The syntheses of 6-*C*-alkyl derivatives of methyl α-isomaltoside for a study of the mechanism of hydrolysis by amyloglucosidase

Ulrike Spohr, Nghia Le, Chang-Chun Ling, and Raymond U. Lemieux

Abstract: The epimeric (6a*R*)- and (6a*S*)-*C*-alkyl (methyl, ethyl and isopropyl) derivatives of methyl α-isomaltoside (**1**) were synthesized in order to examine the effects of introducing alkyl groups of increasing bulk on the rate of catalysis for the hydrolysis of the interunit α-glycosidic bond by the enzyme amyloglucosidase, EC 3.2.1.3, commonly termed glucoamylase (AMG). It was previously established that methyl (6a*R*)-*C*-methyl α-isomaltoside is hydrolysed about 2 times faster than methyl α-isomaltoside and about 8 times faster than its *S*-isomer. The kinetics for the hydrolyses of the ethyl and isopropyl analogs were also recently published. As was expected from molecular model calculations, all the *R*-epimers are good substrates. A rationale is presented for the catalysis based on conventional mechanistic theories that includes the assistance for the decomposition of the activated complex to products by the presence of a hydrogen bond, which connects the 4a-hydroxyl group to the tryptophane and arginine units. It is proposed that activation of the initially formed complex to the transition state is assisted by the energy released as a result of both of the displacement of perturbed water molecules of hydration at the surfaces of both the polyamphiphilic substrate and the combining site and the establishment of intermolecular hydrogen bonds, i.e., micro-thermodynamics. The dissipation of the heat to the bulk solution is impeded by a shell of aromatic amino acids that surround the combining site. Such shields are known to be located around the combining sites of lectins and carbohydrate specific antibodies and are considered necessary to prevent the disruption of the intermolecular hydrogen bonds, which are of key importance for the stability of the complex. These features together with the exquisite stereoelectronic dispositions of the reacting molecules within the combining site offer a rationalization for the catalysis at ambient temperatures and near neutral pH. The syntheses involved the addition of alkyl Grignard reagents to methyl 6-aldehydo-α-D-glucopyranoside. The addition favoured formation of the *S*-epimers by over 90%. Useful amounts of the active *R*-isomers were obtained by epimerization of the chiral centers using conventional methods. Glycosylation of the resulting alcohols under conditions for bromide-ion catalysis, provided methyl (6a*S*)- and (6a*R*)-*C*-alkyl-hepta-*O*-benzyl-α-isomaltosides. Catalytic hydrogenolysis of the benzyl groups afforded the desired disaccharides. ^{1}H NMR studies established the absolute configurations and provided evidence for conformational preferences.

Key words: amyloglucosidase (AMG), *exo*-anomeric effect, 6-*C*-alkyl-α-D-glucopyranosides and isomaltosides, mechanism of enzyme catalysis.

Résumé : On a réalisé la synthèse des dérivés (6a*R*) et (6a*S*)-*C*-alkyles (méthyle, éthyle et isopropyle) de l'α-isomaltoside de méthyle (**1**) afin d'étudier les effets provoqués par l'introduction de groupements alkyles de plus en plus volumineux sur la catalyse de l'hydrolyse de la liaison glycosidique α par l'enzyme amyloglucosidase, EC 3.2.1.3, que l'on dénomme souvent glucoamylase (AMG). Il a été établi antérieurement que le (6a*R*)-*C*-méthyl-α-isomaltoside de méthyle s'hydrolyse environ deux fois plus rapidement que l'α-isomaltoside de méthyle et environ huit fois plus rapidement que son isomère *S*. Les mesures cinétiques pour les hydrolyses des analogues éthyle et isopropyle ont aussi été publiées récemment. Tel qu'on pouvait s'y attendre sur la base de calculs sur des modèles moléculaires, tous les épimères *R* sont de bons substrats. On propose une rationalisation de la catalyse qui est basée sur la théorie mécanistique conventionnelle mais qui, pour la décomposition du complexe activé en produits, implique une aide apportée par la présence dans le complexe d'une liaison hydrogène reliant le groupe 4a-hydroxyle aux unités de tryptophane et d'arginine qui se trouvent loin du centre réactionnel. Il est suggéré que le passage du complexe qui se forme initialement à état de transition se produit avec l'aide de l'énergie libérée en raison à la fois du déplacement des molécules d'eau d'hydratation perturbées sur les surfaces tant du substrat polyamphiphile que du site qui se combine ainsi ce que celle qui résulte de la création de liaisons hydrogènes intermoléculaires, de la microthermodynamique. On indique qu'il y a eu empêchement à la dispersion de la chaleur vers l'ensemble du milieu réactionnel causé par une sorte de coquille

Received June 16, 2000. Published on the NRC Research Press Web site on March 16, 2001.

U. Spohr[1], N. Le[2], C.-C. Ling, and R.U. Lemieux.[3] Department of Chemistry, The University of Alberta, Edmonton, AB T6G 2G2, Canada.

[1]Corresponding author (e-mail: uspohr@aol.com).
[2]Taken in part from a Ph.D. Thesis, University of Alberta, 1990.
[3]Deceased.

 DOI: 10.1139/cjc-79-2-238

formée d'acides aminés aromatiques qui entoure le site où se produit la combinaison. De tels coquilles ont déjà été observées autour des sites où se produit la combinaison des lectines et des anticorps spécifiques aux hydrates de carbone et ils sont considérés comme nécessaires pour empêcher l'interruption des liaisons hydrogènes d'importance capitale à la stabilité du complexe. Ces caractéristiques ainsi que les dispositions stéréoélectroniques exquises des molécules qui réagissent à l'intérieur du site où se produit la combinaison permettent de rationaliser la catalyse à la température ambiante et à un pH pratiquement neutre. Les synthèses impliquent l'addition des réactifs de Grignard alkylés sur le 6-aldéhydo-α-D-glucopyranoside de méthyle. L'addition conduit à la formation préférentielle (90%) des épimères *S*. On a obtenu des quantités utiles des isomères actifs, *R*, par épimérisation des centres chiraux à l'aide de méthodes conventionnelles. La glycosylation des alcools qui en résultent, dans des conditions de catalyse par l'ion bromure, a permis d'obtenir les (6a*R*) et (6a*S*)-*C*-alkyl-*O*-benzyl-α-isomaltosides de méthyle. L'hydrogénolyse catalytique des groupes benzyles fournit ensuite les disaccharides désirés. Des études de RMN du ^{1}H ont permis de déterminer les configurations absolues et ont permis d'obtenir des données relatives aux préférences conformationnelles.

Mots clés : amyloglucidase (AMG), effet anomère *exo*, α-D-glucopyranosides et isomaltosides 6-*C*-alkylés, mécanisme de catalyse enzymatique.

[Traduit par la Rédaction]

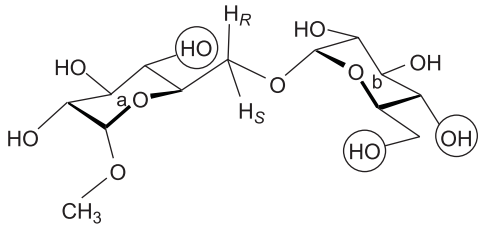
Nomenclature and conventions: The nomenclature follows the general rules of organic chemistry. Thus, the introduction of a methyl group at *C*-6 of methyl α-D-glucopyranoside gives methyl (6*R*)-*C*-methyl-α-D-glucopyranoside not methyl 7-deoxy-β-L-glycero-D-gluco-heptopyranoside. For reasons of clarity and convenience, the so-called "reducing unit" of a disaccharide is termed the *a*-unit. The nonreducing unit is the *b*-unit. The 6a-epimers are given the same compound number but distinguished by their absolute configurations (*R* or *S*). The m, e and i letters that follow the number of a compound are to identify the alkyl substituent: methyl, ethyl or isopropyl, respectively. The rings of glucopyranosides are taken as residing in an essentially rigid $^4C_1$-conformation. The three flexible bonds that link the two glucose units at the 6a-position of an isomaltoside, namely, C-1b-O-6a, O-6a-C-6a and C-6a-C-5a, describe three torsion angles $\phi° =$ O-5b/C-6a, $\psi° =$ C-1b/C-5a, and $\omega° =$ O-6a/O-5a, respectively.

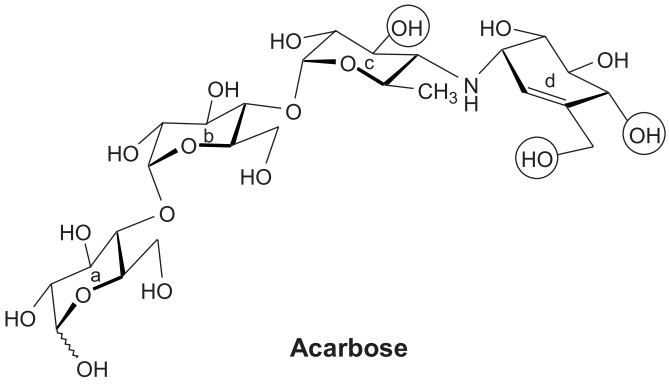
Introduction

The long known (1) industrially important enzyme, amyloglucosidase (EC 3.2.1.3), referred to herein as AMG, is an anomeric-centre inverting exoglycosidase that catalyzes the release of β-D-glucopyranose from the non-reducing end of starch and other related poly- and oligosaccharides (2, 3). AMG catalyzes the hydrolysis of the $1 \rightarrow 4$ linkage of maltose 30–50 times more rapidly than the $1 \rightarrow 6$ linkage of isomaltose. For both substrates three hydroxyl groups were found virtually indispensable, by means of chemical mapping (4, 5), for high enzyme activity. These essential hydroxyl groups circled in Fig. 1 for **1** are located at the 4a, 4b, and 6b positions for both the β- (5) and α- (6) methyl isomaltosides. For methyl β-maltoside these key hydroxyls are located at positions 3a, 4b, and 6b (4). As seen in Table 1, deoxygenation of the 4a-hydroxyl of methyl α-isomaltoside decreases the rate of hydrolysis by about a thousand-fold (6). It is conceivable that the enzyme recognizes the conformation of these substrates that have OH-4a of an isomaltoside (**1**) and OH-3a of a maltoside, which are in similar spatial relationship to the OH-4b and OH-6b of the nonreducing *b*-unit (5).

Chemical mapping (6) of the interaction of methyl α-isomaltoside with AMG proved to be in total agreement with both the kinetic data reported by Bock and co-workers (4, 5)

Fig. 1. The structure of methyl α-isomaltoside that displays the pro-*R* and pro-*S* hydrogen atoms at the *C*-6a position. Acarbose is a strong inhibitor ($\Delta G = -16.3$ kcal mol^{-1}) of the hydrolysis of **1** by the enzyme AMG.





and the X-ray crystal structure of the acarbose–AMG complex (7, 8). Substantially weaker interactions involve the hydroxyl groups at the 3a, 2b, and 3b positions (6). As expected from the X-ray crystal structure of the acarbose–AMG complex the OH-2a (see Table 1) and the aglycon at the 1a position remain in the aqueous phase. The fact that methyl (6a*R*)-*C*-methyl-α-isomaltoside (***R*-2m**) (9) is hydrolyzed 8 times faster than the *S*-epimer (***S*-2m**) suggests, as previously noted (9), that the *C*-methyl group of the *R*-isomer does not impede complex formation. Indeed, Frandsen et al. (10) found that the 6a-*C*-methyl group of ***R*-2m** projects into a surrounding aqueous phase to become hydrophobically associated to the enzyme. In the simulated complex of isomaltoside with AMG (6), it is the pro-*R* hydrogen that

Table 1. The effect of monodeoxygenations and *C*-alkylation on the kinetics for the hydrolysis of methyl α-isomaltoside catalyzed by AMG.

Compound	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (s^{-1} mM^{-1})	$\Delta\Delta G$ (kcal mol^{-1})
Methyl α-isomaltoside (**1**)[a]	0.88	20.0	4.4×10^{-2}	0
Methyl 2a-deoxy-α-isomaltoside[b]	0.5	19.1	2.6×10^{-2}	0.33
Methyl 4a-deoxy-α-isomaltoside[b]	0.00067	8.8	7.6×10^{-5}	4.02
Methyl 2b-deoxy-α-isomaltoside[b]	0.18	21.2	8.5×10^{-3}	1.04
Methyl (6a*R*)-*C*-methyl-α-isomaltoside[c] (***R*-2m**)	0.68	0.71	9.6×10^{-1}	–1.95
Methyl (6a*S*)-*C*-methyl-α-isomaltoside[c] (***S*-2m**)	1.1	90.0	1.2×10^{-2}	0.82
Methyl (6a*R*)-*C*-ethyl-α-isomaltoside[a] (***R*-2e**)	1.2	3.3	3.6×10^{-1}	–1.33
Methyl (6a*R*)-*C*-isopropyl-α-isomaltoside[a] (***R*-2i**)	0.8	47.0	1.7×10^{-2}	0.56

[a]Data from ref. 10.
[b]Data from ref. 6.
[c]Data from ref. 9.

Table 2. Calculated values for the most energetically favorable conformers of methyl α-isomaltoside and *C*-6a alkylated congeners.

Compound	Conformer[a]	Torsional angles for the 1 → 6 linkages (°)			*E*	$\Delta E_{B\text{-}A}$
		φ	ψ	ω	(kcal mol^{-1})	(kcal mol^{-1})
Methyl α-isomaltoside (**1**)	A	–51	–169	–60	–4.2	
	B	–45	167	163	–0.96	3.3
Methyl (6a*R*)-*C*-methyl-α-isomaltoside (***R*-2m)**	A	–54	89	52	–3.7	
	B	–45	167	163	–1.3	2.4
Methyl.(6a*R*)-*C*-isopropyl-α-isomaltoside (***R*-2i**)	A	–54	70	48	–5.8	
	B	–45	167	163	1.56	7.3

[a]Conformers A are the preferred conformations established by GEGOP calculation. These were found to not be compatible with the combining site of the acarbose–AMG complex. Consequently, the values of the φ, ψ, and ω torsion angles were changed in order to achieve a fit. An acceptable fit could not be achieved keeping the *b*-unit in the chair-conformation. However, a fine fit was obtained on changing the conformation of the *b*-unit to the half-chair conformer. The Δ*E* values refer to the differences in energy that are expected to arise from relaxation of the φ, ψ, and ω torsion angles of the B conformers to those of the A conformers.

projects into the channel of water, which in supports the statement that only the *R*-isomers are good substrates (10). Furthermore, this data suggests that a substantial body of bulk water extends from near the 6a-CH_2 group on the side of the complex that includes the catalytic water molecule. It was on this basis that it had seemed likely that the (6a*R*)-*C*-methyl group of ***R*-2m** could be replaced by bulkier groups and still retain good substrate activity. To test this hypothesis, the ***R*-** and ***S*-2e** (6a-*C*-ethyl) and ***R*-** and ***S*-2i** (6a-*C*-isopropyl) analogs were synthesized and made available to Frandsen et al. (10) for kinetic studies.

We considered that should these compounds (***R*-2e** and ***R*-2i**) prove to be good substrates for AMG that it would also follow that all are accepted into the active site with similar φ, ψ, and ω torsion angles for the inter-unit bonds. It was also thought that the effect of the bulky alkyl groups on the rate of hydrolysis could provide insight to the structure of the transition state since the bulkier groups can be expected to better define the conformational requirements for the inter-unit *C*-5a to *C*-1b linkages.

Discussion

Theoretical discussion

GEGOP calculations (11) were used to estimate the preferred conformations for compounds **1**, ***R*-2m**, and ***R*-2i**. The results are presented in Table 2 as conformers A (see also Fig. 6) and are compared against reference compound methyl α-isomaltoside (**1**). It is seen that the different alkylating groups do not have a large effect on the orientation of the agluconic carbon (*C*-6a) (φ torsion angles range from –51° to –54°). The conformational preferences for the more rigid compounds (***R*-2m** and ***R*-2i**) appear to be similar but are significantly different as compared to the global minimum energy conformation of **1**. This inference is supported by the nuclear Overhauser enhancement (NOE) data presented in Table 3. As expected from the GEGOP calculations (Table 2), saturation of H-1b enhanced the signal for H-6a almost equally for the three 6a-alkylated compounds. The NOE data for the *S*-isomers are also recorded in Table 3, but the *S*-isomers are not further discussed because of their poor performance as substrates for AMG (10).

Prior to a consideration of conformers B, a brief review, for the more salient features of the mechanism for the hydrolysis of an α-D-glucopyranoside by AMG, is useful. An outline is presented in Fig. 2.

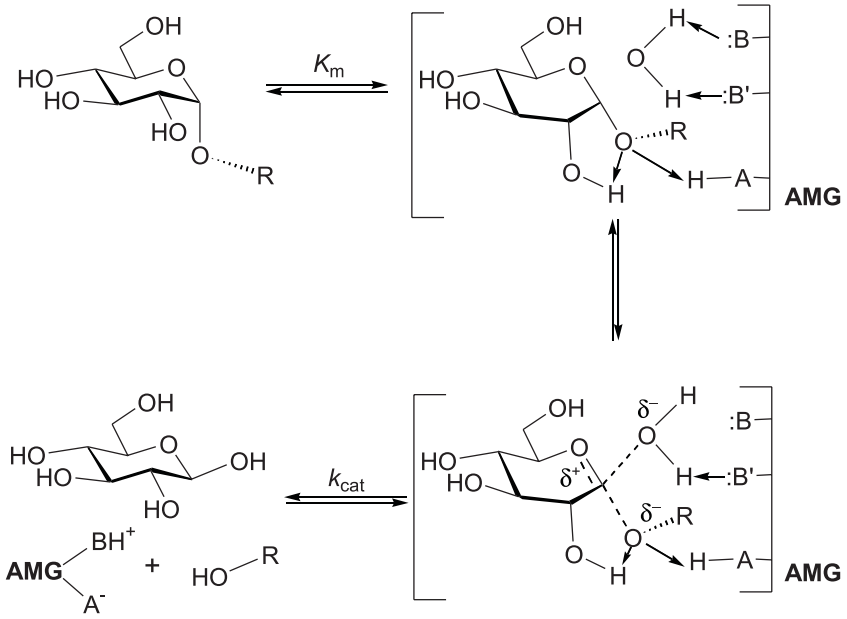
The mechanism (Fig. 2) has been established as an acid-base catalyzed reaction. The X-ray crystal structure of the acarbose–AMG complex (7, 8) displays a well situated "catalytic" water molecule for nucleophilic attack at the anomeric centre of the *b*-unit which results in the displacement of the *a*-unit (see Fig. 3). The nucleophilicity of this water molecule is enhanced by the involvement of both of its hydrogen atoms in hydrogen bonds with two oxygen atoms

Table 3. NOE of methyl 6-*C*-alkyl-α-isomaltosides (**2m**, **2e**, and **2i**).

Compound	6a-*C*-Substituent	Proton saturated	Proton enhancement (%)			
			H-1b	H-2b	H-6a*R*	H-6a*S*
***R*-2m**	CH_3-	H-1b	—	15.2	—	9.9
***R*-2m**	CH_3-	H-6a	10.8	—	—	—
***R*-2e**	CH_3H_2-	H-1b	—	13.2	—	8.7
***R*-2i**	$(CH_3)_2CH$-	H-1b	—	15.5	—	13.3[a]
***S*-2m**	CH_3-	H-1b	—	9.5	2.4	—
***S*-2e**	CH_3H_2-	H-1b	—	17.1	7.6	—
S-2i	$(CH_3)_2CH$-	H-1b	—	17.7	6.7	—

[a]Signal may contain small contributions from H-4, H-3 signals.

Fig. 2. Schematic of key features in the acid-base catalyzed hydrolysis of an α-D-glucopyranoside by the enzyme amyloglucosidase (AMG). The structural features of **1** that are involved in the formation of the complex are not shown.



(see Fig. 3), one with the carboxylate group of Glu 400 and the other with OH-6b. The OH-6b is also hydrogen bonded to the carboxylate of Asp 55 (7, 8). Experimental evidence in support of this type of delocalization of electron density in conjugated hydrogen bonds was established by Lemieux and Pavia (12) and now termed the "cooperativity effect" (13). These and other interactions expected to be present in the transition state were reviewed in a recent publication (6). The acid catalysis is promoted by proton donation from Glu 179 (Fig. 3), where the acidity of the carboxyl group of Glu 179 is expected to be enhanced by electron donation to a proton on Glu 124 (not shown in Fig. 3). It is to be noted (Table 1) that deoxygenation of OH-2b caused little change in the kinetic parameters. This suggestion that OH-2b is not involved in assisting the leaving of the *a*-unit must take into consideration that 2-deoxyglycosides are much more prone to hydrolysis when the 2-substituent is more electronegative, such as a hydroxyl group. Also, in the case of the 2b-deoxy compound, a water molecule may assist in relaying the electrophilicity of the Arg 305 to the 6a-oxygen atom (6). Certainly, a hydrogen bond between OH-2b and the leaving group can be expected to strengthen as the C-1b—O-6a bond is stretched to achieve the transition state.

The conformation of the valienamine moiety of acarbose (Fig. 1), as previously pointed out (6), provides an acceptable working model for the construction of the transition state where the C-5b, O-5b, C-1b, and C-2b atoms of the *b*-unit are coplanar and the oxygen of the catalytic water molecule, C-1b and the leaving O-6a atoms are colinear. Evidence in support of the structure of the transition state for inversions at anomeric centres was obtained in 1955 (14). The X-ray structure also can serve as a model for the structure of the complex displayed in Fig. 2. Although the mole fraction of the half-chair conformation for the *b*-unit of **1** is undoubtedly very small, its formation once **1** is complexed with AMG must be virtually spontaneous, especially since the active site conforms for the acceptance of acarbose. The B conformers listed in Table 2 are based on this consideration. Accordingly, it is expected that formation of the half-chair conformer will substantially weaken the *endo*- and *exo*-anomeric effects (15) and, thereby, render the anomeric centre more susceptible to nucleophilic attack.

As already mentioned, hydrogen bonding of OH-4a to Trp 178 (assisted by Arg 305) greatly enhances the rate of catalysis. Our attempts to establish these hydrogen bonds using both the *a*- and *b*-units in the $^4C_1$ chair conformation proved

unattractive. However, when the *b*-unit was placed in the half-chair conformation (see Table 2 and Figs. 3 and 6), the same hydrogen-bond networks presented in the acarbose–AMG complex (7, 8) are well established.

As seen in Table 2 the ϕ, ψ, and ω torsion angles for the *b*-unit are the same for each compound. However, large differences in conformational energy are indicated and, therefore, complex formation involving substrate ***R*-2m** should be slightly favored over complexation with **1** and much more than with substrate ***R*-2i**. As seen in Table 1, the kinetically determined K_m values are 20 (**1**), 0.7 (***R*-2m**), and 47 (***R*-2i**). The increase in complexation of AMG with ***R*-2m** as compared to AMG with **1** can be attributed to hydrophobic association of the 6a-C-methyl group with both Trp 120 and Trp 52, as shown in Fig. 4. This hydrophobic association is not present during formation of the **1**–AMG complex. Also, the conformational energy of ***R*-2m** in the complex is expected (Table 2) to be about 0.9 kcal mol^{-1} less than that for substrate **1**. These considerations support an increase in the rate of hydrolysis of ***R*-2m** (10). However, this is not the case for ***R*-2i**. As seen in Table 1, the equilibrium for complex formation (K_m) for ***R*-2i** is much less favorable than it is for ***R*-2m** (K_m = 0.71 for ***R*-2m** vs. 47 for ***R*-2i**). This difference is mainly due to the large conformational strain required to form the ***R*-2i**–AMG complex (Table 2). This resistance to complex formation is undoubtedly strongly moderated by important hydrophobic associations of the two methyl groups of the isopropyl group as is illustrated in Fig. 4*b*. Frandsen et al. (10) employed, in part, this synthetically imposed hydrophobic association in a study of site-specific mutagenic changes in the structure of AMG to conclude that "the versatility of AMG catalysis….emphasizes the significance of hydrophobic interactions in protein-carbohydrate complexation." We suggest that this is not necessarily the case with respect to the classical definition of the driving force for hydrophobic associations which involves the release of ordered water molecules to bulk. The strength of hydrophobic interactions was recently observed in the binding of *N*-acetyllactosamine by the lectin, *Erythrina corallodendron* (16). The X-ray crystal structure of the complex shows a strong association of the H-2, H-4, and H-5 on the α-side of the β-D-galactose unit with the appropriately positioned phenylalanine group. In this regard, although Monte Carlo simulations (17) for the hydration of the β-D-Gal unit of the Lewis b-OMe tetrasaccharide suggest that the formation of a shell of disordered perturbed water molecules extends over the hydrophobic α-side, some ordering of the water molecules cannot be discounted. In either event, the association of complementary surfaces should be energetically favorable in aqueous solution whether ordered or perturbed water molecules are released to bulk.

As seen in Table 2, the *b*-unit of the B conformers are highly strained. We suggest that the relaxation of this conformational strain energy provides the necessary assistance for the stretching of the C-1b—C-6a glycosidic bond and thereby promotes decomposition to the products. This structural feature is key for the catalysis of the hydrogen bonds of OH-4a to Trp 178 and Arg 305. Indeed, as previously noted (10), the k_{cat} values for **1**, ***R*-2m**, ***R*-2e**, and ***R*-2i** are remarkably similar.

Prior to a consideration of hydrophobic forces involved in the binding of **1** by AMG, it is of interest to examine the thermodynamics of the binding of the H-type 2-OMe trisaccharide by the lectin, *Ulex europaeus* (UE-1) (18, 19). The main driving force for binding is enthalpic, ΔH = –29 kcal mol^{-1}, which is countered by a large *decrease* in entropy, $T\Delta S$ = –20.5 kcal mol^{-1} (18). Inspection of the X-ray crystal structure for the UE-I–H-type 2-OMe complex reveals, as seen in Fig. 5, that two hydrophobic interactions are present: one involves Tyr 220 with the C-6 methylene group of the GlcNAc unit and the other involves valine 134 with the *C*-6 methyl group of the α-L-Fuc unit. These interactions, if truly hydrophobic in character, are obviously not compatible with the aforesaid thermodynamic parameters that show a decrease in entropy rather than an increase in entropy as expected for hydrophobic association. The proximity of these groups to the trisaccharide requires the displacement of water. Judging from the thoroughly polyamphiphilic surface (20) of the H-type 2-OMe molecule (19), perturbed water molecules must have been displaced, as in the case of Tyr 220. The interaction of Val 134 with the *C*-6-methyl group of the fucosyl group may result in an increase in entropy from the disordering of water molecules in an endothermic process. However, these contributions, if present, are strongly obscured by the large decreases in both enthalpy and entropy that are expected to arise mainly from the return of perturbed water molecules from both the combining site and the epitope of the trisaccharide to bulk (21, 22). This process has been termed the *hydraphobic* effect.

The binding of **1** with AMG is similar in that along with the establishment of numerous attractive polar interactions one hydrophobic group comes into close contact with disaccharide **1**. As seen in Fig. 4, the only nonpolar contact of less than 2.5 Å (omitting the interactions with the *R*-6a-methyl and *R*-6a-isopropyl groups) is between Trp 317 and H-2b. The presence of this large aromatic amino acid unit in the epitope is expected to contribute to the stability of the complex, since, along with the release of the ordered water molecules at its surface, it is also expected to participate in the release of perturbed water molecules from the polyamphiphilic surface of the substrate (**1**). This is clearly evident from the CPK molecular models displayed in Fig. 6.

The thermodynamic parameters for the hydrolysis of **1** catalyzed by AMG are not known. However, in view of the extensive polyamphiphilic surface of **1** and that of the complementary combining site, it is expected that complexation involves the release of a large number of perturbed water molecules (21). The decrease in enthalpy that is expected to result is amplified by the formation of nine intermolecular hydrogen bonds (see Fig. 3). In view of this large polar contribution and the paucity of hydrophobic interactions, the formation of the complex must result in a substantial (perhaps as great as 60 kcal mol^{-1}) decrease in enthalpy. As will be discussed below, the liberation of this heat is expected, as previously discussed (21), to participate in promoting the complex to the transition state (6) (see Fig. 2).

However, Sigurskjold et al. (23) have determined that the binding of the inhibitor acarbose by AMG is driven by both enthalpic (ΔH = –9.7 kcal mol^{-1}) and entropic ($T\Delta S$ = 6.6 kcal mol^{-1}) values. The increase in entropy is expected to arise from an important hydrophobic contribution. This is

Fig. 3. A display of the intermolecular hydrogen bonds that are formed during the binding of **1** in the half-chair conformation and *C*-6a-alkyl derivatives (R = H, Me, Et, or *i*-Pr) into the site occupied by the *c*- and *d*-units of acarbose.

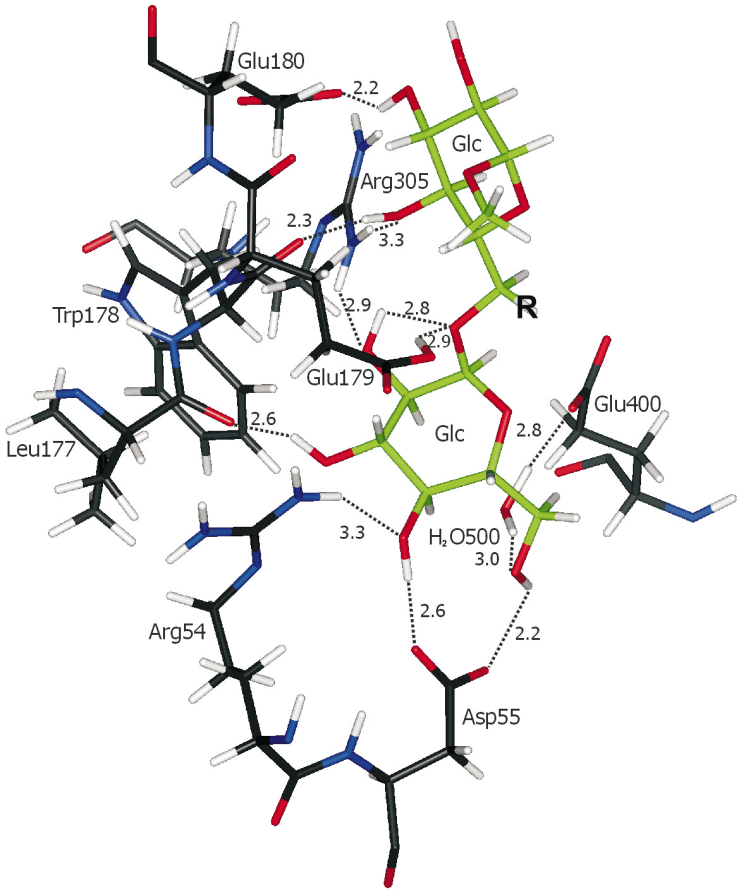
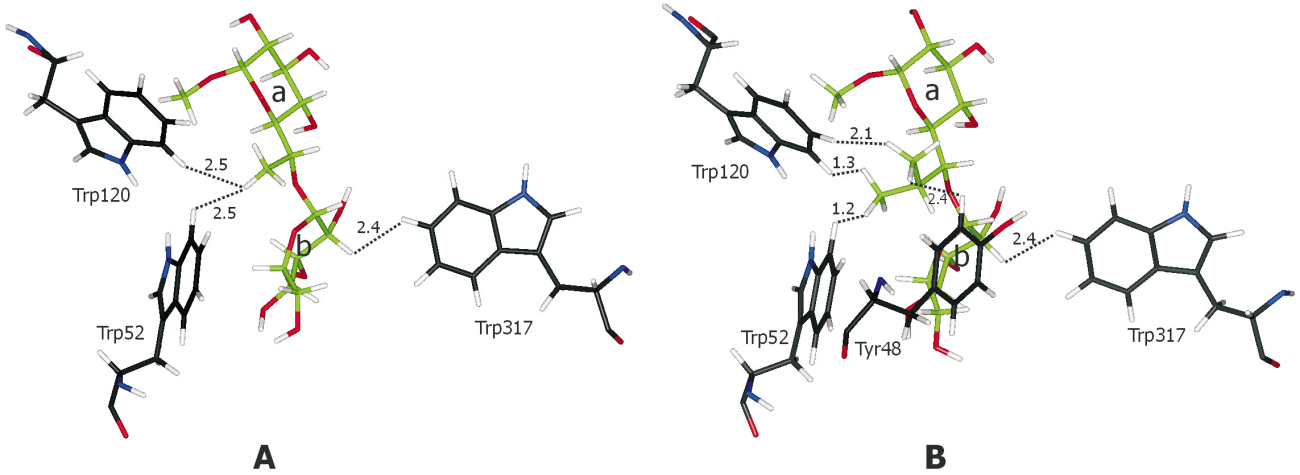


Fig. 4. Display of the near van der Waals' contacts of hydrophobic groups of AMG with nonpolar hydrogen atoms of 6a-alkyl derivatives of **1**. The hydrophobic associations of the methyl group of ***R*-2m** and the isopropyl group of ***R*-2i** are not present in the complex formed with **1**. Consequently, it appears that the only van der Waals contact with **1** involves a hydrogen of Trp 317 that is 2.4 Å away from *H*-2b.



not surprising since the terminal unit of the pseudo-tetrasaccharide (Fig. 1) is a derivative of cyclohexene, which along with the *C*-6a-methyl group of the neighboring 6-deoxy glucose the cyclohexene like unit offers a substantial hydrophobic surface to the aqueous phase (see Fig. 1). The ordered water molecules, along with disordered water molecules hydrogen bonded to the binding site, must be displaced during the binding process. Evidently, the increase in entropy caused by the hydrophobic component dominates the decrease in entropy expected from hydrophobic contributions.

Fig. 5. The structures of the complex of the lectin *Ulex europaeus* (UE-I) and the H-type 2–OMe trisaccharide (α-L-Fuc(1→2)β-D-Gal(1→4)β-D-GlcNAc-OMe) obtained by docking into the combining site of UE-I, which accepts (*R*)-2-methyl-2,4-pentanediol (L.T.J. Delbaere and M. Vandonselaar, private communication). Structure A identifies the key intermolecular hydrogen bonds that are required to established the formation of the complex. Structure B identifies the near van der Waals' contacts established between Tyr 220 and the β-D-GlcNAc and β-D-Gal units and of Val 134 with the α-L-Fuc unit.

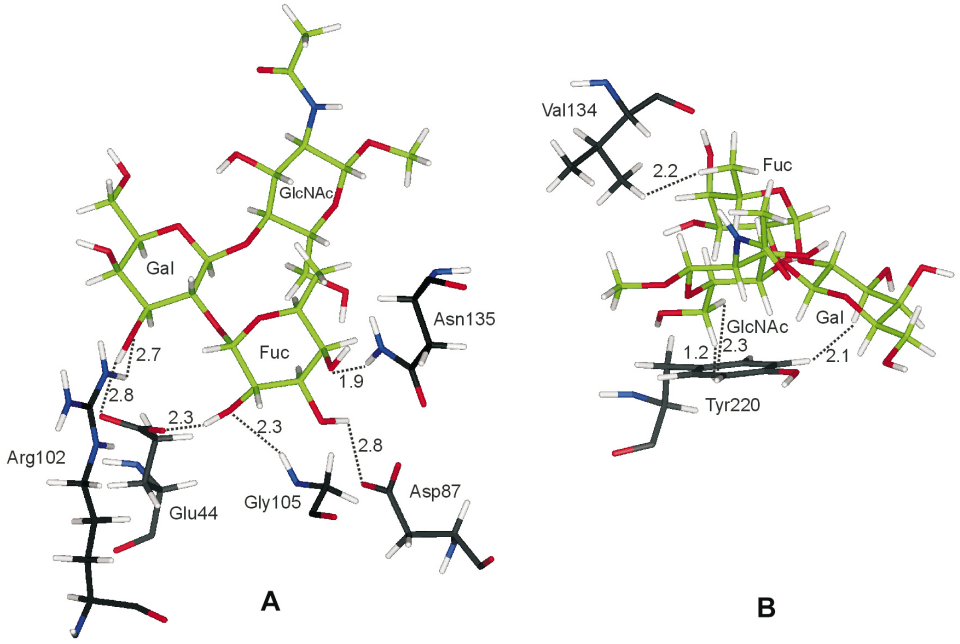


Fig. 6. CPK models based in GEGOP calculations that display the polyamphiphilic surface of substrates **1**, ***R*-2m**, and ***R*-2i**: (*a*) the three preferred conformations in aqueous solution. The pro-*R* hydrogen of **1** is presented in yellow as are the methyl groups of ***R*-2m** and ***R*-2i**. Introduction of the alkyl groups at position 6a results in a similar conformation which is different than that of **1**; (*b*) CPK models representation of the preferred conformation with the *b*-unit in the half-chair conformation with all ***R*-6a** substituents pointing in the same direction. These conformers allow hydrogen bonding between OH-4a and both Trp 178 and Arg 305.

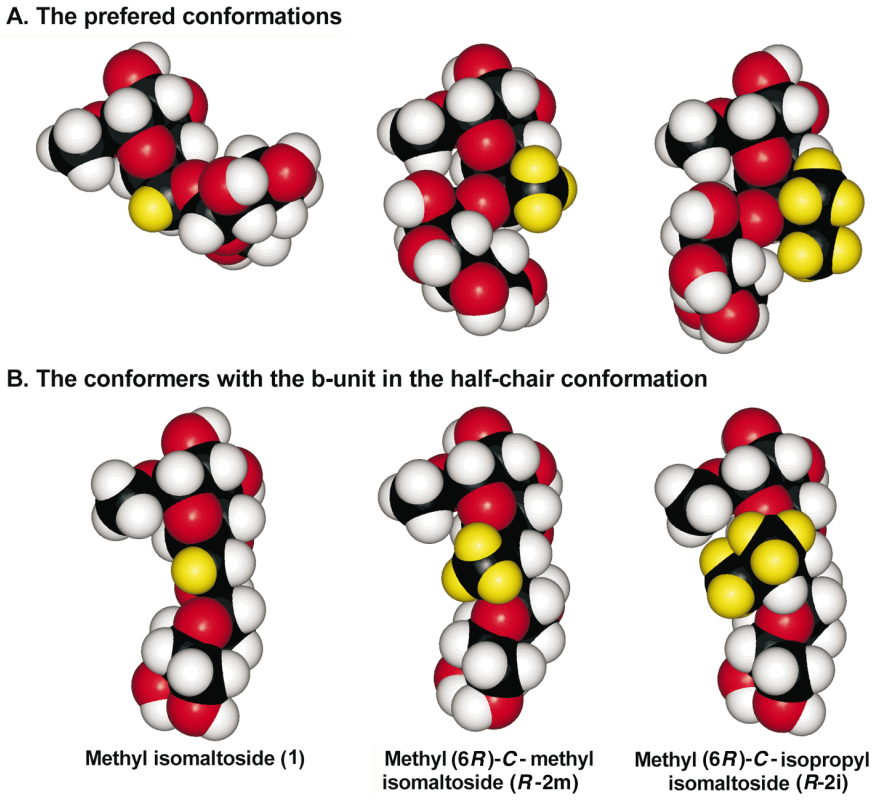


Fig. 7. This graphic is of AMG looking down to the combining site that is occupied by ***R*-2m** (white–hydrogens, green–carbons, and red–oxygen atoms). It is seen that a channel exists for water molecules to access the OH-3a region of the active site. The 6a-methyl group, which remains in the aqueous phase, is visible just below the methoxy group. The yellow atoms belong to aromatic side chains of amino acids that surround the active site, as shown in Fig. 8.

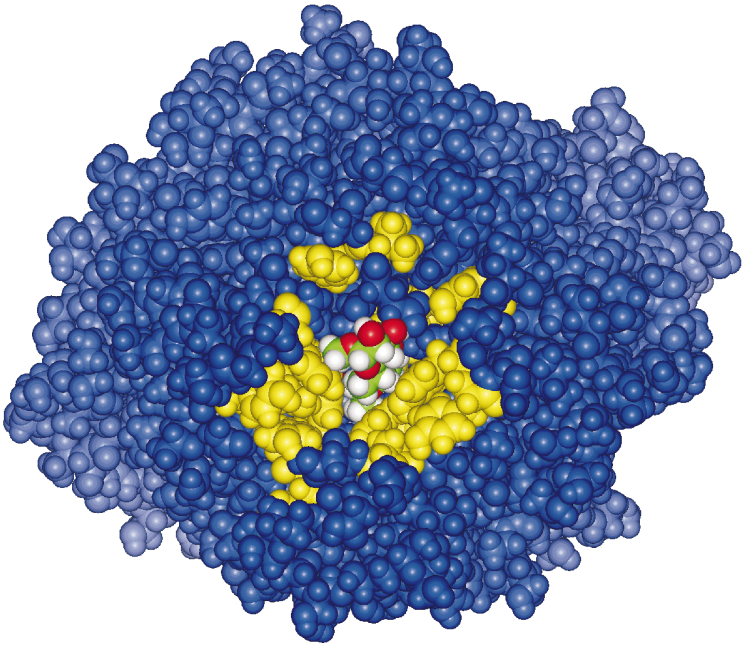
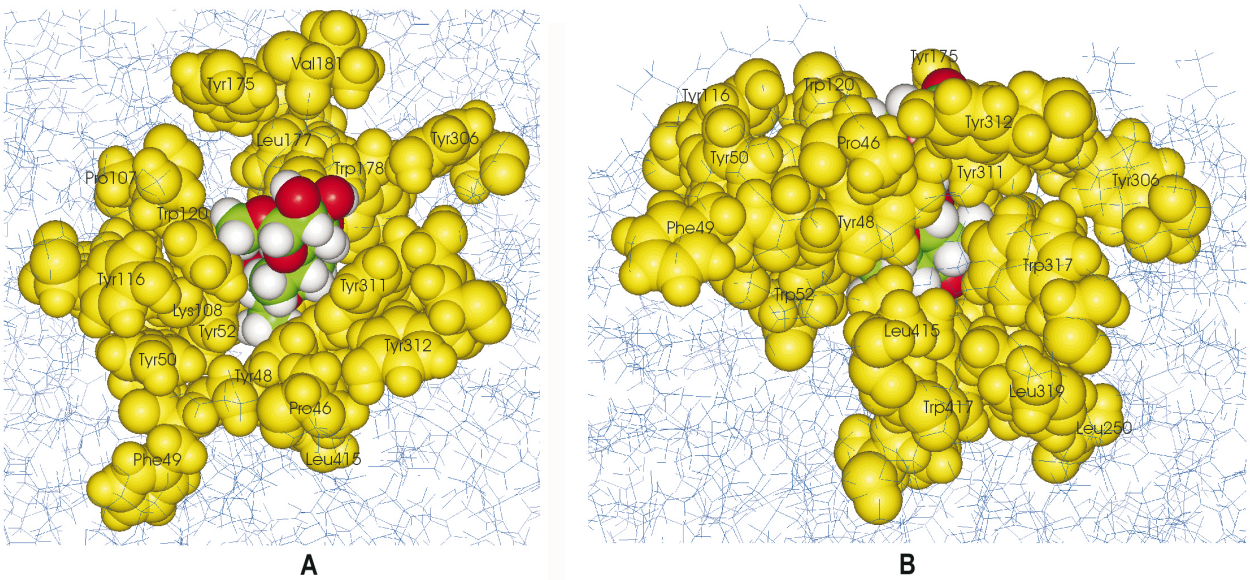


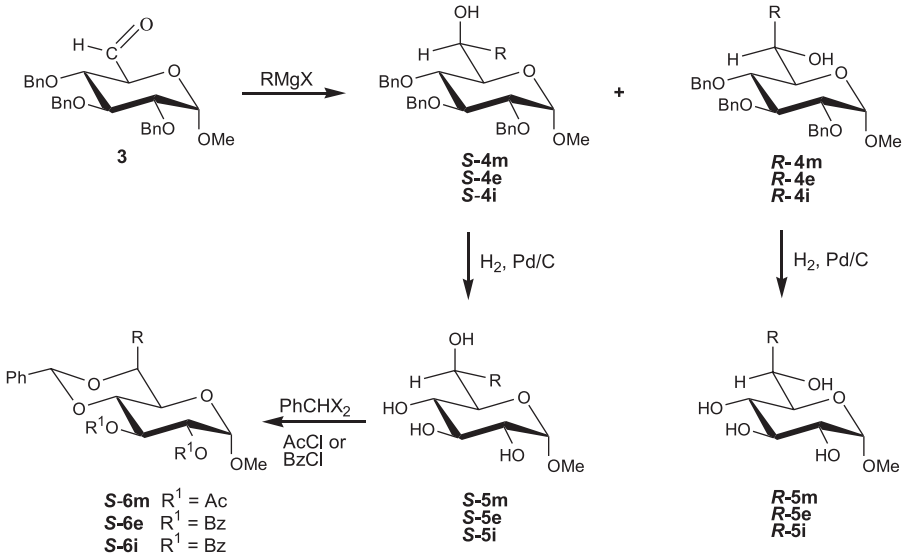
Fig. 8. The aromatic shield that protects the polar interactions (Fig. 2) in the complex from being disrupted by water: (*a*) is a close-up (amino acids are removed for clarity); (*b*) is a side view of the shield of largely nonpolar aromatic groups that is expected to provide thermal insulation of the heat of dehydration to assist achievement of the transition state. The slit in the shield through which a position of the substrate can be viewed does not exist but is occupied by other nonaromatic amino acid residues. The apparent close-packing is not real. However, their proximities are sufficient to provide effective protection of the polar interaction of the complex.



The heat liberated from the latter effect is large enough to cancel the endothermicity of the hydrophobic contribution by 9.7 kcal mol^{-1} and, consequently, the strong exothermicity of the binding of **1** by AMG is supported. Recall that this is the source of heat required to activate the complex to the transition state.

In 1990, Delbaere et al. (24) noted that for the Lewis b–*Griffonia simplicifolia* complex many of the amino acids, except for the diglycine bridge, near the periphery of the polar receptor site have aromatic side chains. These structural features were also observed for antibodies (25) and appear to be necessary for the combining site of all carbohydrate-binding

Scheme 1.



proteins. In general, carbohydrates are held in the cavity mainly by attractive polar interactions in the form of intermolecular hydrogen bonds. Hydrophobic associations are present and important, especially in the case of antibodies (25), but are of minor importance for the binding of oligosaccharides by many lectins. In this regard, the driving force for association is provided by a strong *decrease* in enthalpy and a slight *decrease* in entropy. Clearly, binding occurs in cavities in order to avoid disrupting the association of polar bonds with the bulk water. We suggest that the purpose of the largely aromatic coating surrounding of the polar complex is to provide a strong shield from the bulk water. Not surprisingly, therefore, the active site of the enzyme AMG that binds isomaltose is surrounded by amino acids that contain aromatic side chains (see Fig. 7). These large hydrophobic groups are expected to act as a shield and thus, at high concentrations, prevent water from seeping into the complex. It has been proposed that lower dielectric constants in the binding in a cavity favor the formation of the polar bonds. However, this stabilization of polar bonds must also apply to the water molecules that are displaced by the carbohydrate.

Finally, we propose that the shield of hydrophobic aromatic groups that surround the catalytic site of AMG (see Fig. 8) also serves as thermal insulation to lower the rate of the dissipation of the heat of complex formation (the hydraphobic effect) to bulk. This energy is expected to be the main source of activation for the **1**–AMG complex to the transition state (Fig. 2). This concept of heat being liberated from reacting molecules and being maintained in a suitable submicro-environment to eventually serve as the activation energy for another reaction may be a general feature of enzyme catalysis. This proposal is similar to the energy dissipation in enzyme catalysis proposed by Cramer and Freist (26).

Synthesis

The syntheses of the 6-*C*-alkyl glucosides **4m**, **4e**, and **4i** (Scheme 1) started from the known methyl 6-aldehydo-α-D-glucopyranoside (**3**) (27) that was prepared by oxidation of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (28). Molecule **3** was then reacted with the methyl, ethyl and isopropyl Grignard reagents in the usual fashion to yield mixtures of the *R*- and *S*-epimers, namely, the 6-*C*-methyl, 6-*C*-ethyl, and 6-*C*-isopropyl glucosides, (**4m**, **4e**, and **4i**). In agreement with Cram's rule (29), the *S*-isomers were produced in excess. The ratio of the *S*- to *R*-epimers was about 10:1 for **4m** and **4e** and 20:1 for **4i**.

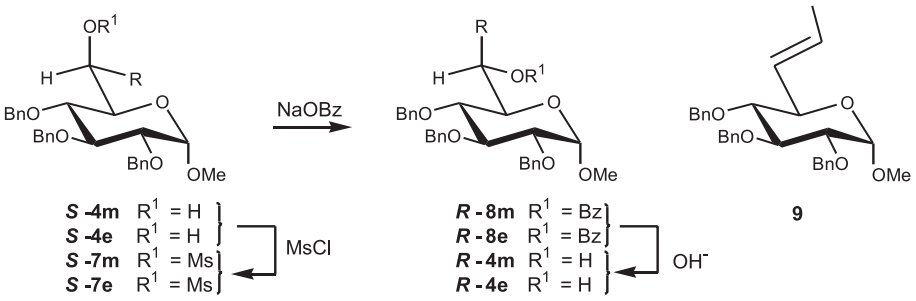
In the case of the methyl Grignard, procedures to prepare ***R*,*S*-4m**, involved converting the crude product into a mixture of 6-*O*-(3,5-dinitrobenzoyl) esters from which the *S*-isomer crystallized and was purified by recrystallization. Zemplen methanolysis then provided the pure ***S*-4m**. The crude products obtained from the ethyl and isopropyl Grignard reagents could be separated by column chromatography into the pure *R*- and *S*-isomers. As will be described below (Schemes 2 and 3) larger amounts of the *R*-isomers, in each case, were obtained by inversion of the C-6 chiral centres of the *S*-compounds.

Both the *R*- and *S*-epimers of **4m**, **4e,** and **4i** were debenzylated by catalytic hydrogenolysis to obtain pure preparations of both the *R*- and *S*-*C*-6-epimers, **5m**, **5e**, and **5i (**Scheme 1).

For the configurational assignments, the *S*-isomers, (***S*-5m**, ***S*-5e**, and ***S*-5i**), were converted into the 4,6-*O*-benzylidene derivatives (***S*-6m**, ***S*-6e**, and ***S*-6i**) by reaction with α,α-dibromo- or α,α-dichlorotoluene in pyridine at elevated temperature followed by acylation. The absolute configuration of compound ***S*-6m** was unequivocally established by the NOE's obtained by saturation of the axial C-6 methyl group (see Table 4). The configurations of compounds ***S*-6e** and ***S*-6i** are assigned on the basis that the $J_{5,6}$ coupling constants (reported in Table 4). The coupling constant of 6.0 Hz is too small for coupling of antiperiplanar hydrogen atoms. Furthermore, the value of $J_{5,6}$ of ***S*-6m**, a compound of known configuration, is essentially the same as those for ***S*-6e** and ***S*-6i**, indicates that all three compounds are configurationally related. It is also relevant that these three *S*-compounds were the major products of the Grignard reactions and, therefore, are surely configurationally related.

For the preparation of larger amounts of ***R*-4m** and ***R*-4e** (Scheme 2), mesylated ***S*-7m** and ***S*-7e** were prepared and reacted with sodium benzoate in *N*,*N*-dimethylformamide at

Scheme 2.



Scheme 3.

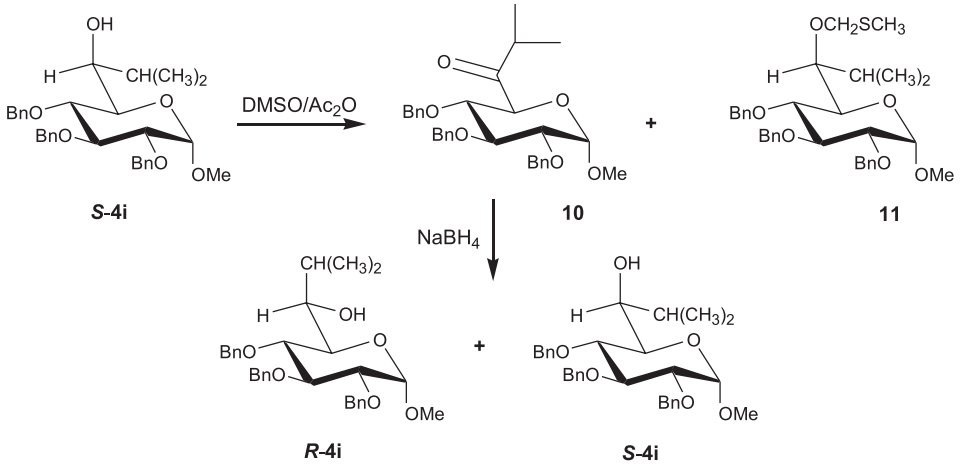


Table 4. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) in $CDCl_3$ for the benzylidene derivatives ***S*-6m**[a], ***S*-6e**, and ***S*-6i**.

	***S*-6m**	***S*-6e**	***S*-6i**
H-1 ($J_{1,2}$)	4.93 (3.5)	5.15 (3.5)	5.16 (3.5)
H-2 ($J_{2,3}$)	4.87 (10.0)	5.19 (9.5)	5.21 (9.5)
H-3 ($J_{3,4}$)	5.57 (9.0)	6.04 (9.5)	6.06 (9.5)
H-4 ($J_{4,5}$)	3.91 (10.0)	4.15 (10.0)	4.21 (10.0)
H-5 ($J_{5,6}$)	4.15 (6.0)	4.33 (6.0)	4.43 (5.5)
H-6 ($J_{6,CH_{1,2,3}}$)	4.50 (7.0)	4.25 (3.5,11.0)	3.88 (10.0)
C*H*Ph	5.80	5.78	5.72
CH_3O	3.41	3.43	3.43
CH_3	1.48	1.13 (7.5)	1.15, 1.13 (6.5)
$CH_{(1,2)}$	—	2.18, 1.79	2.62

[a]NOE experiments performed by irradiation of the CH_3-C6 doublet at 1.48 ppm caused enhancements of the *H*-6, *H*-4, and C*H*Ph signals by 6.2%, 2.9%, and 4.1%, respectively.

120°C followed by saponification of the products. Dehydromesylation of ***S*-7e** also resulted in compound **9**. Its *E*-configuration was assigned by the large $J_{5,6}$ coupling constant (14.5 Hz) (30).

The inversion of configuration at *C*-6 of ***S*-4i** (Scheme 3) to obtain ***R*-4i** was achieved by reduction of ketone **10** with sodium borohydride. Ketone **10** was prepared by oxidation of ***S*-4i** with dimethylsulfoxide and acetic anhydride (31). The crude product was mixture of ketone **10** and methylthiomethylene ether **11** (3:2). The borohydride reduction of **10** provided an epimeric mixture of ***R*-4i** and ***S*-4i** (5:1).

Preparation of the α-linked disaccharides ***R*-2m**, ***R*-2e**, and ***R*-2i** is outlined in Scheme 4. Bromide-ion catalyzed glycosylation conditions using 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**12**), tetraethylammonium bromide (32) and alcohol ***R*-4m** produced ***R*-14m**. Coupling of tetra-*O*-benzyl-α-D-glucopyranosyl bromide (**13**) with alcohols ***R*-4e** and ***R*-4i** was achieved in the presence of the cupric bromide–*N*,*N*-dimethylformamide complex (33). The protecting groups were removed in the usual manner to yield the desired compounds. The *C*-6a epimeric disaccharides ***S*-2m**, ***S*-2e**, and ***S*-2i** were prepared by similar procedures.

The ^{1}H NMR data for the disaccharides are presented in Table 5. The large ^{1}H coupling constants for $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ confirm the $^4C_1$-conformations of the pyranose rings. The $J_{5,6}$ coupling constants are ≤2.5 Hz. This suggests that a positive synclinal (+g) relationship exists between H-5a and H-6a, which is in agreement with the calculated ω angles for the two conformers A and B presented in Table 2.

Experimental

General methods

The ^{1}H NMR spectra were measured at 300 MHz and 360 MHz (Bruker AM 300 and WM 360) with tetramethylsilane as internal standard for $CDCl_3$ solutions. Reference standard for D_2O solutions was acetone (2.23 ppm). The ^{13}C NMR spectra were recorded at 75 MHz

Scheme 4.

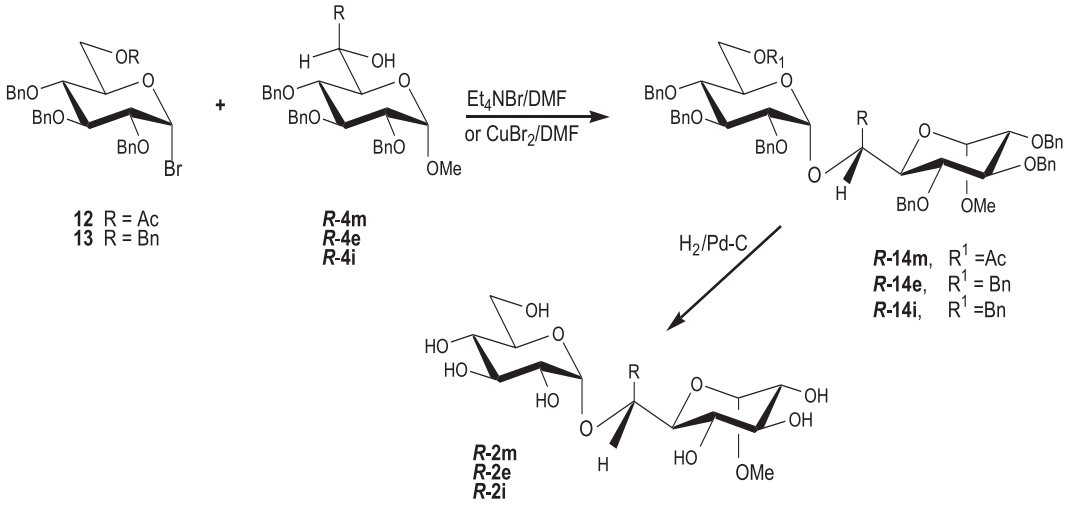


Table 5. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) for methyl 6-*C*-methyl, -ethyl-, and -isopropyl-α-isomaltosides[a].

	(6*R*)-*C*-alkyl-isomaltosides			(6*S*)-*C*-alkyl-isomaltosides		
	***R*-2m**	***R*-2e**	***R*-2i**	***S*-2m**	***S*-2e**	***S*-2i**
a-unit						
H-1 ($J_{1,2}$)	4.80 (4.0)	4.78 (3.5)	4.79 (3.5)	4.85 (4.0)	4.84 (3.5)	4.81 (3.5)
H-2 ($J_{2,3}$)	3.57 (9.5)	3.56 (9.5)	3.58	n.a.	3.55 (9.5)	3.51
H-3 ($J_{3,4}$)	3.67 (9.0)	3.66 (9.0)	3.65	n.a.	3.63	3.63 (9.5)
H-4 ($J_{4,5}$)	3.39 (10.5)	3.47 (9.5)	3.68	n.a.	3.57	3.53 (9.5)
H-5 ($J_{5,6}$)	3.83 (2.5)	3.89 (1.5)	3.92 (1.5)	3.64	3.77 (<1)	3.90 (<1)
H-6 ($J_{6,CH}$)	4.17	3.94 (~5)	3.67 (~6.5)	4.28	3.98 (4.5, 9.5)	3.97 (5.5)
$CH_{(2)}$	—	1.71	2.15	—	1.77	2.29
CH_3	1.29 (6.5)	1.02 (7.5)	1.025 (6.5)	1.28 (6.5)	0.94 (7.2)	1.04 (7.5)
CH_3	—	—	1.018 (6.8)	—	—	1.03 (7.5)
CH_3O	3.44	3.44	3.43	3.43	3.45	3.51
b-unit						
H-1 ($J_{1,2}$)	5.07 (3.5)	5.12 (3.5)	5.20 (3.5)	5.09 (3.5)	5.12 (3.5)	5.17 (3.5)
H-2 ($J_{2,3}$)	3.55 (9.5)	3.57 (10.0)	3.56 (9.5)	3.56 (9.5)	3.54 (10.0)	3.57 (9.5)
H-3 ($J_{3,4}$)	3.73 (10.0)	3.74 (9.5)	3.74	3.69	3.70	3.77 (9.0)
H-4 ($J_{4,5}$)	3.41	3.42 (9.5)	3.41 (9.5)	3.43	3.44 (9.5)	3.46 (9.5)
H-5	n.a.	3.82	3.83	n.a.	3.73	3.74
H-6 ($J_{5,6}$)	3.88 (5.0)	3.85–3.78	n.a.	n.a.	n.a.	3.83
H-6′ ($J_{5,6'}$)	3.76 (4.0)	n.a.	n.a.	n.a.	n.a.	3.80

[a]Spectra recorded in D_2O with acetone as internal reference set at 2.23 ppm at 360 MHz.

with dioxane (67.4 ppm) as reference for D_2O solutions and the $CDCl_3$ signal (77.0 ppm) as reference in $CDCl_3$ solutions. Optical rotations were measured at room temperature (23 ± 1°C) in a 1 dm cell on a Perkin–Elmer 241 polarimeter. Thin-layer chromatography was performed on precoated plates of silica gel (60-F254, E. Merck, Darmstadt) and visualized by spraying with 5% sulfuric acid in ethanol followed by heating. For column chromatography, silica gel 60 (230–400 mesh, E. Merck, Darmstadt) and distilled solvents were used. All solvents and reagents were purified and dried according to standard procedures. Melting points are uncorrected.

The molecular modeling was carried out on an SGI Indigo II. A suite of software from Molecular Simulations Inc., San Diego, was used to build and minimize the (6*R*)-*C*-alkylated glucopyranosyl units using AMBER forcefield. The coordinates of these (6*R*)-*C*-alkylated glucopyranosyl units were then exported to the GEGOP program[4] (11) for use in grid searchs, minimizations, and Monte-Carlo simulations.

[4] R. Stuike-Prill and B. Meyer. Geometry of GlycOProteins. Version 2.6. 1992.

Methyl 6-aldehydo-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3)

Dried chromium trioxide (25.9 g, 259 mmol) was added to a solution of pyridine (40.9 g, 517 mmol) in dichloromethane (400 mL). The mixture was mechanically stirred at room temperature for 30 min. A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (28) (10.0 g, 21.6 mmol) in dichloromethane (22 mL) was rapidly added and the resulting mixture stirred at room temperature for 10 min. The reaction mixture was then decanted and the black tarry precipitate was extracted with ether several times. The combined organic solutions were filtered through a pad of silica gel-G to provide a colorless slightly yellow oily residue which was co-evaporated with toluene (2 × 200 mL) to remove residual pyridine. The oily residue was dried under vacuum overnight before use to give crude **3** (6.94 g, 70%). The relative intensity of the singlet at 9.65 ppm (^{1}H NMR) attributed to H-6 indicated an about 90% content of **3**. $[\alpha]_D$ + 8.2° (*c* 1.0, chloroform), lit. (27) 13.6° (*c* 2.6, chloroform). IR (cm^{-1}): 1741 (CHO). ^{1}H NMR ($CDCl_3$) δ: 9.65 (s, 1H, *H*-6), 7.40–7.10 (m, 15H, 3*Ph*), 5.05–4.50 (m, 7H, 3CH_2Ph, *H*-1), 4.17 (d, $J_{4,5}$ = 10.0 Hz, 1H, *H*-5), 4.08 (dd, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-3), 3.57 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.0 Hz, 1H, *H*-4), 3.50 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.38 (s, 3H, CH_3O).

Methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-methyl-α-D-glucopyranoside (*S*-4m)[5]

A solution of the crude aldehyde **3** (6.94 g, 15.0 mmol) in ether (30 mL) was added dropwise to a 0.6 N methylmagnsesium iodide solution in ether (50 mL, 30 mmol) and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by the dropwise addition of aq sat. ammonium chloride. The mixture was diluted with water, extracted with ether and the organic solution was dried and evaporated to provide a yellow oil (5.96 g). The ^{1}H NMR spectrum was consistent with the presence of the desired compounds ***S*-4m** and ***R*-4m** in the ratio of about 10:1.

The crude mixture of ***S*-4m** and ***R*-4m** (0.59 g, 1.2 mmol) was reacted in pyridine (10 mL) with 3,5-dinitrobenzoyl chloride (0.51 g, 2.25 mmol) in the presence of a catalytic amount of 4-dimethylaminopyridine (few milligrams). The reaction mixture was stirred at room temperature until analysis by TLC indicated the reaction was completed. The usual work-up provided a yellow residue which crystallized spontaneously and was recrystallized from methanol. The compound characterized as the 6-*O*-(3,5-dinitrobenzoyl) derivative of ***S*-4m**, namely methyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,5-dinitrobenzoyl)-(6S)-*C*-methyl-α-D-glucopyranoside, was obtained in 74% yield (0.612 g), mp 68–70°C. $[\alpha]_D$ + 52.2° (c 0.85, chloroform).

The 6-*O*-(3,5-dinitrobenzoyl) derivative of ***S*-4m** (1.00 g, 1.49 mmol) was dissolved in dioxane (50 mL) followed by the addition of 1 N sodium hydroxide solution (20 mL). After stirring for 1 h, the mixture was diluted with dichloromethane and filtered over a pad of silica gel-G, the organic solution was washed with water, dried and evaporated to give ***S*-4m** (0.69 mg, 97%) as a solid, mp 82–84°C. $[\alpha]_D$ + 26.9° (c 1.0, chloroform). ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 15H, 3*Ph*), 5.01–4.63 (3ABq, 6H, 3CH_2Ph), 4.58 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.05 (dq, $J_{5,6}$ = 1.5 Hz, $J_{6,CH3}$ = 6.5 Hz, 1H, *H*-6), 3.99 (dd, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-3), 3.62 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-4), 3.50 (dd, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 3.5 Hz, 1H, *H*-2), 3.42 (dd, $J_{5,6}$ = 1.5 Hz, $J_{4,5}$ = 10.0 Hz, 1H, *H*-5), 3.34 (s, 3H, CH_3O), 1.25 (d, $J_{6,7}$ = 6.5 Hz, 3H, CH_3C-6). Anal. calcd. for $C_{29}H_{34}O_6$: C 72.78, H 7.16; found: C 72.94, H 6.82.

Methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-ethyl-α-D-glucopyranoside (*S*-4e) and -(6*R*)-C-ethyl-α-D-glucopyranoside (*R*- 4e*)*

A solution of crude methyl 6-aldehydo-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**3**) (1.6 g, 3.46 mmol) in tetrahydrofuran (20 mL) was transferred slowly into a 0.5 N ethylmagnesium chloride solution in tetrahydrofuran (21 mL, 10.1 mmol) with stirring. After 1 h, the reaction was processed as described for ***S*-4m**. The crude material was applied to a column of silica gel (hexane–ethyl acetate, 5:1, 4:1) to provide in the main fraction the title compound ***S*-4e** (1.043 g, 61%) as a syrup. $[\alpha]_D$ + 12° (c 1.4, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.38–7.22 (m, 15H, 3*Ph*), 5.01–4.64 (3ABq, 6H, 3CH_2Ph), 4.56 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 3.99 (t, $J_{2,3} \sim J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.70 (m, *H*-6), 3.66 (t, $J_{3,4} \sim J_{4,5}$ = 9.5 Hz, 1H, *H*-4), 3.53 (br d, $J_{5,6}$ < 1 Hz, 1H, *H*-5), 3.49 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.35 (s, 3H, CH_3O), 1.67 (m, 1H, CH_2CH$_3$), 1.50 (m, 1H, CH_2CH$_3$), 0.95 (t, *J* = 7.5 Hz, 3H, CH_3CH$_2$). Anal. calcd. for $C_{30}H_{36}O_6$: C 73.15, H 7.37; found: C 73.26, H 7.32. Continued development of the column eluted epimer methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-ethyl-α-D-glucopyranoside (109 mg, 6.4%) as a crystallizing syrup. $[\alpha]_D$ + 35° (*c* 0.4, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 15H, 3*Ph*), 5.04–4.61 (3ABq, 6H, 3CH_2Ph), 4.55 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.02 (t, $J_{2,3} \sim J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.70–3.63 (m, 1H, *H*-5, *H*-6), 3.50 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.49 (t, $J_{3,4} \sim J_{4,5}$ = 9.0 Hz, 1H, *H*-4), 3.38 (s, 3H, CH_3O), 1.54 (m, 1H, CH_2CH$_3$), 1.42 (m, 1H, CH_2CH$_3$), 0.93 (t, *J* = 7.5 Hz, 3H, CH_3CH$_2$). Anal. calcd. for $C_{30}H_{36}O_6$: C 73.15, H 7.37; found: C 72.47, H 7.47.

Methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-isopropyl-α-D-glucopyranoside (*S*-4i) and -(6*R*)-*C*-isopropyl-α-D-glucopyranoside (*R*-4i*)*

Crude aldehyde **3** (2.47 g, 5.34 mmol) in tetrahydrofuran (30 mL) was reacted with isopropylmagnesium chloride as described for the preparation of ***S*-4e** The crude reaction product was purified on a column of silica gel (hexane–ethyl acetate, 5:1 → 3:1) to provide in the first fraction the minor epimer ***R*-4i** (73.8 mg, 2.7%) as a slowly crystallizing syrup. $[\alpha]_D$ + 26.4° (*c* 0.4, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.23 (m, 15H, 3*Ph*), 5.06–4.62 (3ABq, 6H, 3CH_2Ph), 4.53 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.03 (t, $J_{2,3} \sim J_{3,4}$ = 9.5 Hz, 1H, *H*-3), 3.69 (dd, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.0 Hz, 1H, H-5), 3.60 (t, $J_{3,4} \sim J_{4,5}$ = 9.0 Hz, 1H, *H*-4), 3.53 (dd, $J_{6,CH}$ = 4.0 Hz, $J_{5,6}$ = 6.0 Hz, 1H, *H*-6), 3.50 (dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.40 (s, 3H, CH_3O), 1.96 (m, 1H, C*H*(CH$_3$)$_2$), 0.95 (d, *J* = 7.0 Hz, 3H, CH(CH_3)$_2$), 0.89 (d, *J* = 7.0 Hz, 3H, CH(CH_3)$_2$). Anal. calcd. for $C_{31}H_{38}O_6$: C 73.49, H 7.56; found: C 73.27, H 7.72. Continued development of the

[5] Methyl 2,3,4-tri-*O*-benzyl-7-deoxy-β-L-glycero-D-gluco-heptopyranoside.

column eluted the major epimer ***S*-4i** (1.46 g, 54%) as a syrup. $[\alpha]_D$ + 12.1° (*c* 0.7, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.23 (m, 15H, 3Ph), 5.00–4.64 (3ABq, 6H, 3CH_2Ph), 4.55 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 3.99 (tX, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.75 (br d, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ < 1 Hz, 1H, *H*-5), 3.67 (t, $J_{3,4} \sim J_{4,5}$ = 9.0 Hz, 1H, *H*-4), 3.48 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.38 (s, 3H, CH_3O), 3.33 (br d, $J_{6,CH}$ = 8.5 Hz, 1H, *H*-6), 1.83 (m, 1H, CH(CH_3)$_2$), 1.01 (d, *J* = 7.0 Hz, 3H, (CH_3)$_2$CH), 0.89 (d, *J* = 7.0 Hz, 3H, (CH_3)$_2$CH). Anal. calcd. for $C_{31}H_{38}O_6$: C 73.49, H 7.56; found: C 73.53, H 7.67.

General procedure for the catalytic hydrogenolysis of *O*-benzyl derivatives

A mixture of the benzyl derivative (200 mg, 0.2–0.4 mmol) and 5% palladium-on-carbon (200 mg) in methanol (10–15 mL) was hydrogenated in the hydrogen stream until completion (TLC). The catalyst was removed by filtration and the solvent evaporated. The resultant material was applied to a column of Iatrobeads® (chloroform-methanol-water) followed by gel filtration on a column of Sephadex LH 20 (50% ethanol) to provide the deprotected material.

*Methyl (6*S*)*-C-*methyl*-α-D-*glucopyranoside (***S*-5m**)

Compound ***S*-5m** was prepared from ***S*-4m** in quantitative yield by catalytic hydrogenolysis following the general procedure. The solid material could be recrystallized from 98% ethanol/hexane to give needle like crystals; mp 136–137°C; $[\alpha]_D$ + 150° (*c* 0.2, water). ^{1}H NMR (D_2O) δ: 4.80 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.16 (dq, $J_{5,6}$ = 1.5 Hz, $J_{6,CH3}$ = 6.5 Hz, 1H, *H*-6), 3.62 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.55 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, 1H, *H*-2), 3.46 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-4), 3.39 (s, 3H, CH_3O), 3.38 (dd, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 1.5 Hz, 1H, *H*-5), 1.29 (d, *J* = 6.5 Hz, 3H, CH_3C-6). ^{13}C NMR (D_2O) δ: 100.24 (C-1), 74.49, 74.41, 72.31, 70.74, 65.26, 55.89 (CH_3O), 19.72 (*C*H_3C-6). Anal. calcd. for $C_8H_{16}O_6$: C 46.15, H 7.75; found: C 46.04, H 7.46.

*Methyl (6*S*)*-C-*ethyl*-α-D-*glucopyranoside (***S*-5e**)

Compound ***S*-5e** was prepared from ***S*-4e** by catalytic hydrogenolysis following the general procedure and obtained as a white solid (89%) after lyophilization of the aq solution. $[\alpha]_D$ + 150° (*c* 0.2, water). ^{1}H NMR (D_2O) δ: 4.80 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 3.85 (m, 1H, *H*-6), 3.64 (dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.55 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.53 – 3.50 (m, 2H, *H*-4, *H*-5), 3.41 (s, 3H, CH_3O), 1.64 (m, 2H, CH_2CH$_3$), 0.95 (t, *J* = 7.5 Hz, 3H, CH_3CH$_2$); ^{13}C NMR (D_2O) δ: 100.17 (C-1), 74.27, 72.60, 72.06, 70.47, 70.30, 55.87 (CH_3O), 26.70 (CH_3CH_2), 10.67 (*C*H_3CH$_2$).

*Methyl (*6S*)*-C-*isopropyl*-α-D-*glucopyranoside (***S*-5i**)

Compound ***S*-5i** was prepared from ***S*-4i** by catalytic hydrogenolysis following the general procedure and obtained as a white solid (95%) after lyophilization of its aq solution. $[\alpha]_D$ + 150° (*c* 0.3, water). ^{1}H NMR (D_2O) δ: 4.79 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 3.70 (dd, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ < 1 Hz, 1H, *H*-5), 3.64 (dd, $J_{3,4}$ = 8.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-3), 3.56 (dd, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.5 Hz, 1H, *H*-4), 3.54 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.47 (dd, $J_{6,CH}$ = 9.5 Hz, $J_{5,6}$ < 1 Hz, 1H, *H*-6), 3.44 (s, 3H, CH_3O), 1.92 (m, 1H, CH(CH_3)$_2$), 1.01 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$), 0.91 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$). ^{13}C NMR (D_2O) δ: 100.48 (C-1), 74.36, 74.14, 71.99, 71.13, 70.39, 56.50 (CH_3O), 30.61 (*C*H(CH_3)$_2$), 19.84, 19.32 (*C*H_3)$_2$CH).

*Methyl (*6R*)*-C-*methyl*-α-D-*glucopyranoside (***R*-5m**)

Compound ***R*-5m** was prepared from ***R*-4m** in quantitative yield by catalytic hydrogenolysis following the general procedure; mp 135–136°C. $[\alpha]_D$ + 148° (*c* 1.0, water). ^{1}H NMR (D_2O) δ: 4.82 (d, $J_{1,2}$ = 4.0 Hz, 1H, *H*-1), 4.20 (dq, $J_{5,6}$ = 2.5 Hz, $J_{6,CH3}$ = 6.0 Hz, 1H, *H*-6), 3.71 (dd, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 2.5 Hz, 1H, *H*-5), 3.68 (dd, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 10.0 Hz, 1H, *H*-3), 3.58 (dd, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.0 Hz, 1H, *H*-2), 3.44 (s, 3H, CH_3O), 3.38 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.0 Hz, 1H, *H*-4), 1.25 (d, *J* = 6.5 Hz, 3H, CH_3C-6). ^{13}C NMR (D_2O) δ: 100.02 (C-1), 74.17, 74,12, 72.07, 71.73, 67.14, 55.76 (*C*H_3O), 15.85 (*C*H_3C-6). Anal. calcd. for $C_8H_{16}O_6$: C 46.15, H 7.75; found: C 45.99, H 7.50.

*Methyl (*6R*)*-C-*ethyl*-α-D-*glucopyranoside (***R*-5e**)

Compound ***R*-5e** was prepared from ***R*-4e** by catalytic hydrogenolysis following the general procedure and obtained as a foam after freeze-drying its aqueous solution (87%). $[\alpha]_D$ + 152° (*c* 0.4, water). ^{1}H NMR (D_2O) δ: 4.78 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 3.78 (m, 1H, *H*-6), 3.69 (dd, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 1.5 Hz, 1H, *H*-5), 3.64 (dd, $J_{3,4}$ = 8.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-3), 3.54 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.43 (dd, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 10.0 Hz, 1H, *H*-4), 3.41 (s, 3H, CH_3O), 1.64 (m, 1H, CH_2CH$_3$), 1.53 (m, 1H, CH_2CH$_3$), 0.98 (t, *J* = 7.5 Hz, 3H, CH_3CH$_2$). ^{13}C NMR (D_2O) δ: 100.00 (C-1), 74.26, 74.05, 73.39, 72.01, 71.55, 55.78 (*C*H_3O), 23.94 (*C*H_2CH$_3$), 10.84 (*C*H_3CH$_2$).

*Methyl (*6R*)*-C-*isopropyl*-α-D-*glucopyranoside (***R*-5i**)

Compound ***R*-5i** was prepared from ***R*-4i** by catalytic hydrogenolysis following the general procedure and obtained as a white solid (85%) after lyophilization of its aqueous solution. $[\alpha]_D$ + 123.4° (*c* 0.2, water). ^{1}H NMR (D_2O) δ: 4.78 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 3.74 (dd, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 4.0 Hz, 1H, *H*-5), 3.67 (dd, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-3), 3.58 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.5 Hz, 1H, *H*-4), 3.57 (dd, $J_{6,CH} \sim$ 6.5 Hz, *J* = 40 Hz, 1H, *H*-6), 3.54 (dd, $J_{1,2}$ = 3.5 Hz,$J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.42 (s, 3H, CH_3O), 2.04 (m, 1H, CH(CH_3)$_2$), 0.96 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$), 0.95 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$). ^{13}C NMR (D_2O) δ: 100.10 (C-1), 78.63, 74.16, 72.33, 71.86, 71.49, 56.12 (*C*H_3O), 30.11 (*C*H(CH_3)$_2$), 20.09, 17.96 ((*C*H_3)$_2$CH).

Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-(6*S*)-*C*-methyl-α-D-glucopyranoside (*S*-6m)

A stirred solution of ***S*-5m** (16 mg, 0.053 mmol) and α,α-dibromotoluene (112 mg, 0.45 mmol) in pyridine (15 mL) was heated at reflux for 2 h. The mixture was cooled and then acetic anhydride (3 mL) was added. After 48 h at room temperature, the mixture was poured into ice water followed by extraction with chloroform, washing and drying of the organic solution and evaporation. The resultant product was purified by thin layer chromatography on silica gel (hexane–ethyl acetate, 3:2) to give ***S*-6m** (15 mg, 51%) as a solid, mp 132–134°C. $[\alpha]_D$ + 50.2° (*c* 0.3, chloroform). The ^{1}H NMR

data are reported in Table 4. ^{13}C NMR ($CDCl_3$) δ: 170.48, 169.76 (2CO), 137.40, 128.92, 128.22, 126.24 (Ph), 97.59 (C-1), 94.07 (*C*HPh), 72.74, 71.50, 70.49, 69.66 (C-2, C-3, C-4, C-5), 64.45 (C-6), 55.34 (CH_3O), 20.79, 20.65 ($2CH_3CO$), 11.28 (CH_3C-6). Anal. calcd. for $C_{19}H_{24}O_8$: C 59.99, H 6.36; found: C 59.55, H 6.06.

Methyl 2,3-di-*O*-benzoy-4,6-*O*-benzylidene-(6*S*)-*C*-ethyl-α-D-glucopyranoside (*S*-6e)

A solution of ***S*-5e** (49 mg, 0.22 mmol) and α,α-dichlorotoluene (100 μL, 0.78 mmol) in pyridine (7 mL) was heated at reflux for 4 h. Then more reagent (75 μL, 0.58 mmol) was added and heating was continued for 4.5 h. The mixture was cooled to ice bath temperature and benzoyl chloride (100 μL, 0.86 mmol) was added. After 2 h at this temperature, the mixture was poured into ice water, extracted with dichloromethane, followed by washing of the organic solution with water, drying and evaporation. The resultant material was purified on a column of silica gel (hexane-ethyl acetate, 9:1, 0.05% triethylamine) to provide labile, solid ***S*-6e** (56 mg, 49%) which was not analytically pure and was only characterized by ^{1}H NMR spectroscopy. The ^{1}H NMR data are reported in Table 5.

Methyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-(6*S*)-*C*-isopropylidene-α-D-glucopyranoside (*S*-6i)

***S*-5i** (37 mg, 0.16 mmol) was converted into ***S*-6i (**41 mg, 49%) as described for the preparation of ***S*-6e.** Labile ***S*-6i** contained a minor component (13%) that could not be removed. The ^{1}H NMR data are reported in Table 4.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-methanesulfonyl-(6*S*)-*C*-methyl-α-D-glucopyranoside (*S*-7m*)*

To a solution of ***S*-4m** (135 mg, 0.28 mmol) in pyridine (5 mL) at 0°C was added methanesulfonyl chloride (87 mg, 0.76 mmol). After 2 h at room temperature, the mixture was poured into ice-water, extracted with dichloromethane and the organic solution washed with saturated aqueous sodium bicarbonate and water, followed by drying and evaporation. Purification of the resultant material on a column of silica gel (toluene–acetone, 6:1) provided mesylate ***S*-7m** (104 mg, 66%) as a syrup. $[\alpha]_D$ + 9.3° (*c* 1.2, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.25 (m, 15H, 3*Ph*), 5.32 (dq, J_{6,CH_3} = 6.5 Hz, $J_{6,5}$ = 1.0 Hz, 1H, *H*-6), 5.06–4.62 (m, 7H, $3CH_2$Ph, *H*-1), 4.02 (m, 1H, *H*-5), 3.65–3.53 (m, 3H, *H*-3, *H*-4, *H*-2), 3.36 (s, 3H, CH_3O), 3.04 (s, 3H, CH_3SO_2), 1.53 (d, *J* = XX Hz, 3H, CH_3C-6). ^{13}C NMR ($CDCl_3$) δ: 138.54–127.74 (Ph), 98.26 (C-1), 82.30, 79.71, 77.17, 73.55, 72.38 (C-2, C-3, C-4, C-5, C-6), 75.74, 75.31, 75.00 ($3CH_2$Ph), 55.33 (CH_3O), 39.54 (CH_3SO_2-), 17.73 (CH_3C-6). Anal. calcd. for $C_{30}H_{36}O_8S$: C 64.73, H 6.52, S 5.76; found: C 64.85, H 6.55, S 5.52.

Methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-ethyl-6-*O*-methanesulfonyl-α-D-glucopyranoside (*S*-7e)

***S*-4e** (419 mg, 0.85 mmol) was reacted with methanesulfonyl chloride (100 μL, 1.29 mmol) as described for the preparation of ***S*-7m**. Purification of the resultant material on a column of silica gel (toluene–ethyl acetate, 10:1) provided mesylate ***S*-7e** (439 mg, 90%) as a syrup. $[\alpha]_D$ – 15.2° (*c* 0.4, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 15H, 3*Ph*), 5.12 (br t, J_{6,CH_2} = 7.5 Hz, 1H, *H*-6), 5.06–4.63 (3ABq, 6H, $3CH_2$Ph), 4.62 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.02 (t, $J_{2,3} \sim J_{3,4}$ = 9.5 Hz, 1H, *H*-3), 3.76 (br d, $J_{4,5}$ = 9.5 Hz, 1H, *H*-5), 3.64 (t, $J_{3,4} \sim J_{4,5}$ = 9.5 Hz, 1H, *H*-4), 3.55 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.38 (s, 3H, CH_3O), 3.07 (s, 3H, CH_3SO_2), 1.94 (m, 2H, CH_2CH_3), 0.99 (t, *J* = 7.5 Hz, 3H, CH_3CH_2). Anal. calcd. for $C_{31}H_{38}O_8S$: C 65.24, H 6.71; found: C 64.86, H 7.03.

Methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-methyl-α-D-glucopyranoside (*R*-4m)

A stirring mixture of mesylate ***S*-7m** (600 mg, 1.08 mmol) and sodium benzoate (1.0 g, 7.0 mmol) in *N*,*N*-dimethylformamide (20 mL) was heated at 120° for 17 h. It was diluted with dichloromethane, filtered and the solution washed with water, dried and evaporated. The resultant material was purified by HPLC (hexane–acetone, 10:1) to give methyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-(6*R*)-*C*-methyl-α-D-glucopyranoside (***R*-8m**) (294 mg, 47%) as a syrup. $[\alpha]_D$ + 9.8° (*c* 1.04, chloroform). ^{1}H NMR ($CDCl_3$) δ: 8.40–7.20 (m, 20H, 3*Ph*, *Bz*), 5.47 (dq, $J_{5,6}$ = 2.0 Hz, J_{6,CH_3} = 7.0 Hz, 1H, *H*-6), 5.03–4.65 (m, 6H, $3CH_2$Ph), 4.64 (d, $J_{1,2}$ = 4.0 Hz, 1H, *H*-1), 4.04 (dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.93 (dd, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 2.0 Hz, 1H, *H*-5), 3.52 (dd, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.42 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.0 Hz, 1H, *H*-4), 3.38 (s, 3H, CH_3O), 1.18 (d, *J* = 7.0 Hz, 3H, CH_3C-6). Anal. calcd. for $C_{36}H_{38}O_7$: C 74.20, H 6.57; found: C 74.33, H 6.55.

Benzoate ***R*-8m** (137 mg, 0.24 mmol) was dissolved in dioxane (10 mL) and 1 N sodium hydroxide solution (6 mL) was added. After stirring for 48 h at 60°C, the mixture was diluted with dichloromethane and filtered over a pad of silica gel-G. The dichloromethane solution was washed with water, dried and evaporated to give solidifying ***R*-4m** (80 mg, 71%), mp 89–90°C. $[\alpha]_D$ + 28° (*c* 0.4, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.34–7.16 (m, 15H, 3*Ph*), 4.98–4.53 (m, 6H, $3CH_2$Ph), 4.50 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 3.95 (dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.88 (m, 1H, *H*-6), 3.55 (dd, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 4.5 Hz, 1H, *H*-5), 3.43 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.35 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.0 Hz, 1H, *H*-4), 3.31 (s, 3H, CH_3O), 2.49 (d, $J_{6,OH}$ = 6.0 Hz, 1H, OH), 1.05 (d, J_{6,CH_3} = 6.5 Hz, 3H, CH_3C-6). Anal. calcd. for $C_{29}H_{34}O_6$: C 72.78, H 7.16; found: C 72.81, H 7.07.

Methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-ethyl-α-D-glucopyranoside (*R*-4e)

A stirring mixture of mesylate ***S*-7e** (430 mg, 0.75 mmol) and sodium benzoate (600 mg, 4.16 mmol) in *N*,*N*-dimethylformamide (20 mL) was heated at 120° for 20 h. The solution was diluted with dichloromethane, filtered and the solution washed with water, dried and evaporated. The resultant material (methyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-(6*R*)-*C*-ethyl-α-D-glucopyranoside (***R*-8e**)) and potassium hydroxide (2.5 g) in 90% ethanol (50 mL) was kept at reflux for 3 h. The mixture was neutralized with acetic acid, evaporated, and the remainder taken up in dichloromethane. Washing of the solution with water, drying and evaporation was followed by column chromatography of the crude product on a column of silica gel (hexane–ethyl acetate, 4:1 → 3:1). Evaporation of the first fraction provided (*E*)-methyl

2,3,4-tri-*O*-benzyl-6,7,8-trideoxy-α-D-gluco-oct-6-enopyranoside (**9**) (105 mg, 22%) as a crystallizing syrup. $[\alpha]_D$ + 1.8°, $[\alpha]_{365}$ –33.4° (*c* 0.5, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 15H, 3*Ph*), 5.85 (m, 1H, *H*-7), 5.42 (ddd, $J_{6,5}$ = 7.5 Hz, J_{6,CH_3} = 1.5 Hz, $J_{6,7}$ = 14.5 Hz, 1H, *H*-6), 4.98–4.59 (3ABq, 6H, 3CH_2Ph), 4.56 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.01 (m, 1H, *H*-5), 3.96 (t, $J_{2,3} \sim J_{3,4}$ = 9.5 Hz, 1H, *H*-3), 3.51 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.37 (s, 3H, CH_3O), 3.23 (t, $J_{3,4} \sim J_{4,5}$ = 9.5 Hz, 1H, *H*-4), 1.73 (m, 3H, CH_3CH=CH). Anal. calcd. for $C_{30}H_{34}O_5$: C 75.92, H 7.22; found: C 76.05, H 7.31. Continued development eluted ***R*-4e** (159 mg, 43%), identical to the minor Grignard product of aldehyde **3**.

Methyl 2,3,4-tri-*O*-benzyl-7,8-dideoxy-7-*C*-methyl-α-D-gluco-octopyranos-6-uloside (10)

A solution of ***S*-4i** (100 mg, 0.20 mmol) in dimethylsulfoxide (1.5 mL) and acetic anhydride (0.75 mL) was kept at room temperature for 15 h. The solution was evaporated and the residue was taken up in dichloromethane, washed with water, and evaporated to dryness. The ^{1}H NMR spectrum indicated a product mixture of ketone **10** and ether **11** in the ratio 1.6:1. Column chromatography on silica gel (toluene–ethyl acetate, 49:1) provided pure ketone **10** (55 mg, 55%) as an oil. However, a crude product mixture was used for the subsequent reduction because of the difficult separation. $[\alpha]_D$ + 6.4° (*c* 0.6, chloroform). IR ($CHCl_3$) cm^{-1}: 1730 (CO). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.15 (m, 15H, 3*Ph*), 5.00–4.50 (3ABq, 6H, 3CH_2Ph), 4.58 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.35 (d, $J_{4,5}$ = 9.5 Hz, 1H, *H*-5), 4.02 (t, $J_{3,4} \sim J_{2,3}$ = 9.5 Hz, 1H, *H*-3), 3.72 (t, $J_{3,4} \sim J_{4,5}$ = 9.5 Hz, 1H, *H*-4), 3.54 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.42 (s, 3H, CH_3O), 2.85 (m, 1H, CH(CH_3)$_2$), 1.12 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$), 1.07 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$). Anal. calcd. for $C_{31}H_{36}O_6$: C 73.79, H 7.19; found: C 73.45, H 7.11.

Methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-isopropyl-α-D-glucopyranoside (*R*-4i)

To a solution of crude ketone **10** (0.40 mmol) in methanol (10 mL) was added, with stirring, sodium borohydride (65 mg, 1.72 mmol) at ice bath temperatures. After 30 min, the solution was evaporated, and the residue was taken up in dichloromethane, washed with water, dried, and evaporated. The product mixture was applied to a column of silica gel (hexane–ethyl acetate, 6:1 → 5:1 → 4:1) to provide in the first fraction methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-isopropyl-6-*O*-methylthiomethylene-α-D-glucopyranoside (**11**) (63 mg, 28%). $[\alpha]_D$ + 30° (*c* 0.8, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 15H, 3*Ph*), 5.06–4.58 (m, 8H, CH_2S, 3CH_2Ph), 4.65 (d, $J_{1,2}$ = 3.5 Hz, 1H, *H*-1), 4.02 (dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, *H*-3), 3.83 (br d, $J_{4,5}$ = 9.5 Hz, 1H, *H*-5), 3.73 (br d, $J_{6,CH}$ = 7.0 Hz, 1H, *H*-6), 3.65 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.5 Hz, 1H, *H*-4), 3.56 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, 1H, *H*-2), 3.43 (s, 3H, CH_3O), 2.20 (br s, 3H, CH_3S), 2.17 (m, 1H, CH(CH_3)$_2$), 1.01 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$), 0.99 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$). Anal. calcd. for $C_{33}H_{42}O_6S$: C 69.94, H 7.47, S 5.66; found: C 69.81, H 7.65, S 5.73. Continued elution afforded *R*-epimer ***R*-4i** (90 mg, 44%) identical to the minor Grignard product of aldehyde **3**. Further development eluted *S*-epimer ***S*-4i** (22 mg, 11%) identical to the major Grignard product of aldehyde **3**.

Methyl 6-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-(6*R*)-*C*-methyl-α-D-glucopyranoside (*R*-14m)

A solution of 6-*O*-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl bromide (**12**) (34) (2.1 mmol) in dichloromethane (5 mL) was added to a stirring mixture of ***R*-4m** (500 mg, 1.04 mmol), tetraethylammonium bromide (420 mg, 2.00 mmol), *N*,*N*-dimethylformamide (1 mL) and 4 Å molecular sieves (powdered, 2 g) in dichloromethane (5 mL). The mixture was stirred under nitrogen for several days until completion (TLC), diluted with dichloromethane and filtered. The solution was washed with sat. aq $NaHCO_3$ and water, followed by drying and evaporation. The crude product was applied to a column of silica gel (hexane–ethyl acetate, 4:1) to provide ***R*-14m** (587 mg, 59%). $[\alpha]_D$ + 78.2° (*c* 1.1, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 30H, 6*Ph*), 5.00 (d, $J_{1b,2b}$ = 3.5 Hz, 1H, *H*-1b), 4.67 (d, $J_{1a,2a}$ = 3.5 Hz, 1H, *H*-1a), 5.03–4.51 (m, 12H, 6CH_2Ph), 4.27 (dd, $J_{5b,6b}$ = 4.5 Hz, $J_{6b,6b'}$ = 11.5 Hz, 1H, *H*-6b), 4.21 (dd, $J_{5b,6b'}$ = 2.5 Hz, $J_{6b,6b'}$ = 11.5 Hz, 1H, *H*-6b′), 4.08–3.91 (m, 4H, *H*-3a, *H*-3b, *H*-6a, *H*-5b), 3.78 (dd, $J_{5a,6a}$ = 0.5 Hz, $J_{4a,5a}$ = 10.0 Hz, 1H, *H*-5a), 3.57–3.43 (m, 3H, *H*-4a, *H*-4b, *H*-2a), 3.41 (dd, $J_{2b,3b}$ = 9.5 Hz, $J_{1b,2b}$ = 3.5 Hz, 1H, *H*-2b), 3.29 (3, 3H, CH_3O), 1.99 (s, 3H, CH_3CO), 1.16 (d, $J_{6a,CH3}$ = 6.5 Hz, 3H, CH_3C-6a). Anal. calcd. for $C_{58}H_{64}O_{12}$: C 73.09, H 6.77; found: C 73.46, H 6.61.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl bromide (13) (32)

Oxalyl bromide (190 μL, ~1.8 mmol) was added to a stirring solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (650 mg, 1.20 mmol) in dichloromethane (10 mL) and *N*,*N*-dimethylformamide (0.5 mL, 7.0 mmol). After 20 min at room temperature, the mixture was poured into ice water and the organic solution was washed twice with ice-cold water, dried and evaporated to provide **13** (quant.) as a syrup.

Methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-ethyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (*R*-14e) and -β-D-glucopyranoside

A solution of bromide **13** (1.12 mmol) in 1,2-dichloroethane (4 mL) was transferred into a stirring mixture of ***R*-4e** (159 mg, 0.32 mmol), copper(II) bromide (260 mg, 1.16 mmol), *N*,*N*-dimethylformamide (350 μL, 4.93 mmol) in 1,2-dichloroethane (2 mL) containing powdered 4 Å molecular sieves (1 g). After 44 h, toluene (15 mL) and moist pyridine (7.5 mL) were added. After stirring for 30 min, the mixture was filtered and the solvent evaporated. The resultant material was applied to a column of silica gel (hexane–ethyl acetate, 5:1) to first provide the β-anomer of ***R*-14e** (50 mg, 15%) as a syrup. $[\alpha]_D$ + 26.4° (*c* 0.6, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.15 (m, 35H, 7*Ph*), 5.01–4.51 (m, 35H, 7CH_2Ph), 4.60 (d, overlapped, *H*-1a), 4.45 (d, $J_{1b,2b}$ = 7.5 Hz, 1H, *H*-1b), 4.00 (t, $J_{2a,3a} \sim J_{3a,4a}$ = 9.5 Hz, 1H, *H*-3a), 3.48 (dd, $J_{1a,2a}$ = 3.5 Hz, $J_{2a,3a}$ = 9.5 Hz, 1H, *H*-2a), 3.40 (s, overlapped, CH_3O), 1.63 (m, 1H, C$H_2$$CH_3$), 1.40 (m, 1H, C$H_2$$CH_3$), 0.88 (t, *J* = 7.5 Hz, 3H, C$H_3$$CH_2$). Anal. calcd. for $C_{64}H_{70}O_{11}$: C 75.71, H 6.95; found: C 75.40, H 6.91.

Continued elution of the column provided the α-anomer ***R*-14e** (240 mg, 73%) as a syrup. $[\alpha]_D$ + 57° (*c* 1.0, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.10 (m, 35H, 7*Ph*), 5.12 (d, $J_{1b,2b}$ = 3.5 Hz, 1H, *H*-1b), 4.97–4.42 (m, 14H, 7CH_2Ph), 4.52 (d, overlapped, $J_{1a,2a}$ = 3.5 Hz, 1H, *H*-1a), 4.01 (t, $J_{2b,3b} \sim J_{3b,4b}$ = 9.5 Hz, 1H, *H*-3b), 3.97 (t, $J_{2a,3a} \sim J_{3a,4a}$ = 9.5 Hz, 1H, *H*-3a), 3.93 (m, 1H, *H*-5b), 3.85 (br d, $J_{4a,5a}$ = 9.5 Hz, $J_{5a,6a}$ < 1 Hz, 1H, *H*-5a), 3.73 (m, 3H, *H*-4a, *H*-6a, *H*-6b), 3.64 (t, $J_{3b,4b} \sim J_{4b,5b}$ = 9.5 Hz, 1H, *H*-4b), 3.60 (dd, $J_{6b,6b'}$ = 11.0 Hz, $J_{6b',5b}$ = 1.5 Hz, 1H, *H*-6b′), 3.53 (dd, $J_{1b,2b'}$ = 3.5 Hz, $J_{2b,3b}$ = 9.5 Hz, 1H, *H*-2b), 3.35 (dd, $J_{1a,2a}$ = 3.5 Hz, $J_{2a,3a}$ = 9.5 Hz, 1H, *H*-2a), 3.32 (s, 3H, CH_3O), 1.61 (m, 2H, CH_2CH$_3$), 0.89 (t, *J* = 7.5 Hz, 3H, *C*H$_3$CH_2). Anal. calcd. for $C_{64}H_{70}O_{11}$: C 75.71, H 6.95; found: C 75.38, H 6.88.

Methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-isopropyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (*R*-14i)

Alcohol ***R*-4i** (150 mg, 0.30 mmol) was reacted with bromide **13** (1.24 mmol) for 6 days as described for the preparation of ***R*-14e**. Column chromatography (hexane–ethyl acetate, 8:1 → 7:1) provided ***R*-14i** (178 mg, 58%) as a thick syrup. $[\alpha]_D$ + 56.4° (*c* 0.3, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.10 (m, 35H, 7*Ph*), 5.26 (d, $J_{1b,2b}$ = 3.5 Hz, 1H, *H*-1b), 4.96–4.42 (m, 14H, 7CH_2Ph), 4.52 (d, $J_{1a,2a}$ = 3.5 Hz, 1H, *H*-1a), 4.01 (t, $J_{2b,3b} \sim J_{3b,4b}$ = 9.5 Hz, 1H, *H*-3b), 3.96 (t, overlapped, $J_{2a,3a} \sim J_{3a,4a}$ = 9.5 Hz, 1H, *H*-3a), 3.74, (dd, $J_{6b,6b'}$ = 11.0 Hz, $J_{6b,5b}$ = 3.5 Hz, 1H, *H*-6b), 3.61 (dd, $J_{6b',5b}$ = 1.5 Hz, $J_{6b,6b'}$ = 11.0 Hz, overlapped,1H, *H*-6b′), 3.54 (dd, $J_{1b,2b}$ = 3.5 Hz, $J_{2b,3b}$ = 9.5 Hz, 1H, *H*-2b), 3.51 (d, $J_{6a,CH}$ = 8.5 Hz, 1H, *H*-6a), 3.34 (dd, $J_{1a,2a}$ = 3.5 Hz, $J_{2a,3a}$ = 9.5 Hz, 1H, *H*-2a), 3.32 (s, 3H, CH_3O), 2.08 (m, 1H, C*H*(CH$_3$)$_2$), 0.98 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$), 0.89 (d, *J* = 6.5 Hz, 3H, CH(CH_3)$_2$). Anal. calcd. for $C_{65}H_{72}O_{11}$: C 75.85, H 7.05; found: C 75.78, H 7.27.

Methyl 6-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-(6*R*)-*C*-methyl-α-D-glucopyranoside (*S*-14m)

Compound ***S*-4m** (100 mg, 0.21 mmol) was reacted with bromide **12** (0.42 mmol) as described for the preparation of ***R*-14m**. Usual processing and chromatography provided ***S*-14m** (101 mg, 51%) $[\alpha]_D$ + 0.5° (*c* 1.06, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 30H, 6*Ph*), 5.09 (d, $J_{1b,2b}$ = 3.5 Hz, 1H, *H*-1b), 5.02–4.49 (m, 7H, 3CH_2Ph, *H*-1a), 4.26–4.06 (m, 3H, 2*H*-6b, *H*-1a), 4.06–3.94 (m, 3H, *H*-3b, *H*-3a, *H*-5b), 3.77 (dd, $J_{3a,4a}$ = 9.5 Hz, $J_{4a,5a}$ = 9.0 Hz, 1H, *H*-4a), 3.56 (dq, $J_{5a,6a}$ = 2.0 Hz, $J_{4a,5a}$ = 9.0 Hz, 1H, *H*-5a), 3.56–3.47 (m, 2H, *H*-2a, *H*-2b), 3.46 (dd, $J_{3b,4b}$ = 9.0 Hz, $J_{4b,5b}$ = 10.0 Hz, 1H, *H*-4b), 3.38 (s, 3H, CH_3O), 1.92 (s, 3H, CH_3CO), 1.33 (d, J_{6a,CH_3} = 6.0 Hz, 3H, CH_3C-6a). Anal. calcd. for $C_{57}H_{64}O_{12}$: C 73.09, H 6.77; found: C 72.84, H 6.85.

Methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-ethyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (*S*-14e)

Alcohol ***S*-4e** (170 mg, 0.35 mmol) was reacted with bromide **13** (1.24 mmol) for 4 days as described for the preparation of ***R*-14e**. Column chromatography (hexane–dichloromethane-ethyl acetate, 20:10:1–2) provided ***S*-14e** (243 mg, 69%) as a syrup. $[\alpha]_D$ + 48° (*c* 0.5, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.35–7.10 (m, 35H, 7*Ph*), 5.02 (d, $J_{1b,2b}$ = 3.5 Hz, 1H, *H*-1b), 4.96–4.43 (m, 14H, 7CH_2Ph), 4.59 (d, $J_{1a,2a}$ = 3.5 Hz, 1H, *H*-1a), 3.98–3.96 (m, 3H, *H*-3b, *H*-5b, *H*-3a), 3.84 (t, $J_{3a,4a} \sim J_{4a,5a}$ = 9.0 Hz, 1H, *H*-4a), 3.81 (br t, overlapped, J_{6a,CH_2} = 7.0 Hz, 1H, *H*-6a), 3.68–3.66 (m, 2H, *H*-5a, *H*-4b), 3.64 (dd, overlapped, $J_{6b,6b'}$ = 11.0 Hz, $J_{6b,5b}$ = 2.5 Hz, 1H, *H*-6b), 3.52–3.51 (m, 2H, *H*-2b, *H*-6b′), 3.45 (dd, $J_{2a,3a}$ = 9.5 Hz, $J_{1a,2a}$ = 3.5 Hz, 1H, *H*-2a), 3.37 (s, 3H, CH_3O), 1.78 (m, 2H, CH$_3$CH_2), 0.94 (t, *J* = 7.5 Hz, 3H, CH_3CH$_2$). Anal. calcd. for $C_{64}H_{70}O_{11}$: C 75.71, H 6.95; found: C 75.52, H 7.08.

Methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-isopropyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (*S*-14i)

Alcohol ***S*-4i** (160 mg, 0.32 mmol) was reacted with bromide **13** (1.29 mmol) for 5 days as described for the preparation of ***R*-14e**. Column chromatography (hexane–ethyl acetate, 6:1, 5:1) provided ***S*-14i** (207 mg, 64%) as a tough syrup. $[\alpha]_D$ + 57.4° (*c* 0.4, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.35–7.10 (m, 35H, 7Ph), 5.02–4.36 (m, 16H, 7CH_2Ph, *H*-1a, *H*-1b), 4.04 (t, $J_{2b,3b} \sim J_{3b,4b}$ = 9.5 Hz, 1H, *H*-3b), 4.00 (m, overlapped, 1H, *H*-5b), 3.95 (t, $J_{2a,3a} \sim J_{3b,4b}$ = 9.0 Hz, 1H, *H*-4a), 3.90 (t, $J_{3a,4a} \sim J_{4a,5a}$ = 9.0 Hz, 1H, *H*-4a), 3.74 (br d, $J_{5a,6a}$ < 1 Hz, 1H, *H*-5a), 3.68 (t, $J_{3a,4a} \sim J_{4b,5b}$ = 9.0 Hz, 1H, *H*-4b), 3.62 (dd, $J_{6b,6b'}$ = 11.0 Hz, $J_{6b,5b}$ = 2.5 Hz, 1H, *H*-6b), 3.57 (dd, $J_{6b',5b}$ = 1.5 Hz, $J_{6b,6b'}$ = 11.0 Hz, 1H, *H*-6b′), 3.55 (d, $J_{6a,CH}$ = 6.5 Hz, 1H, *H*-6a), 3.51 (dd, $J_{1b,2b}$ = 3.5 Hz, $J_{2b,3b}$ = 9.5 Hz, 1H, *H*-2b), 3.41 (s, 3H, CH_3O), 3.38 (dd, $J_{1a,2a}$ = 3.5 Hz, $J_{2a,3a}$ = 9.0 Hz, 1H, *H*-2a), 2.03 (m, 1H, C*H*(CH$_3$)$_2$), 1.03 (d, 3H, *J* = 6.5 Hz, CH(CH_3)$_2$), 1.00 (d, 3H, *J* = 6.5 Hz, CH(CH_3)$_2$). Anal. calcd. for $C_{65}H_{72}O_{11}$: C 75.85, H 7.05; found: C 75.67, H 7.14.

Methyl 6-*O*-(α-D-glucopyranosyl)-(6*R*)-*C*-methyl-α-D-glucopyranoside (*R*-2m)

To a solution of compound ***R*-14m** (440 mg, 0.46 mmol) in methanol (20 mL) was added a catalytic amount of sodium methoxide. After stirring overnight, it was deionized with Amberlite IRC-50 H^+. Evaporation of the methanol provided methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-methyl-6-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (347 mg, 83%) as an oil. $[\alpha]_D$ + 65.2° (*c* 0.34, water). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 30H, 6*Ph*), 5.00 (d, $J_{1b,2b}$ = 4.5 Hz, 1H, *H*-1b), 4.98–4.53 (m, 13H, 6CH_2Ph, *H*-1a), 4.08–3.91 (m, 3H, *H*-3a, *H*-3b, *H*-6a), 3.81–3.67 (m, 4H, *H*-5a, *H*-5b, H$_2$-6b), 3.56–3.37 (m, 4H, *H*-4a, *H*-4b, *H*-2a, *H*-2b), 3.29 (s, 3H, CH_3O), 1.13 (d, $J_{6a,CH3}$ = 6.5 Hz, 3H, CH_3C-6a). Anal. calcd. for $C_{56}H_{62}O_{11}$: C 73.82, H 6.86; found: C 73.23, H 7.23.

The deacetylation product of ***R*-14m**, namely methyl 2,3,4-tri-*O*-benzyl-(6*R*)-*C*-methyl-6-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside, (247 mg, 0.27 mmol) was debenzylated following the general procedure to give solid ***R*-2m** (80 mg, 80%), mp 231–232°C. $[\alpha]_D$ + 228.1° (*c* 0.43, water). The ^{1}H NMR data are reported in Table 5. ^{13}C NMR (D_2O) δ: 100.13 (C-1a), 97.98 (C-1b), 73.89, 73.73, 73.37, 72.98, 72.02, 71.89, 71.80, 71.48, 70.51, 56.09

(CH_3O), 15.12 (CH_3C-6a). Anal. calcd. for $C_{14}H_{25}O_{11}$: C 45.53, H 6.82; found: C 45.18, H 6.97.

Methyl (6*R*)-*C*-ethyl-6-*O*-(α-D-glucopyranosyl)-α-D-glucopyranoside (*R*-2e)

Compound ***R*-2e** was prepared from ***R*-14e** by catalytic hydrogenolysis following the general procedure and obtained as a white solid (89%) after lyophilization of an aqueous solution. $[\alpha]_D$ + 182.5° (*c* 0.3, water). The ^{1}H NMR data are reported in Table 5. ^{13}C NMR (D_2O) δ: 100.36 (C-1a), 97.01 (C-1b), 78.21, 73.94, 73.73, 73.10, 72.09, 71.89, 71.28, 70.92, 70.47, 61.43 (C-6b), 56.46 (CH_3O), 22.73 (CH_2CH_3), 11.48 (CH_3CH_2).

Methyl 6-*O*-(α-D-glucopyranosyl)-(6*R*)-*C*-isopropyl-α-D-glucopyranoside (*R*-2i)

Compound ***R*-2i** was prepared from ***R*-14i** by catalytic hydrogenolysis following the general procedure and obtained as a white solid (87%) after lyophilization of an aqueous solution. $[\alpha]_D$ + 190.5° (*c* 0.2, water). The ^{1}H NMR data are reported in Table 5. ^{13}C NMR (D_2O) δ: 100.23 (C-1a), 97.86 (C-1b), 82.69, 73.99, 73.64, 73.27, 72.36, 71.88, 71.73, 71.34, 70.47, 61.45 (C-6b), 56.39 (CH_3O), 30.23 ($CH(CH_3)_2$), 20.75, 19.94 ($CH(CH_3)_2$).

Methyl 6-*O*-(α-D-glucopyranosyl)-(6*S*)-*C*-methyl-α-D-glucopyranoside (*S*-2m)

Compound ***S*-14m** (292 mg, 0.31 mmol) was deacetylated as described for ***R*-14m** to provide methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-methyl-6-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (269 mg, 96%) as an oil. $[\alpha]_D$ + 47.6° (*c* 2.0, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.10 (m, 30H, 6*Ph*), 5.03 (d, $J_{1b,2b}$ = 3.5 Hz, 1H, *H*-1b), 5.01–4.57 (m, 13H, 6CH_2Ph, *H*-1a), 4.32 (dq, $J_{5a,6a}$ = 1.5 Hz, J_{6a,CH_3} = 6.5 Hz, 1H, *H*-6a), 4.01 (dd, $J_{2b,3b}$ = 9.5 Hz, $J_{3b,4b}$ = 9.0 Hz, 1H, *H*-3b), 3.98 (dd, $J_{2a,3a}$ = 9.5 Hz, $J_{3a,4a}$ = 10.0 Hz, 1H, *H*-3a), 3.84–3.72 (m, 2H, *H*-5b, *H*-4a), 3.66–3.49 (m, 5H, 2*H*-6a, *H*-5a, *H*-4b, *H*-2a), 3.46 (dd, $J_{1b,2b}$ = 3.5 Hz, $J_{2b,3b}$ = 9.5 Hz, 1H, *H*-2b), 3.36 (s, 3H, CH_3O), 1.32 (d, J_{6a,CH_3} = 6.5 Hz 3H, CH_3C-6). Anal. calcd. for $C_{56}H_{62}O_{11}$: C 73.82, H 6.86; found: C 73.20, H 6.95.

The deacetylation product of ***S*-14m**, namely methyl 2,3,4-tri-*O*-benzyl-(6*S*)-*C*-methyl-6-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside, (126 mg, 0.138 mmol) was debenzylated following the general procedure to give crystalline ***S*-2m** (40 mg, 78%), mp 127–128°. $[\alpha]_D$ + 175.3° (*c* 0.3, water). The ^{1}H NMR data are reported in Table 5. ^{13}C NMR (D_2O) δ: 100.03 (C-1a), 95.56 (C-1b), 74.49, 74.15, 73.68, 73.04, 72.25, 71.99, 70.24, 68.50, 61.17, 55.67 (CH_3O), 14.76 (CH_3-6a).

Methyl-(6*S*)-*C*-ethyl-6-*O*-(α-D-glucopyranosyl)-α-D-glucopyranoside (*S*-2e)

Compound ***S*-2e** was prepared from ***S*-14e** by catalytic hydrogenolysis following the general procedure and obtained as a white solid (94%) after lyophilization of an aqueous solution. $[\alpha]_D$ + 198° (*c* 0.2, water). The ^{1}H NMR data are reported in Table 5. ^{13}C NMR (D_2O) δ: 100.31 (C-1a), 96.31 (C-1b), 74.77, 74.47, 73.85. 73.15, 72.37, 72.01, 70.93, 70.29, 70.14, 61.21 (C-6b), 56.38 (CH_3O), 21.67 (CH_2CH_3), 10.02 (CH_3CH_2).

Methyl 6-*O*-(α-D-glucopyranosyl)-(6*S*)-*C*-isopropyl-α-D-glucopyranoside (*S*-2i)

Compound ***S*-2i** was prepared from ***S*-14i** by catalytic hydrogenolysis following the general procedure and obtained as a white solid (98%) after lyophilization of an aqueous solution. $[\alpha]_D$ + 192° (*c* 0.2, water). The ^{1}H NMR data are reported in Table 5. ^{13}C NMR (D_2O) δ: 100.63 (C-1a), 96.52 (C-1b), 76.45, 74.20, 73.70, 73.05, 72.67, 71.90, 70.86, 70.51, 70.33, 61.15 (C-6b), 57.43 (CH_3O), 28.42 ($CH(CH_3)_2$, 19.04, 17.72 ($CHCH_3)_2$).

Acknowledgements

This research was supported by the Pharmaceutical Manufacturers Association of Canada (Prize to R.U.L.) and in part by the Natural Sciences and Engineering Council of Canada. We are deeply grateful to Professor D.R. Bundle for the use of his computer graphic facilities and the collaboration of his Research Associate, Dr. Chang-Chun Ling.

References

1. R.U. Lemieux and U. Spohr. Adv. Carbohydr. Chem. Biochem. **50**, 1 (1994).
2. J.H. Pazur and T. Ando. J. Biol. Chem. **237**, 297 (1962).
3. K. Hiromi, M. Kawai, and S. Ono. J. Biochem. (Tokyo), **59**, 476 (1966).
4. K. Bock and H. Pedersen. Acta Chem. Scand. Ser. B, **41**, 617 (1987).
5. K. Bock, T. Skrydstrup, and S. Refn. J. Carbohydr. Chem. **10**, 969 (1991).
6. R.U. Lemieux, U. Spohr, M. Bach, D.R. Cameron, T.B. Frandsen, B.B. Stoffer, B. Svensson, and M.M. Palcic. Can. J. Chem. **74**, 319 (1996).
7. A.E. Aleshin, L.M. Firsov, and R.B. Honzatko. J. Biol. Chem. **269**, 15 631 (1994).
8. A.E. Aleshin, B. Stoffer, L.M. Firsov, B. Svensson, and R.B. Honzatko. Biochemistry, **35**, 8319 (1996).
9. M.M. Palcic, T. Skrydstrup, K. Bock, N. Le, and R.U. Lemieux. Carbohydr. Res. **250**, 87 (1993).
10. T.P. Frandsen, M.M. Palcic, C. Dupont, and B. Svensson. Carbohydr. Res. **314**, 127 (1998).
11. R. Stuike-Prill and B. Meyer. Eur. J. Biochem. **194**, 903 (1990).
12. R.U. Lemieux and A.A. Pavia. Can. J. Chem. **47**, 4441 (1969).
13. J.E. Del Bene and J.A.J. Pople. J. Chem. Phys. **58**, 3605 (1973).
14. R.U. Lemieux and G. Huber. Can. J. Chem. **33**, 128 (1955).
15. J.P. Praly and R.U. Lemieux. Can. J. Chem. **65**, 213 (1987).
16. R.U. Lemieux, C.-C. Ling, H. Streicher, and N. Sharon. Israel J. Chem, in press (2001).
17. H. Beierbeck, L.T.J. Delbaere, M. Vandonselaar, and R.U. Lemieux. Can. J. Chem. **72**, 463 (1994).
18. O. Hindsgaul, D.P. Khare, M. Bach, and R.U. Lemieux. Can. J. Chem. **63**, 2653 (1985).
19. U. Spohr, E. Paszkiewicz-Hnatiw, N. Morishima, and R.U. Lemieux. Can. J. Chem. **70**, 254 (1992).
20. R.U. Lemieux, L.T.J. Delbaere, H. Beierbeck, and U. Spohr. Involvement of water in host–guest interactions. Ciba Foundation Symposium no. 158. Host–Guest Molecular Interactions: From Chemistry to Biology, July 3–5, 1990. Wiley, Chichester, London, U.K. 1991. pp. 231–248.
21. R.U. Lemieux. Acc. Chem. Res. **29**, 373 (1996).
22. R.U. Lemieux. How proteins recognize and bind oligosaccharides. *In* Carbohydrate antigens, ACS Symposium Series 519.

Edited by Per J. Garegg and Alf A. Lindberg. American Chemical Society, Washington, D.C. 1993.
23. B.W. Sigurskjold, C.R. Berland, and B. Svensson. Biochemistry, **33**, 10191 (1994).
24. L.T.J. Delbaere, M. Vandonselaar, L. Prasad, J.W. Quail, P.V. Nikrad, J.R. Pearlstone, M.R. Carpenter, L.B. Smillie, U. Spohr, and R.U. Lemieux. Can. J. Chem. **68**, 1116 (1990).
25. M. Cygler, D.R. Rose, and D.R. Bundle. Science (Washington, D.C.), **253**, 442 (1991).
26. F. Cramer and W. Freist. Acc. Chem. Res. **20**, 79 (1987).
27. H. Hashimoto, K. Asano, F. Fujii, and J. Yoshimura. Carbohydr. Res. **104**, 87 (1982).
28. A. Liptak, I. Jodal, and P. Nanasi. Carbohydr. Res. **44**, 1 (1975).
29. D.J. Cram and F.A.A. Elhafez. J. Am. Chem. Soc. **74**, 5828 (1952).
30. D.H. Williams and J. Fleming. Spectroscopic methods in organic chemistry. MacGraw-Hill, U.K. 1980.
31. J.D. Albright and L. Goodman. J. Am. Chem. Soc. **87**, 4217 (1965).
32. R.U. Lemieux, K.B. Hendricks, R.V. Stick, and K. James. J. Am. Chem. Soc. **97**, 4056 (1975).
33. O.P. Srivastava, R. Mendez, and A.P. Venot. Abstracts of the XVth International Carbohydrate Symposium. Yokohama, Japan. August 12–17. 1990. p. 84.
34. G. Zemplén, Z. Csürös, and S. Angyal. Chem. Ber. **70**, 1848 (1937).