interaction is somewhat weaker in the reactant ground state, and that at least part of its observed reactivity must derive from the higher ground state energy of the boat conformation.



2 **29**

Figure 5. Structure of compounds **2** and **29**.

Though both α- and β-constrained compounds possess similar synperiplanar lone pair/leaving group n_O-σ^*_{C-OR} interactions in their ground states the rates of hydrolysis of the α-constrained compounds are higher than those of the β-constrained compounds, by 41 and 83%, respectively, for the *p*-nitrophenyl and isopropyl compounds. It is unlikely that these small effects have a simple, single explanation. Possible contributions from the different patterns of functionalities surrounding the glycosidic center are the enhanced β-effect of the C(2)–O of the C(6)–O bridge, which is antiperiplanar to the bond to the leaving group in the β-anomers, and internal hydrogen-bonding between the C(3)–OH and the incoming water nucleophile (similar to that suggested by Sinnott and Jencks to explain differences in reactivity to solvolysis of α- and β-glucopyranosyl fluorides).[28]

3.3. Ground state effects

Bond length (*x*)—leaving group (pK_a of ROH) correlations for the series of tetrahydropyranyl acetals **29** (R=Ar), constrained in the symmetrical boat conformation by the 3-carbon bridge, are consistent with a substantial ground state n_O-σ^*_{C-OR} interaction, though one which is weaker in

the synperiplanar than in the antiperiplanar geometry[17] (observed bond-length changes are considered a 'more or less uncomplicated measure of stereoelectronic effects' in this system). However, the rates of (spontaneous) hydrolysis are significantly more sensitive to the leaving group for the constrained system. The rate of acid catalyzed hydrolysis of the methyl acetal (**29**, R=Me) is similar to that of 2-methoxytetrahydropyran (corresponding conformationally to an α-glucoside), as observed for the isopropyl derivative **4** described in this paper.

Of the present series of compounds we have a crystal structure only for the constrained α-*p*-nitrophenyl derivative **2** (Fig. 4). The data are not of high accuracy, and conclusions based on a single structure must be very tentative. However, the pattern of bond lengths at the anomeric center is consistent with the operation of substantial (and comparable) n_O-σ^*_{C-OR} interactions in **2** and in the corresponding α-D-glucopyranoside, but little or none in the case of the β-D-glucopyranoside. Thus the length of the exocyclic C–O bond *x* at the acetal center is 1.413(8) Å in **2**, the same as observed for *p*-nitrophenyl α-D-glucopyranoside (1.415(3) Å[29] and significantly greater than the value of 1.404 Å (based on the good bond length-leaving group correlation established for a series of β-D-glucopyranosides)[29] expected for the corresponding bond in *p*-nitrophenyl β-D-glucopyranoside.

3.4. Glycosidase inhibition

Cellobiose analogue **28** was first assayed against a variety of commercially available glycosidases. Compound **28** did not show inhibition at a concentration of 0.2 mmol mL^{-1} on yeast α-glucosidase, almond β-glucosidase, Jack bean α-mannosidase, green coffee bean α-galactosidase, bovine liver α-galactosidase, bovine kidney β-*N*-acetylglucosaminidase, *Penicillium decumbens* naringinase, *Aspergillus niger* amyloglucosidase and human placenta α-L-fucosidase. We then investigated the inhibition of compound **28** on barley β-D-glucan glucohydrolase, a family GH3 glycoside hydrolase that catalyses hydrolytic removal of non-reducing glucosyl residues from β-D-glucans.[30] We expected compound **28** to fit in the two −1 and +1 subsite-binding sites of this glycosidase recently proved to perform substrate distortion.[31] Disaccharide **28** was found to be a weak competitive inhibitor (K_i 16.1 mM) of this enzyme, probably because this glycosidase does not perform a substrate distorsion toward a $B_{2,5}$ conformation but rather a $^4H_3$ conformation as suggested by the very tight binding of a glucophenylimidazole adopting a $^4H_3$ conformation.[31] Finally, compound **28** was tested against Cel6A, Cel7A and Cel7B cellobiohydrolases from *Trichoderma Reesei*,[32] which degrade crystalline cellulose very efficiently, releasing cellobiose from one or the other chain end. Vasella et al. showed that glycosidase inhibitors based on a lactone motif and adopting a half-chair conformation happened to be weak inhibitors of these enzymes, their shape being probably not complementary to the −1 subsite of these cellulases.[33] This is in keeping with the distorsion of the glucosyl unit in the −1 subsite towards a skew-boat conformation observed in the crystal structure of one of the members of the family-7 glycosidases, EGI of *Fusarium oxysporum*, in complex with a thioglucoside.[34]

Disaccharide **28**, displaying a distorted $B_{2,5}$ glucose unit, was only found to inhibit weakly Cel7B (K_i 3.4 mM) and did not inhibit nor was cleaved by Cel6A and Cel7A. The limited size of disaccharide **28** makes it unable to span in the important −2, −1, +1 and +2 subsites of the active site and is probably responsible for its lack of inhibition.[35] These results could also be rationalized by a possible steric clash between the nucleophile in the active site and the bridge present in the disaccharide.

4. Conclusion

We have synthesized five monosaccharides **1**–**5** locked in a $B_{2,5}$ conformation. Compounds **1**–**4** were hydrolyzed in acid at similar rates, close to those reported for the corresponding unlocked glucosides. This confirms that $B_{2,5}$ transient conformations of glycosides can indeed be acceptable candidates for direct acid hydrolysis, despite the fact that ALPE is not operating. Incorporation of this glucosyl unit into a disaccharide **28** yielded a cellobiose analogue which displayed only weak inhibition of cellobiohydrolases and barley β-D-glucan glucohydrolase. Nevertheless, these new glucosyl scaffolds adopting a boat conformation are interesting candidates to probe glycosidases that distort their substrate towards a $B_{2,5}$ conformation during or prior to hydrolysis. This is in keeping with the design of an increasing number of inhibitors adopting a boat conformation.[36] Furthermore, inhibitory results for several inhibitors have now been rationalized by invoking such a boat conformation.[37]

5. Experimental

5.1. Kinetics of hydrolysis

The hydrolyses of the *p*-nitrophenyl glucosides and the constrained compounds **1** and **2** were followed spectrophotometrically 348.6 nm, the wavelength of maximum change in absorbance for the hydrolysis of *p*-nitrophenyl glucosides to *p*-nitrophenol occurs. Stock solutions (containing approximately 6 mg mL^{-1}) were prepared in water (*p*-nitrophenyl glucosides) and 20% v/v 1,4-dioxane/water (for constrained compounds **1** and **2**, which are insoluble in pure water). 'Constant pH' solutions 1.0, 0.9 and 0.8 M in hydrochloric acid were made from Convol® stock solutions and their ionic strength adjusted to a value of 1.0 M (where necessary) by the addition of an appropriate volume of 2.0 M potassium chloride solution. For kinetic runs aliquots (20 or 60 μL) were injected into 2.4 mL volumes of the constant pH solutions, to give final 1,4-dioxane concentrations <0.5% v/v in the case of the constrained compounds. Reactions were followed over a range of temperatures for each glycoside and pseudo-first-order rate constants (k_{obs}, Table 3) obtained by non-linear least squares fitting of $A_{348.6}$ vs. time data to a standard first order equation. Derived second order rate constants are given in Table 3.

The isopropyl glucosides and the constrained compounds **3** and **4** do not contain strong chromophores, and their acid catalyzed hydrolysis reactions cannot be followed by UV

spectroscopy. The hydrolysis of alkyl glycosides is most often followed by polarimetry, observing a change in optical rotation of with time. Choice of a suitable wavelength, at which a large change in specific rotation occurs over the course of a reaction, allows relatively low (mg mL^{-1}) concentrations of glycosides to be used. Compounds **3** and **4** were available in only small quantities, but a variable wavelength polarimeter was not available. So the rates of acid catalyzed hydrolysis of the isopropyl glycosides and their hydrolyses were followed by ^1H NMR. This method also required relatively large amounts of material (ca. 30 mg per kinetic run), so a maximum of two runs could be carried out for each compound. Reactions were followed in 1 M DCl in D_2O (containing 10% 1,4-dioxane-d_8 for the constrained compounds), so the rates of reaction obtained are not exactly comparable with those of the *p*-nitrophenylglucosides (obtained in protic media): though the opposite effects of the solvent deuterium isotope effect and the 10 °C difference in temperature partly cancel out.

The ^1H NMR spectra obtained for these hydrolysis experiments were less than ideal for the purpose. Peaks that could be readily assigned to starting material or product were often not well resolved, and in such cases it was not possible to derive kinetic data from changes in integrated peak areas. However, the reactions of the isopropyl-glucosides could be followed by observing the variation in chemical shift for a number of protons in the spectra. The data obtained in this way are shown in Table 4.

Table 4. Rate constants for the hydrolysis of isopropyl glycosides, in 1 M HCl at 80 °C and ionic strength 1.0[a]

Compound	$k_{D+}\times10^5/M^{-1}\ s^{-1}$ at 80 °C	No. of peaks followed (no. of runs carried out)
α-*i*-Pr-Glu	69±4	3 (1)
4 α-constr	46±17	4 (2)
3 β-constr	23±5	5 (2)
β-*i*-Pr-Glu	16±1	2 (1)

[a] Notes. Rates followed by ^1H NMR (see the text).

5.2. General methods

Melting points were determined with a Büchi model 535 mp apparatus and are uncorrected. Optical rotations were measured at 20±2 °C with a Perkin Elmer Model 241 digital polarimeter, using a 10 cm, 1 mL cell. Chemical Ionisation Mass Spectra (CI-MS ammonia) and Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained with a JMS-700 spectrometer. Elemental analyses were performed by Service de Microanalyse de l'Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker AC 250 or a Bruker DRX 400. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with sulfuric acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).

5.2.1. 1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-5-*C*-vinyl-β-D-glucopyranoside 7. Vinyl derivative **6** (14.66 g, 0.032 mol) was dissolved in a 1:1 mixture of 1,4-dioxane/water (200 mL). Ion exchange resin IR-120 (20 g) was added and the solution was stirred for 18 h at 90 °C. The reaction mixture was cooled to rt and the resin was filtered, washed with water (100 mL). The solvent was evaporated and the residue was dried on a vacuum pump to afford the crude tetrol, which was directly used for the next step. Crude tetrol was dissolved in dry pyridine (100 mL) under argon and the solution cooled to 0 °C. Acetic anhydride (30 mL) and DMAP (50 mg) were added and the reaction mixture was stirred for 15 h at rt. The solvent was removed under reduced pressure and the residue co-evaporated with toluene (2×50 mL). Purification by column chromatography (EtOAc/cyclohexane 1:4→1:3) afforded compound **7** (11.5 g, 0.025 mol, 76%) as a crystalline solid.

$[\alpha]_D^{22}=-73$ ($c=1$ in $CHCl_3$); mp 111 °C (ethyl acetate/*n*-pentane); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.37–7.25 (m, 5H, Ph), 5.98–5.89 (m, 3H, H-1, H-7, H-8), 5.67 (dd, J=3.0, 9.2 Hz, 1H, H-8′), 5.46 (d, J=10.1 Hz, 1H, H-4), 5.26 (dd, J=8.4, 9.6 Hz, 1H, H-2), 4.63 (s, 2H, CH_2Ph), 4.18 (d, J=12.5 Hz, 1H, H-6), 3.76 (t, J=9.8 Hz, 1H, H-3), 3.72 (d, J=12.5 Hz, 1H, H-6′), 2.21 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.14 (s, 3H, OAc); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 170.61, 169.44, 168.97, 168.90, (4×C=O), 137.56 (C*ipso*), 129.66 (CH-7), 128.41, 127.82, 127.56 (Ph), 121.98 (CH_2-8), 88.67 (CH-1), 78.36 (C-5), 77.83 (CH-3), 74.54 (CH_2Ph), 72.44 (CH-2), 69.28 (CH-4), 64.99 (CH_2-6), 20.85, 20.78, 20.69, 20.62 (4×OAc); MS (CI, NH_3): *m*/*z* (%): 482 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{23}H_{28}O_{10}$ (464.47): C 59.47, H 6.07; found C 59.59, H 6.17.

5.2.2. Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-5-*C*-vinyl-1-thio-β-D-glucopyranoside 8. Tetraacetate **7** (11.5 g, 0.025 mol) was dissolved in dry CH_2Cl_2 (150 mL) and the solution cooled to 0 °C. Thiophenol (3.1 mL) was added dropwise followed by $BF_3{\cdot}OEt_2$ (9.35 mL) and the reaction mixture was stirred at rt. After 10 h, the reaction mixture was diluted with CH_2Cl_2 (150 mL), washed with saturated aq. $NaHCO_3$ (150 mL) and water (150 mL). Organic extracts were dried over $MgSO_4$ and concentrated. Purification by column chromatography (EtOAc/cyclohexane 1:4) afforded the thiophenyl derivative **8** (10.83 g, 0.021 mol, 84% yield) as an oil.

$[\alpha]_D^{20}=-64$ ($c=1$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.63–7.23 (m, 10H, 2×Ph), 5.91 (dd, J=11.2, 17.8 Hz, 1H, H-7), 5.49 (d, J=11.2 Hz, 1H, H-8), 5.36 (d, J= 10.2 Hz, 1H, H-1), 5.26 (dd, J=0.9, 17.8 Hz, 1H, H-8′), 5.07 (dd, J=9.3, 10.1 Hz, 1H, H-3), 4.85 (d, J=10.1 Hz, 1H, H-4), 4.62 (d, J=11.6 Hz, 1H, CH_2Ph), 4.56 (d, J=11.6 Hz, 1H, CH_2Ph), 4.09 (d, J=12.2 Hz, 1H, H-6), 3.84 (d, J= 12.2 Hz, 1H, H-6′), 3.72 (dd, J=9.4, 10.1 Hz, 1H, H-2), 2.12, 2.06, 1.99 (3×s, 9H, 3×OAc); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=170.70, 169.02, 168.91 (3×C=O), 137.70, 130.86 (2×C*ipso*), 134.82, 128.65, 128.40, 127.77, 127.58 (Ph), 130.62 (CH-7), 121.62 (CH_2-8), 80.88 (CH-4), 79.42 (C-5), 79.17 (CH-2), 74.54 (CH_2Ph), 72.13 (CH-3), 69.56 (CH-1), 65.45 (CH_2-6), 20.92, 20.84, 20.73 (3×OAc); MS (CI, NH_3): *m*/*z* (%): 532 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{27}H_{30}O_8S$ (514.60): C 63.02, H 5.88; found C 63.05, H 6.06.

5.2.3. Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-5-*C*-vinyl-1-thio-β-D-glucopyranoside 9. Compound **8** (10.83 g, 0.021 mol) was dissolved in CH_3OH (200 mL) and sodium (500 mg) was added. The solution was stirred for 1 h at rt, ion exchange resin IR-120 (20 g) was added and the reaction mixture stirred for 1 h. The resin was filtered and washed with CH_3OH (100 mL). The solvent was evaporated and the resulting oil was dried on a vacuum pump. The crude diol was dissolved in dry DMF (100 mL) under argon. Benzaldehyde dimethyl acetal (11.5 mL, 0.084 mol) and camphorsulphonic acid (100 mg) were added under argon and the soluton stirred at rt for 14 h. The reaction was quenched by addition of triethylamine (2 mL). The solvent was removed under vacuum and co-evaporated with toluene (2×50 mL). Purification by column chromatography (EtOAc/cyclohexane 1:6) afforded the alcohol **9** (8.28 g, 0.016 mol, 74% yield) as an oil.

$[\alpha]_D^{20}=-8$ ($c=1$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.19–7.48 (m, 15H, 3×Ph), 6.28 (dd, J=11.4, 17.9 Hz, 1H, H-7), 5.69 (s, 1H, CHPh), 5.51 (d, J=11.3 Hz, 1H, H-8), 5.39 (d, J=17.9 Hz, 1H, H-8′), 5.03 (d, J=9.8 Hz, 1H, H-1), 4.98 (d, J=11.5 Hz, 1H, CH_2Ph), 4.82 (d, J=11.5 Hz, 1H, CH_2Ph), 4.13 (d, J=9.7 Hz, 1H, H-6), 3.96 (d, J=9.7 Hz, 1H, H-6′), 3.81 (m, 2H, H-3, H-4), 3.60 (m, 1H, H-2), 2.77 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=138.03, 137.08, 130.93 (3×C*ipso*), 134.85 (CH-7), 128.98, 128.85, 128.43, 128.33, 128.17, 127.98, 127.75, 126.02 (Ph), 119.16 (CH_2-8), 102.30 (CHPh), 83.64 (CH-1), 83.00 (CH-4), 78.27 (CH-3), 77.25 (CH_2-6), 74.62 (CH_2Ph), 73.23 (CH-2), 72.51 (C-5); MS (CI, NH_3): m/z (%): 494 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{28}H_{28}O_5S$ (476.17): C 70.57, H 5.92; found C 70.56, H 5.97.

5.2.4. Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-5-*C*-hydroxymethyl-1-thio-β-D-glucopyranoside 10. Compound **9** (1.1 g, 2.12 mmol) was dissolved in CH_2Cl_2 (50 mL). The solution was cooled to −78 °C. Ozone was bubbled through the solution until appearance of a pale blue colour (2 min). The reaction was then quenched by addition of dimethylsulfide (0.2 mL). The solution was allowed to warm to rt for 1 h, the solvent was evaporated to afford the crude aldehyde which was used directly for the next step. The crude aldehyde was dissolved in EtOH (50 mL) at 0 °C, sodium borohydride (92 mg, 2.54 mmol) was added slowly to the solution and the reaction mixture was stirred at rt for 18 h. The reaction was quenched with methanol (20 mL) and the solvent was removed under reduced pressure and co-evaporated with methanol (2×20 mL). The residue was preadsorbed on silica. Purification by column chromatography (EtOAc/cyclohexane 1:3) afforded the diol **10** (858 mg, 1.79 mmol, 84% yield) as an oil.

$[\alpha]_D^{20}=-34$ ($c=0.7$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz): δ=7.53–7.07 (m, 15H, 3×Ph), 5.54 (s, 1H, CHPh), 4.85 (d, J=9.9 Hz, 1H, H-1), 4.77 (d, J=11.5 Hz, 1H, CH_2Ph), 4.63 (d, J=11.5 Hz, 1H, CH_2Ph), 4.33 (d, J=10.2 Hz, 1H, H-6), 4.04 (m, 1H, H-7), 3.94 (m, 1H, H-7′), 3.78–3.66 (m, 2H, H-3, H-4), 3.48 (d, J=10.2 Hz, 1H, H-6′), 3.43 (ddd, 1H, H-2), 2.73 (s, 1H, OH), 1.77 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=139.08, 138.03, 132.02 (3×C*ipso*), 130.65, 129.97, 128.95, 128.54, 128.28, 128.22, 128.12, 128.07, 127.90, 125.94 (3×Ph), 102.69 (CHPh), 83.65 (CH-1), 82.92 (CH-4), 77.72 (CH-3), 74.74 (CH_2Ph), 73.13 (CH-2), 72.90 (C-5), 70.62 (CH_2-6), 57.1 (CH_2-7); (CI, NH_3): m/z (%): 498 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{27}H_{28}O_6S$ (480.58): C 67.48, H 5.87; found C 67.13, H 6.08.

5.2.5. Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*,5-*C*-methylene-1-thio-β-D-glucopyranoside 11. Diol **10** (0.76 g, 1.58 mmol) was dissolved in anhydrous pyridine (10 mL) under argon. The solution was cooled to 0 °C. Tosyl chloride (0.6 g, 3.16 mmol) and DMAP (20 mg) were added under argon and the solution stirred at rt for 15 h. Tosyl chloride (300 mg) was further added to complete the reaction. After 7 h, the solvent was removed under reduced pressure and co-evaporated with toluene (2×10 mL). The crude tosylate was dried under vacuum for 1 h and was then dissolved in anhydrous DMF (15 mL) under argon. Sodium hydride (380 mg, 15.8 mmol) was added slowly and the suspension was stirred at rt for 18 h. The reaction was quenched with methanol (30 mL) and the solvent was removed under vacuum and co-evaporated with toluene (2×20 mL). The residue was dissolved in ethyl acetate (80 mL) and washed with water (80 mL). Organic extracts were dried over $MgSO_4$ and the solution concentrated. Purification by column chromatography (EtOAc/cyclohexane 1:4) afforded the bicycle **11** (0.65 g, 1.40 mmol, 88% yield) as an oil.

5.2.6. Spectroscopic data for phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-5-*C*-(2-tosyloxymethyl)-1-thio-β-D-glucopyranoside. $[\alpha]_D^{20}=-7$ ($c=1$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.81–7.30 (m, 19H, 4×Ph), 5.60 (s, 1H, CHPh), 4.89 (d, J=10.0 Hz, 1H, H-1), 4.84 (d, J=11.4 Hz, 1H, CH_2Ph), 4.74 (d, J=11.4 Hz, 1H, CH_2Ph), 4.62 (d, J=11.2 Hz, 1H, H-7), 4.54 (d, J=11.2 Hz, 1H, H-7′), 4.34 (d, J=10.8 Hz, 1H, H-6), 3.80 (d, J=10.5 Hz, 1H, H-4), 3.72 (m, 1H, H-3), 3.65 (d, J=10.8 Hz, 1H, H-6′), 3.55 (dd, 1H, H-2), 2.48 (s, 3H, PhCH_3 tosyl), 2.09 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=[145.13, 137.87, 136.55, 131.97, 131.53 (5×C*ipso*)], [132.15, 130.00, 129.97, 129.19, 129.12, 128.98, 128.62, 128.43, 128.18, 128.13, 128.07, 128.00, 127.96, 127.94, 127.89, 125.95 (4×Ph)], 102.69 (CHPh), 83.90 (CH-1), 82.46 (CH-4), 77.64 (CH-3), 74.86 (CH_2Ph), 73.17 (CH-2), 71.47 (C-5), 70.48 (CH_2-6), 63.35 (CH_2-7); (CI, NH_3): m/z (%): 652 (100) [M+NH_4^+].

5.2.7. Data for bicycle 11. $[\alpha]_D^{20}=-218$ ($c=1$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.58–7.30 (m, 15H, 3×Ph), 5.81 (dd, J=2.3, 1.5 Hz, 1H, H-1), 5.64 (s, 1H, CHPh), 4.32 (t, J=2.7 Hz, 1H, H-2), 4.08 (ddd, J=1.5, 2.7, 4.3 Hz, 1H, H-3), 4.04 (d, J=11.3 Hz, 1H, H-6), 5.98 (d, J=11.3 Hz, 1H, H-6′), 3.91 (dd, J=1.9, 9.4 Hz, 1H, H-7), 4.85 (d, J=11.8 Hz, 1H, CH_2Ph), 4.78 (d, J=11.8 Hz, 1H, CH_2Ph), 4.51 (dd, J=1.7, 4.1 Hz, 1H, H-4), 4.51 (d, J=9.4 Hz, 1H, H-7′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=137.44, 136.95, 136.59 (3×C*ipso*), 130.61, 129.25, 129.03, 128.97, 128.36, 127.81, 127.76, 127.16, 126.15, 126.12 (3×Ph), 101.35 (CHPh), 86.54 (CH-1), 81.23 (CH-4), 78.31 (CH-3), 71.54 (CH_2Ph), 69.97 (CH_2-6), 67.99 (CH-2), 66.45 (CH_2-7), 66.01 (C-5); (CI, NH_3): m/z (%): 480 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{27}H_{26}O_5S$ (462.57): C 70.10, H 5.67; found C 70.07, H 5.79.

5.2.8. *para*-Nitrophenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*,5-*C*-methylene-β-D-glucopyranoside 12, *para*-nitrophenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*,5-*C*-methylene-α-D-glucopyranoside 13. Bicycle **11** (652 mg, 1.41 mmol), *para*-nitrophenol (235 mg, 1.69 mmol), 4 Å molecular sieves (1.3 g) were suspended in dry CH_2Cl_2 (20 mL) under argon and the suspension was stirred for 30 min and then cooled to −40 °C. *N*-Iodosuccinimide (381 mg, 1.69 mmol) and triflic acid (19 μL, 0.211 mmol) were added and the solution was stirred at −40 °C to afford a red coloured solution. After 1 h, the reaction mixture was quenched with aq. sat. $NaHCO_3$ (30 mL) and diluted with Et_2O (50 mL). The organic layer was separated, washed with sat. $Na_2S_2O_3$ (50 mL). The aqueous layer was extracted with Et_2O (80 mL). Organic extracts were combined, dried over $MgSO_4$ and the solution concentrated. Purification by column chromatography (EtOAc/cyclohexane 1:10) afforded the α-*para*-nitrophenyl derivative **13** (500 mg, 1.18 mmol, 72% yield) as an oil.

$[\alpha]_D^{22}=+44$ ($c=0.34$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): $\delta=8.25$ (d, $J=9.2$ Hz, 2H, $PhNO_2$), 7.40–7.55 (m, 10H, 2×Ph), 7.18 (d, $J=9.2$ Hz, 2H, $PhNO_2$), 5.95 (d, $J=1.1$ Hz, 1H, H-1), 5.65 (s, 1H, CHPh), 4.81 (d, $J=11.6$ Hz, 1H, CH_2Ph), 4.69 (d, $J=11.6$ Hz, 1H, CH_2Ph), 4.57 (d, $J=9.7$ Hz, 1H, H-7), 4.22 (dd, $J=1.1$, 3.6 Hz, 1H, H-2), 4.12 (m, 3H, H-3, H-4, H-7′), 4.03 (d, $J=11.2$ Hz, 1H, H-6), 3.91 (d, $J=11.2$ Hz, 1H, H-6′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=161.59$, 142.43, 136.97, 136.61, (4×C*ipso*), 129.38, 128.52, 128.35, 128.14, 127.86, 126.12, 125.74, 116.29 (3×Ph), 101.90 (CHPh), 95.97 (CH-1), 81.92, 77.00 (CH-3, CH-4), 71.86 (CH_2Ph), 69.48 (CH_2-6), 68.19 (CH-2), 67.35 (C-5), 66.16 (CH_2-7); MS (CI, NH_3): *m*/*z* (%): 509 (100) [$M+NH_4^+$]; elemental analysis: calcd (%) for $C_{27}H_{25}O_8N$ (491.50): C 65.98, H 5.13, N 2.85; found C 65.81, H 5.32, N 2.75.

Further elution afforded the β-*para*-nitrophenyl derivative **12** (130 mg, 0.26 mmol, 19% yield) as an oil.

$[\alpha]_D^{22}=-265$ ($c=0.21$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): $\delta=8.26$ (d, $J=9.3$ Hz, 2H, $PhNO_2$), 7.38–7.51 (m, 10H, 2×Ph), 7.13 (d, $J=9.3$ Hz, 2H, $PhNO_2$), 5.96 (dd, $J=1.3$, 2.9 Hz, 1H, H-1), 5.59 (s, 1H, CHPh), 4.82 (d, $J=11.7$ Hz, 1H, CH_2Ph), 4.76 (d, $J=11.7$ Hz, 1H, CH_2Ph), 4.50 (d, $J=9.4$ Hz, 1H, H-7), 4.41 (dd, $J=1.8$, 5.0 Hz, H-4), 4.23 (t, $J=2.7$ Hz, 1H, H-2), 4.13 (ddd, $J=1.3$, 2.7 Hz, $J=5.0$ Hz, 1H, H-3), 4.10 (d, $J=11.5$ Hz, 1H, H-6), 3.93 (d, $J=11.5$ Hz, 1H, H-6′), 3.81 (dd, $J=1.9$, 9.4 Hz, H-7′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=161.15$, 142.51, 137.78, 136.73 (4×C*ipso*), 129.32, 128.41, 128.34, 127.83, 127.59, 126.07, 125.81, 116.23 (3×Ph), 101.26 (CHPh), 96.82 (CH-1), 81.12 (CH-4), 78.20 (CH-3), 71.74 (CH_2Ph), 69.66 (CH_2-6), 66.09 (C-5), 75.72 (CH_2-7), 75.56 (CH-2); MS (CI, NH_3): *m*/*z* (%): 509 (100) [$M+NH_4^+$]; elemental analysis: calcd (%) for $C_{27}H_{25}O_8N$ (491.50): C 65.98, H 5.13, N 2.85; found C 65.80, H 5.25, N 2.72.

5.2.9. *para*-Nitrophenyl 4-*O*-benzoyl-2-*O*,5-*C*-methylene-β-D-glucopyranoside 14 and *para*-nitrophenyl 6-*O*-benzoyl-2-*O*,5-*C*-methylene-β-D-glucopyranoside 15. The β-*para*-nitrophenyl derivative **12** (78 mg, 0.158 mmol) was dissolved in EtOAc (2 mL). A solution of $NaBrO_3$ (144 mg, 0.953 mmol) in water (1.5 mL) was then added at rt followed by dropwise addition over 10 min under vigorous stirring of an aqueous solution (3 mL) of $Na_2S_2O_4$ (150 mg). After 24 h, the reaction mixture was diluted with EtOAc (20 mL). The organic phase was washed with a saturated aqueous solution of $Na_2S_2O_3$ (10 mL). Organic extracts were dried over $MgSO_4$ and concentrated. The residue was preadsorbed on silica gel. Purification by column chromatography (EtOAc/cyclohexane 1:2→1:1→EtOAc) afforded the diol **14** (28 mg, 0.067 mmol, 42% yield) as an oil.

$[\alpha]_D^{22}=-120$ ($c=0.4$ in CH_3OH); ^1H NMR (CD_3OD, 400 MHz): $\delta=8.42$ (d, $J=9.3$ Hz, 2H, $PhNO_2$), 8.26 (m, 2H, Ph), 7.83 (m, 1H, Ph), 7.68 (m, 2H, Ph), 7.53 (d, $J=9.3$ Hz, 2H, $PhNO_2$), 6.23 (dd, $J=1.1$, 2.7 Hz, 1H, H-1), 5.63 (dd, $J=1.8$, 4.8 Hz, 1H, H-4), 4.48 (m, 1H, H-3), 4.28 (m, 2H, H-2, H-7), 4.11 (dd, $J=1.8$, 9.7 Hz, 1H, H-7′), 3.85 (d, $J=12.3$ Hz, 1H, H-6), 3.74 (d, $J=12.3$ Hz, 1H, H-6′); ^{13}C NMR (CD_3OD, 100 MHz): $\delta=167.30$, 144.20, 131.10, (3×C*ipso*), 163.32 (C=O), 134.98, 131.06, 130.04 (Ph), 126.89, 118.22 (2×$PhNO_2$), 98.80 (CH-1), 77.40 (CH-4), 77.14 (C-5), 74.92 (CH-3), 69.96 (CH-2), 64.65 (CH_2-7), 62.31 (CH_2-6); MS (CI, NH_3): *m*/*z* (%): 435 (100) [$M+NH_4^+$]; HRMS (positive-ion CI, NH_3): calcd for $C_{20}H_{23}O_9N_2$ ($M+NH_4^+$) 435.1404, found 435.1394.

Further elution afforded the diol **15** (17 mg, 0.040 mmol, 26% yield) as an oil.

$[\alpha]_D^{22}=-122$ ($c=0.6$ in CH_3OH); ^1H NMR (CD_3OD, 400 MHz): $\delta=8.29$ (d, $J=9.3$ Hz, 2H, $PhNO_2$), 8.10 (m, 2H, Ph), 7.72 (m, 1H, Ph), 7.72 (m, 2H, Ph), 7.56 (m, 2H, Ph), 7.38 (d, $J=9.3$ Hz, 2H, $PhNO_2$), 6.16 (d, $J=1.3$, 2.8 Hz, 1H, H-1), 4.72 (d, $J=11.9$ Hz, 1H, H-6), 4.57 (d, $J=11.9$ Hz, 1H, H-6′), 4.32 (dd, $J=1.8$, 5.3 Hz, 1H, H-4), 4.23 (m, 1H, H-3), 4.21 (d, $J=9.3$ Hz, 1H, H-7), 4.18 (t, $J=2.8$ Hz, 1H, H-2), 3.93 (dd, $J=1.8$, 9.3 Hz, 1H, H-7′); ^{13}C NMR (CD_3OD, 100 MHz): $\delta=167.81$, 144.01, 131.35, (3×C*ipso*), 162.89 (C=O), 134.60, 130.84, 129.77 (Ph), 126.77, 118.23 (2×$PhNO_2$), 98.41 (CH-1), 77.10 (C-5), 76.88 (CH-3), 75.13 (CH-4), 69.98 (CH-2), 63.82 (CH_2-6), 63.72 (CH_2-7); MS (CI, NH_3): *m*/*z* (%): 435 (100) [$M+NH_4^+$]; HRMS (positive-ion CI, NH_3): calcd for $C_{20}H_{23}O_9N_2$ ($M+NH_4^+$) 435.1404, found 435.1397.

5.2.10. *para*-Nitrophenyl 2-*O*,5-*C*-methylene-β-D-glucopyranoside 1. *Compound* **1** *from diol* **14**. Diol **14** (20 mg, 0.048 mmol) was dissolved in CH_3OH (10 mL) and CH_3ONa (200 μL of a 1 M methanolic solution) was added. After 30 min, the reaction was complete and was quenched by stirring with resin IR-120 (1 g) for 1 h. The resin was filtered and washed with CH_3OH (20 mL). The solvent was removed under reduced pressure and purification by column chromatography (EtOAc) afforded the triol **1** (11 mg, 0.035 mmol, 73% yield) as a foam.

Compound **1** *from diol* **15**. The same procedure as the one used above afforded triol **1** (12 mg, 0.038 mmol, 70% yield).

$[\alpha]_D^{22}=-128$ ($c=0.55$ in CH_3OH); ^1H NMR (400 MHz, D_2O): $\delta=8.21$ (d, $J=9.0$ Hz, 2H, $PhNO_2$), 7.21 (d,

J=9.0 Hz, 2H, $PhNO_2$), 6.01 (dd, *J*=1.0, 2.6 Hz, 1H, H-1), 4.12 (t, *J*=2.7 Hz, 1H, H-2), 4.06 (m, 1H, H-3), 4.02 (dd, *J*=1.6, 5.1 Hz, 1H, H-4), 3.91 (d, *J*=9.8 Hz, 1H, H-7), 3.76 (dd, *J*=1.6, 9.8 Hz, 1H, H-7′), 3.68 (d, *J*=12.8 Hz, 1H, H-6), 3.64 (d, *J*=12.8 Hz, 1H, H-6′); ^{13}C NMR (100 MHz, D_2O): δ=161.51, 142.67 (2×C*ipso*), 126.37 (Ph), 116.97 (Ph), 97.09 (CH-1), 76.76 (C-5), 74.62, 73.59, 67.99 (CH-2, CH-3, CH-4), 62.46, 60.48 (CH_2-6, CH_2-7); MS (CI, NH_3): *m*/*z* (%): 331 (100) [M+NH_4^+]; HRMS (positive-ion CI, NH_3): calcd for $C_{13}H_{19}O_8N_2$ (M+NH_4^+) 331.1141, found 331.1140.

5.2.11. *para*-Nitrophenyl 4-*O*-benzoyl-2-*O*,5-*C*-methylene-α-D-glucopyranoside 16 and *para*-nitrophenyl 6-*O*-benzoyl-2-*O*,5-*C*-methylene-α-D-glucopyranoside 17. The same procedure as the one used to obtain compounds **14** and **15** was applied to the α-*para*-nitrophenyl derivative **13** (304 mg, 0.619 mmol) to afford the diol **16** (137 mg, 0.328 mmol, 53% yield) as an oil.

$[\alpha]_D^{22}$=+100 (*c*=0.25 in CH_3OH); ^1H NMR (CD_3OD, 400 MHz): δ=8.44 (d, *J*=9.3 Hz, 2H, $PhNO_2$), 8.25 (m, 2H, Ph), 7.83 (m, 1H, Ph), 7.72 (m, 2H, Ph), 7.48 (d, *J*=9.3 Hz, 2H, $PhNO_2$), 6.20 (d, *J*=1.4 Hz, 1H, H-1), 5.31 (m, 2H, H-1, H-4), 4.34–4.30 (m, 4H, H-2, H-3, H-7, H-7′), 3.81 (d, *J*=12.5 Hz, 1H, H-6), 3.73 (d, *J*=12.5 Hz, 1H, H-6′); ^{13}C NMR (CD_3OD, 100 MHz): δ=167.32, 144.08, 131.20 (3×C*ipso*), 163.79 (C=O), 135.00, 131.01, 130.08 (Ph), 127.02, 118.10 (2×$PhNO_2$), 97.16 (CH-1), 78.21 (C-5), 76.17 (CH-4), 73.21, 71.45 (CH-2, CH-3), 65.84 (CH_2-7), 62.80 (CH_2-6); MS (CI, NH_3): *m*/*z* (%): 435 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{20}H_{19}O_9N$ (417.37): C 57.55, H 4.59, N 3.35; found C 57.64, H 4.77, N 3.22.

Further elution afforded the diol **17** (102 mg, 0.244 mmol, 39% yield) as an oil.

$[\alpha]_D^{22}$=+111 (*c*=0.3 in CH_3OH); ^1H NMR (CD_3OD, 400 MHz): δ=8.27 (d, *J*=9.3 Hz, 2H, $PhNO_2$), 7.97 (m, 2H, Ph), 7.68 (m, 1H, Ph), 7.50 (m, 2H, Ph), 7.31 (d, *J*=9.3 Hz, 2H, $PhNO_2$), 6.12 (d, *J*=1.4 Hz, 1H, H-1), 4.74 (d, *J*=12.1 Hz, 1H, H-6), 4.50 (d, *J*=12.1 Hz, 1H, H-6′), 4.29 (m, 2H, H-4, H-7), 4.24 (m, 2H, H-2, H-7′), 4.02 (m, 1H, H-3); ^{13}C NMR (CD_3OD, 100 MHz): δ=167.65, 143.79, 131.20, (3×C*ipso*), 163.28 (C=O), 134.54, 130.73, 129.70 (Ph), 126.87, 117.97 (2×$PhNO_2$), 96.43 (CH-1), 78.16 (C-5), 75.40, 75.31, 71.96 (CH-2, CH-3, CH-4), 65.35 (CH_2-6), 64.79 (CH_2-7); MS (CI, NH_3): *m*/*z* (%): 435 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{20}H_{19}O_9N$ (417.37): C 57.55, H 4.59, N 3.35; found C 57.70, H 4.89, N 3.13.

5.2.12. *para*-Nitrophenyl 2-*O*,5-*C*-methylene-α-D-glucopyranoside 2. The same procedure as the one used to obtain compound **1** was applied to diol **17** (102 mg, 0.244 mmol) to afford the triol **2** (70 mg, 0.223 mmol, 92% yield), which was recrystallized from EtOAc.

$[\alpha]_D^{22}$=+106 (*c*=0.94 in CH_3OH); mp 229–230 °C (EtOAc); ^1H NMR (400 MHz, CD_3OD): δ=8.38 (d, *J*=9.2 Hz, 2H, $PhNO_2$), 7.44 (d, *J*=9.2 Hz, 2H, $PhNO_2$), 6.07 (d, *J*=1.4 Hz, 1H, H-1), 4.18 (dd, *J*=1.6, 4.5 Hz, 1H, H-3), 4.16 (dd, *J*=1.4, 4.5 Hz, 1H, H-2), 4.16 (d, *J*=9.4 Hz, 1H, H-7), 4.12 (dd, *J*=1.0, 9.4 Hz, 1H, H-7′), 3.97 (m, 1H, H-4), 3.79 (d, *J*=12.3 Hz, 1H, H-6), 3.75 (d, *J*=12.3 Hz, 1H, H-6′); ^{13}C NMR (100 MHz, CD_3OD): δ=[164.01, 143.91, (2×C*ipso*)], 126.97 (Ph), 118.02 (Ph), 97.16 (CH-1), 79.34 (C-5), 75.36, 74.38, 71.92 (CH-2, CH-3, CH-4), 65.07, 63.18 (CH_2-6, CH_2-7); MS (CI, NH_3): *m*/*z* (%): 331 (52) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{13}H_{15}O_8N$ (313.26): C 49.84, H 4.83, N 4.47; found C 49.87, H 4.87, N 4.30.

5.2.13. Isopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*,5-*C*-methylene-α-D-glucopyranoside 18. Thiophenyl derivative **11** (500 mg, 1.08 mmol) was dissolved in dry isopropanol (50 mL) and NBS (98 mg, 5.5 mmol) was added under argon. The slurry solution was stirred for 48 h at rt, filtered and concentrated. The crude product was purified by column chromatography (EtOAc/cyclohexane 1:10) to afford compound **18** (290 mg, 0.070 mmol, 64% yield) as a colourless oil.

$[\alpha]_D^{20}$=−38 (*c*=0.6 in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.53–7.30 (m, 10H, 2×Ph), 5.58 (s, 1H, CHPh), 5.30 (s, 1H, H-1), 4.77 (d, *J*=11.7 Hz, 1H, CHPh), 4.64 (d, *J*=11.7 Hz, 1H, CHPh), 4.45 (d, *J*=9.3 Hz, 1H, H-7), 4.05 (dd, *J*=0.7, 9.3 Hz, 1H, H-7′), 4.01 (t, *J*=6.2 Hz, 1H, H-8), 3.99 (m, 2H, H-3, H-4), 3.98 (d, *J*=11.1 Hz, 1H, H-6), 3.91 (m, 1H, H-2), 3.83 (d, *J*=11.1 Hz, 1H, H-6′), 1.33 (d, *J*=6.2 Hz, 3H, CH_3-*i*Pr),1.22 (d, *J*=6.2 Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ=138.3, 137.7 (2×C*ipso*), 129.2–126.2 (2×Ph), 101.8 (CHPh), 95.8 (CH-1), 82.3 (CH-3 or CH-4), 77.6 (CH-3 or CH-4), 71.6 (CH_2Ph), 70.5 (CH-8), 70.0 (CH_2-6), 69.0 (CH-2), 66.2 (CH_2-7), 64.9 (C-5), 23.8, 21.9 (2×CH_3-*i*Pr); (CI, NH_3): *m*/*z* (%): 413 (100) [M+H^+]; HRMS (positive-ion CI, NH_3): calcd for $C_{24}H_{29}O_6$ (M+H^+) 413.1964, found 413.1963.

5.2.14. Isopropyl 2-*O*,5-*C*-methylene-α-D-glucopyranoside 3. A round-bottom flask, fitted with an ammonia condenser, was charged with **18** (200 mg, 0.49 mmol), dry THF (50 mL) and ammonia (~10 mL) and was cooled to −78 °C. A small amount of lithium was added and the reaction was stirred for 2 min and quenched with NH_4Cl. The reaction mixture was allowed to warm to rt and concentrated. The crude product was purified by column chromatography (EtOAc/cyclohexane 3:1) to afford compound **3** (60 mg, 0.26 mmol, 52% yield) as a colourless oil.

$[\alpha]_D^{22}$=−58 (*c*=1.65 in CH_3OH); ^1H NMR (CD_3OD, 400 MHz): δ=5.47 (dd, *J*=1.5, 2.7 Hz, 1H, H-1), 4.24 (m, *J*=6.2 Hz, 1H, H-8), 4.05 (dd, *J*=1.8, 4.3 Hz, 1H, H-4), 3.99 (d, *J*=9.2 Hz, 1H, H-7), 3.92 (m, 1H, H-3), 3.87 (t, *J*=2.7 Hz, 1H, H-2), 3.78 (s, 2H, H-6, H-6′), 3.77 (dd, *J*=1.8, 9.2 Hz, 1H, H-7′), 1.43 (d, *J*=6.2 Hz, 3H, CH_3-*i*Pr), 1.34 (d, *J*=6.2 Hz, 3H, CH_3-*i*Pr); ^{13}C NMR (D_2O, 100 MHz): δ=99.20 (CH-1), 77.71 (CH-3), 76.79 (C-5), 76.77 (CH-4), 72.15 (CH-8), 69.64 (CH-2), 63.62 (CH_2-7), 62.96 (CH_2-6), 24.33 (CH_3-*i*Pr), 22.24 (CH_3-*i*Pr); MS (CI, NH_3): *m*/*z* (%): 252 (100) [M+NH_4^+]; HRMS (positive-ion CI, NH_3): calcd for $C_{10}H_{22}O_6N$ (M+NH_4^+) 252.1447, found 252.1450.

5.2.15. Isopropyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-5-*C*-vinyl-β-D-glucopyranoside 19. Tetraacetate **7** (20 g,

43.1 mmol) was dissolved in dry CH_2Cl_2 (230 mL) and extra dry isopropanol (4.83 mL) and powdered 4 Å molecular sieves (32 g) were added. The solution was stirred for 2 h, cooled to −78 °C and TMSOTf (11.7 mL, 64.6 mmol) was added slowly. The reaction mixture was allowed to warm up to room temperature under stirring and was stirred for another 5 h. The reaction mixture was then quenched with Et_3N, filtered through celite and washed with water. The organic layer was dried over $MgSO_4$ and concentrated. Purification by column chromatography (EtOAc/cyclohexane 1:3) afforded the isopropyl derivative **19** (15.3 g, 33 mmol, 76% yield) as a colourless syrup.

$[\alpha]_D^{20}=-79$ ($c=0.86$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=7.26-7.35$ (m, 5H, Ph), 6.02 (dd, $J=11.1$, 17.8 Hz, 1H, H-7), 5.62 (dd, $J=1.2$, 17.8 Hz, 1H, H-8), 5.59 (dd, $J=0.9$, 11.1 Hz, 1H, H-8′), 5.44 (d, $J=10.1$ Hz, 1H, H-4), 5.08 (dd, $J=8.0$, 9.5 Hz, 1H, H-2), 4.75 (d, $J=8.0$ Hz, 1H, H-1), 5.07 (dd, $J=9.3$, 10.1 Hz, 1H, H-3), 4.63 (d, $J=11.7$ Hz, 1H, CHPh), 4.59 (d, $J=11.7$ Hz, 1H, CHPh), 4.08 (d, $J=12.2$ Hz, 1H, H-6), 3.88 (m, $J=6.2$ Hz, 1H, H-9), 3.83 (d, $J=12.2$ Hz, 1H, H-6′), 2.12, 2.01 (2×s, 9H, 3×OAc), 1.43 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr), 1.34 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=170.88$, 169.03, 168.99 (3×C=O), 137.87 (C*ipso*), 132.19 (CH-7), 128.36, 127.71, 127.62 (Ph), 120.49 (CH_2-8), 95.49 (CH-1), 77.84 (CH-3), 76.63 (C-5), 73.89 (CH_2Ph), 73.70 (CH-2), 72.25 (CH-9), 69.77 (CH-4), 65.68 (CH_2-6), 23.34 (CH_3-*i*Pr), 22.06 (CH_3-*i*Pr), 20.88, 20.78 (3×OAc); MS (CI, NH_3): *m*/*z* (%): 582 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{24}H_{32}O_9$ (564.20): C 62.06, H 6.94; found C 62.08, H 6.93.

5.2.16. Isopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-5-*C*-vinyl-β-D-glucopyranoside 20. Compound **19** (2.3 g, 4.96 mmol) was dissolved in dry CH_3OH (70 mL) under argon and sodium (50 mg) was added. The solution was stirred for 16 h, ion exchange resin IR-120 (5 g) was added and the reaction mixture stirred for 1 h. The resin was filtered and washed with CH_3OH (30 mL). The solvent was evaporated and the resulting oil was dissolved in ethyl acetate (50 mL) and washed with water (30 mL). The organic layer was dried over $MgSO_4$ and evaporated. To a solution of crude triol in dry CH_2Cl_2 (75 mL) was added benzaldehyde dimethyl acetal (1.1 mL, 7.2 mmol) and camphorsulphonic acid (50 mg) under argon and the solution was stirred at rt for 12 h. The reaction mixture was quenched with $NaHCO_3$. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated. Purification by column chromatography (EtOAc/cyclohexane 1:6) afforded alcohol **20** (1.7 g, 3.99 mmol, 81%) as a white needles.

$[\alpha]_D^{20}=-35$ ($c=0.62$ in $CHCl_3$); mp 99 °C (*n*-pentane/EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=7.93-7.56$ (m, 10H, 2×Ph), 6.37 (dd, $J=11.2$, 18.0 Hz, 1H, H-7), 5.70 (s, 1H, CHPh), 5.65 (dd, $J=1.2$, 18.0 Hz, 1H, H-8), 5.57 (dd, $J=1.2$, 11.2 Hz, 1H, H-8′), 4.97 (d, $J=11.7$ Hz, 1H, CH_2Ph), 4.84 (d, $J=7.7$ Hz, 1H, H-1), 4.83 (d, $J=11.7$ Hz, 1H, CH_2Ph), 4.07 (d, $J=9.7$ Hz, 1H, H-6), 4.03 (m, $J=6.2$ Hz 1H, H-9), 3.95 (d, $J=9.7$ Hz, 1H, H-6′), 3.88 (d, $J=10.1$ Hz, 1H, H-4), 3.75 (dd, $J=8.8$, 10.1 Hz, 1H, H-3), 3.61 (dt, $J=2.4$, 7.9 Hz, 1H, H-2), 2.48 (d, $J=2.1$ Hz, 1H, OH),1.34 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr),1.25 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=138.33$, 137.24 (2×C*ipso*), 136.23 (CH-7), 128.98, 128.33, 128.20, 127.91, 127.67, 126.10 (Ph), 118.25 (CH_2-8), 102.38 (CHPh), 97.47 (CH-1), 83.24 (CH-4), 77.46 (CH_2-6), 77.28 (CH-3), 75.36 (CH-2), 74.41 (CH_2Ph), 71.97 (CH-9), 69.75 (C-5), 23.40, 22.00 (2×CH_3-*i*Pr); MS (CI, NH_3): *m*/*z* (%): 444 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{25}H_{30}O_6$ (426.5): C 70.40, H 7.09; found C 70.50, H 7.22.

5.2.17. Isopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-5-*C*-hydroxymethyl-β-D-glucopyranoside 21. Ozone was passed through a stirred solution of olefin **20** (1.0 g, 2.3 mmol) in anhydrous CH_2Cl_2 (50 mL) cooled to −78 °C until the appearance of a pale blue colour. The reaction mixture was quenched with $(CH_3)_2S$ (0.2 mL) and allowed to warm to room temperature. The solvent was evaporated. The residue was dissolved in methanol (20 mL) and the solution was cooled to 0 °C, $NaBH_4$ (250 mg, 6.9 mmol) was added and the reaction mixture was warmed to room temperature and stirred for 1 h, cooled to 0 °C and quenched with NH_4Cl. The reaction mixture was evaporated, the residue dissolved in ethyl acetate, washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (EtOAc/cyclohexane 1:3) to afford the diol **21** (0.7 g, 70%) as a solid.

$[\alpha]_D^{20}=-46$ ($c=0.86$ in $CHCl_3$); mp 141 °C (*n*-pentane/ethyl acetate); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=7.52-7.30$ (m, 10H, 2×Ph), 5.68 (s, 1H, CHPh), 4.92 (d, $J=11.7$ Hz, 1H, CH_2Ph), 4.88 (d, $J=7.7$ Hz, 1H, H-1), 4.81 (d, $J=11.7$ Hz, 1H, CH_2Ph), 4.36 (d, $J=10.4$ Hz, 1H, H-6), 4.22 (dd, $J=7.1$, 12.0 Hz, 1H, H-7), 4.09 (dd, $J=2.7$, 12.0 Hz, 1H, H-7′), 4.03 (m, $J=6.2$ Hz, 1H, H-8), 3.97 (d, $J=10.2$ Hz, 1H, H-4), 3.88 (dd, $J=8.2$, 10.2 Hz, 1H, H-3), 3.66 (dd, $J=1.2$, 10.4 Hz, 1H, H-6′), 3.62 (dd, $J=2.7$, 7.9 Hz, 1H, H-2), 2.62 (d, $J=$ 7.9 Hz, 1H, OH-2), 1.82 (dd, $J=3.8$, 7.1 Hz, 1H, OH-7), 1.32 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr),1.27 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=138.31$, 137.13 (2×C*ipso*), 129.07–126.00 (2×Ph), 102.59 (CHPh), 97.79 (CH-1), 83.08 (CH-4), 77.02 (CH-3), 75.40 (CH-2), 74.35 (CH_2Ph), 72.43 (CH-8), 70.90 (CH_2-6), 70.35 (C-5), 58.30 (CH_2-7), 23.39, 21.97 (2×CH_3-*i*Pr); (CI, NH_3): *m*/*z* (%): 448 (35) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{24}H_{30}O_7$ (430,49): C 66.96, H 7.02; found C 66.69, H 7.21.

5.2.18. Isopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*,5-*C*-methylene-β-D-glucopyranoside 22. To a stirred solution of diol **21** (700 mg, 1.6 mmol) in anhydrous pyridine (10 mL) was added TsCl (370 mg, 1.95 mmol), followed by DMAP (50 mg). The reaction mixture was stirred for 12 h at 60 °C, cooled to room temperature and quenched with water. Ethyl acetate was added and the organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (EtOAc/cyclohexane 1:5) yielding the corresponding tosylate as a colourless oil (700 mg, 1.2 mmol, 74% yield).

5.2.19. Spectroscopic data for tosylate. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=7.86$ (d, 2H, aromatic H), 7.84–7.30 (m, 12H, aromatic H), 5.59 (s, 1H, CHPh), 4.87 (d, $J=11.7$ Hz, 1H,

CHPh), 4.86 (d, J=7.9 Hz, 1H, H-1), 4.78 (d, J=11.7 Hz, 1H, CHPh), 4.54 (s, 2H, H-7, H-7′), 4.26 (d, J=10.7 Hz, 1H, H-6), 4.06 (m, J=6.2 Hz, 1H, H-8), 3.89 (d, J=10.3 Hz, 1H, H-4), 3.72 (dd, J=8.7, 10.3 Hz, 1H, H-3), 3.65 (d, J=10.7 Hz, 1H, H-6′), 3.60 (dt, J=2.2, 8.2 Hz, 1H, H-2), 2.47 (s, 3H, CH_3-Ts), 2.44 (d, J=2.5 Hz, 1H, OH-2), 1.31 (d, J=6.2 Hz, 3H, CH_3-*i*Pr), 1.26 (d, J=6.2 Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=145.13, 136.70 (2× C*ipso* tosyl), 138.18, 132.29 (2×C*ipso*), 129.98–125.94 (2×Ph), 102.67 (CHPh), 97.62 (CH-1), 82.83 (CH-4), 77.01 (CH-3), 75.33 (CH-2), 74.60 (CH_2Ph), 72.82 (CH-8), 70.68 (CH_2-6), 68.89 (C-5), 64.29 (CH_2-7), 23.31, 21.99 (2×CH_3-*i*Pr), 21.67 (CH_3 tosyl); (CI, NH_3): *m*/*z* (%): 602 (100) [M+NH_4^+]; HRMS (positive-ion CI, NH_3): calcd for $C_{31}H_{40}O_9NS$ (M+NH_4^+) 602.2424, found 602.2430.

The tosylate (500 mg, 0.86 mmol) was dissolved in dry DMF (5 mL) under argon. The solution was cooled to 0 °C and sodium hydride (100 mg, 60% in oil, 2.5 mmol) was added and the reaction mixture was stirred for 1 h at room temperature and quenched with NH_4Cl. Ethyl acetate was added and the organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (EtOAc/cyclohexane 1:8) yielding compound **22** (340 mg, 95%) as a colourless oil.

$[\alpha]_D^{20}$=−42 (c=0.8 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.49–7.30 (m, 10H, aromatic H), 5.57 (s, 1H, CHPh), 5.34 (dd, J=1.2, 2.5 Hz, 1H, H-1), 4.72 (s, 2H, CH_2Ph), 4.40 (d, J=9.2 Hz, 1H, H-7), 4.35 (dd, J=1.8, 4.8 Hz, 1H, H-3 or H-4), 4.03 (d, J=11.2 Hz, 1H, H-6), 4.01 (t, J=6.1 Hz, 1H, H-3 or H-4), 3.99 (m, J=6.2 Hz, 1H, H-8), 3.97 (t, J=2.5 Hz, 1H, H-2), 3.89 (d, J=11.2 Hz, 1H, H-6′), 3.69 (dd, J=1.9, 9.2 Hz, 1H, H-7′), 1.34 (d, J=6.2 Hz, 3H, CH_3-*i*Pr),1.24 (d, J=6.2 Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=138.31, 137.18 (2×C*ipso*), 129.14–126.12 (2×Ph), 101.14 (CHPh), 97.91 (CH-1), 81.46 (CH-3 or CH-4), 78.57 (CH-3 or CH-4), 71.22 (CH_2Ph), 70.43 (CH-8), 70.24 (CH_2-6), 66.56 (CH-2), 65.91 (CH_2-7), 64.89 (C-5), 23.65, 21.77 (2×CH_3-*i*Pr); (CI, NH_3): *m*/*z* (%): 430 (100) [M+NH_4^+]; elemental analysis: calcd (%) for $C_{24}H_{28}O_6$ (412.48): C 69.88, H 6.84; found C 70.08, H 7.10.

5.2.20. Isopropyl 2-*O*,5-*C*-methylene-β-D-glucopyranoside 4. Compound **22** (300 mg, 0.728 mmol) was dissolved in dry methanol (10 mL) and 10% Pd/C (30 mg) was added. The solution was purged with hydrogen and stirred at rt overnight. After completion of the reaction, the reaction mixture was filtered through celite and washed with methanol. The solvent was concentrated and the crude product was purified by column chromatography (EtOAc/cyclohexane 2:1) to afford compound **4** (140 mg, 0.598 mmol, 82% yield) as colourless oil.

$[\alpha]_D^{22}$=+20 (c=0.44 in CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ=5.36 (d, J=1.6 Hz, 1H, H-1), 4.27 (m, J=6.2 Hz, 1H, H-8), 4.13 (dd, J=1.3, 9.0 Hz, 1H, H-7), 4.07 (dd, J=1.6, 4.5 Hz, 1H, H-3), 4.03 (dd, J=0.9, 9.0 Hz, 1H, H-7′), 3.85 (dd, J=1.6, 4.5 Hz, 1H, H-2), 3.83 (m, 1H, H-4), 3.73 (s, 2H, H-6, H-6′), 1.47 (d, J=6.2 Hz, 3H, CH_3-*i*Pr), 1.35 (d, J=6.2 Hz, 3H, CH_3-*i*Pr); ^{13}C NMR (100 MHz, CD_3OD): δ=95.86 (CH-1), 77.83 (C-5), 75.72 (CH-3), 74.88 (CH-4), 72.69 (CH-2), 71.12 (CH-8), 65.00 (CH_2-7), 63.77 (CH_2-6), 24.56 (CH_3-*i*Pr), 22.41 (CH_3-*i*Pr); MS (CI, NH_3): *m*/*z* (%): 252 (100) [M+NH_4^+]; HRMS (positive-ion CI, NH_3): calcd for $C_{10}H_{22}O_6N$ (M+NH_4^+) 252.1447, found 252.1448.

5.2.21. Isopropyl 3-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-4,6-*O*-isopropylidene-5-*C*-vinyl-β-D-glucopyranoside 23. Sodium (600 mg, 26.1 mmol) was added at 0 °C to a solution of compound **19** (15.1 g, 32.3 mmol) in methanol (300 mL). After 8 h of stirring at rt, the reaction mixture was neutralized with ion exchange resin IR-120 H^+ stirring for 1 h. The mixture was filtered, eluted with methanol and the solvent removed under vacuum to afford the corresponding triol (10.43 g, 30.86 mmol, 95%), which was used directly for the next reaction.

Triol (8.89 g, 26.3 mmol) was dissolved in dry acetone (39 mL), and 2,2′-dimethoxypropane (39 mL) followed by camphorsulphonic acid (610 mg, 2.63 mmol) were added. The reaction was stirred at rt overnight under argon, then quenched by addition of a saturated aqueous solution of $NaHCO_3$ and extracted with dichloromethane. The organic layer was dried over $MgSO_4$, concentrated and the residue was purified by column chromatography (cyclohexane/EtOAc 6:1) to afford the corresponding 4,6-*O*-isopropylidene derivative (8.6 g, 22.75 mmol, 86%) as a white powder.

This alcohol (8.23 g, 21.77 mmol) was dissolved in dry DMF (55 mL) and TBDMSCl (4.27 g, 28.3 mmol) followed by imidazole (1.92 g, 28.3 mmol) were added under argon. The reaction mixture was stirred at 60 °C for 3.5 h, then cooled to rt, and finally poured in a water–ice mixture and extracted with ether. The organic layer was dried over $MgSO_4$, concentrated and the residue was purified by column chromatography (cyclohexane/EtOAc 4:1) to afford compound **23** (10.45 g, 21.2 mmol, 97%) as a crystalline solid.

$[\alpha]_D^{20}$=−44 (c=0.92 in $CHCl_3$); mp 113 °C (*n*-pentane/EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.50–7.30 (m, 5H, Ph), 6.33 (dd, J=11.3, 17.9 Hz, 1H, H-7), 5.60 (dd, J=1.5, 17.9 Hz, 1H, H-8′), 5.53 (dd, J=1.3, 11.3 Hz, 1H, H-8), 4.85 (d, J=11.1 Hz, 1H, CHPh), 4.71 (d, J=7.2 Hz, 1H, H-1), 4.69 (d, J=11.1 Hz, 1H, CHPh), 4.02 (m, J=6.2 Hz, 1H, H-9), 3.93 (d, J=10.1 Hz, 1H, H-6′), 3.85 (d, J=9.5 Hz, 1H, H-4), 3.64 (d, J=10.1 Hz, 1H, H-6), 3.50 (m, 2H, H-2, H-3), 1.47 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.29 (d, J=6.2 Hz, 3H, CH_3-*i*Pr), 1.20 (d, J=6.2 Hz, 3H, CH_3-*i*Pr), 0.93 (s, 9H, tBu), 0.12 (s, 3H, CH_3), 0.08 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=138.87 (Cipso), 136.58 (CH-7), 128.12, 128.06, 127.34 (Ph), 117.70 (CH_2-8), 99.96 ($C(CH_3)_2$), 97.45 (CH-1), 78.91 (CH-2), 73.33 (CH-4), 75.96 (CH-3), 74.61 (CH_2Ph), 71.51 (CH_2-6), 70.58 (CH-9), 72.43 (CH-8), 69.90 (C-5), 29.18, 18.92 (2×CH_3), 25.92 (tBu), 23.48, 21.56 (2×CH_3-*i*Pr), −4.08, −4.31 (2×CH_3-Si); (CI, NH_3): *m*/*z* (%): 510 (10) [M+NH_4^+], 493 (20) [M+H^+], 392 (100); HRMS (positive-ion CI, NH_3): calcd for $C_{27}H_{45}O_6Si$ (M+H^+) 493.2985, found 493.2982.

5.2.22. Isopropyl 3-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-4,6-*O*-isopropylidene-5-*C*-methanoate-β-D-glucopyranoside 24. Ozone was passed through a stirred solution of olefin **23** (1.0 g, 2.03 mmol) in CH_2Cl_2 (50 mL) cooled to −78 °C for 4 h. The reaction mixture was quenched with $(CH_3)_2S$ (0.2 mL) and allowed to warm to room temperature. The solvent was evaporated. The crude carboxylic acid was dissolved in dry DMF (20 mL). Iodomethane (8 mL) and $KHCO_3$ (570 mg) were added and the reaction mixture was stirred under argon for 16 h. The solvent was removed under reduced pressure and the residue dissolved in EtOAc and washed with water and brine. The organic layer was dried over $MgSO_4$ and concentrated. Purification by column chromatography (cyclohexane/EtOAc 10:1) afforded the methyl ester derivative **24** (744 mg, 1.42 mmol, 70% yield) as an oil.

$[\alpha]_D^{20}=-18$ ($c=1.2$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): $\delta=7.38-7.30$ (m, 5H, Ph), 4.93 (d, $J=10.8$ Hz, 1H, CHPh), 4.72 (d, $J=10.8$ Hz, 1H, CHPh), 4.36 (d, $J=7.8$ Hz, 1H, H-1), 4.18 (d, $J=9.9$ Hz, 1H, H-6), 4.13 (dd, $J=9.8, 8.1$ Hz, 1H, H-3), 4.00 (m, $J=6.2$ Hz, 1H, H-8), 3.89 (d, $J=10.1$ Hz, 1H, H-4), 3.88 (s, 3H, CO_2CH_3), 3.86 (d, $J=9.9$ Hz, 1H, H-6′), 3.49 (t, $J=7.9$ Hz, 1H, H-2), 1.48 (s, 1H, CH_3-isopropylidene), 1.44 (s, 1H, CH_3-isopropylidene), 1.25 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr), 1.18 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr), 0.91 (s, 9H, tBu), 0.09 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=169.88$ (C=O), 138.96 (C*ipso*), 129.68, 128.11, 128.06, 127.31 (Ph), 100.20 ($C(CH_3)_2$), 99.54 (CH-1), 78.98 (CH-3), 76.02 (CH-4), 75.18 (CH-2), 74.66 (CH_2Ph), 71.71 (C-5), 71.34 (CH-8), 67.02 (CH_2-6), 52.17 (CO_2CH_3), 29.10, 18.51 (2×CH_3-isopropylidene), 25.87 (tBu), 23.16, 21.29 (2×CH_3-*i*Pr), −4.18, −4.40 (2×$SiCH_3$); (CI, NH_3): *m/z* (%): 542 (100) [$M+NH_4^+$]; HRMS (positive-ion CI, NH_3): calcd for $C_{27}H_{48}O_8NSi$ ($M+NH_4^+$) 542.3149, found 542.3143.

5.2.23. Isopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*,5-*C*-carbonyl-β-D-glucopyranoside 25. Tetrabutylammonium fluoride (190 mg, 0.6 mmol) was added to a solution of methyl ester derivative **24** (104 mg, 0.2 mmol) in dry THF (10 mL) under argon and the solution was stirred for 3 h at rt. The reaction mixture was then poured in ice–water, extracted with EtOAc, the organic layer was dried over $MgSO_4$ and concentrated. Purification by column chromatography (cyclohexane/EtOAc 9:1) afforded the lactonized compound **25** (68 mg, 0.18 mmol, 90% yield) as a crystalline compound.

$[\alpha]_D^{20}=-74$ ($c=2.31$ in $CHCl_3$); mp 91–92 °C (*n*-pentane/EtOAc); ^1H NMR ($CDCl_3$, 400 MHz): $\delta=7.43-7.30$ (m, 5H, Ph), 5.28 (dd, $J=1.3$, 2.8 Hz, 1H, H-1), 4.73 (d, $J=12.1$ Hz, 1H, CHPh), 4.69 (d, $J=12.1$ Hz, 1H, CHPh), 4.58 (t, $J=2.8$ Hz, 1H, H-2), 4.44 (d, $J=5.0$ Hz, 1H, H-4), 4.27 (d, $J=11.8$ Hz, 1H, H-6), 4.01 (m, $J=6.2$ Hz, 1H, H-7), 3.85 (d, $J=11.8$ Hz, 1H, H-6′), 3.83 (ddd, $J=1.3$, 5.0, 2.8 Hz, 1H, H-3), 1.48 (s, 3H, CH_3-isopropylidene), 1.42 (s, 3H, CH_3-isopropylidene), 1.34 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr), 1.24 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=168.42$ (C=O), 137.65 (C*ipso*), 128.30, 127.78, 127.56 (Ph), 99.65 ($C(CH_3)_2$), 96.40 (CH-1), 78.90 (CH-3), 72.91 (CH-2), 72.31 (CH-4), 72.17 (CH-7), 72.14 (CH_2Ph), 67.05 (C-5), 60.73 (CH_2-6), 27.94, 19.15 (2×CH_3-isopropylidene), 23.42, 21.69 (2×CH_3-*i*Pr); (CI, NH_3): *m/z* (%): 396 (100) [$M+NH_4^+$]; HRMS (positive-ion CI, NH_3): calcd for $C_{20}H_{27}O_7$ ($M+H^+$) 379.1757, found 379.1759.

5.2.24. Isopropyl 2-*O*,5-*C*-carbonyl-β-D-glucopyranoside 5. Compound **25** (53 mg, 0.14 mmol) was dissolved in AcOH/H_2O (3:2, 2 mL) and stirred at 60 °C for 3.5 h. The solvent was removed under reduced pressure and the residue co-evaporated with toluene (2×5 mL) to afford the crude diol, which was used directly in the next step. The diol (47 mg, 0.139 mmol) was dissolved in EtOAc (10 mL) and Pd/C (10 mg) was added. The suspension was stirred under H_2 for 1 h at rt, filtered through celite (eluted with EtOAc) and the solvent was removed under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc 1:1) afforded compound **5** (29 mg, 0.121 mmol, 87% yield) as crystalline compound.

$[\alpha]_D^{20}=-110$ ($c=0.3$ in $CHCl_3$); mp 118 °C (*n*-pentane/ethyl acetate); ^1H NMR ($CDCl_3$, 400 MHz): $\delta=5.39$ (dd, $J=1.5$, 2.8 Hz, 1H, H-1), 4.62 (t, $J=2.8$ Hz, 1H, H-2), 4.33 (d, $J=3.9$ Hz, 1H, H-4), 4.17 (d, $J=12.6$ Hz, 1H, H-6), 4.10 (m, $J=6.2$ Hz, 1H, H-7), 3.98 (d, $J=12.6$ Hz, 1H, H-6′), 3.94 (m, 1H, H-3), 3.64 (d, $J=11.9$ Hz, 1H, OH), 3.30 (s, 1H, OH), 1.64 (s, 1H, OH), 1.33 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr), 1.26 (d, $J=6.2$ Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=169.0$ (C=O), 95.90 (CH-1), 75.91 (CH-4), 75.15 (CH-3), 73.46 (CH-2), 73.06 (CH-7), 61.94 (CH_2-6), 23.53, 21.57 (2×CH_3-*i*Pr); (CI, NH_3): *m/z* (%): 266 (100) [$M+NH_4^+$]; HRMS (positive-ion CI, NH_3): calcd for $C_{10}H_{17}O_7$ ($M+H^+$) 249.0974, found 249.0979.

5.2.25. Methyl (3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*,5-*C*-methylene-β-D-glucopyranosyl)-(1,4)-*O*-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside 27. Thiophenyl derivative **11** (110 mg, 0.24 mmol), alcohol **26** (133 mg, 0.29 mmol) and powdered 4 Å molecular sieves (350 mg) were suspended in dry CH_2Cl_2 (13 mL) under argon and the suspension was stirred for 30 min at rt. The reaction mixture was then cooled to −30 °C, NIS (108 mg, 0.48 mmol) followed by triflic acid (4 μL, 0.036 mmol) were added to give a red solution. After 15 min of stirring at −30 °C, the reaction mixture was neutralized with sat. aq. $NaHCO_3$, diluted with Et_2O (50 mL), washed with sat. aq. $Na_2S_2O_3$, brine, and dried over $MgSO_4$. Purification by column chromatography (cyclohexane/EtOAc 5:1) afforded the protected disaccharide **27** (93 mg, 0.114 mmol, 48% yield) as an oil.

$[\alpha]_D^{20}=-37$ ($c=1$ in $CHCl_3$); ^1H RMN ($CDCl_3$, 400 MHz): $\delta=7.48-7,30$ (m, 5H, Ph), 5.37 (dd, $J=2.5$, 1.1 Hz, 1H, H-1′), 5.07 (s, 2H, CH_2Ph), 4.97 (s, 1H, CHPh), 4.82 (d, $J=12.3$ Hz, 1H, CHPh), 4.71 (d, $J=12.3$ Hz, 1H, CHPh), 4.67 (d, $J=12.0$ Hz, 1H, CHPh), 4.66 (d, $J=3.4$ Hz, 1H, H-1), 4.64 (d, $J=11.3$ Hz, 1H, CHPh), 4.56 (d, $J=12.0$ Hz, 1H, CHPh), 4.45 (d, $J=11.3$ Hz, 1H, CHPh), 4.29 (d, $J=9.5$ Hz, 1H, H-7), 4.12 (dd, $J=4.8$, 1.6 Hz, 1H, H-4′), 3.97 (t, $J=9.1$ Hz, 1H, H-3), 3.90 (t, $J=9.2$ Hz, 1H, H-4), 3.87 (m, 1H, H-3′), 3.76 (d, $J=11.2$ Hz, 1H, H-6a′), 3.74 (m, $J=9.4$ Hz, 1H, H-5), 3.69 (t, $J=2.6$ Hz, 1H, H-2′), 3.68 (dd, $J=3.0$, 10.4 Hz, 1H, H-6a), 3.62 (dd, $J=3.4$, 9.1 Hz, 1H, H-2), 3.56 (d, $J=1.9$ Hz, 1H, H-6b), 3.55 (dd, $J=1.7$,

9.5 Hz, 1H, H-7b′), 3.46 (d, *J*=11.2 Hz, 1H, H-6b′), 3.43 (s, 3H, OCH_3); ^{13}C RMN ($CDCl_3$, 400 MHz): δ=139.64, 138.05, 137.98, 137.62, 137.14 (5×C*ipso*), 129.05–126.03 (5×Ph), 100.6 (CHPh), 100.08 (C-1′), 98.27 (C-1), 81.02 (C-4′), 80.16 (C-3), 79.21 (C-2), 78.36 (C-3′), 76.42 (C-4), 74.76 (CH_2Ph), 73.51 (CH_2Ph), 73.35 (CH_2Ph), 71.35 (CH_2Ph), 69.65 (C-5), 69.61 (C-6′), 68.16 (C-6), 66.45 (C-2′), 65.66 (C-7′), 65.02 (C-5′), 55.22 (OCH_3); (CI, NH_3): *m/z* (%): 834 (100) [M+NH_4^+]; $C_{49}H_{52}O_{11}$ (816.95): calcd C 72.04, H 6.42; found C 71.81, H 6.65.

5.2.26. Methyl (2-*O*,5-*C*-methylene-β-D-glucopyranosyl)-(1,4)-α-D-glucopyranoside 28. Disaccharide **27** (25 mg, 0.030 mmol) was dissolved in methanol (5 mL) and 10% Pd/C (10 mg) was added. The suspension was stirred under H_2 for 1 h at rt, filtered through celite eluted with methanol and concentrated. Purification by column chromatography (10% CH_3OH in EtOAc) afforded the disaccharide **28** (10 mg, 0.027 mmol, 90% yield) as a foam.

$[\alpha]_D^{20}$=+61 (*c*=1.05 in H_2O); ^1H RMN (D_2O, 500 MHz): δ=5.33 (dd, *J*=2.7, 1.3 Hz, 1H, H-1′), 4.81 (d, *J*=3.8 Hz, 1H, H-1), 4.07 (dd, *J*=5.2, 1.7 Hz, 1H, H-4′), 3.98 (t, *J*=2.7 Hz, 1H, H-2′), 3.95 (dd, *J*=2.7, 5.2 Hz, 1H, H-3′), 3.84 (d, *J*=12.0 Hz, 1H, H-6a), 3.83 (dd, *J*=9.2, 9.8 Hz, 1H, H-3), 3.85 (d, *J*=9.8 Hz, 1H, H-7′a), 3.77 (dd, *J*=4.5, 12.0 Hz, 1H, H-6b), 3.76 (ddd, *J*=4.5, 9.8, 12.0 Hz, 1H, H-5), 3.73 (d, *J*=12.7 Hz, 1H, H-6a′), 3.65 (dd, *J*=1.7, 9.8 Hz, 1H, H-7b′), 3.62 (dd, *J*=3.8, 9.8 Hz, 1H, H-2), 3.59 (dd, *J*=9.2, 9.8 Hz, 1H, H-4), 3.64 (d, *J*=12.7 Hz, 1H, H-6b′), 3.39 (s, 3H, OCH_3); ^{13}C RMN (D_2O, 100 MHz): δ=100.06 (CH-1′), 99.42 (CH-1), 78.92 (CH-4), 73.33 (C-5′), 74.72 (CH-3′), 73.53 (CH-4′), 71.81 (CH-3), 71.78 (CH-2), 70.50 (CH-5), 68.31 (CH-2′), 62.22 (CH_2-7′), 60.38 (CH_2-6, CH_2-6′), 55.39 (OCH_3); (CI, NH_3): *m/z* (%): 386 (100) [M+NH_4^+]; HRMS (positive-ion CI, NH_3): calcd for $C_{14}H_{28}O_{11}N$ (M+NH_4^+) 386.1662, found 386.1654.

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References and notes

1. Yang, J. K.; Yoon, H.-J.; Ahn, H. J.; Lee, B. I.; Pedelacq, J.-D.; Liong, E. C.; Berendzen, J.; Laivenieks, M.; Vieille, C.; Zeikus, G. J.; Vocadlo, D. J.; Withers, S. G.; Suh, S. W. *J. Mol. Biol.* **2004**, *335*, 155–165, and references cited herein.
2. Bennet, A. J.; Kitos, T. E. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1207–1222, and selective relevant references therein.
3. Capon, B. *Chem. Rev.* **1969**, *69*, 407–496.
4. Jensen, H. H.; Bols, M. *Org. Lett.* **2003**, *5*, 3419–3421.
5. Edward, J. T. *Chem. Ind. (London)* **1955**, 1102–1104.
6. Namchuk, N. M.; McCarter, J. D.; Becalski, A.; Andrews, T.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 1270–1277.
7. Dean, K. E. S.; Kirby, A. J.; Komarov, I. V. *J. Chem. Soc., Perkin Trans. 2* **2002**, 337–341.
8. Barnes, J. A.; Williams, I. H. *Chem. Commun.* **1996**, 193–194.
9. Bennet, A. J.; Sinnott, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 7287–7294.
10. Indurugalla, D.; Bennet, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 10889–10898.
11. For a preliminary communication on this work, see: Blériot, Y.; Vadivel, S. K.; Greig, I. R.; Kirby, A. J.; Sinaÿ, P. *Tetrahedron Lett.* **2003**, *44*, 6687–6690.
12. Chandrasekhar, S.; Kirby, A. J.; Martin, R. J. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1619–1626. Ley, S. V.; Baeschlin, K. K.; Dixon, D. J.; Foster, A. C.; Ince, S. J.; Priepke, H. W. M.; Reynolds, D. J. *Chem. Rev.* **2001**, *101*, 53–80.
13. Stoddart, J. F. *Stereochemistry of Carbohydrates*. Wiley-Interscience: New York, 1971; p 47.
14. Hosie, L.; Sinnott, M. L. *Biochem. J.* **1985**, *226*, 437–446. Tanaka, K. S. E.; Bennet, A. J. *Can. J. Chem.* **1998**, *76*, 431–436. Berti, P. J.; Tanaka, K. S. E. *Adv. Phys. Org. Chem.* **2002**, *37*, 239–314.
15. Sabini, E.; Sulzenbacher, G.; Dauter, M.; Dauter, Z.; Jorgensen, P. L.; Schülein, M.; Dupont, C.; Davies, G. J.; Wilson, K. S. *Chem. Biol.* **1999**, *6*, 483–492. Sidhu, G.; Withers, S. G.; Nguyen, N. T.; McIntosh, L. P.; Ziser, L.; Brayer, G. D. *Biochemistry* **1999**, *38*, 5346–5354. Ducros, V. M.-A.; Zechel, D. L.; Murshudov, G. N.; Gilbert, H. J.; Szabo, L.; Stoll, D.; Withers, S. G.; Davies, G. J. *Angew. Chem.* **2002**, *114*, 2948–2951, *Angew. Chem. Int. Ed.*, **2002**, *41*, 2824–2827. Vasella, A.; Davies, G. J.; Böhm, M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 619–629. Varrot, A.; MacDonald, J.; Stick, R. V.; Pell, G.; Gilbert, H. J.; Davies, G. J. *Chem. Commun.* **2003**, 946–947.
16. Deslongchamps, P. In *Anomeric effect and associated stereoelectronic effects*. *ACS Symposium Series*, Thatcher, G. R. J., Ed.; 1993; Vol. 539, pp 27–54.
17. Deslongchamps, P.; Jones, P. G.; Li, S.; Kirby, A. J.; Kuusela, S.; Ma, Y. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2621–2626. Li, S.; Kirby, A. J.; Deslongchamps, P. *Tetrahedron Lett.* **1993**, *34*, 7757–7758. Li, S.; Deslongchamps, P. *Tetrahedron Lett.* **1993**, *34*, 7759–7762.
18. Sinnott, M. L. *Advances in Physical Organic Chemistry*, 1988; Vol. 24. pp 113–204.
19. We have previously developed such an oxymethylene bridge to lock flexible L-iduronic acid derivatives Das, S. K.; Mallet, J.-M.; Esnault, J.; Driguez, P.-A.; Duchaussoy, P.; Sizun, P.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Angew. Chem.* **2001**, *113*, 1723–1726, *Angew. Chem. Int. Ed. Engl.*, **2001**, *40*, 1670–1673. Das, S. K.; Mallet, J.-M.; Esnault, J.; Driguez, P.-A.; Duchaussoy, P.; Sizun, P.; Herault, J.-P.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Chem. Eur. J.* **2001**, *7*, 4821–4834.
20. Rama Rao, A. V.; Gurjar, M. K.; Rama Devi, T.; Ravi Kumar, K. *Tetrahedron Lett.* **1993**, *34*, 1653–1656.
21. Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **1999**, *40*, 8439–8441.
22. Nieman, C. E.; Wong, A. W.; He, S.; Clarke, L.; Hopwood, J. J.; Withers, S. G. *Biochemistry* **2003**, *42*, 8054–8065.

23. Haasnoot, C. A. G.; de Leewuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792. Walaszek, Z.; Horton, D.; Ekiel, I. *Carbohydr. Res.* **1982**, *106*, 193–201.
24. Selected crystal structure data for **5**; crystal system orthorhombic; Space Group $P\ 2_12_12_1$; Z=4; cell parameters: a=7.1735 (11), b=7.3340 (9), c=21.210 (3), α=90, β=90, γ=90; radiation (Mo Kα) λ=0.71073 Å; 155 variables for 1342 reflections; final R=0.0517, R_W=0.0536; atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK, allocated the deposition number no. CCDC 234021 and are available on request. Requests should be accompanied by a full citation for this paper.
25. Küster, J. M.; Dyiong, I. *Justus Liebigs Ann. Chem.* **1975**, 2179–2189. Garegg, P. J.; Iversen, T.; Oscarsson, S. *Carbohydr. Res.* **1976**, *50*, C12–C14. Petit, J. M.; Jacquinet, J.-C.; Sinaÿ, P. *Carbohydr. Res.* **1980**, *82*, 130–134. Garegg, P. J.; Hultberg, H. *Carbohydr. Res.* **1981**, *93*, C10–C11. Mallet, J.-M.; Meyer, G.; Yvelin, F.; Jutand, A.; Amatore, C.; Sinaÿ, P. *Carbohydr. Res.* **1993**, *244*, 237–246.
26. *para*-Nitrophenyl glucopyranosides were purchased from Acros and isopropyl glucopyranosides were synthesized using standard procedures.
27. For another study of the acid catalyzed cleavage of more simple mimics of *para*-nitrophenyl glucosides see: Kirby, A. J.; Martin, R. J. *Chem. Commun.* **1978**, 803–804.
28. Sinnott, M. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 2026–2032.
29. Briggs, A. J.; Glenn, R.; Jones, P. G.; Kirby, A. J.; Ramaswamy, P. *J. Am. Chem. Soc.* **1984**, *106*, 6200–6206.
30. Hrmova, M.; Harvey, A. J.; Wang, J.; Shirley, N. J.; Jones, G. P.; Stone, B. A.; Hoj, P. B.; Fincher, G. B. *J. Biol. Chem.* **1996**, *271*, 5277–5286.
31. Hrmova, M.; De Gori, R.; Smith, B. J.; Vasella, A.; Varghese, J. N.; Fincher, G. B. *J. Biol. Chem.* **2004**, *279*, 4970–4980.
32. Barr, B. K.; Hsieh, Y.-L.; Ganem, B.; Wilson, D. B. *Biochemistry* **1996**, *35*, 586–592.
33. Vonhoff, S.; Piens, K.; Pipelier, M.; Braet, C.; Claeyssens, M.; Vasella, A. *Helv. Chim. Acta* **1999**, *82*, 963–980.
34. Sulzenbacher, G.; Driguez, H.; Henrissat, B.; Schülein, M.; Davies, G. J. *Biochemistry* **1996**, *35*, 15280–15287.
35. Divne, C.; Stahlberg, J.; Teeri, T. T.; Jones, T. A. *J. Mol. Biol.* **1998**, *275*, 309–325.
36. Böhm, M.; Lorthiois, E.; Meyyappan, A. *Helv. Chim. Acta* **2003**, *86*, 3787–3817. Böhm, M.; Lorthiois, E.; Meyyappan, A. *Helv. Chim. Acta* **2003**, *86*, 3818–3835. Lorthiois, E.; Meyyappan, A. *Chem. Commun.* **2000**, *83*, 1829–1830. Tanaka, K. S. E.; Bennet, A. J. *Can. J. Chem.* **1998**, *76*, 431–436.
37. Heck, M. P.; Vincent, S. P.; Murray, B. W.; Bellamy, F.; Wong, C.-H.; Mioskowski, C. *J. Am. Chem. Soc.* **2004**, *126*, 1971–1979. Shah, N.; Kuntz, D. A.; Rose, D. R. *Biochemistry* **2003**, *42*, 13812–13816.