SYNTHESIS AND STRUCTURE ASSIGNMENT OF 2-(4-METHOXYBENZYL)CYCLOHEXYL β-D-GALACTOPYRANOSIDE STEREOISOMERS

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Dedicated to Professor Antonín Holý, former Director of this Institute, on the occasion of his 70th birthday.

Several promoters were used in the Koenigs–Knorr synthesis of the title alkyl β-D-galactopyranosides, both in their diastereoisomeric forms (**5a**/**5b** and **6a**/**6b**), resulting from the synthesis performed with the respective racemic *cis* and *trans* isomers of 2-(4-methoxybenzyl)cyclohexan-1-ol, and in their enantiomerically pure forms **5a** and **6a**, starting only from the (1*S*,2*S*)- and (1*S*,2*R*)-enantiomers of 2-(4-methoxybenzyl)cyclohexan-1-ol. The aim of the study was to find convenient modification(s) of the Koenigs–Knorr synthesis of alkyl β-D-galactopyranosides from more hindered and more complex 2-substituted cycloalkanols. Separation of the diastereoisomeric compounds using HPLC on a chiral Nucleodex-β-OH column was used to obtain small quantities of all possibly existing enantiomerically pure products for unambiguous structure assignment by NMR analysis. The (1*S*,2*S*)- and (1*S*,2*R*)-enantiomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a** and **2a**) were prepared by a reduction of 2-(4-methoxybenzyl)cyclohexan-1-one with *Saccharomyces cerevisiae* in enantiomeric purities: ee = 98.5% ((1*S*,2*S*)-enantiomer (**1a**)), and ee ≥ 99% ((1*S*,2*R*)-enantiomer (**2a**)).
Keywords: Koenigs–Knorr synthesis; Glycosylations; Alkyl β-D-galactopyranoside; Glycosides; Stereoselective reactions; Chiral HPLC; Juvenoids; Juvenogens.

2-(4-Methoxybenzyl)cycloalkan-1-ols have been used as model compounds[1] in our research focused on insect juvenile hormone bioanalogs. The model compounds keep important structural features of the biologically active compounds of our interest, and are generally convenient for investigation of chemical reactions to be performed with the biologically active compounds[2]. Their transformation into juvenogens, hormonogenic compounds, is a very important chemical step[2]. The roles of juvenogens are to change physico-chemical properties of the original compound (due to a

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better focusing on a selected target insect pest), and to assist in slow liberation of the biologically active compound by the action of enzymes or environmental factors, i.e. to assist in prolongation of the effect of the biologically active compounds[2]. In fact, juvenogens act as slow release formulations of insect juvenile hormone bioanalogs, and can be understood as biochemically activated complex chemical substances.

In this investigation, attention has been paid to the galactoside derivatives of model alcohols, both racemic and enantiomerically pure isomers of 2-(4-methoxybenzyl)cyclohexan-1-ol. Alkyl galactosides are more polar substances than parent secondary alcohols, and the juvenogens of this type can potentially be used as systemic agents against phytophagous insect species[3].

Biotechnology is an advantageous and environmentally friendly tool for synthetic chemist in the synthesis of single enantiomers of compounds bearing chiral center(s) in their molecules[4]. Two possible ways of application of biotechnological approaches in synthetic organic chemistry have been most widespread: (a) using isolated enzymes or (b) using whole cells as natural bioreactors, which contain all necessary auxiliaries (e.g., co-factors, if required by the enzyme)[4,5]. Application of whole-cell bioreactors (e.g., microorganisms) in the synthesis of enantiomerically pure compounds represents a convenient methodology. We had performed a number of enzymic reductions employing different strains of yeasts[5] (*Saccharomyces cerevisiae* or *Geotrichum candidum*). In that investigation, we have found that only a synthesis of the only enantiomer can be realized with high enantiomeric purity of the product[5].

The Koenigs–Knorr method of synthesis of alkyl glycosides is a very old synthetic procedure, however, still often used due to its broad applicability[6,7]. The need for using up to five-fold molar excess of heavy metal salts (promoters), and limited stability of 2,3,4,6-tetra-*O*-acetyl-α-D-glycosyl halides at higher temperature, are its greatest disadvantages[6,7]. During this reaction, the C-1 chiral centre of the alcohol to be galactosylated is not involved in the reaction, and, therefore, this reaction may also be used on enantiomerically pure alcohols without changing absolute configuration at the C-1 carbon centre[7].

In this partial investigation, we synthesized two of the four possible enantiomers of 2-(4-methoxybenzyl)cyclohexan-1-ol through the reduction of 2-(4-methoxybenzyl)cyclohexan-1-one with whole cells of *Saccharomyces cerevisiae* affording enantiomerically pure alcohols for the synthesis of reference enantiomerically pure alkyl galactosides. In addition, we used reverse phase HPLC microseparation on a chiral Nucleodex-β-OH column (β-cyclo-

dextrin-modified silica gel) for separation of small quantities of alkyl galactosides from their diastereoisomeric mixtures to compare the NMR spectra of synthesized and separated enantiomeric alkyl galactosides.

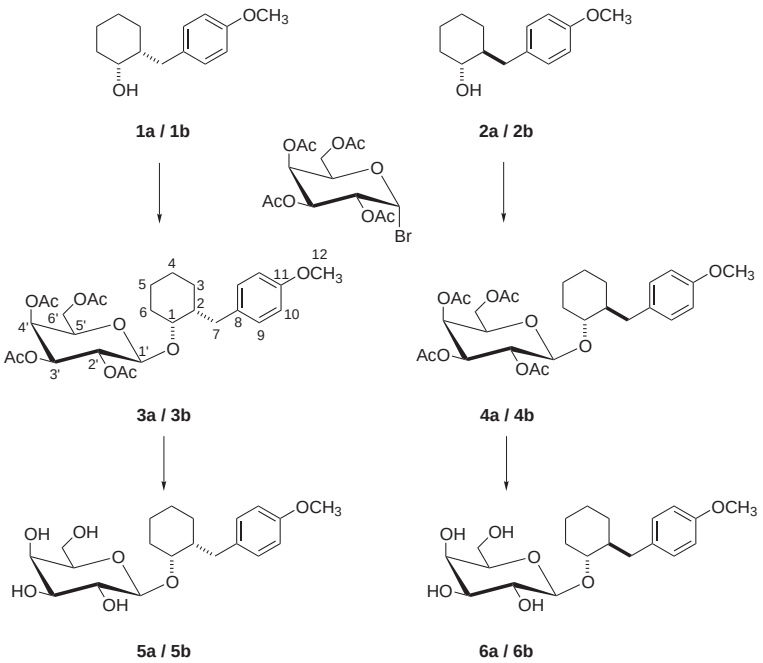
RESULTS AND DISCUSSION

Racemic *cis* and *trans* isomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a**/**1b** and **2a**/**2b**, respectively) were obtained by a simple reduction of the parent ketone with lithium aluminum hydride[8]. In turn, *Saccharomyces cerevisiae*, strain DBM 2115 [9], employed previously in the synthesis of enantiomerically pure 2-substituted cyclohexanols[9], was used in this study to obtain the respective (1*S*,2*S*)- and (1*S*,2*R*)-enantiomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a** and **2a**) in almost quantitative yields and with ee > 98.5% and ee ≥ 99%, starting from the racemic 2-(4-methoxybenzyl)-cyclohexan-1-one.

In the synthesis of 2-(4-methoxybenzyl)cyclohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosides (**3a**/**3b** and **4a**/**4b**; Schemes 1 and 2), four promoters $CdCO_3$, Ag_2CO_3, Ag_2O and Ag_3PO_4 were employed, using toluene as the solvent. The promoter/alcohol molar ratio used was 3:1. Traces of moisture were removed from the mixture containing a solution of the alcohol **1a**/**1b** or **2a**/**2b** in its racemic or enantiomerically pure form (cf. Schemes 1 and 2) in toluene, and a promoter by azeotropic distillation. Thereafter, a solution of an excess of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (commercially available as pure α-anomer from Fluka) in toluene was added dropwise within several minutes under stirring and heating the reaction mixture to the boiling point of toluene (110 °C). When the reaction was complete (monitored by TLC), the reaction mixture was worked-up by filtration and evaporation of the organic solution. The obtained crude residue was purified by column chromatography on silica gel, affording the pure product, either in the form of a diastereoisomeric mixture or single enantiomers (**3a**/**3b** and **4a**/**4b**; Schemes 1 and 2). Based on the yields of the products **3a**/**3b** and **4a**/**4b**, a decrease in the promoter efficiency was observed in the following order of promoters: $CdCO_3$ > Ag_3PO_4 > Ag_2O > Ag_2CO_3 (Table I). Protecting acetyl groups were removed from **3a**/**3b** and **4a**/**4b** by alkaline hydrolysis with potassium carbonate in water/methanol (1:2, v/v). Products **5a**/**5b** and **6a**/**6b** obtained again as diastereoisomeric mixtures or in forms of single enantiomers (Schemes 1 and 2), were purified by column chromatographies on silica gel, using chloroform/methanol (15:1 to 5:1).

A cyclodextrin-based chiral Nucleodex-β-OH column is used with isocratic mobile phase methanol/water (9:1, v/v) for the HPLC analysis of target products **5a**/**5b** and **6a**/**6b**. Retention times of **5a** (16.2 min), **5b** (13.5 min), **6a** (14.2 min) and **6b** (16. 6 min) enabled separation of small quantities of **5b** and **6b**. The protected compounds **3a**/**3b** and **4a**/**4b** could not be separated either on the Nucleodex-β-OH column or on a Reprosil Chiral NR column (Watrex, Ltd., Prague). The NMR experiments led to unambiguous structure assignment of all alkyl β-D-galactopyranosides prepared (**3a**–**6b**).

The NMR data were determined on the basis of both 1D and 2D NMR experiments[10]. The critical analysis of the 1D ^{1}H NMR and ^{1}H,^{1}H PFG COSY spectra[10] of pure enantiomers **3a**–**6a** allowed to obtain the ^{1}H chemical shifts and coupling constants. However, chemical shifts of the cyclohexane

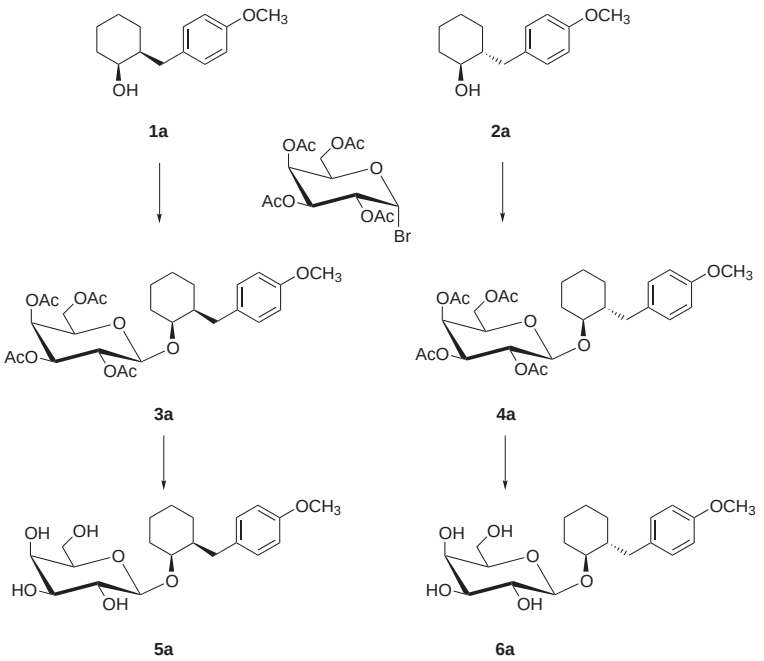


SCHEME 1

Reaction pathways performed with racemic isomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a**/**1b** and **2a**/**2b**). Carbon atom numbers shown in the formula **3a**/**3b** explain those given in Tables II and III for unambiguous assignment of the ^{1}H and ^{13}C NMR signals. The structures **1a**/**1b** and **2a**/**2b** in this scheme show both enantiomers; this is valid for alkyl parts all other structures shown in this scheme.

TABLE I
Conditions for the Koenigs–Knorr synthesis of 2-(4-methoxybenzyl)cyclohexyl galactopyranosides

Alcohol	Promoter	Temperature/Time °C/h	Product	Yield %
1a/**1b**	$CdCO_3$	110/5	**3a**/**3b**	84
2a/**2b**	$CdCO_3$	110/5	**4a**/**4b**	75
1a/**1b**	Ag_2CO_3	110/7	**3a**/**3b**	30
2a/**2b**	Ag_2CO_3	110/7	**4a**/**4b**	43
1a/**1b**	Ag_2O	110/7	**3a**/**3b**	35
2a/**2b**	Ag_2O	110/7	**4a**/**4b**	55
1a/**1b**	Ag_3PO_4	110/7	**3a**/**3b**	41
2a/**2b**	Ag_3PO_4	110/7	**4a**/**4b**	63
1a	$CdCO_3$	110/5	**3a**	90
2a	$CdCO_3$	110/5	**4a**	82



SCHEME 2
Reaction pathways performed with enantiomers **1a** and **2a** of 2-(4-methoxybenzyl)-cyclohexan-1-ol

protons could not be extracted either directly from the 1D NMR spectra or from the ^{1}H,^{1}H PFG COSY spectra. It was necessary to estimate these values from the ^{1}H,^{13}C PFG HSQC spectra[10] using the knowledge of the ^{13}C chemical shifts from the model compounds with similar structure[8]. The 2D ^{1}H,^{13}C PFG HSQC spectra were used for unambiguous assignment of the ^{13}C NMR chemical shifts of the remaining carbon atoms. The NMR data of compounds **3a**–**6b** are summarized in Tables II and III.

CONCLUSION

The protected alkyl galactosides **3a**/**3b** and **4a**/**4b**, and the unprotected alkyl galactosides **5a**/**5b** and **6a**/**6b** were prepared. The structures of single enantiomers **3a**–**6b** were assigned, based mainly on the ^{1}H and ^{13}C NMR analysis, combined with 1D and 2D NMR experiments, and analysis of the ^{1}H,^{1}H PFG COSY and 2D ^{1}H,^{13}C PFG HSQC spectra. Chiral HPLC analysis resulted in separation of small amounts of the diastereoisomeric mixtures **5a**/**5b** and **6a**/**6b** and obtaining pure **5b** and **6b**. A convenient methodology for preparation of cycloalkyl glycosides derived from more complex and sterically hindered cycloalkanols was designed.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were recorded on a Bruker AVANCE 500 spectrometer (in FT mode) at 500.1 and 125.8 MHz, respectively, in $CDCl_3$ or in CD_3OD using either tetramethylsilane (δ 0.0 for ^{1}H NMR) or a solvent signal ($CDCl_3$, δ 77.00 for ^{13}C NMR; CD_3OD, δ 3.31 for ^{1}H NMR and δ 49.50 for ^{13}C NMR) as internal reference at 303 K, the typical spectral width of ^{1}H/^{13}C spectra was 9/180 ppm, for processing 128 k data points were used (for both ^{1}H and ^{13}C). 2D NMR experiments were measured using following characteristic parameters: ^{1}H,^{1}H PFG COSY – spectral width 9 ppm in both f_1, f_2 dimensions, delay 1 s, data matrix for processing 2048 × 2048 data points; ^{1}H,^{13}C PFG HSQC – spectral width 9 ppm in f_2 and 180 ppm in f_1, delay 1 s, data matrix for processing 2048 × 2048 data points. IR spectra (wavenumbers in cm^{-1}) were recorded in solution (CCl_4) or by the KBr technique on a Bruker IFS 88 instrument. MS (FAB; matrix thioglycerol/glycerol (3:1)) were recorded on a VG analytical 70–250 SE mass spectrometer, ZAB-EQ (BEQQ configuration) at 70 eV. An Autopol IV polarimeter (Rudolph Research Analytical, U.S.A.) was used for measurement of optical rotation; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. A Unimax 1010 incubator (Heidolph, Germany) equipped with controlled heating and shaking plate. Preparative column chromatography was performed on a silica gel type 60 (particle size 0.04–0.063 mm; Fluka, Switzerland). TLC was performed on aluminum sheets precoated with silica gel type 60 (Merck, Germany). Analytical HPLC was carried out on a TSP (Thermoseparation Products, U.S.A.) instrument equipped with a ConstaMetric 4100 Bio pump and a SpectroMonitor 5000 UV DAD. The analyses of the products were performed on a chiral Nucleodex β-OH

TABLE II
^{1}H and ^{13}C NMR data of 2-(4-methoxybenzyl)cyclohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosides **3a**, **3b**, **4a** and **4b**[a]

Position	^{1}H NMR spectra				^{13}C NMR spectra			
	3a	**3b**	**4a**	**4b**	**3a**	**3b**	**4a**	**4b**
1	3.66 ddd *J* = 2.6, 2.6, 4.6	3.81 ddd *J* = 2.3, 2.3, 4.5	3.22 ddd *J* = 4.6, 9.5, 10.5	3.32 ddd *J* = 4.1, 9.5, 9.5	80.00 d	75.17 d	85.81 d	81.25 d
2[b]	1.59–1.63 m	1.58–1.61 m	1.59–1.61 m	1.57–1.57 m	43.75 m	43.81 m	45.12 d	44.28 d
3[b]	1.28–1.43 m	1.28–1.43 m	0.86–0.89 m 1.62–1.65 m	0.86–0.89 m 1.62–1.65 m	26.55 t	26.73 t	29.75 t	29.60 t
4[b]	1.18–1.23 m 1.59–1.65 m	1.16–1.21 m 1.57–1.64 m	1.18–1.21 m 1.69–1.72 m	1.18–1.21 m 1.69–1.72 m	25.34 t	24.96 t	24.75 t	24.41 t
5[b]	1.28–1.43 m	1.28–1.43 m	1.05–1.08 m 1.50–1.53 m	1.05–1.08 m 1.50–1.53 m	20.87 t	20.98 t	24.92 t	24.81 t
6[b]	1.32–1.36 m 1.95–2.00 m	1.23–1.28 m 1.80–1.86 m	1.18–1.21 m 1.94–1.97 m	1.41–1.44 m 2.15–2.17 m	31.85 t	28.88 t	33.80 t	31.26 t
7	2.40 dd *J* = 8.0, 13.6 2.53 dd *J* = 6.9, 13.6	2.44 dd *J* = 7.0, 13.8 2.74 dd *J* = 7.4, 13.8	2.00–2.20 m 3.08 dd *J* = 2.8, 13.3	2.09–2.14 m 3.19 dd *J* = 3.8, 13.4	37.23 t	37.03 t	37.28 t	37.37 t
8	–	–	–	–	132.96 s	133.51 s	132.44 s	132.99 s
9	7.02 m	7.13 m	7.03 m	7.09 m	129.83 d	130.27 d	130.11 d	130.35 d
10	6.84 m	6.80 m	6.82 m	6.80 m	113.65 d	113.40 d	113.59 d	113.44 d
11	–	–	–	–	157.68 s	157.83 s	157.75 s	157.66 s
12	3.80 s	3.78 s	3.79 s	3.78 s	55.20 q	55.24 q	55.20 q	55.20 q

TABLE II
(*Continued*)

Position	^{1}H NMR spectra				^{13}C NMR spectra			
	3a	**3b**	**4a**	**4b**	**3a**	**3b**	**4a**	**4b**
1′	4.51 d *J* = 7.9	4.50 d *J* = 7.9	4.60 d *J* = 8.0	4.56 d *J* = 7.9	102.25 d	98.85 d	102.55 d	99.24 d
2′	5.30 dd *J* = 7.9, 10.6	5.30 dd *J* = 7.9, 10.5	5.32 dd *J* = 8.0, 10.5	5.20 dd *J* = 7.9, 10.5	69.15 d	69.34 d	69.25 d	69.32 d
3′	5.04 dd *J* = 3.6, 10.6	5.05 dd *J* = 3.5, 10.5	5.04 dd *J* = 3.5, 10.5	5.04 dd *J* = 3.5, 10.5	71.08 d	71.16 d	71.13 d	71.18 d
4′	5.40 dd *J* = 1.3, 3.6	5.38 dd *J* = 1.2, 3.5	5.40 dd *J* = 1.2, 3.5	5.39 dd *J* = 1.3, 3.5	67.11 d	67.19 d	67.17 d	67.13 d
5′	3.85 dt *J* = 1.3, 3.7, 6.7	3.87 dt *J* = 1.2, 6.7, 6.7	3.92 dt *J* = 1.2, 6.8, 6.8	3.89 dt *J* = 1.3, 6.3, 6.8	70.30 d	70.45 d	70.46 d	70.52 d
6′	4.08 dd *J* = 6.8, 11.2 4.16 dd *J* = 6.6, 11.2	4.16 d *J* = 6.7	4.10 dd *J* = 6.8, 11.2 4.22 dd *J* = 6.8, 11.2	4.11 dd *J* = 6.3, 11.1 4.22 dd *J* = 6.2, 11.1	61.36 t	61.37 t	61.36 t	61.36 t

[a] Signals of the acetoxy groups: ^{1}H NMR spectra: **3a**: 2.00 (s), 2.00 (s), 2.03 (s), 2.06 (s); **3b**: 2.02 (s), 2.09 (s), 2.09 (s), 2.17 (s); **4a**: 2.05 (s), 2.09 (s), 2.12 (s), 2.14 (s); **4b**: 2.03 (s), 2.04 (s), 2.06 (s), 2.16 (s). ^{13}C NMR spectra: **3a**: 20.67 (q), 20.69 (q), 20.73 (q), 20.82 (q), 169.42 (s), 169.50 (s), 170.08 (s), 170.26 (s); **3b**: 20.61 (q), 20.62 (q), 20.71 (q), 20.90 (q), 169.95 (s), 170.21 (s), 170.39 (s), 170.48 (s); **4a**: 20.57 (q), 20.65 (q), 20.72 (q), 20.82 (q), 169.90 (s), 169.98 (s), 170.38 (s), 170.52 (s); **4b**: 20.60 (q), 20.69 (q), 20.80 (q), 20.83 (q), 169.40 (s), 169.86 (s), 170.22 (s), 170.40 (s). [b] The ^{1}H NMR chemical shifts were assigned on the basis of the ^{1}H,^{13}C HSQC spectra (cf. Results and Discussion).

TABLE III
^{1}H and ^{13}C NMR data of 2-(4-methoxybenzyl)cyclohexyl β-D-galactopyranosides **5a**, **5b**, **6a** and **6b**

Position	^{1}H NMR spectra				^{13}C NMR spectra			
	5a	**5b**	**6a**	**6b**	**5a**	**5b**	**6a**	**6b**
1	3.74 ddd *J* = 2.4, 2.4, 4.8	3.91 ddd *J* = 2.4, 2.4, 4.9	3.31 ddd *J* = 4.1, 9.8, 9.8	3.48 ddd *J* = 4.2, 9.7, 9.7	80.56 d	76.48 d	85.82 d	81.26 d
2[a]	1.76–1.80 m	1.73–1.76 m	1.58–1.62 m	1.55–1.58 m	45.32 d	45.71 d	47.43 d	46.73 d
3[a]	1.29–1.33 m 1.50–1.54 m	1.29–1.33 m 1.50–1.54 m	0.87–0.90 m 1.62–1.64 m	0.86–0.89 m 1.59–1.62 m	28.31 t	28.27 t	31.39 t	31.58 t
4[a]	1.22–1.25 m 1.61–1.65 m	1.22–1.25 m 1.61–1.65 m	1.22–1.26 m 1.70–1.76 m	1.22–1.26 m 1.70–1.76 m	26.15 t	26.55 t	26.49 t	26.22 t
5[a]	1.31–1.35 m 1.64–1.69	1.27–1.31 m 1.72–1.76 m	1.05–1.09 m 1.53–1.57 m	1.04–1.08 m 1.51–1.55 m	23.27 t	22.37 t	26.70 t	26.85 t
6[a]	1.35–1.39 m 2.00–2.07 m	1.29–1.33 m 1.93–1.98 m	1.35–1.38 m 2.21–2.24 m	1.29–1.33 m 2.17–2.21 m	33.29 t	30.18 t	35.72 t	32.78 t
7	2.53 dd *J* = 8.4, 13.6 2.85 dd *J* = 6.5, 13.6	2.42 dd *J* = 7.8, 13.7 2.81 dd *J* = 6.6, 13.7	2.14 dd *J* = 10.0, 13.4 3.40 dd *J* = 3.3, 13.4	2.21 dd *J* = 9.5, 13.7 3.24 dd *J* = 3.6, 13.7	37.44 t	38.25 t	39.03 t	39.01 t
8	–	–	–	–	135.50 s	135.65 s	135.01 s	135.13 s
9	7.15 m	7.14 m	7.09 m	7.08 m	131.69 d	131.81 d	131.76 d	131.86 d
10	6.80 m	6.79 m	6.80 m	6.80 m	115.05 d	143.93 d	115.00 d	114.93 d
11	–	–	–	–	159.69 s	159.62 s	159.70 s	159.65 s
12	3.76 s	3.75 s	3.75 s	3.75 s	56.13 q	56.12 q	56.14 q	56.12 q

TABLE III
(*Continued*)

Position	^{1}H NMR spectra				^{13}C NMR spectra			
	5a	**5b**	**6a**	**6b**	**5a**	**5b**	**6a**	**6b**
1′	4.29 d *J* = 7.7	4.30 d *J* = 7.7	4.36 d *J* = 7.7	4.36 d *J* = 7.5	106.31 d	102.98 d	106.80 d	102.54 d
2′	3.60 dd *J* = 7.7, 9.8	3.60 dd *J* = 7.7, 9.7	3.57 dd *J* = 7.7, 9.6	3.55 dd *J* = 7.5, 9.7	73.55 d	73.24 d	73.59 d	73.13 d
3′	3.48 dd *J* = 3.5, 9.8	3.48 dd *J* = 3.5, 9.7	3.47 dd *J* = 3.4, 9.8	3.48 dd *J* = 3.3, 9.7	75.72 d	75.74 d	75.66 d	75.64 d
4′	3.85 dd *J* = 1.1, 3.5	3.88 dd *J* = 1.2, 3.5	3.85 dd *J* = 1.0, 3.4	3.86 dd *J* = 1.1, 3.3	70.76 d	70.76 d	70.76 d	70.81 d
5′	3.44 dt *J* = 1.1, 6.2, 6.2	3.48 dt *J* = 1.2, 6.2, 6.2	3.50 dt *J* = 1.0, 6.2, 6.2	3.50 dt *J* = 1.1, 6.2, 6.2	76.86 d	76.90 d	76.92 d	77.01 d
6′	3.69 dd *J* = 6.4, 11.2 3.72 dd *J* = 6.0, 11.2	3.76 dd *J* = 6.1, 11.0 3.80 dd *J* = 6.3, 11.0	3.72 dd *J* = 6.3, 11.2 3.75 dd *J* = 6.1, 11.2	3.75 dd *J* = 6.2, 11.2 3.78 dd *J* = 6.3, 11.2	62.84 t	62.84 t	62.89 t	62.92 t

[a] The ^{1}H NMR chemical shifts were assigned on the basis of the ^{1}H,^{13}C HSQC spectra (cf. Results and Discussion).

column (150 × 4 mm; Macherey-Nagel, Germany) using methanol/water (9:1, v/v) as mobile phase at 0.3 ml min^{-1}. The eluate was monitored at 220, 254 and 275 nm, and the UV spectra were run from 200 to 300 nm.

(1*S*,2*S*)- and (1*S*,2*R*)-2-(4-Methoxybenzyl)cyclohexan-1-ol (**1a** and **2a**)

Bioreduction of 2-(4-methoxybenzyl)cyclohexan-1-one was performed by a new strain DBM 2115 of *Saccharomyces cerevisiae*, obtained from the Research Institute of Fermentation Industry (Prague, Czech Republic). Yeast was cultivated in a cultivation medium[9] (100 ml) at 27 ± 1 °C for 48 h. The ketone (150 mg per flask, 0.688 mmol), dissolved in ethanol (0.5 ml), was added to the yeast in five 250-ml flasks, and the reaction was maintained under shaking at 27 ± 1 °C for 7 days using a Unimax incubator, and worked-up as described earlier[9]. Yields: **1a** 48.5%, ee = 98.5%; **2a** 45.4%, ee ≥ 99%. Both ^{1}H and the ^{13}C NMR spectra of **1a** and **2a** were compared with the literature data[11]. Their absolute configurations were assigned by transforming alcohols **1a** and **2a** into their diastereoisomeric esters with (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid and analyzing their the ^{1}H and ^{19}F NMR spectra as described earlier[11].

2-(4-Methoxybenzyl)cyclohexyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (**3a**/**3b** and **4a**/**4b** or **4a** and **4b**)

In a typical experiment, the respective isomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a**/**1b** or **2a**/**2b**, and **1a** or **2a**; 0.136 g, 0.62 mmol) were dissolved in toluene (15 ml), and a promoter (1.86 mmol) was added. A portion of the toluene (7 ml) was distilled off under azeotropic conditions to remove all traces of water from the system. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (0.764 g, 1.86 mmol) in toluene (2 ml) was then added over 5 min to the reaction mixture, which was further stirred and heated under azeotropic conditions for additional 6 h. A mixture of inorganic salts was removed by filtration and the solvent was evaporated from the filtrate. Column chromatography of the residue on silica gel (light petroleum/ether (3:1 to 1:1)) yielded pure products in the respective yields given in Table I. The ^{1}H and ^{13}C NMR data of compounds **3a**, **3b**, **4a** and **4b** are summarized in Table II, and their additional analytical data are shown below:

3a/**3b**: IR (CCl_4): 3063 (w), 3030 (w), 2936 (m), 2859 (w), 2836 (w), 1757 (s), 1613 (w), 1513 (s), 1441 (w), 1369 (s), 1300 (w), 1246 (s), 1221 (s), 1173 (s), 1056 (s), 1045 (s), 898 (w), 842 (w). MS (FAB), *m/z* (%): $[M]^+$ 550 (1), 331 (10), 203 (12), 169 (24), 121 (100), 109 (28), 91 (10). For $C_{28}H_{38}O_{11}$ (550.6) calculated: 61.08% C, 6.96% H; found: 61.00% C, 6.92% H.

4a/**4b**: IR (CCl_4): 3063 (w), 3031 (w), 2935 (m), 2859 (m), 2836 (w), 1757 (s), 1613 (w), 1513 (s), 1441 (w), 1369 (s), 1300 (w), 1245 (s), 1221 (s), 1172 (s), 1044 (s), 880 (w), 856 (w). MS (FAB), *m/z* (%): $[M]^+$ 550 (1), 331 (16), 202 (18), 169 (36), 121 (100), 109 (34), 81 (10). For $C_{28}H_{38}O_{11}$ (550.6) calculated: 61.08% C, 6.96% H; found: 60.99% C, 6.99% H.

2-(4-Methoxybenzyl)cyclohexyl β-D-Galactopyranoside (**5a**/**5b** and **6a**/**6b**)

In a typical experiment, a solution of the respective mixtures of diastereoisomers **3a**/**3b** or **4a**/**4b**, or of the respective pure enantiomers **3a** or **4a** (0.103 g, 0.187 mmol) in a mixture of methanol (10 ml) and water (2 ml) was heated to reflux in the presence of potassium carbonate (0.15 g) for 2 h. Methanol and water were removed under reduced pressure, the resi-

due was applied onto the top of a column filled with silica gel and purified by elution with a chloroform/methanol mixture (15:1 to 5:1), affording pure products in the respective yields: **5a**/**5b** 90%, **6a**/**6b** 80%, **5a** 93% and **6a** 86%. Small samples of the diastereoisomeric mixtures **5a**/**5b** and **6a**/**6b** were separated by analytical HPLC on a chiral Nucleodex β-OH column, with methanol/water (9:1, v/v) as a mobile phase, to get a small quantities of compounds **5b** and **6b** in their enantiomerically pure forms. The ^{1}H and ^{13}C NMR data of compounds **5a**, **5b**, **6a** and **6b** are summarized in the Table III. Their additional analytical data are shown below:

5a/**5b**: IR (KBr): 3413 (s), 2923 (s), 2908 (m), 1611 (w), 1512 (s) 1105 (m), 1090 (s), 1082 (s), 1049 (s), 1038 (s), 848 (w). MS (FAB), *m/z* (%): $[M + H]^+$ 383 (1), 231 (2), 203 (9), 121 (100), 91 (8), 79 (17). For $C_{20}H_{30}O_7$ (382.5) calculated: 62.81% C, 7.91% H; found: 62.87% C, 7.85% H. HPLC analysis (methanol/water (9:1, v/v)): **5a**: 16.2 min, **5b**: 13.5 min. **5a**: $[\alpha]_D^{20}$ +35.0 (*c* 0.075, CH_3OH).

6a/**6b**: IR (KBr): 3520 (s), 3420 (s), 2990 (w), 1613 (m), 1514 (s) 1100 (m), 1084 (s), 1078 (m), 1052 (s), 1035 (s), 880 (w). MS (FAB), *m/z* (%): $[M + H]^+$ 383 (1), 231 (1), 203 (10), 121 (100), 91 (8), 79 (11). For $C_{20}H_{30}O_7$ (382.5): 62.81% C, 7.91% H; found: 62.78% C, 7.87% H. HPLC analysis (methanol/water (9:1, v/v)): **6a**: 14.2 min, **6b**: 16.5 min. **6a**: $[\alpha]_D^{20}$ –9.3 (*c* 0.078, CH_3OH).

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