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A stereodivergent, two-directional synthesis of stereoisomeric *C*-linked disaccharide mimetics

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Dipyranones, such as 1,2-bis[(2*R*,3*S*,6*S*)-3-hydroxy-6-methoxy-3-oxo-6*H*-pyran-2-yl]ethane, were exploited as templates for the synthesis of some novel *C*-linked disaccharide analogues. Efficient methods, such as stereoselective reduction and dihydroxylation, were developed for two-directional functionalisation of these templates. Peracetylated derivatives of ten stereoisomeric disaccharide analogues {acetic acid 4,5-diacetoxy-6-methoxy-[(3′,4′,5′-triacetoxy-6′-methoxytetrahydropyran-2′-yl)ethyl]tetrahydropyran-3-yl esters} were synthesised from a virtual library of 136 compounds; furthermore, an additional eight stereoisomers could have been synthesised simply by using the enantiomeric ligand in the enantioselective step. The ability of (2*S*,3*S*,4*R*,5*R*,6*R*)- 6-methoxy-2-[2′-((2′*R*,3′*R*,4′*S*,5′*R*,6′*S*)-3′,4′,5′-trihydroxy-6′-methoxytetrahydropyran-2′-yl)ethyl]tetrahydropyran-3,4,5-triol to bind to the repressor protein, LacI, was estimated to be similar to that of isopropyl-β-thiogalactoside. The disaccharide mimetics were concluded to be a new and interesting class of *C*-linked disaccharide mimetics with promising, though largely unstudied, biological activity.

Introduction

Libraries of stereo- and regioisomeric oligosaccharides can probe large areas of conformational space, and can be used to identify unnatural ligands for carbohydrate receptors.[1] A key milestone in this area has been the synthesis of a 1 300 member library of acylated amino di- and trisaccharides.[2] This approach enabled the identification of two compounds which exhibited a higher affinity for a bacterial lectin from *Bauhinia purpurea* than the known natural ligand.

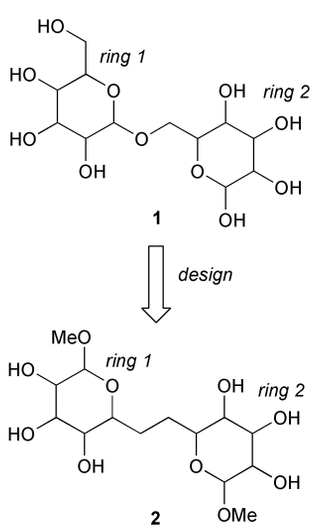
C-Linked glycosides, in which *exo*-anomeric oxygen atoms are replaced by methylene groups, are a particularly interesting class of carbohydrate mimetic which are resistant to enzymatic degradation and have potential as inhibitors of glycosidases[3] and glycosyl transferases.[4] A number of reliable methods have been developed for the synthesis of *C*-linked disaccharides.[5] For example, most methods for the synthesis of *C*-(1⟶6)-linked disaccharides (**3**) involve the coupling of two monosaccharide derivatives using a key connective reaction, such as the Kishi,[6] Ramberg–Bäcklund,[7] Wittig,[8] Henry[9] or metathesis[10] reactions. This is an excellent approach to the synthesis of *C*-glycosides which can be synthesised from cheap, available monosaccharides.

There is considerable debate in the literature concerning the similarity between the conformations of *C*-glycosides and their parent *O*-linked oligosaccharides, particularly concerning ground state conformational preferences around the aglyconic bond. The ground state and bound conformations of *C*- and *O*-lactose have been studied in detail by the Kishi[11] and the Schmidt and Jiménez-Barbero groups.[12] Kishi has concluded that *C*- and *O*-lactose both populate the same two conformers in solution, and that the same high-energy conformation ligates to peanut lectin.[11] Schmidt and Jiménez-Barbero assert that *Escherichia coli* β-galactosidase binds a high energy conformation of *C*-lactose which is barely present in solution for *C*-lactose, and absent for lactose itself.[12]

Several studies have shown that the biological activity of *C*-linked mimetics often does mirror that of the corresponding *O*-linked structure in a remarkable way. For example, the β-galactosidase-catalysed cleavage of *p*-nitrophenyl galactose was similarly competitively inhibited by both *O*-lactose ($K_i = 1.2$ mM) and *C*-lactose (K_i = 3.3 mM),[12] and the free energy of association of some *C*-linked oligo-β-1,6-galacosides with three monoclonal immunoglobulins were remarkably similar to those of the parent immunodeterminants.[4]

Synthetic methods have been developed for the preparation of *C*-linked stereoisomeric di- and trisaccharides. These methods enable the synthesis of mimetics which would otherwise need to be made from monosaccharides which are either expensive or unavailable, for example *C*-linked analogues of disaccharides formed from D and L hexoses.[13] Twelve stereochemically and structurally diverse *C*-trisaccharides have been synthesised; at three steps of the synthesis, this approach relied on the separation of two diastereoisomers which were subsequently functionalised.

We have designed a new class of *C*-(1⟶6)-linked disaccharide mimetic **2** in which *C*-6 of ring 1 has been replaced with a methoxy group (see Scheme 1). In this paper, we describe our



Scheme 1

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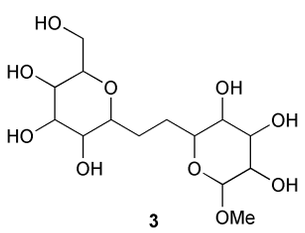
Table 1 Synthesis of some of the possible stereoisomers of general structure 2

Ring 2 stereochemistry \ Ring 1 stereochemistry	← H, G, F	E	D	C	B	A	A'	B'	C'	D'	E'	→ F', G', H'
↑ F, G, H												
E							√[a]					
D							√					
C				√	√[a]	√			√[a]			
B					√	√						
A						√	√			*b*	*b*	
A'							*b*	*b*	*b*			
B'								*b*	*b*			
C'									*b*			
D'												
E'												
↓ F', G', H'												

[a] Formed in low yield (<40%). [b] Enantiomeric compound prepared.

progress towards a general synthesis of these compounds which mimic both natural and unnatural disaccharides. A library of the mimetics **2** would probe large regions of conformational space and would be valuable in the identification of therapeutic agents[15] based on carbohydrates.



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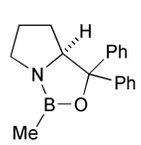
Results and discussion

At the start of our investigation, we envisaged that the difuryl diols **4** could be expanded to give dipyranones such as **5** which would serve as a template for further stereoselective functionisation. It was hoped that minor changes to a general reaction sequence would enable different relative stereochemistry to be installed in the products at will. A strength of the approach is that a large number of stereoisomers could, in principle, be made from a single precursor, so the choice of the configuration of the products would be made at a late stage in each synthesis. Ignoring anomers, there are eight possible relative configurations in each ring of the mimetics **2** (see Fig. 1) and we have developed methods for installing six of these combinations (Table 1).[16]† Our approach lends itself to a two-directional synthetic strategy,[17] which would greatly reduce the number of synthetic steps required.[18]

There are 136 possible stereoisomeric mimetics **2** (ignoring anomers), and these can be divided into two groups according to the 1,4-stereochemical relationship between the two pyran rings (Scheme 2). This distinction is strategically very important since it governs the synthetic approach which must be used. There are 72 stereoisomers which would need to be derived from the chiral diastereoisomer **4**; all of these analogues are chiral and would need to be made from either optically active diol (*R*,*R*)-**4** or (*S*,*S*)-**4**. In contrast, the enantioselective step required for the synthesis of the remaining 56 chiral stereoisomers would need to be delayed, and strategies for the desymmetrisation of *meso* compounds derived from *meso*-**4** have been described.[19] The remaining eight stereoisomers are not chiral.

Asymmetric synthesis of the C_2-symmetric diol 9

Reduction of the diketone **7** using borane–dimethyl sulfide complex and 10 mol% of the catalyst **10** gave the diol **4** as a 85 : 15 mixture of diastereoisomers in 80% yield; (*R*,*R*)-**4** had >98% ee (Scheme 3). The ratio of diastereomeric products was determined by careful examination of the ^{13}C NMR spectrum of the crude reaction mixture, and the enantiomeric excess of the chiral diastereoisomer was measured by analytical chiral HPLC. The observation of moderate diastereoselectivity accompanied by excellent enantioselectivity has also been observed in other asymmetric reactions of bifunctional compounds (for example, in similar reductions[20]); this "proof-reading" behaviour stems from the fact that most additions to the *Si*-face of either ketone lead to *meso*-**4** rather than (*S*,*S*)-**4**.



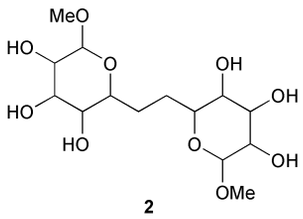
10

Oxidative ring expansion of the furan rings of (*R*,*R*)-**4**, using VO(acac)$_2$–^{t}BuOOH, and acetalisation, gave the dipyranone **8** as a 75 : 25 mixture of C_2-symmetric and unsymmetrical anomers. This mixture **8** was reduced with sodium borohydride to give an anomeric mixture of diols from which C_2-symmetric diastereoisomer **9** was isolated by careful column chromatography.

Two-directional synthesis of *C*-linked disaccharides

The stage was now set for the two-directional synthesis[18] of some *C*-linked disaccharides (Scheme 4). The C_2-symmetric diol **9** was converted into its C_2-symmetric diastereoisomer **11**

† In this paper, we have labelled the eight possible relative configurations of the rings A–H; rings labelled A′–H′ belong to the other enantiomeric series.



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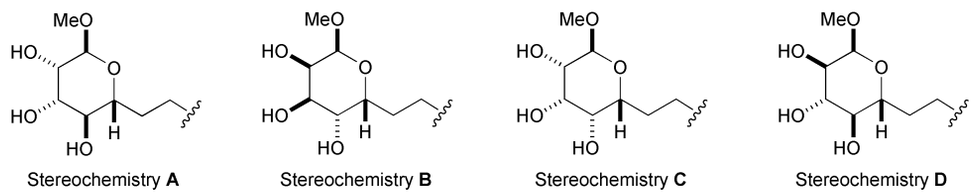
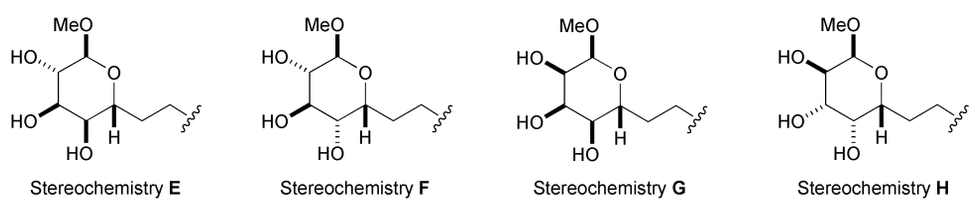
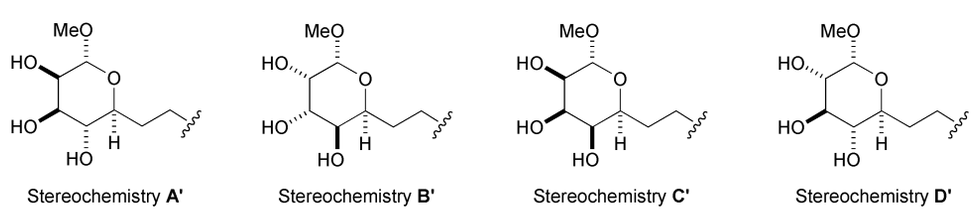


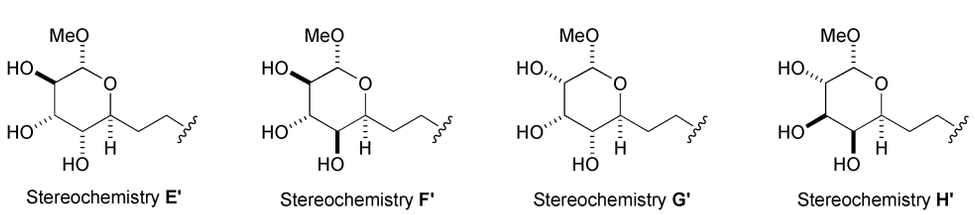
Stereochemistry **A** Stereochemistry **B** Stereochemistry **C** Stereochemistry **D**



Stereochemistry **E** Stereochemistry **F** Stereochemistry **G** Stereochemistry **H**



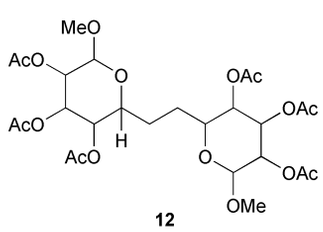
Stereochemistry **A'** Stereochemistry **B'** Stereochemistry **C'** Stereochemistry **D'**



Stereochemistry **E'** Stereochemistry **F'** Stereochemistry **G'** Stereochemistry **H'**

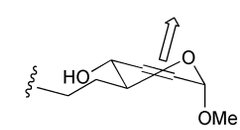
Fig. 1

by double Mitsunobu inversion and methanolysis, and into its unsymmetrical diastereoisomer **14** by statistical benzoylation of one of its homotopic alcohols (⟶ **13**), inversion and methanolysis.



12

Subjection of the diols **9**, **11** and **14** to Upjohn's dihydroxylation conditions (OsO_4, NMO) resulted in double dihydroxylation of each alkene to give, after peracetylation, predominantly the hexaacetates **12AA**, **12BB** and **12AB** respectively. The new *syn* 1,2-diol was introduced predominantly *anti* to the neighbouring hydroxy group regardless of the configuration of the starting material, though the process was markedly more diastereoselective when the pre-existing hydroxy group was *pseudo* equatorial (*i.e.* when installing stereochemistry **A**, Fig. 2). Even in the most troublesome case, the double dihydroxylation of **11** with its two *pseudo* axial hydroxy groups (see Fig. 3), a 53% yield of the carbohydrate mimetic **12BB** was



→ **Stereochemistry A**

Fig. 2

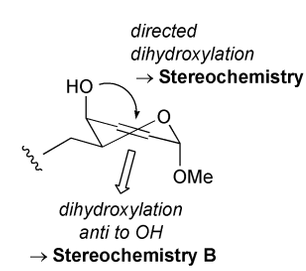
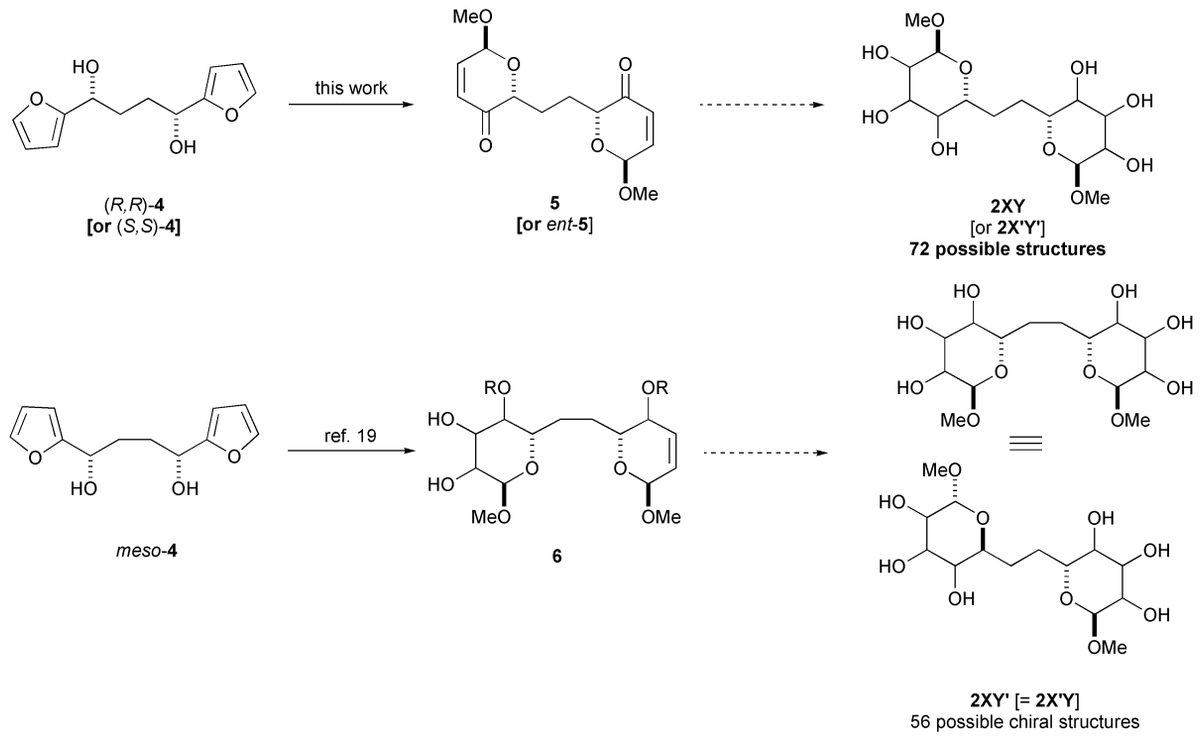
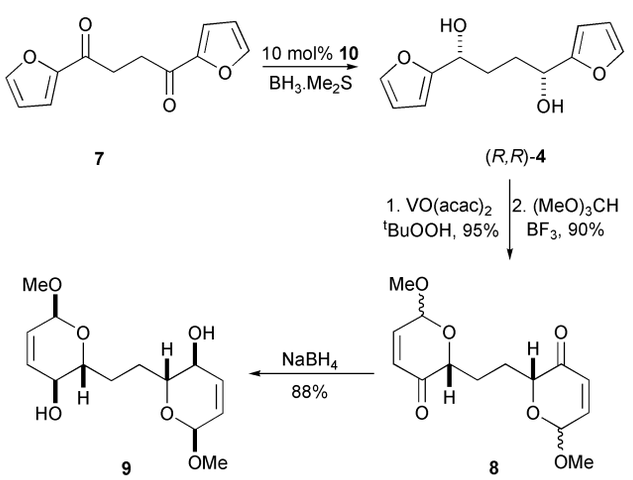


Fig. 3

obtained along with its diastereoisomer **12BC** (23% yield). In the case of **12AA**, the two-directional strategy is very efficient indeed: in the reaction sequence **8** ⟶ **9** ⟶ **12AA**, six new stereogenic centres were introduced with almost complete diastereoselectivity in just two steps. The two-directional approach was not restricted to symmetrical diastereoisomers, since the hexaacetate **12AB** was obtained in 60% yield from the diol **14** (which



Scheme 2



Scheme 3

has both an *pseudo* axial and an *pseudo* equatorial hydroxy group). The stereochemical outcome of these reactions is consistent with previous observations that dihydroxylations under Upjohn's conditions generally proceed *anti* to a preexisting allylic oxygen substituent (Figs. 2 and 3).[21]

Donohoe's dihydroxylation reaction,[22] in which the TMEDA–OsO_4 complex is delivered to the reacting double bond by an allylic hydroxy group, was an extremely valuable alternative to Upjohn's conditions. Hence, simply by changing the reagent used at the final step of the divergent synthesis, two further diastereoisomers could be prepared (see Fig. 2). In the case of **11**, both of its *pseudo* axial hydroxy groups delivered the reagent to the alkene, giving **12CC** as a single diastereoisomer. More remarkably, however, was the reaction of the unsymmetrical diol **14** under Donohoe's conditions; **14** was elaborated in a two-directional fashion such that the stereochemical outcome of dihydroxylation was different in each of the rings. The *pseudo* axial hydroxy group directed the reagent to the alkene as before but the *pseudo* equatorial hydroxy group was unable to deliver the reagent, resulting in *anti*-selective dihydroxylation (⟶ **12AC**) as before (see Figs. 2 and 3). This behaviour has been noted previously in the synthesis of some monosaccharide derivatives.[23] The stereochemical outcomes of two-directional double dihydroxylation of the diol **14** are summarised in Fig. 4.

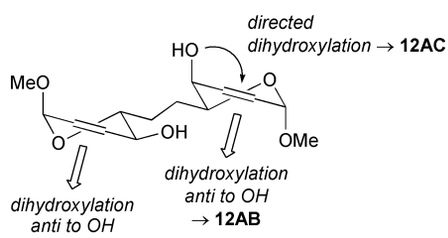


Fig. 4

The relative configuration of the products was determined using a combination of three approaches: (a) analysis of the coupling constants around the six-membered rings, (b) the observation of nuclear Overhauser enhancements, and (c) comparison of the ^{1}H spectra of compounds containing rings with the same relative configuration. Details of the ^{1}H NMR spectra of the hexaacetates **12** and related compounds are summarised in Table 2, and important NOE observations are shown in Figs. 5–7.

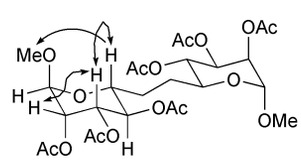


Fig. 5

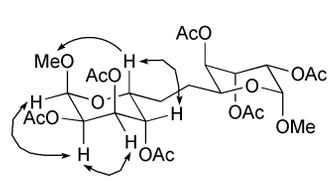
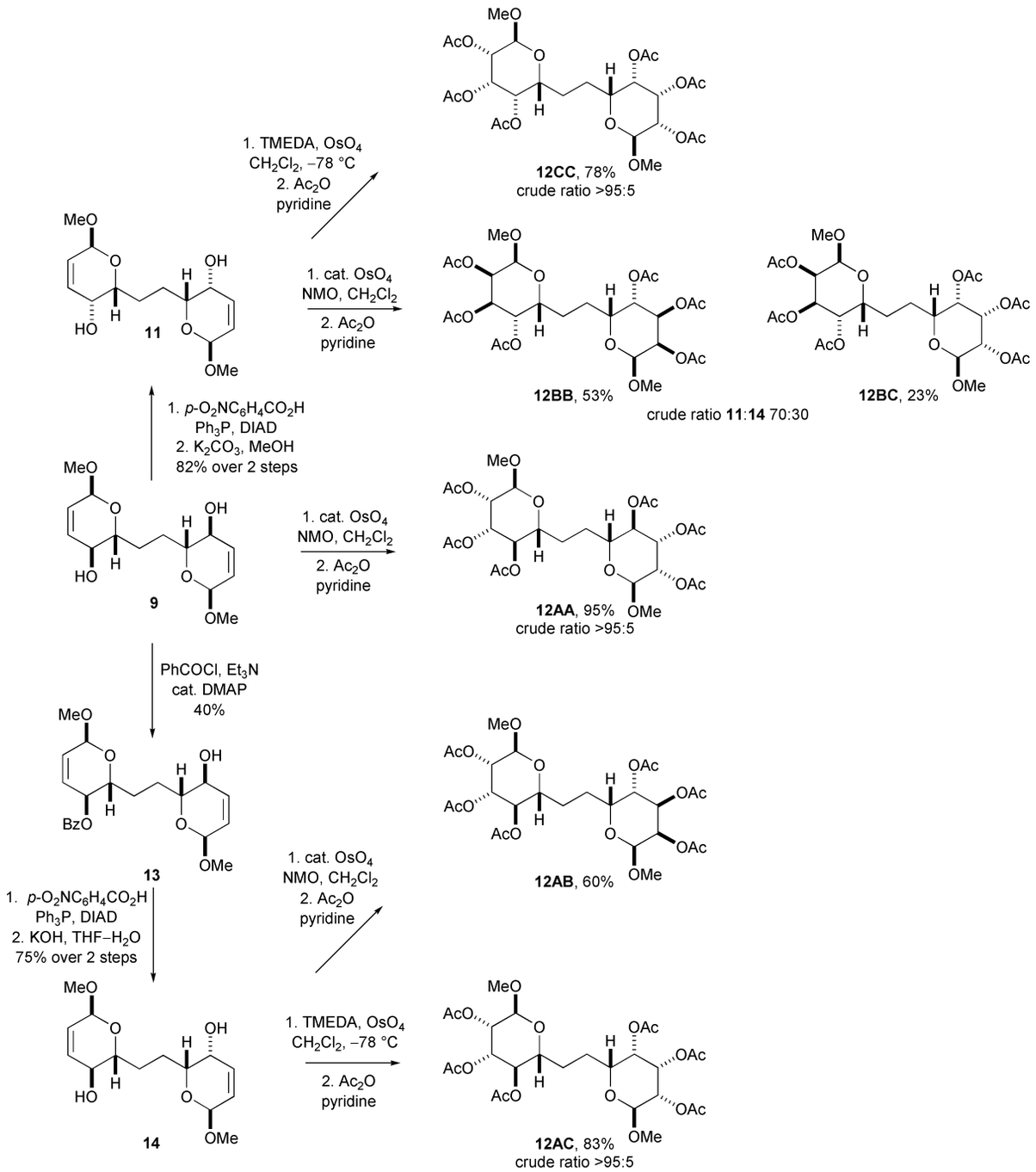


Fig. 6



Scheme 4

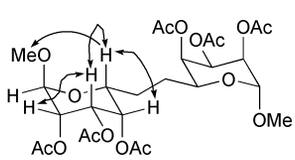


Fig. 7

have led to the formation of a mixture of esters **17** and **18**. Hydrolysis of the esters **17** and **18** with aqueous potassium hydroxide solution, and ring-closure, would give the corresponding epoxide (see Scheme 6); opening of this epoxide by hydroxide ion (conformation **19** ⟶ **12A′E** and conformation **20** ⟶ **12A′D**), and peracetylation, gave a 75 : 25 mixture of diastereoisomers. Purification by preparative HPLC gave the hexaacetates **12A′D** and **12A′E** in 64% and 11% yield respectively.

In an unsuccessful desymmetrisation reaction, the *meso* diol **21** was treated with OsO_4·**22** complex (Scheme 7). Double dihydroxylation of **21**, *anti* to each hydroxy group, followed by peracetylation of the products, gave the *meso* hexaacetate **12AA′** in 40% yield.

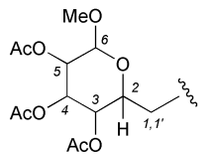
Synthetic transformations of desymmetrised dihydroxylation products

We have described some desymmetrisation reactions in which compounds similar to **6** (Scheme 2) were prepared in moderate to good enantiomeric excess;[19] stereoselective functionalisation of the remaining double bond in these compound would lead to mimetics **2XY′** (Scheme 2). For example, acetylation of the diol **15** (which had 60% ee) gave **16**, which was treated with iodine and silver benzoate in dry carbon tetrachloride (Scheme 5); this reaction, by analogy with the transformation of similar model compounds under identical reaction conditions, is presumed to

Comparison of the mimetics 2 with disaccharides: conformation of the rings and the linking chain

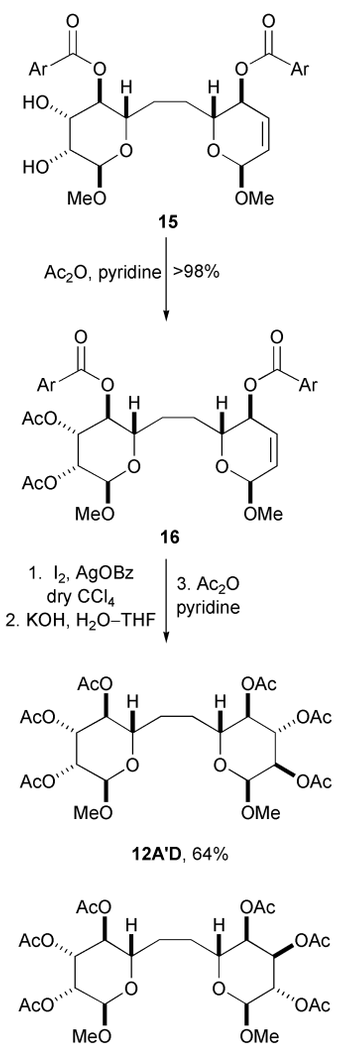
The mimetics **2** are *C*-linked analogues of (1⟶6)-linked disaccharides in which the free *C*-6 has been replaced by a

Table 2 Coupling constants and chemical shifts of the hexaacetates **12**



Compound	ring	$J(H^2H^{1'})$/ Hz	$J(H^2H^3)$/ Hz	$J(H^3H^4)$/ Hz	$J(H^4H^5)$/ Hz	$J(H^5H^6)$/ Hz	^4J/Hz	δ (H^2)- (ppm)	δ (H^3)- (ppm)	δ (H^4)- (ppm)	δ (H^5)- (ppm)	δ (H^6)- (ppm)
12AA	A	[a], 10.0	10.0	10.0	3.5	1.7	—	3.77	5.12	5.28	5.22	4.66
12AB	A	2.9, 9.6	9.6	9.5	3.4	1.6	—	3.71	5.10	5.28	5.22	4.65
	B	5.3, 7.6	2.3	3.6	4.1	4.0	—	4.14	4.93	5.20	5.16	4.81
12AC	A	2.9, 9.3	9.3	10.0	3.6	1.7	—	3.68	5.11	5.28	5.22	4.63
	C	[a], 7.3	[a],	3.8	3.8	1.5	[a], (H^3H^5)	3.95	5.21	5.25	5.08	4.74
12BB	B	[a], 9.1	1.3	3.8	3.8	3.8	—	4.17	4.92	5.21	5.16	4.81
12BC	B	[a], 9.0	1.3	3.8	3.8	3.9	—	4.18	4.91	5.20	5.14	4.84
	C	[b],	[a],	3.9	3.9	1.7	1.7 (H^3H^5)	3.91	5.18	5.26	5.06	4.72
12CC	C	[b],	[a],	3.9	3.9	1.4	1.4 (H^3H^5)	3.91	5.18	5.25	5.08	4.74
12A′D	A	1.9, 9.7	9.7	10.0	3.5	1.7	—	3.71	5.09	5.28	5.23	4.63
	D	2.2, 9.6	9.6	9.6	10.1	3.7	—	3.76	4.84	5.43	4.83	4.89
12A′E	A	2.1, 9.8	9.8	10.0	3.6	1.5	—	3.72	5.10	5.29	5.24	4.58
	E	3.1, 9.5	9.5	3.7	3.7	1.7	—	4.04	4.98	5.19	4.94	4.65
12AA′	A	[a], 8.9	10.0	10.0	3.5	1.7	—	3.72	5.10	5.28	5.23	4.64

[a] Broad peak: small coupling constant(s) not measured [b] Not determined.

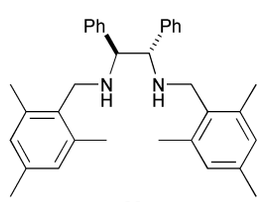


Scheme 5

methoxy group. These compounds may be divided into two groups according to the 1,4 stereochemical relationship between the THP ring systems (compounds **2XY** and

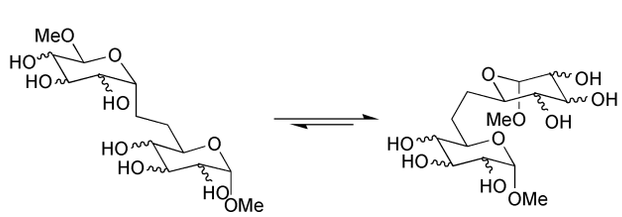
Table 3 Classification of *C*-linked disaccharide mimetics **2**

Compound	Parent disaccharide(s)
2AA	L-Gal-β(1⟶6)-D-Man
2BB	L-Alt-β(1⟶6)-D-Gul
2CC	L-Tal-β(1⟶6)-D-Tal
2BC	L-Alt-β(1⟶6)-D-Tal or L-Tal-β(1⟶6)-D-Gul
2AB	L-Alt-β(1⟶6)-D-Man or L-Gal-β(1⟶6)-D-Gul
2AC	L-Tal-β(1⟶6)-D-Man or L-Gal-β(1⟶6)-D-Tal
2AA′	D-Gal-β(1⟶6)-D-Man
2A′D	L-Gal-β(1⟶6)-L-Glu or L-Glu-β(1⟶6)-L-Man
2A′E	L-Gal-β(1⟶6)-L-Alt or L-Gul-β(1⟶6)-L-Man
2CC′	D-Tal-β(1⟶6)-D-Tal



22

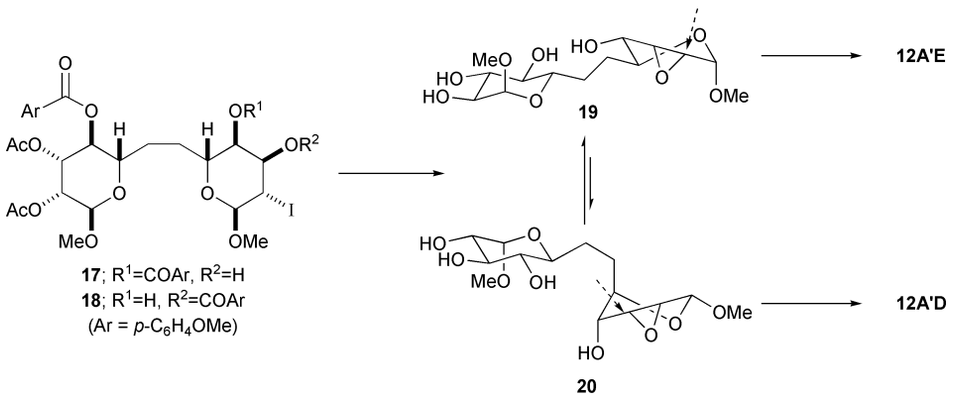
compounds **2XY′**; Scheme 2). The relationship between the analogues **2** and the disaccharides which they mimic are summarised in Table 3. Compounds **2XY** are likely to predominantly populate conformation **24** which resembles a β-linked disaccharide formed from a D and an L sugar (Fig. 8).‡ Higher lying conformations such as **23**, which resembles an α(1⟶6)-linked disaccharide formed from two D sugars, can be stabilised by complexation to a carbohydrate receptor.[12] Compounds



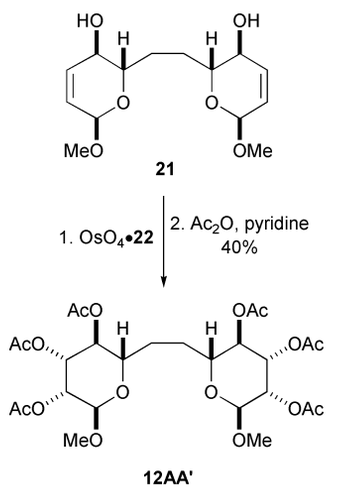
23 **24**

Fig. 8

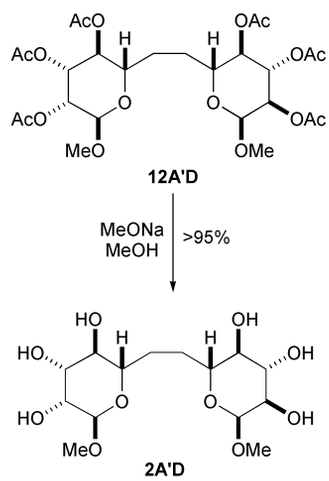
‡ Analysis of the coupling constants of the hexaacetates **12** (Table 2) indicates that the protected mimetics exhibit this conformational behaviour.



Scheme 6



Scheme 7



Scheme 8

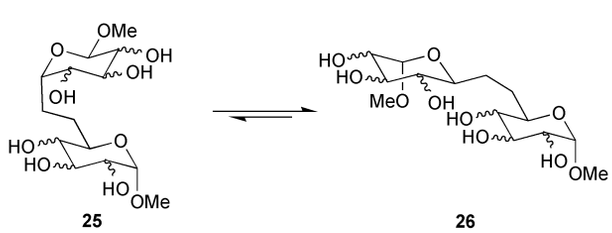


Fig. 9

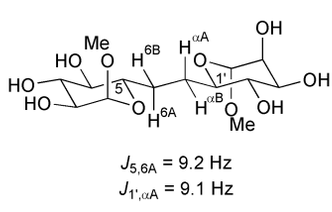
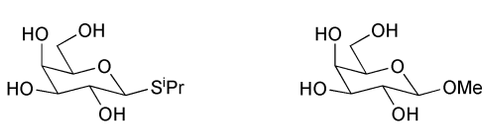
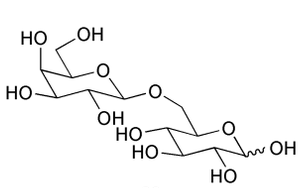


Fig. 10





2XY′, on the other hand, presumably populate the conformation **26** which resembles a β(1⟶6)-linked disaccharide formed from two D sugars (Fig. 9).

The mimetic **2A′D** was synthesised by deprotection of the hexaacetate **12A′D** (Scheme 8); some selected coupling constants for this compound are summarised (Fig. 10).§ We conclude that **2A′D** exists predominantly in the $C_{2'}$–$C_{1'}$–C_{α}–C_6–C_5–C_4 extended form shown in Fig. 10. In order to minimise 1,3-interactions, the central C_{α}–C_6 bond is antiperiplanar to *C*–*C* bonds in both pyanose rings, adopting the *exo*-anomeric conformation relative to the galactose ring. The enantiomeric compound **2AD′** may be considered to be a *C*-linked analogue of the disaccharide, allolactose.

Evaluation of the hexaol 2AD′ as an allolactose mimetic

The ability of the hexaol **2AD′** to control repression by LacI was investigated to evaluate its capacity to mimic allolactose.¶ The LacI repression system is used widely in molecular genetics to control the expression of heterologous genes. The product of the *lacI* gene is able to bind an operator sequence in DNA, thereby blocking transcription from an overlapping promoter; this repression can be inactivated by the binding of allolactose to another site in the LacI protein resulting in the induction of transcription. The product of the *lacZ* gene, β-galactosidase, is commonly used as a reporter of gene expression as its activity can be readily assayed *in vivo* and *in vitro*.

We had prepared a sample of **2A′D** which had 60% ee; this sample was, therefore, an 80 : 20 mixture of the enantiomeric mimetics **2A′D** and **2AD′**. However, a limited quantity (<1 mg)

§ In Table 4, conventional disaccharide numbering is used, with primed numbers being used to describe positions in the galactose ring.

¶ It has previously been suggested that *C*-linked analogues of allolactose may also suppress repression of the lactose (*lac*) operon (refs. 8*a* and 9).

of material was available and a sensitive assay needed to be developed. A two-gene system was used to assess the ability of **2AD′** to induce the *lac* promoter relative to that of the commonly used inducer isopropyl-β-thiogalactoside (IPTG, **27**). In this system, LacI represses the expression of the essential *rne* gene[25] that itself represses expression of a β-galactosidase-based reporter construct. This assay is highly sensitive, for example, to micromolar concentrations of IPTG: the higher the concentration of the inducer, the lower the level of β-galactosidase activity observed. The results of this assay, in which the biological activities of IPTG and **2AD′** are directly compared, are given in Fig. 11.

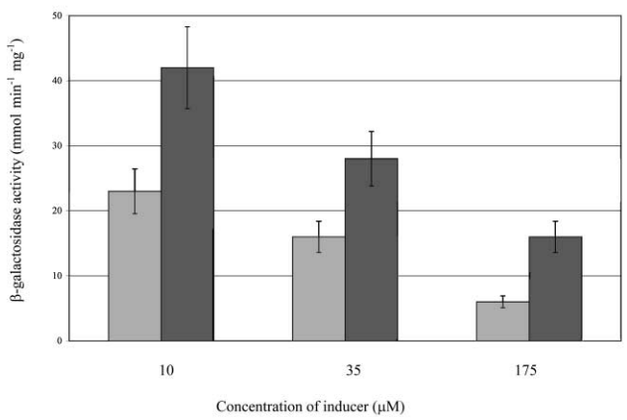


Fig. 11 Assay of inactivation of LacI-mediated repression. The light grey columns correspond to IPTG whereas the dark grey correspond to **2A′D** (which had 60% ee and was, therefore, an 80 : 20 mixture of **2A′D** and **2A′D**). Each measurement was made in triplicate. Bars indicate the standard error of each value.

The hexaol **2AD′** was found to be an inducer of the *lac* promoter since increasing its concentration resulted in decreasing levels of β-galactosidase activity. A concentration of 175 μM of **2A′D** resulted in the same level of β-galactosidase activity as 35 μM of the inducer IPTG: since the sample of **2A′D** used had 60% ee, this corresponds to a 35 μM concentration of the *C*-linked allolactose mimetic **2AD′**. The mimetic **2AD′** and IPTG may, therefore, bind with similar affinity to the repressor protein LacI.

The free energies of binding of many inducers to the *lac* repressor protein, LacI, have been compared, and the contribution of some hydroxy groups has been estimated.[26] The binding constants, K_d, for the inducers **27**–**29** are: IPTG (**27**) 0.83 μM; methyl β-galactoside (**28**) 100 μM; and allolactose (**29**) 0.59 μM. The *C*-linked allolactose mimetic **2AD′**, which we estimate to have a K_d similar to IPTG, has some important structural differences compared to allolactose: the THP which mimics the galactose ring of allolactose lacks a 6-hydroxy group and has an additional axial methoxy group at *C*-5. In view of the fact that the 6-hydroxy group of the galactose ring has been estimated to contribute *ca.* 7 kJ mol^{-1} to the binding energy, the potency of the mimetic **2AD′** is remarkable. It is clear that **2AD′** binds LacI at least 10 kJ mol^{-1} more tightly than does methyl β-galactoside (**28**): this indicates that the interactions of both of THP rings with LacI contribute significantly to the binding energy. We conclude that **2AD′** is a very good mimic of the disaccharide allolactose.

Summary

Efficient two-directional synthetic methods have been developed for the preparation of some *C*-linked disaccharide analogues **2**. The approach is unusual in that neither of the sugar rings derived directly from a sugar,‖ a strategy which is particularly valuable in the synthesis of analogues of unusual sugars. The methods developed were particularly suited to the synthesis of a library of stereoisomeric compounds, since diastereomeric analogues could be prepared by minor variation of a common reaction sequence. In total, ten mimetics were prepared from a virtual library of 136 compounds (see Table 1 for details). Furthermore, an additional eight mimetics could have been synthesised simply by using the enantiomeric ligand in the enantioselective step. The reliability of the methods[16] which we have developed for the functionalisation of polyhydroxylated THPs should mean that a wide range of other stereoisomeric mimetics **2** could be prepared using this general approach.

The analogue **2AD′** was found to be a good mimic of the disaccharide allolactose: **2AD′** was estimated to bind the repressor protein, LacI, with similar affinity to IPTG and only slightly less tightly (*ca.* 1 kJ mol^{-1}) than allolactose itself. The polyols **2**, therefore, represent a new and interesting class of *C*-linked disaccharide mimetics with promising, though largely unstudied, biological activity. Stereisomeric mimetics **2**, including analogues of unnatural disaccharides, may be synthesised efficiently in a two-directional manner from a common precursor and may prove to be valuable tools for glycobiology.

Experimental

General experimental methods have been previously described.[16,19] Optical rotations are given in 10^{-1} deg cm^{-2} g^{-1} and *J* values are given in Hz.

(1*R*,4*R*)-1,4-Di-furan-2′-ylbutane-1,4-diol 4

To a 1 M solution of (*S*)-1-methyl-3,3-diphenyltetrahydro-3*H*-pyrrolo[1,2-*c*][1,3,2]oxazaborole **10** in toluene (0.83 ml, 0.83 mmol) at ambient temperature was added a 1 M solution of borane–tetrahydrofuran complex in tetrahydrofuran (1.66 ml, 1.66 mmol). After 10 minutes a solution of the diketone **7** (1.83 g, 8.32 mmol) in tetrahydrofuran (15 ml) and a 1 M solution of borane–tetrahydrofuran complex in tetrahydrofuran (8.32 ml, 8.32 mmol) was added simultaneously over 30 minutes and the reaction mixture was left to stir at ambient temperature. After 2 hours methanol (30 cm^3) was added and the volatiles were removed under reduced pressure to give a crude product which was purified by flash column chromatography, eluting with 1 : 1 petrol–ethyl acetate to give the *diol*. Analysis of the crude reaction mixture by 75 MHz ^{13}C NMR spectroscopy revealed it to be an 85 : 15 mixture of diastereoisomers. Crystallisation of the *meso* isomer from chloroform, followed by evaporation of the filtrate under reduced pressure, afforded the *diol* (1.48 g, 80%) as a colourless oil, R_f 0.43 (1 : 1 EtOAc–Petrol); $[a]_D^{20}$ +21.8 (*c* 1.1 in $CHCl_3$); (Found: C, 64.6; H, 6.65; $C_{12}H_{14}O_4$ requires C, 64.9; H, 6.35%); ν_{max}/cm^{-1} (thin film) 3356, 2928, 1505, 1149, 1066, 1009, 738, 599; δ_H (300 MHz; d_6-DMSO) 7.52 (2H, d, *J* = 1.7, 5′-H), 6.31 (2H, dd, *J* = 3.2 and 1.7, 4′-H), 6.14 (2H, d, *J* = 3.2, 3′-H), 5.22 (2H, d, *J* = 5.4, OH), 4.48–4.40 (2H, m, 1-H and 4-H), 1.77–1.50 (4H, m, 2-H$_2$ and 4-H$_2$); δ_C (75 MHz; $CDCl_3$) 158.4 (C=O), 141.9, 110.4, 105.5, 66.0 and 32.1; *m*/*z* (EI) 222 (14%, M$^+$), 205 (80, M$^+$ − OH), 187 (18), 137 (100) and 110 (29).

1,2-Bis[(2*R*)-6-hydroxy-3-oxo-6*H*-pyran-2-yl]ethane

To a solution of the diol (*R*,*R*)-**4** (775 mg, 3.49 mmol) in dichloromethane (30 cm^3) was added a 4.6 M solution of *tert*-butyl hydroperoxide (1.90 ml, 8.72 mmol) in toluene and vanadium(III) acetylacetonate (46 mg, 0.17 mmol). After 6 hours a white precipitate was observed which was collected by filtration to give the *dipyranone* (842 mg, 95%) as colourless prisms as a 75 : 25 mixture of anomers, mp 164.1–165.8 °C (from MeOH); R_f 0.58 (4 : 1 EtOAc–petrol); $[a]_D^{20}$ −50 (*c* 0.2 in MeOH); (Found: C, 56.5; H, 5.60; $C_{12}H_{14}O_6$ requires C, 56.7; H,

‖ Vogel has reported the use of a non-carbohydrate based template to introduce one of the rings of disaccharide mimetics.[24]

5.55%); ν_{max}/cm^{-1} (thin film) 3383 (OH), 1655, 1596, 1261 and 799; δ_H (300 MHz; d_6-DMSO) 7.28 (0.5H, d, J = 7.3, OH), 7.02 (1.5H, dd, J = 10.2 and 3.5, 5-H), 7.01 (0.5H, m, 5-H), 6.98 (1.5H, d, J 6.6, OH), 6.06 (0.5H, br d, J 10.2, 4-H), 5.98 (1.5H, d, J = 10.2, 4-H), 5.57 (0.5H, m, 6-H), 5.48 (1.5H, d, J = 9.4 and 3.5, 6-H), 4.50 (1.5H, br d, J = 8.4, 2-H), 4.40 (0.5H, br d, J = 6.6, 2-H), 2.00–1.81 (2H, m, CH_2) and 1.61–1.52 (2H, m, CH_2); δ_C (75 MHz; d_4-MeOH) 198.9^{maj}, 198.4^{min}, 151.6^{min}, 148.1^{maj}, 129.2^{min}, 127.6^{maj}, 92.3^{min}, 88.7^{maj}, 79.1^{maj}, 75.0^{min}, 26.7^{min} and 26.3^{maj}; *m*/*z* (EI) 236 (7%, M^{+-}CO), 151 (30), 123 (30), 110 (26), 95 (80), 85 (86), 84 (63), 55 (100) and 44 (63).

1,2-Bis[(2*R*,6*S*)-6-methoxy-3-oxo-6*H*-pyran-2-yl]ethane 8

To a solution of 1,2-bis[(2*R*)-6-hydroxy-3-oxo-6*H*-pyran-2-yl]-ethane (780 mg, 3.07 mmol) in dichloromethane (25 cm^3) at ambient temperature was added trimethylorthoformate (1.34 ml, 12.28 mmol) followed by boron trifluoride–diethyl ether (40 µl, 0.31 mmol). After 4 hours, water (20 cm^3) was added, the organic layer was removed and the aqueous layer was extracted with dichloromethane (3 × 15 cm^3). The combined organics were washed with brine (50 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product. Analysis of the crude reaction mixture by 300 MHz ^{1}H NMR spectroscopy revealed a 75 : 25 mixture of diastereoisomers. Purification by flash column chromatography, eluting with 4 : 1 petrol–ethyl acetate gave the *di-acetal* **8** (649 mg, 75%; >90 : 10 mixture of diastereoisomers) as colourless needles, mp 79.6–81.1 °C (from EtOAc); R_f 0.3 (4 : 1 EtOAc–Petrol); $[\alpha]_D^{20}$ +15.7 (*c* 0.4 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2924, 1695 (C=O), 1100 and 1046; δ_H (300 MHz; $CDCl_3$) 6.86 (2H, dd, J = 10.3 and 3.5, 5-H), 6.09 (2H, d, J = 10.3, 4-H), 5.12 (2H, d, J = 3.5, 6-H), 4.45 (2H, br d, J = 8.2, 2-H), 3.52 (6H, s, OCH_3), 2.23–2.05 (2H, m, CH_2) and 1.95–1.75 (2H, m, CH_2); δ_C (75 MHz; $CDCl_3$) 147.0, 128.1, 94.5, 73.8, 57.0 and 25.5 (one peak missing); *m*/*z* 305 (ES) (100%, MNa$^+$); (Found: 305.0993; $C_{14}H_{18}O_6$ requires *MNa*, 305.1001).

1,2-Bis[(2*R*,3*S*,6*S*)-3-hydroxy-6-methoxy-2,3-dihydro-6*H*-pyran-2-yl]ethane 9

To a solution of the di-acetal **8** (250 mg, 0.89 mmol; 75 : 25 mixture of diastereoisomers) in dichloromethane (5 cm^3) at −78 °C was added a solution of cerium(III) chloride heptahydrate (727 mg, 1.95 mmol) in methanol. After stirring for 10 minutes sodium borohydride (70 mg, 1.86 mmol) was added. The reaction was left for a further 30 minutes and quenched by addition of water (10 cm^3). The organic layer was removed and the aqueous layer was extracted with dichloromethane (3 × 15 cm^3). The combined organics were washed with brine (20 cm^3), dried ($MgSO_4$) and evaporated under pressure to give the crude product as a mixture of diastereoisomers which were purified by flash column chromatography, eluting with 4 : 1 ethyl acetate–petrol to give the *diol* **9** (190 mg, 75%) as colourless needles, mp 169.1–170.3 °C; R_f 0.3 (4 : 1 EtOAc–petrol); $[\alpha]_D^{20}$ +76.3 (*c* 0.8 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 3321 (OH), 1403, 1186, 1041 and 970; δ_H (300 MHz; $CDCl_3$) 5.88 (2H, d, J = 10.0, 4-H), 5.68 (2H, dt, J = 10.0 and 2.3, 5-H), 4.79 (2H, br s, 6-H), 3.86 (2H, br d, J = 9.0, 3-H), 3.53 (6H, br t, J = 9.0, 2-H), 3.38 (6H, s, OCH_3), 2.00–1.85 (2H, m, CH_2) and 1.78–1.64 (2H, m, CH_2); δ_C (75 MHz; $CDCl_3$) 134.2, 126.8, 95.7 (6-C), 71.8, 68.4, 56.4 (CO*C*H$_3$) and 28.2; *m*/*z* (ES) 309 (100 %); (Found: 309.1328; $C_{14}H_{22}O_6$ requires [*MNa*], 309.1314).

Acetic acid (2*R*,3*R*,4*R*,5*R*,6*S*)-4,5-diacetoxy-6-methoxy-2-[2′-((2′*R*,3′*R*,4′*R*,5′*R*,6′*S*)-3′,4′,5′-triacetoxy-6′-methoxy-tetrahydropyran-2′-yl)-ethyl]tetrahydropyran-3-yl ester 12AA

To a solution of the diol **9** (117 mg, 0.41 mmol) in acetone–water (4 : 1, 3 cm^3) at 0 °C was added 4-methylmorpholine *N*-oxide (138 mg, 1.02 mmol) followed by osmium tetraoxide (10 mg, 0.04 mmol). The reaction mixture was allowed to warm to ambient temperature. After 24 hours a saturated aqueous solution of sodium sulfite (1 cm^3) was added and the reaction mixture was evaporated under reduced pressure. Pyridine (1 cm^3) and acetic anhydride (1 cm^3) were added to the residue and the reaction was left to stir for 24 hours at ambient temperature and then evaporated under reduced pressure. The crude product was purified by flash column chromatography, eluting with 4 : 1 petrol–ethyl acetate to give the *hexaacetate* **12AA** (236 mg, 95%) as a colourless oil, R_f 0.32 (3 : 2 petrol–EtOAc); $[\alpha]_D^{20}$ +28.4 (*c* 0.3 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2925, 1751, 1373, 1224, 1134, 1085 and 1050; δ_H (300 MHz; $CDCl_3$) 5.28 (2H, d, J = 10.0 and 3.5 4-H), 5.22 (2H, dd, J = 10.0 and 1.7, 5-H), 5.12 (2H, t, J = 10.0, 3-H), 4.66 (2H, d, J = 1.7, 6-H), 3.77 (2H, br t, J = 10.0, 2-H), 3.39 (6H, s, OCH_3), 2.15 (6H, s, Ac), 2.03 (6H, s, Ac), 1.98 (6H, s, Ac) and 1.82–1.62 (4H, m, CH_2); δ_C (75 MHz; $CDCl_3$) 170.5, 170.4, 170.3, 98.7 (6-C), 70.1, 70.0, 69.8, 69.1, 55.6 (OCH_3), 26.6 (CH_2), 21.3, 21.2 and 21.1; *m*/*z* (ES) 629 (100, MNa$^+$); (Found: 629.2079; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082).

The relative stereochemistry was determined using a series of NOE experiments.

1,2-Bis[(2*R*,3*R*,6*S*)-3-hydroxy-6-methoxy-2,3-dihydro-6*H*-pyran-2-yl]ethane 11

To a solution of triphenylphosphine (303 mg, 1.15 mmol) in tetrahydrofuran (2 cm^3) at 0 °C was added diisopropyl azodicarboxylate (0.18 ml, 1.15 mmol). After stirring for 60 minutes a creamy white precipitate was observed. A solution of the diol **9** (110 mg, 0.38 mmol) and *p*-nitrobenzoic acid (193 mg, 1.15 mmol) in tetrahydrofuran (2 cm^3) was added to the reaction mixture and left to stir at 0 °C for 1 hour and then ambient temperature for 1 hour. The resulting homogeneous solution was evaporated under reduced pressure and purified by flash column chromatography eluting with 5 : 1 petrol-ethyl acetate to give the *dibenzoate* as a mixture of products. The crude product was dissolved in methanol–tetrahydrofuran (1 : 1, 2 cm^3) and anhydrous potassium carbonate was added and the reaction mixture was left to stir at ambient temperature for 16 hours. The solids were removed by filtration and the volatiles were removed *in vacuo* to afford the crude product which was purified by flash column chromatography, eluting with 4 : 1 ethyl acetate–petrol to give the *diol* **11** (236 mg, 95%)*as colourless needles*, mp 148.2–150.1 °C (EtOAc–petrol); R_f 0.35 (9 : 1 EtOAc–petrol); $[\alpha]_D^{20}$ −73.3 (*c* 0.3 in $CHCl_3$), ν_{max}/cm^{-1} (thin film) 3336 (OH), 2922, 1655, 1405, 1186, 1079, 1041 and 969; δ_H (300 MHz; $CDCl_3$) 6.12 (2H, ddd, J = 10.0, 5.6 and 0.8, 4-H), 5.84 (2H, dd, J = 10.0, and 3.1, 5-H), 4.82 (2H, d, J = 3.1, 6-H), 3.95–3.88 (2H, m, 2-H), 3.70–3.62 (2H, m, 3-H), 3.44 (3H, s, OCH_3) and 1.99–1.66 (4H, m, CH_2CH_2); δ_C (75 MHz; $CDCl_3$) 130.4, 128.7, 95.8 (C-6), 70.5, 63.2, 56.0 (OMe) and 26.7 (*C*H$_2$); *m*/*z* (ES) 629 (100, MNa$^+$); (Found: 629.2085; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082).

Acetic acid (2*R*,3*R*,4*R*,5*R*,6*S*)-4,5-diacetoxy-6-methoxy-2-[2′-((2′*R*,3′*R*,4′*R*,5′*R*,6′*S*)-3′,4′,5′-triacetoxy-6′-methoxy-tetrahydropyran-2′-yl)ethyl]tetrahydropyran-3-yl ester 12BB

To a solution of the diol **11** (6 mg, 0.02 mmol) in acetone–water (4 : 1, 1 cm^3) at 0 °C was added 4-methylmorpholine *N*-oxide (9 mg, 0.07 mmol) followed by osmium tetraoxide (0.6 mg, 0.002 mmol). The reaction mixture was allowed to warm to ambient temperature. After 24 hours a saturated aqueous solution of sodium sulfite (1 cm^3) was added and the reaction mixture was evaporated under reduced pressure. Pyridine (1 cm^3) and acetic anhydride (1 cm^3) were added to the residue and the reaction was left to stir for 24 hours at ambient temperature and then evaporated under reduced pressure. The crude product was purified by flash column chromatography, eluting with 4 : 1 petrol–ethyl acetate to give the *hexaacetate* **12BB** (6 mg, 53%) as a colourless oil, R_f 0.38 (4 : 1 EtOAc–petrol); $[\alpha]_D^{20}$ +25.7

(*c* 0.4 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2920, 1749 (C=O), 1654, 1224 and 1046; δ_H (500 MHz; $CDCl_3$) 5.21 (2H, t, *J* = 3.8, 4-H), 5.16 (2H, t, *J* = 3.8, 5-H), 4.92 (2H, dd, *J* = 3.8 and 1.3, 3-H), 4.81 (2H, d, *J* = 3.8, 6-H), 4.18–4.16 (2H, br d, *J* = 9.1, 2-H), 3.41 (3H, s, OCH_3), 2.14 (6H, s, Ac), 2.12 (6H, s, Ac), 2.08 (6H, s, Ac), 1.76–1.66 (2H, m, CH_2) and 1.54–1.44 (2H, m, CH_2); δ_C (126 MHz; $CDCl_3$) 170.1 (C=O), 169.7, 97.3 ($COCH_3$), 70.2, 66.9, 66.3, 64.9, 56.0 ($COCH_3$), 26.0, 21.0, 20.8 and 20.8 (Ac); *m*/*z* (ES) 629 (100, MNa^+); (Found: 629.2083; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082).

The relative stereochemistry was determined using a series of NOE experiments.

Also obtained was the *hexaacetate* **12BC** (2.9 mg, 23%) as a colourless oil, R_f 0.34 (4 : 1 EtOAc–petrol); $[\alpha]_D^{20}$ +10.1 (*c* 0.2 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2920, 1749 (C=O), 1654, 1224 and 1046; δ_H (500 MHz; $CDCl_3$) 5.26 (1H, t, *J* = 3.9, 4′-H), 5.20 (1H, t, *J* = 3.8, 4-H), 5.18 (1H, br d, *J* = 3.9, 3′-H), 5.14 (1H, t, *J* = 3.9, 5-H), 5.06 (1H, dt, *J* = 3.9 and 1.7, 5′-H), 4.91 (1H, dd, *J* = 3.8 and 1.3, 3-H), 4.84 (1H, d, *J* = 3.9, 6-H), 4.72 (1H, d, *J* = 1.7, 6′-H), 4.18 (1H, br d, *J* = 9.0, 2-H), 3.91 (1H, m, 2′-H), 3.40 (3H, s, OCH_3), 3.30 (3H, s, OCH_3), 2.14 (3H, s, Ac), 2.12 (3H, s, Ac), 2.09 (3H, s, Ac), 2.08 (6H, s, Ac), 1.92 (6H, s, Ac), 1.88–1.77 (2H, m, CH_2) and 1.59–1.47 (2H, m, CH_2); δ_C (126 MHz; $CDCl_3$) 170.1 (C=O), 169.7, 97.3 ($COCH_3$), 70.2, 66.9, 66.3, 64.9, 56.0 ($COCH_3$), 26.0, 21.0, 20.8 and 20.8 (Ac); *m*/*z* (ES) 629 (100, MNa^+); (Found: 629.2082; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082).

The relative stereochemistry was determined using a series of NOE experiments.

Acetic acid (2*R*,3*R*,4*S*,5*S*,6*S*)-4,5-diacetoxy-6-methoxy-2-[2′-((2′*R*,3′*R*,4′*S*,5′*S*,6′*S*)-3′,4′,5′-triacetoxy-6′-methoxytetrahydropyran-2′-yl)ethyl]tetrahydropyran-3-yl ester 12CC

To a solution of the diol **11** (4 mg, 0.014 mmol) in dichloromethane (1 cm^3) at −78 °C was added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (6.3 µl, 0.04 mmol) followed by osmium tetraoxide (11 mg, 0.04 mmol). The resulting homogeneous orange solution was left to stir at −78 °C for 2 hours. The reaction mixture was then allowed to warm to ambient temperature and evaporated under reduced pressure . A saturated aqueous solution of sodium sulfite (1 cm^3) was added and the mixture was heated at reflux for 4 hours then evaporated under reduced pressure. Pyridine (1 cm^3) and acetic anhydride were added to the residue and the reaction was left to stir for 24 hours at ambient temperature and then evaporated under reduced pressure. The crude product was purified by flash column chromatography, eluting with 7 : 3 ethyl acetate–petrol to give the *hexaacetate* **12CC** (7 mg, 78%) as a colourless oil, R_f 0.62 (9 : 1 EtOAc–petrol); $[\alpha]_D^{20}$ +33.7 (*c* 0.3 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2924, 1742 (C=O), 1374, 1256, 1226, 1131 and 1073; δ_H (300 MHz; $CDCl_3$) 5.25 (2H, t, *J* = 3.9, 4-H), 5.18 (2H, br d, *J* = 3.9, 3-H), 5.08 (2H, dt, *J* = 3.9 and 1.4, 5-H), 4.74 (2H, d, *J* = 1.4, 6-H), 3.91 (2H, m, 2-H), 3.30 (3H, s, OCH_3), 2.09 (6H, s, Ac), 2.08 (6H, s, Ac), 1.92 (6H, s, Ac), 1.88–1.77 (2H, m, CH_2) and 1.59–1.47 (2H, m, CH_2); δ_C (125 MHz; $CDCl_3$) 170.4 (C=O), 170.1, 169.7, 99.4, 68.3, 68.2, 67.9, 55.3, 26.3, 21.0, 20.7 and 20.5 ($C{=}OCH_3$); *m*/*z* (ES) 629 (100, MNa^+); (Found: 629.2079; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082). The relative stereochemistry was determined using a series of NOE experiments.

Benzoic acid (2*R*,3*R*,6*S*)-2-{2-[(2′*R*,3′*S*,6′*S*)-3′-hydroxy-6′-methoxy-2′,3′-dihydro-6*H*-pyran-2′-yl]ethyl}-6-methoxy-3,6-dihydro-2*H*-pyran-3-yl ester 13

To a solution of the diol **9** (285 mg, 1.00 mmol) in dichloromethane (4 cm^3) at 0 °C was added benzoyl chloride (0.13 ml, 1.10 mmol), triethylamine (0.31 ml, 2.19 mmol) and 4-dimethylaminopyridine (12 mg, 0.10 mmol). After 4 hours, water (10 cm^3) was added, the organic layer was removed and the aqueous layer was extracted with ethyl acetate (3 × 15 cm^3). The combined organics were washed with brine (20 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give the crude product which was purified by flash column chromatography, eluting with 1 : 1 petrol–ethyl acetate, to give the *alcohol* **13** (155 mg, 40%) as a colourless oil, R_f 0.40 (1 : 1 petrol–EtOAc); $[\alpha]_D^{20}$ +111 (*c* 1.5 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 3447 (OH), 2932, 1719 (C=O), 1266, 1110, 1045, 964 and 731; δ_H (300 MHz; $CDCl_3$ 7.97 (2H, dd, *J* = 8.2 and 1.1, Ar*H*), 7.52 (1H, tt, *J* = 7.5 and 1.1, Ar*H*), 7.38 (2H, t, *J* = 7.5, Ar*H*), 5.94–5.77 (3H, m, 4-H, 5-H and 4′-H), 5.69 (1H, dt, *J* = 10.2 and 2.4, 5′-H), 5.38 (1H, br d, *J* = 9.1, 3-H), 4.88 (1H, br s, 6-H), 4.73 (1H, br s, 6′-H), 3.95 (1H, m, 2-H), 3.90 (1H, m, 2′-H), 3.53 (6H, br t, *J* = 9.1, 3′-H), 3.43 (3H, s, OCH_3), 3.26 (3H, s, OCH_3) and 2.05–1.64 (4H, m, CH_2); δ_C (75 MHz; $CDCl_3$) 166.4, 134.2, 133.3, 130.3, 130.1, 128.9, 128.2, 127.2, 127.0, 95.8, 95.6, 71.4, 70.5, 68.4, 68.3, 56.5, 56.3, 28.0 and 27.7; *m*/*z* 391 (M^+ + H); (Found: MH^+ 391.1766; $C_{21}H_{26}O_7$ requires *MH*, 391.1756).

(2*R*,3*R*,6*S*)-2-{2-[(2′*R*,3′*S*,6′*S*)-3′-hydroxy-6′-methoxy-2′,3′-dihydro-2*H*-pyran-2′-yl]ethyl}-3-hydroxy-6-methoxy-3,6-dihydro-2*H*-pyran 14

To a solution of triphenylphosphine (113 mg, 0.43 mmol) in tetrahydrofuran (1 cm^3) at 0 °C was added diisopropylazodicarboxylate (85 µl, 0.43 mmol). After stirring for 60 minutes a creamy white precipitate was observed. A solution of the diol **13** (67 mg, 0.17 mmol) and *p*-nitrobenzoic acid (72 mg, 0.43 mmol) in tetrahydrofuran (0.5 cm^3) was added to the reaction mixture and left to stir at 0 °C for 1 hour and then ambient temperature for 2 hours. The resulting homogeneous solution was evaporated under reduced pressure and purified by flash column chromatography eluting with 4 : 1 petrol–ethyl acetate to give a crude product. The crude product was dissolved in methanol–tetrahydrofuran (1 : 1, 1 cm^3) and anhydrous potassium carbonate (94 mg, 0.68 mmol) was added and the reaction mixture was left to stir at ambient temperature for 20 hours. The solids were removed by filtration and the volatiles were removed *in vacuo* to afford the crude product which was purified by flash column chromatography, eluting with 9 : 1 ethyl acetate–petrol to give *diol* **14** (36 mg, 75%) as colourless needles, mp 95.3–97.1 °C; R_f 0.32 (9 : 1 EtOAc–petrol); $[\alpha]_D^{20}$ −24.2 (*c* 1.0 in $CHCl_3$), ν_{max}/cm^{-1} (thin film) 3395 (OH), 2929, 1398, 1187, 1040 and 964; δ_H (300 MHz; $CDCl_3$) 6.19 (2H, dd, *J* = 10.0 and 5.6, 4′-H), 5.95 (1H, br d, *J* = 10.4, 4-H), 5.89 (1H, dd, *J* = 10.0, and 3.1, 5′-H), 5.76 (1H, dt, *J* = 10.0 and 2.6, 5-H), 4.90–4.82 (2H, m, 6-H and 6′-H), 4.02–3.93 (2H, m, 2-H and 2′-H), 3.72–3.58 (2H, m, 3-H and 3′-H), 3.45 (6H, s, OCH_3) and 2.08–1.59 (4H, m, CH_2CH_2); δ_C (75 MHz; $CDCl_3$) 134.1, 130.7, 128.6, 126.8, 95.8 ($CHOCH_3$), 95.7($CHOCH_3$), 71.8, 70.7, 68.2, 63.5, 56.3($CHOCH_3$), 56.1($CHOCH_3$), 28.1 (CH_2) and 26.7 (CH_2); *m*/*z* (%) 277 (33), 100 (100), 71 (40), 57 (29); (Found: 286.1422; $C_{14}H_{22}O_6$ requires *M*, 286.1416).

Acetic acid (2*R*,3*S*,4*S*,5*S*,6*S*)-4,5-diacetoxy-6-methoxy-2-[2′-((2′*R*,3′*R*,4′*R*,5′*R*,6′*S*)-3′,4′,5′-triacetoxy-6′-methoxytetrahydropyran-2′-yl)ethyl]tetrahydropyran-3-yl ester 12AB

To a solution of the diol **14** (4 mg, 0.014 mmol) in acetone–water (4 : 1, 1 cm^3) at 0 °C was added 4-methylmorpholine *N*-oxide (6 mg, 0.04 mmol) followed by osmium tetraoxide (0.4 mg, 0.001 mmol). The reaction mixture was allowed to warm to ambient temperature. After 48 hours a saturated aqueous solution of sodium sulfite (1 cm^3) was added and the reaction mixture was evaporated under reduced pressure. Pyridine (1 cm^3) and acetic anhydride (1 cm^3) were added to the residue and the reaction was left to stir for 24 hours at ambient temperature and then evaporated under reduced pressure. The crude product was purified by flash column chromatography, eluting with 1 : 1 petrol–ethyl acetate to give the *hexaacetate* **12AB** (5 mg, 60%) as a colourless oil, R_f 0.14 (3 : 2 petrol–

EtOAc); $[a]_D^{20}$ + 65 (*c* 0.3 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2922, 1749 (C=O), 1374, 1222 and 1046; δ_H (500 MHz; $CDCl_3$) 5.28 (1H, dd, *J* = 9.5 and 3.4, 4-H), 5.22 (1H, dd, *J* = 3.4 and 1.6, 5-H), 5.20 (1H, app br t, *J* = 3.8, 4′-H), 5.16 (1H, app t, *J* = 4.1, 5′-H), 5.10 (1H, app t, *J* = 9.6, 3-H), 4.93 (1H, dd, *J* = 3.6 and 2.3, 3′-H), 4.81 (1H, d, *J* = 4.0, 6′-H), 4.65 (1H, d, *J* = 1.6, 6-H), 4.14 (1H, ddd, 7.6, 5.3 and 2.3, 2′-H), 3.71 (1H, td, *J* = 9.6 and 2.9, 2-H), 3.35 (3H, s, OC*H*$_3$), 3.31 (3H, s, OC*H*$_3$), 2.08 (3H, s, Ac), 2.07 (3H, s, Ac), 2.06 (3H, s, Ac), 2.01 (3H, s, Ac), 1.98 (3H, s, Ac), 1.92 (3H, s, Ac) and 1.77–1.44 (4H, m, CH_2); δ_C (125 MHz; $CDCl_3$) 170.2, 170.1, 170.0, 170.0, 169.8, 169.7 (C=O), 98.5, 97.3 (*C*OCH$_3$), 70.0, 69.7, 69.7, 69.5, 69.2, 66.9, 66.3, 64.7, 56.0, 55.1 (OC*H*$_3$), 27.0, 25.6 (C*H*$_2$), 21.0, 20.9, 20.9, 20.8, 20.7 and 20.7 (C=O*C*H$_3$); *m*/*z* (ES) 629 (100, MNa^+); (Found: 629.2080; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082).

Acetic acid (2*R*,3*S*,4*S*,5*S*,6*S*)-4,5-diacetoxy-6-methoxy-2-[2′-((2′*R*,3′*R*,4′*S*,5′*S*,6′*S*)-3′,4′,5′-triacetoxy-6′-methoxytetrahydropyran-2′-yl)ethyl]tetrahydropyran-3-yl ester 12AC

To a solution of the diol **14** (8 mg, 0.03 mmol) in dichloromethane (0.5 cm^3) at −78 °C was added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (12.7 μl, 0.04 mmol) followed by osmium tetraoxide (21.3 mg, 0.08 mmol). The resulting homogeneous orange solution was left to stir at −78 °C for 4 hours. The reaction mixture was then allowed to warm to ambient temperature and evaporated under reduced pressure. A saturated aqueous solution of sodium sulfite (1 cm^3) was added and the mixture was heated at reflux for 4 hours then evaporated under reduced pressure. Pyridine (1 cm^3) and acetic anhydride were added to the residue. The reaction was left to stir for 24 hours at ambient temperature and then evaporated under reduced pressure. The crude product was purified by flash column chromatography, eluting with 1 : 1 ethyl acetate–petrol to give the *hexaacetate* **12AC** (15 mg, 83%) as a colourless oil, R_f 0.27 (1 : 1 EtOAc–petrol); $[a]_D^{20}$ +25.0 (*c* 0.2 in $CHCl_3$); ν_{max}/cm^{-1} (thin film) 2922, 1750 (C=O), 1374, 1223, 1132 and 1083; δ_H (500 MHz; $CDCl_3$) 5.28 (1H, dd, *J* = 10.0 and 3.6, 4-H), 5.25 (1H, t, *J* = 3.8, 4′-H), 5.22 (1H, dd, *J* = 3.6 and 1.7, 5-H), 5.21 (1H, br d, *J* = 3.8, 3′-H), 5.11 (1H, m, 3-H), 5.08 (1H, m, 5′-H), 4.74 (1H, d, *J* = 1.5, 6′-H), 4.63 (1H, d, *J* = 1.7, 6-H), 3.95 (1H, app br t, *J* = 7.5, 2′-H), 3.68 (1H, td, *J* 9.3 and 2.9, 2-H), 3.39 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 2.15 (6H, s, Ac), 2.14 (3H, s, Ac), 2.04 (3H, s, Ac), 1.98 (6H, s, Ac) and 1.67–1.45 (4H, m, C*H*$_2$); δ_C (126 MHz; $CDCl_3$) 170.4 (C=O), 170.1, 170.1, 170.0, 169.6, 99.4, 98.4, 69.7, 69.6, 69.4, 69.2, 68.2, 67.9, 67.5, 66.0, 55.4, 55.2, 29.7, 26.7, 26.3, 21.0, 21.0, 20.7 and 20.6 (C=O*C*H$_3$); *m*/*z* (ES) 629 (100, MNa^+); (Found: 629.2086; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082).

Acetic acid (2*R*,3*R*,4*S*,5*S*,6*S*)-4,5-diacetoxy-6-methoxy-2-[2′-((2′*S*,3′*S*,4′*R*,5′*S*,6′*R*)-3′,4′,5′-triacetoxy-6′-methoxytetrahydropyran-2′-yl)ethyl]tetrahydropyran-3-yl ester 12A′D

The diacetate **16** (18 mg, 0.027 mol) was dried azeotropically from toluene and dissolved in dry carbon tetrachloride. To the stirred solution under N_2 freshly prepared and azeotropically dried silver benzoate (30.7 mg, 0.134 mmol) was added, followed by iodine (17.0 mg, 0.134 mmol). The suspension was stirred at room temperature with protection from light for 4 days. The suspension was diluted with chloroform (10 ml) and the silver residues removed by centrifuge. The residue was washed with a further portion of chloroform (5 ml) and the combined organic extracts washed successively with saturated aqueous sodium bicarbonate (2 × 5 ml), 10% aqueous sodium sulfite (2 × 5 ml), followed by brine (5 ml). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was dissolved in potassium hydroxide solution (2.0 M, 3 ml), stirred at room temperature for 2 h, and refluxed for 2 days. The solution was evaporated to dryness under reduced pressure and the residue dissolved in acetic anhydride–pyridine (2 : 1, 3 ml). After stirring at room temperature under N_2 for 6 h, the reagents were removed by evaporation under reduced pressure. The residue was pre-absorbed onto silica gel and purified by flash column chromatography (gradient elution: 3 : 7⟶1 : 1 EtOAc–petrol) to give the *hexaacetate* **12A′D** (11.4 mg, 72%, **12A′D** : **12A′E** 75 : 25). The mixture was purified by HPLC (gradient elution: 95 : 5⟶85 : 15 hexane–isopropanol, 24 °C, λ_{max} 225 nm) to give the *hexaacetate* **12A′D** (10.1 mg, 64%) as a colourless viscous oil, retention time 23.8 min, $[a]_D$ = −20.6 (*c* = 0.253, $CHCl_3$); R_f 0.21 (3 : 7 EtOAc–petrol); ν_{max}/cm^{-1} 2940, 1750 (C=O), 1442, 1371, 1246, 1224 and 1043; δ_H (500 MHz; $CDCl_3$) 5.43 (1H, dd, *J* = 10.1 and 9.6, 4′-H), 5.28 (1H, dd, *J* = 10.0 and 3.5, 4-H), 5.23 (1H, dd, *J* = 3.5 and 1.8, 5-H), 5.09 (1H, t, *J* = 9.7, 3-H), 4.89 (1H, d, *J* = 3.7, 6′-H), 4.84 (1H, t, *J* = 9.6, 3′-H), 4.83 (1H, dd, *J* = 10.1 and 3.7, 5′-H), 4.63 (1H, d, *J* = 1.7, 6-H), 3.76 (1H, td, *J* = 9.6 and 2.2, 2′-H), 3.71 (1H, td, *J* = 9.7 and 1.9, 2-H), 3.37 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 2.14 (3H, s, OAc), 2.07 (3H, s, OAc), 2.05, (3H, s, OAc), 2.03 (3H, s, OAc), 2.00 (3H, s, OAc), 1.98 (3H, s, OAc), 1.78 (2H, m, CH_2) and 1.50–1.44 (2H, m, CH_2); *m*/*z* (ES) 629 (100, MNa^+); (Found: 629.2083; $C_{26}H_{38}O_{18}$ requires *MNa*, 629.2082).

Also obtained was the *hexaacetate* **12A′E** (1.7 mg, 11%) as a colourless viscous oil, retention time 22.8 min, $[a]_D$ = −2.73 [*c* = 0.078, $CHCl_3$]; R_f 0.21 (3 : 7 EtOAc–petrol); δ_H (300 MHz; $CDCl_3$) 5.29 (1H, dd, *J* = 10.0 and 3.6, 4-H), 5.24 (1H, dd, *J* = 3.6 and 1.7, 5-H), 5.19 (1H, t, *J* = 3.7, 4′-H), 5.10 (1H, t, *J* = 9.8, 3-H), 4.98 (1H, dd, *J* = 9.2 and 3.6, 3′-H), 4.94 (1H, dd, *J* = 3.8 and 1.5, 5′-H), 4.65 (1H, d, *J* = 1.7, 6-H), 4.58 (1H, d, *J* = 1.5, 6′-H), 4.04 (1H, td, *J* = 9.5 and 3.1, 2′-H), 3.72 (1H, td, *J* = 9.8 and 2.1, 2-H), 2.15 (3H, s, OAc), 2.12 (3H, s, OAc), 2.10, (3H, s, OAc), 2.04 (3H, s, OAc), 1.99, (3H, s, OAc), 1.98 (3H, s, OAc), 1.78 (2H, m, CH_2) and 1.50–1.44 (2H, m, CH_2).

(2*R*,3*R*,4*S*,5*S*,6*S*)- 6-Methoxy-2-[2′-((2′*S*,3′*S*,4′*R*,5′*S*,6′*R*)-3′,4′,5′-trihydroxy-6′-methoxytetrahydropyran-2′-yl)ethyl]-tetrahydropyran-3,4,5-triol

Sodium methoxide (1 mg) was added to a stirred solution of allolactose mimetic [X] (0.29 mg) in dry methanol (0.4 ml) and the solution stirred for 7 days at room temperature under nitrogen. The solvent was removed under reduced pressure and the residue dissolved in D_2O and stirred for 30 min with pre-activated Dowex-15 (0.4 mg). After filtration the solution was evaporated to dryness and the residue re-dissolved in D_2O. δ_H (500 MHz; D_2O) 4.63 (1H, d, *J* = 1.6, 6-H′), 4.63 (1H, d, *J* = 3.8, 6-H), 3.92 (1H, dd, *J* = 3.4 and 1.6, 5-H′), 3.70 (1H, dd, *J* = 9.1 and 3.4, 4-H′), 3.61 (1H, t, *J* = 9.2, 3-H), 3.59 (1H, br t, *J* = 9.1, 2-H′), 3.56 (1H, dd, *J* = 9.2 and 3.8, 5-H), 3.49 (1H, t, *J* = 9.2, 4-H), 3.48 (1H, t, *J* = 9.1, 3-H′), 3.33 (3H, s, OMe), 3.31 (3H, s, OMe), 3.22 (1H, br t, *J* = 9.2, 2-H), 2.21 (2H, m) and 1.51 (2H, m); δ_C (125 MHz; D_2O) 101.2, 99.5, 73.6, 73.5, 72.6, 71.8, 71.7, 70.9, 70.8, 70.3, 55.4, 55.1, 27.9 and 27.2; *m*/*z* (ES) 377.2 (100, MNa^+).

Assay of inactivation of LacI-mediated repression

The strain used for this assay was ENS2401 (Δ*lac12*, *malP*-pΔ534::P$_{rne}$-*rne*-*lacZ*-*lacY*-TerT7-Ter*trp*, P*lac*-*rne,zce*-726::-*Tn10, recA*::*cat*), which was kindly provided by Dr Marc Dreyfus (Paris). This strain contains the *rne* gene under the control of the P$_{lac}$ promoter[27] and the *ez1* reporter construct.[28] A 5 ml aliquot of Luria broth (Sigma) was inoculated with scrapings from a frozen glycerol stock of ENS2401 and incubated at 37 °C with shaking until the culture reached an optical density (600 nm) of 0.4. It was then placed in an ice–water bath for 15 min and stored overnight at 4 °C. 100 μl Aliquots of Luria broth containing 10, 35 and 175 μM of the allolactose or IPTG were inoculated with 1 μl of the overnight culture and incubated at 37 °C with shaking (225 rpm). Each culture condition was set up in triplicate. When the OD_{600} of the cultures was

between 0.4 to 0.6, the cultures were placed in an ice–water bath for 15 min before the cells were harvested by centrifugation (13,000 × *g* for 1 min at 4 °C). The cells were resuspended in 500 µl of Z buffer[28] and lysed by brief sonication using an MSE instrument equipped with a microtip (10 s, 6 microns peak to peak). The cell debris was pelleted by centrifugation (13,000 × *g* for 10 min) and the supernatant transferred to a new tube. β-Galactosidase activity in each cell extract was measured by a modification of the method of Miller.[29] Briefly, 200 µl of cell extract was added to a 1 ml cuvette containing 800 µl of 1 mg ml^{-1} *o*-nitrophenyl-β-D-galactopyranoside (ONPG, an chromogenic substrate) that had been recently dissolved in Z buffer and equilibrated to 37 °C. The content was mixed by repeated pipetting and the cuvette placed immediately in a temperature-controlled spectrophotometer at 37 °C. The rate of formation of ONP was determined by continuously measuring the increase in absorbance at 420 nm over a period of 10 min. To control for differences in cell growth and lysis, the amount of protein in 200 µl aliquots of each cell extract was determined using a kit (Bio-Rad) that followed the method of Bradford.[30] The activity of β-galactosidase in the extracts was expressed as the mmoles of ONP formed per min per mg of total protein.

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