SYNTHESIS AND STRUCTURE ASSIGNMENT OF 2-(4-METHOXYBENZYL)CYCLOHEXYL β-D-GLUCOPYRANOSIDE ENANTIOMERS

David Šaman[a1], Pavel Kratina[a,b], Jitka Moravcová[b1], Martina Wimmerová[a] and Zdeněk Wimmer[a2,*]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague 6, Czech Republic; e-mail: [1] saman@uochb.cas.cz, [2] wimmer@uochb.cas.cz*

[b] *Institute of Chemical Technology, Prague, Technická 5, CZ-16628 Prague 6, Czech Republic; e-mail: [1] jitka.moravcova@vscht.cz*

Received August 3, 2006
Accepted September 30, 2006

Glucosylation of the *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a**/**1b**, **2a**/**2b**, **1a** or **2a**) was performed to prepare the corresponding alkyl β-D-glucopyranosides, mainly to get analytical data of pure enantiomers of the glucosides (**3a**–**6b**), required for subsequent investigations of related compounds with biological activity. One of the employed modifications of the Koenigs–Knorr synthesis resulted in achieving 85–95% yields of pure β-anomers **3a**/**3b**, **4a**/**4b**, **3a** or **4a** of protected intermediates, with several promoters and toluene as solvent, yielding finally the deprotected products **5a**/**5b**, **6a**/**6b**, **5a** or **6a** as pure β-anomers. To obtain enantiomerically pure β-anomers of the target structure (**3a**, **4a**, **5a** and **6a**) for unambiguous structure assignment, an enzymic reduction of 2-(4-methoxybenzyl)cyclohexan-1-one by *Saccharomyces cerevisiae* whole cells was performed to get (1*S*,2*S*)- and (1*S*,2*R*)-enantiomers (**1a** and **2a**) of 2-(4-methoxybenzyl)cyclohexan-1-ol. The opposite enantiomers of alkyl β-D-glucopyranosides (**5b** and **6b**) were obtained by separation of the diastereoisomeric mixtures **5a**/**5b** and **6a**/**6b** by chiral HPLC. All stereoisomers of the products (**3a**–**6b**) were subjected to a detailed ^{1}H NMR and ^{13}C NMR analysis.

Keywords: Glycosides; Glycosidations; Glucosylations; Koenigs–Knorr synthesis; Enzymatic reduction; Insect juvenile hormon analogs; Juvenoids; Chiral HPLC; NMR spectroscopy.

Formation of the glycosidic linkage is a result of various factors that include, among others, electronic, stereoelectronic, conformational, substituent and reactivity effects generally associated with the incipient oxocarbenium ions derived from carbohydrates. These factors mostly concern the glycosyl donor molecule. Additional parameters to be considered in the formation of alkyl glycosides involve the nature of the alcohol acceptor, polarity of the solvent, and the type of catalyst or promoter activating

doi:10.1135/cccc20061470

the leaving group of the anomeric carbon of the donor molecule[1,2]. Due to the above-mentioned reasons, glycosylation of sterically hindered cycloalkanols requires a powerful synthetic method to get the products in high chemical yields.

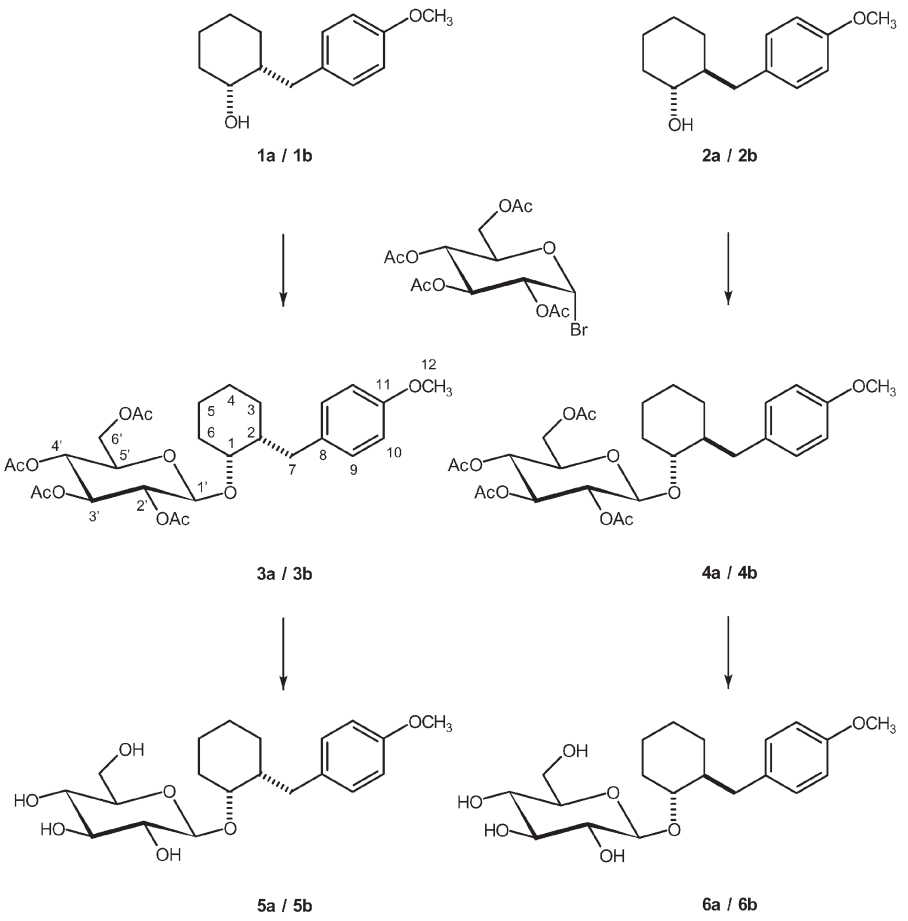
Even a well known procedure, which the Koenigs–Knorr synthesis[3] undoubtedly is, can become a powerful method leading to strong glycosyl donor properties in the activated species through exchange of the anomeric hydroxyl functionality by bromine or chlorine atom in the activation step. An α-halosubstituted carbohydrate generated can be readily activated in the glycosylation step by halophilic promoters, i.e. heavy metal salts, resulting in irreversible glycosyl transfer to the acceptor. This method has always been valuable in the synthesis of complex alkyl glycosides, oligosaccharides or glycoconjugates. As such, it has been continuously developed and widely employed[4]. In spite of the versatility of the Koenigs–Knorr method, the requirement for up to 4 equivalents of heavy metal salts as promoters could be a limiting factor for large-scale preparations. Nevertheless, the method was considered as reliable for the present study dealing with a synthesis and subsequent analytical study of the title alkyl β-D-glucopyranosides.

The racemic and enantiomerically pure *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a**/**1b**, **2a**/**2b**, **1a** and **2a**) were used as glycosyl acceptors in this study because they are general key intermediates in the synthesis of a large series of compounds mimicking the mode of action of the insect juvenile hormone[5]. Glycosylation of the cyclic alcohols **1a**/**1b**, **2a**/**2b**, **1a** and **2a** resulted in preparations of the target alkyl β-D-glucopyranosides **6a**, **6b**, **7a** and **7b** (Schemes 1 and 2).

To achieve enantiomerically pure alkyl β-D-glucopyranosides, two of the four possible enantiomers of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a** and **2a**) were synthesized through the reduction of 2-(4-methoxybenzyl)-cyclohexan-1-one by *Saccharomyces cerevisiae* whole cells. Biotechnology is a convenient methodology for the synthesis of enantiomers of chiral compounds[6]. Two biotechnological methods have been the most widespread: (i) the use of isolated enzymes or (ii) the use of whole cells as natural bioreactors, which contain all necessary auxiliaries (e.g. co-factors)[6,7]. We performed a number of enzymic reductions employing various microorganisms, e.g. *Saccharomyces cerevisiae* or *Geotrichum candidum*, and found that the enzymic reduction proceeds with high enantioselectivity[7]. A reverse phase HPLC microseparation on a chiral Nucleodex-β-OH column (β-cyclodextrin-modified silica gel) was performed to obtain small quantities of pure enantiomers of alkyl β-D-glucopyranosides from their diastereoisomeric

mixtures to be able to unambiguously assign the signals in their ^{1}H and ^{13}C NMR spectra.

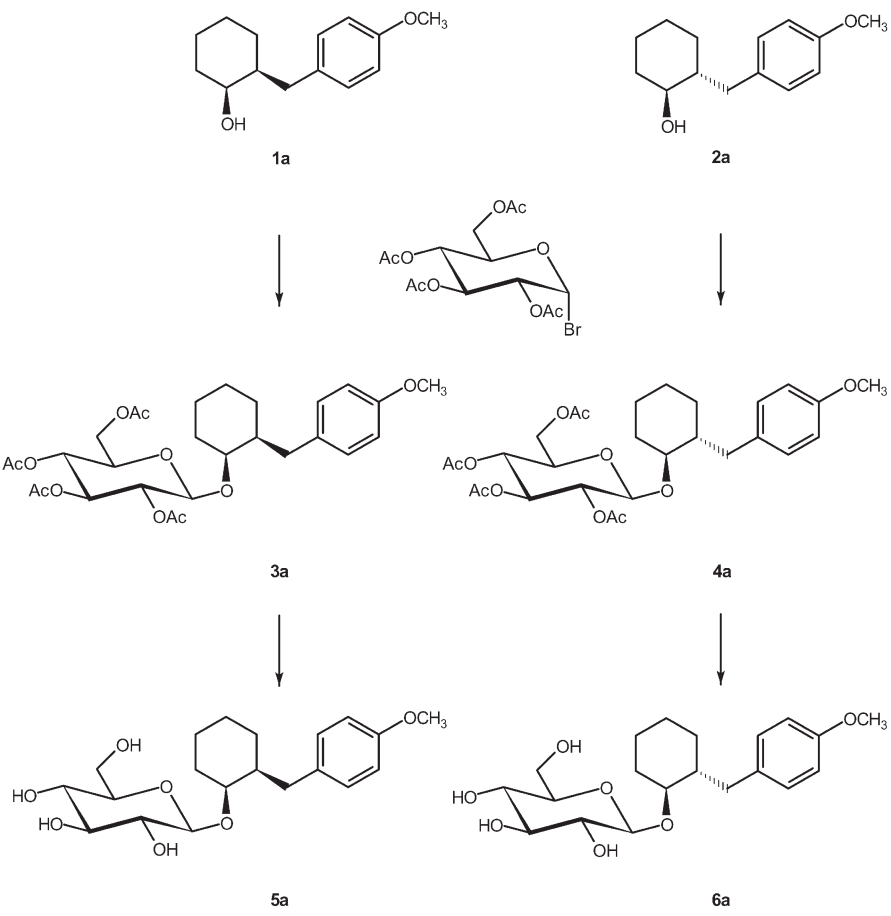
The objective of the present investigation was (i) to synthesize model alkyl β-D-glucopyranosides in their enantiomerically pure forms, (ii) to study conditions for their separation by chiral HPLC, provided that the synthesis



SCHEME 1

Reaction pathways performed with racemates of 2-(4-methoxybenzyl)cyclohexan-1-ol (**1a**/**1b** and **2a**/**2b**). The carbon atom numbering shown in the formula of **3a**/**3b** corresponds to that used 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

starts from racemic alcohols, and (iii) to perform as detailed as possible structure assignment of the protected and unprotected alkyl glucosides by NMR measurement, especially due to the fact that reliable model systems are required for a detailed investigation of more complicated molecules of insect juvenile hormone analogs (juvenoids) of this series (cf. ref.[5]). Similar study has recently been performed with alkyl β-D-galactopyranosides[8].



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

RESULTS AND DISCUSSION

Method A: Using a modified Koenigs–Knorr synthesis, reaction mixtures were heated to 110 °C in boiling toluene for about 5–7 h (monitored by TLC). Four promoters ($CdCO_3$, Ag_2CO_3, Ag_2O and Ag_3PO_4) were employed. The promoter/alcohol molar ratio used in the reaction was 3:1. Based on the yields of the products **3a**/**3b** and **4a**/**4b**, the promoter efficiency decreased in the order: $CdCO_3$ > Ag_3PO_4 > Ag_2O > Ag_2CO_3 (Table I).

Method B: With dichloromethane as the solvent, reaction mixtures were stirred at room temperature for 24 h, and five promoters ($CdCO_3$, Ag_3PO_4, AgOTf, $AgClO_4/Ag_2CO_3$ (1:1) and $LiClO_4$) were employed, using again a 3:1 molar excess of the promoter/acceptor (**1a**/**1b** or **2a**/**2b**). When these promoters were compared, their efficiency decreased in the order AgOTf > $AgClO_4/Ag_2CO_3$ (1:1) > $CdCO_3$ > Ag_3PO_4 > $LiClO_4$ based again on the yield

TABLE I
Koenigs–Knorr glucosylation of 2-(4-methoxybenzyl)cyclohexan-1-ol

Method	Promoter	Acceptor	Solvent	Temperature/Time °C/h	Product	Yield %
A	$CdCO_3$	**1a**/**1b**	toluene	110/5	**3a**/**3b**	87
A	$CdCO_3$	**2a**/**2b**	toluene	110/5	**4a**/**4b**	95
A	Ag_2CO_3	**1a**/**1b**	toluene	110/7	**3a**/**3b**	20
A	Ag_2CO_3	**2a**/**2b**	toluene	110/7	**4a**/**4b**	33
A	Ag_2O	**1a**/**1b**	toluene	110/7	**3a**/**3b**	25
A	Ag_2O	**2a**/**2b**	toluene	110/7	**4a**/**4b**	35
A	Ag_3PO_4	**1a**/**1b**	toluene	110/7	**3a**/**3b**	21
A	Ag_3PO_4	**2a**/**2b**	toluene	110/7	**4a**/**4b**	43
A	$CdCO_3$	**1a**	toluene	110/5	**3a**	85
A	$CdCO_3$	**2a**	toluene	110/5	**4a**	92
B	$AgClO_4/Ag_2CO_3$ (1:1)	**1a**/**1b**	CH_2Cl_2	r.t./24	**3a**/**3b**	24
B	$AgClO_4/Ag_2CO_3$ (1:1)	**2a**/**2b**	CH_2Cl_2	r.t./24	**4a**/**4b**	30
B	AgOTf	**1a**/**1b**	CH_2Cl_2	r.t./24	**3a**/**3b**	40
B	AgOTf	**2a**/**2b**	CH_2Cl_2	r.t./24	**4a**/**4b**	40
B	$LiClO_4$	**1a**/**1b**	CH_2Cl_2	r.t./24	**3a**/**3b**	20
B	$LiClO_4$	**2a**/**2b**	CH_2Cl_2	r.t./24	**4a**/**4b**	25
B	$CdCO_3$	**1a**/**1b**	CH_2Cl_2	r.t./24	**3a**/**3b**	29
B	$CdCO_3$	**2a**/**2b**	CH_2Cl_2	r.t./24	**4a**/**4b**	35
B	Ag_3PO_4	**1a**/**1b**	CH_2Cl_2	r.t./24	**3a**/**3b**	23
B	Ag_3PO_4	**2a**/**2b**	CH_2Cl_2	r.t./24	**4a**/**4b**	30

of the products **4a**/**4b** and **5a**/**5b** (Table I). Comparing both methods, method *A* employing $CdCO_3$ as promoter was evaluated as the most satisfactory. The exclusive presence of the required β-anomers of the products **3a**/**3b** and **4a**/**4b** was found, which was also reported by Schmidt[3,9].

When the starting alcohols were racemates (**1a**/**1b** and **2a**/**2b**), alkyl glycosides **3a**/**3b** and **4a**/**4b** formed during the reaction, were obtained as diastereoisomeric mixtures (Scheme 1, Table I). However, when using pure enantiomers of the starting alcohol (**1a** and **2a**), pure enantiomers of the products (**3a** and **4a**) were prepared (Scheme 2, Table I).

Protecting acetyl groups of **3a**/**3b**, **4a**/**4b**, **3a** and **4a** were removed by alkaline hydrolysis with potassium carbonate in a water/methanol (1:2, v/v) system. Each of the final products (**5a**/**5b**, **6a**/**6b**, **5a** and **6a**), obtained again either in a form of a diastereoisomeric mixture or as single enantiomer (Schemes 1 and 2), was purified by column chromatography on silica gel, using chloroform/methanol (15:1–5:1) as a gradient mobile phase.

A cyclodextrin-based chiral Nucleodex-β-OH column was used for analyzing the target products **5a**/**5b** and **6a**/**6b**. Isocratic mobile phase (methanol/water 9:1) was used for their HPLC analysis. The determined retention times of **5a** (11.47 min), **5b** (9.65 min), **6a** (9.99 min) and **6b** (10.71 min) enabled separation of small quantities of the respective enantiomers of the compounds. No separation of the enantiomers of the protected compounds **3a**/**3b** and **4a**/**4b** was observed under the applied chromatographic conditions. The NMR experiments employed in this study though enabled finally unambiguous structure assignment of all alkyl β-D-glucopyranosides prepared (**3a**–**6b**).

The NMR data were determined on the basis of both, 1D and 2D NMR experiments. The critical analysis of the 1D ^1H NMR and $^1H,^1H$ PFG COSY spectra[10] of pure enantiomers **3a**, **4a**, **5a** and **6a** allowed extracting the ^1H chemical shifts and coupling constants. However, positions of the ^1H signals of the cyclohexane cycle could be extracted neither directly from the 1D NMR spectra nor from the $^1H,^1H$ PFG COSY spectra, and it was necessary to estimate these values from the $^1H,^{13}C$ PFG HSQC spectra[10] using the knowledge of the ^{13}C NMR chemical shifts from the model compounds with similar structure[11]. The 2D $^1H,^{13}C$ PFG HSQC spectra were used for unambiguous assignment of the ^{13}C NMR chemical shifts of the remaining carbon atoms. The NMR data of the protected and unprotected alkyl glucosides **3a**/**3b**–**6a**/**6b** are summarized in Tables II and III.

TABLE II
The ^{1}H and ^{13}C NMR data of the protected alkyl β-D-glucopyranosides **3a**, **3b**, **4a** and **4b** (measured in $CDCl_3$)[a]

Position	^{1}H NMR				^{13}C NMR			
	3a	**3b**	**4a**	**4b**	**3a**	**3b**	**4a**	**4b**
1	3.65 dt	3.81 dt	3.20 ddd	3.32 dt	79.71 d	74.65 d	85.62 d	80.94 d
	J = 2.4, 2.4, 4.5	*J* = 2.4, 2.4, 4.3	*J* = 4.3, 9.6, 10.4	*J* = 4.2, 9.5, 9.5				
2[b]	1.57–1.65 m	1.63–1.68 m	1.57–1.62 m	1.53–1.57 m	43.04 d	43.10 d	45.14 d	44.32 d
3[b]	1.17–1.43 m	1.33–1.42 m	0.85–0.89 m	0.86–0.91 m	26.66 t	26.82 t	29.75 t	29.68 t
			1.62–1.69 m	1.60–1.67 m				
4[b]	1.17–1.43 m	1.17–1.43 m	1.17–1.22 m	1.15–1.20 m	25.56 t	25.05 t	24.76 t	24.43 t
	1.57–1.63 m	1.57–1.63 m	1.49–1.53 m	1.47–1.55 m				
5[b]	1.17–1.43 m	1.32–1.43 m	0.98–1.08 m	0.97–1.05 m	20.86 t	20.70 t	24.91 t	24.88 t
		1.63–1.68 m	1.51–1.55 m	1.49–1.53 m				
6[b]	1.17–1.43 m	1.17–1.43 m	1.35–1.43 m	1.33–1.41 m	31.90 t	28.78 t	33.72 t	31.23 t
	1.84–1.89 m	1.98–2.01 m	2.06–2.11 m	2.03–2.08 m				
7	2.39 dd	2.41 dd	2.09 dd	2.19 dd	37.28 t	37.17 t	37.29 t	37.25 t
	J = 7.7, 13.6	*J* = 6.4, 13.8	*J* = 9.0, 13.1	*J* = 9.3, 13.6				
	2.52 dd	2.72 dd	3.06 dd	3.13 dd				
	J = 7.0, 13.6	*J* = 7.8, 13.8	*J* = 3.2, 13.1	*J* = 3.6, 13.6				

TABLE II
(*Continued*)

Position	^{1}H NMR				^{13}C NMR			
	3a	**3b**	**4a**	**4b**	**3a**	**3b**	**4a**	**4b**
8	–	–	–	–	132.98 s	133.43 s	132.42 s	132.91 s
9	7.07 m	7.13 m	7.02 m	7.09 m	129.84 d	130.30 d	130.08 d	130.40 d
10	6.84 m	6.77 m	6.81 m	6.80 m	113.74 d	113.34 d	113.62 d	113.41 d
11	–	–	–	–	157.68 s	157.83 s	157.78 s	157.66 s
12	3.80 s	3.77 s	3.78 s	3.78 s	55.23 q	55.19 q	55.20 q	55.20 q
1′	4.54 d	4.56 d	4.63 d	4.62 d	101.74 d	98.13 d	102.04 d	98.49 d
	J = 7.9	*J* = 7.9	*J* = 7.9	*J* = 8.0				
2′	5.11 dd	5.09 dd	5.08 dd	5.01 dd	71.69 d	71.77 d	71.76 d	71.67 d
	J = 7.9, 9.9	*J* = 7.9, 9.8	*J* = 7.9, 10.0	*J* = 8.0, 9.6				
3′	5.22 t	5.23 t	5.22 t	5.22 t	73.06 d	73.10 d	73.02 d	73.09 d
	J = 9.5	*J* = 9.5	*J* = 9.6	*J* = 9.6				
4′	5.15 t	5.06 t	5.08 dd	5.10 t	68.86 d	68.94 d	68.70 d	68.77 d
	J = 9.8	*J* = 9.8	*J* = 9.4, 10.1	*J* = 9.8				
5′	3.64 ddd	3.65 ddd	3.71 ddd	3.68 ddd	71.47 d	71.59 d	71.58 d	71.60 d
	J = 2.6, 5.5, 10.1	*J* = 2.6, 4.7, 10.0	*J* = 2.6, 5.4, 10.1	*J* = 2.7, 4.7, 10.0				
6′	4.11 dd	4.14 dd	4.12 dd	4.17 dd	62.24 t	62.10 t	62.25 t	62.10 t
	J = 2.6, 12.2	*J* = 2.6, 12.2	*J* = 2.6, 12.1	*J* = 2.6, 12.1				
	4.21 dd	4.28 dd	4.26 dd	4.27 dd				
	J = 5.5, 12.2	*J* = 4.7, 12.2	*J* = 5.4, 12.2	*J* = 4.7, 12.1				

[a] Signals of the acetoxy groups. ^{1}H NMR spectra: **3a**: 2.02 s, 2.06 s, 2.08 s, 2.08 s; **3b**: 2.02 s, 2.03 s, 2.03 s, 2.06 s; **4a**: 1.95 s, 2.00 s, 2.03 s, 2.07 s; **4b**: 2.01 s, 2.03 s, 2.05 s, 2.08 s. ^{13}C NMR spectra: **3a**: 20.57 q, 20.62 q, 20.72 q, 20.76 q, 169.16 s, 169.31 s, 170.44 s, 170.63 s; **3b**: 20.61 q, 20.63 q, 20.74 q, 20.80 q, 169.90 s, 170.19 s, 170.38 s, 170.49 s; **4a**: 20.61 q, 20.62 q, 20.70 q, 20.71 q, 169.28 s, 169.42 s, 170.37 s, 170.64 s; **4b**: 20.60 q, 20.67 q, 20.78 q, 20.81 q, 169.39 s, 169.56 s, 170.20 s, 170.45 s. [b] The ^{1}H NMR chemical shifts were assigned on the basis of the ^{1}H,^{13}C PFG HSQC spectra (cf. Results and Discussion).

TABLE III
The ^{1}H and ^{13}C NMR data of the alkyl β-D-glucopyranosides **5a**, **5b**, **6a** and **6b** (measured in CD_3OD)[a]

Position	^{1}H NMR				^{13}C NMR			
	5a	**5b**	**6a**	**6b**	**5a**	**5b**	**6a**	**6b**
1	3.74 dt	3.92 dt	3.27 dt	3.49 dt	80.55 d	76.58 d	85.88 d	81.20 d
	J = 2.4, 2.4, 4.8	*J* = 2.5, 2.5, 4.9	*J* = 4.1, 9.5, 9.5	*J* = 4.2, 9.6, 9.6				
2	1.76–1.80 m	1.68–1.72 m	1.59–1.63 m	1.56–1.60 m	45.24 d	45.61 d	47.42 d	46.70 d
3	1.32–1.35 m	1.32–1.35 m	0.86–0.91 m	0.88–0.92 m	28.32 t	28.23 t	31.38 t	31.58 t
	1.50–1.56 m	1.52–1.56 m	1.59–1.62 m	1.59–1.62 m				
4	1.21–1.25 m	1.22–1.26 m	1.24–1.27 m	1.22–1.25 m	26.11 t	26.61 t	26.42 t	26.20 t
	1.63–1.67 m	1.63–1.67 m	1.68–1.73 m	1.72–1.74 m				
5	1.39–1.44 m	1.34–1.37 m	1.03–1.12 m	1.05–1.09 m	23.31 t	22.35 t	26.70 t	26.85 t
	1.69–1.75 m	1.73–1.76 m	1.53–1.58 m	1.53–1.58 m				
6	1.37–1.92 m	1.31–1.34 m	1.33–1.37 m	1.19–1.22 m	33.25 t	30.13 t	35.69 t	32.71 t
	2.01–2.07 m	1.96–2.00 m	2.22–2.27 m	2.11–2.14 m				
7	2.52 dd	2.42 dd	2.15 dd	2.22 dd	37.44 t	38.35 t	39.00 t	39.02 t
	J = 8.4, 13.6	*J* = 7.9, 13.6	*J* = 9.9, 13.3	*J* = 9.4, 13.7				
	2.84 dd	2.81 dd	3.39 dd	3.23–3.41 m				
	J = 6.6, 13.6	*J* = 6.5, 13.6	*J* = 3.2, 13.3					

TABLE III
(*Continued*)

Position	^{1}H NMR				^{13}C NMR			
	5a	**5b**	**6a**	**6b**	**5a**	**5b**	**6a**	**6b**
8	–	–	–	–	135.45 s	135.62 s	134.96 s	135.12 s
9	7.15 m	7.15 m	7.09 m	7.08 m	131.69 d	131.79 d	131.76 d	131.84 d
10	6.80 m	6.79 m	6.80 m	6.80 m	115.06 d	114.96 d	115.01 d	114.95 d
11	–	–	–	–	159.71 s	159.64 s	159.72 s	159.66 s
12	3.75 s	3.75 s	3.75 s	3.75 s	56.14 q	56.14 q	56.14 q	56.14 q
1′	4.33 d	4.34 d	4.40 d	4.41 d	105.66 d	102.24 d	106.18 d	101.79 d
	J = 7.8	*J* = 7.7	*J* = 7.9	*J* = 7.8				
2′	3.27 dd	3.25 dd	3.24 dd	3.22 dd	76.09 d	75.77 d	76.15 d	75.70 d
	J = 7.8, 9.2	*J* = 7.7, 9.1	*J* = 7.9, 9.1	*J* = 7.8, 9.2				
3′	3.38 t	3.35 dd	3.35 dd	3.23–3.41 m	78.85 d	78.85 d	78.78 d	78.76 d
	J = 9.0	*J* = 9.2, 9.8	*J* = 9.1, 10.1					
4′	3.31 dd	3.27 t	3.30 dd	3.23–3.41 m	72.31 d	72.39 d	72.23 d	72.40 d
	J = 8.8, 9.5	*J* = 9.7	*J* = 9.5, 10.1					
5′	3.20 ddd	3.25 ddd	3.27 ddd	3.23–3.41 m	78.22 d	78.29 d	78.29 d	78.39 d
	J = 2.5, 5.6, 9.5	*J* = 2.5, 5.6, 9.7	*J* = 2.4, 5.3, 9.5					
6′	3.66 dd	3.72 dd	3.68 dd	3.70 dd	63.38 t	63.47 t	63.34 t	63.52 t
	J = 5.6, 11.8	*J* = 5.6, 11.7	*J* = 2.4, 11.9	*J* = 5.6, 11.7				
	3.82 dd	3.89 dd	3.85 dd	3.90 dd				
	J = 2.5, 11.8	*J* = 2.5, 11.7	*J* = 5.3, 11.9	*J* = 2.3, 11.7				

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

CONCLUSION

The Koenigs–Knorr synthesis was found to be a convenient synthetic procedure for the preparation of 2-(4-methoxybenzyl)cyclohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides. Several heavy metal salts were tested as promoters of this reaction, together with two modifications of the Koenigs–Knorr method. Using method *A*, cadmium carbonate was found to be a reliable promoter for glycosylation of 2-(4-methoxybenzyl)cyclohexan-1-ol (Table I). Higher yields of the protected alkyl β-D-glucopyranosides **3a**/**3b**, **4a**/**4b**, **3a** and **4a** were obtained with cadmium carbonate compared with three silver salts and in shorter reaction time under otherwise identical reaction conditions. When evaluating the use of method *B*, then employing of silver triflate as the promoter resulted in the highest yield of the products. However, method *A* was much more reliable synthetic procedure than method *B* due to the almost quantitative chemical yields achieved of the required products.

EXPERIMENTAL

General

The ^{1}H and the ^{13}C NMR spectra (chemical shifts in ppm (δ-scale), coupling constants (*J*) in Hz) were recorded on a Bruker AVANCE 500 spectrometer (in FT mode) at 500.1 MHz 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. 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 a solution (CCl_4) or by the KBr technique on a Bruker IFS 88 instrument. Mass spectra (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 was used for performing the yeast reactions. 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 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. Analyses of the products were performed on a chiral Nucleodex β-OH column (150 × 4 mm; Macherey–Nagel, Germany) using a methanol/water mixture (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.

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

Method A: In a typical experiment, a promoter (2.05 mmol, 3 equiv.) was added to a solution of the alcohol (**1a**/**1b**, **2a**/**2b**, **1a** or **2a**; 0.15 g, 0.682 mmol) in dry toluene (20 ml), and the mixture was heated under azeotropic conditions to remove traces of moisture from the system. Then a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.84 g, 2.05 mmol) in toluene (15 ml) was added to this mixture, which was then heated under reflux for 5–7 h (monitored by TLC). Then the mixture was filtered, the solvent was evaporated and the product was purified by column chromatography.

Method B: Powdered molecular sieves (0.3 g; 4 Å) and the promoter (0.681 mmol) were added to a solution of alcohol **1a**/**1b** or **2a**/**2b** (0.05 g, 0.227 mmol), and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.25 g, 0.6 mmol) in dry dichloromethane (5 ml) at –50 °C. The reaction mixture was then stirred at room temperature overnight, and the reaction course was monitored by TLC. The solid was filtered off, and the solvent was evaporated. The residue was purified by column chromatography. The yields of the products **3a**/**3b**, **4a**/**4b**, **3a** and **4a** obtained by using both modifications of the Koenigs–Knorr procedure are given in Table I. The ^{1}H and ^{13}C NMR data of the products **3a**–**4b** are given in Table II, while their additional analytical data are summarized below.

3a/**3b**: IR (CCl_4): 3027 (w), 2960 (w), 2936 (w), 2836 (w), 1756 (s), 1613 (w), 1513 (m), 1441 (w), 1388 (m), 1300 (w), 1245 (s), 1220 (s), 1042 (s), 895 (w), 843 (w). MS (FAB), *m/z* (%): $[M]^+$ 550 (4), 331 (28), 289 (5), 229 (6), 203 (24), 169 (56), 121 (100), 109 (40), 77 (8). For $C_{28}H_{38}O_{11}$ (550.6) calculated: 61.08% C, 6.96% H; found: 60.92% C, 6.95% H.

4a/**4b**: IR (CCl_4): 3031 (w), 2935 (m), 2859 (w), 2853 (w), 1761 (s), 1753 (s), 1613 (w), 1513 (m), 1442 (w), 1300 (w), 1246 (s), 1225 (s), 1040 (s), 880 (w), 856 (w). MS (FAB), *m/z* (%): $[M]^+$ 550 (2), 331 (25), 289 (10), 229 (8), 202 (14), 169 (62), 121 (100), 109 (46), 91 (16). For $C_{28}H_{38}O_{11}$ (550.6) calculated: 61.08% C, 6.96% H; found: 61.05% C, 6.98% H.

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

In a typical experiment, potassium carbonate (0.3 g, 1.8 mmol) was added to a solution of the protected glycosides **3a**/**3b**, **4a**/**4b**, **3a** or **4a** (0.25 g, 0.45 mmol) in a methanol/water mixture (5:1; 12 ml). The mixture was heated to reflux for 2 h. Methanol was removed under reduced pressure, the water phase was saturated with ammonium sulfate, and the products were extracted with ethyl acetate. After drying the extract over anhydrous sodium sulfate, the solvent was evaporated, and the product was purified by column chromatography. Yields: **5a**/**5b**, 92%; **6a**/**6b**, 85%; **5a**, 94%; **6a**, 88%. The ^{1}H and ^{13}C NMR data of the products **5a**–**6b** are given in Table III, while their additional analytical data are summarized below.

5a/**5b**: IR (KBr): 3401 (s), 2994 (w), 2932 (s), 1612 (m), 1513 (s), 1177 (s), 1096 (s), 1074 (s), 1036 (s), 1018 (s), 895 (w), 842 (w). MS (FAB), *m/z* (%): $[M + H]^+$ 383 (1), 325 (1), 273 (1), 261 (1), 241 (1), 203 (16), 121 (100), 91 (5), 79 (7). For $C_{20}H_{30}O_7$ (382.5) calculated: 62.81% C, 7.91% H; found: 62.75% C, 7.92% H. **5a**: $[\alpha]_D^{20}$ –14.6 (*c* 0.064, CH_3OH).

6a/**6b**: IR (KBr): 3429 (s), 2995 (w), 2927 (m), 1612 (m), 1513 (m), 1099 (m), 1074 (m), 1036 (s), 1024 (s), 879 (w). MS (FAB), *m/z* (%): $[M + H]^+$ 383 (1), 261 (1), 241 (2), 203 (8), 121 (100), 91 (5), 79 (16). For $C_{20}H_{30}O_7$ (382.5) calculated: 62.81% C, 7.91% H; found: 62.85% C, 7.88% H. **6a**: $[\alpha]_D^{20}$ +1.0 (*c* 0.137, CH_3OH).

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

Saccharomyces cerevisiae, strain DBM 2115, obtained from the Research Institute of Fermentation Industry (Prague, Czech Republic), was cultivated in the cultivation medium (100 ml) at 27 ± 1 °C for 48 h [12]. 2-(4-Methoxybenzyl)cyclohexanone (150 mg per flask, 0.688 mmol), dissolved in ethanol (0.5 ml), was added to the yeast cells in five 250-ml shake-flasks. The reaction was maintained under shaking at 27 ± 1 °C for 7 days using a Unimax incubator, and worked-up as described previously[12]. **1a**: yield 49.0%, ee (1*S*,2*S*) 98.7%; **2a**: yield 46.5%, ee (1*S*,2*R*) ≥ 99%. Both ^{1}H and the ^{13}C NMR spectra of **1a** and **2a** were compared with the literature data[13]. Their absolute configuration was assigned by transforming the samples of the alcohols **1a** and **2a** into their diastereoisomeric esters with (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid and their ^{1}H and ^{19}F NMR spectra were analyzed as described previously[12]. **1a**: $[\alpha]_D^{20}$ +21.1 (*c* 0.178, $CHCl_3$); **2a**: $[\alpha]_D^{20}$ +32.1 (*c* 0.187, $CHCl_3$).

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic, project COST D29.001, a part of the COST D29/0014/04 European Network.

REFERENCES

1. Toshima K., Tatsuta K.: *Chem. Rev.* **1993**, *93*, 1503.
2. Hanessian S. in: *Preparative Carbohydrate Chemistry* (S. Hanessian, Ed.), pp. 381–388. Dekker, New York 1997.
3. Schmidt R. R.: *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212.
4. Binkley E. R., Binkley R. W. in: *Preparative Carbohydrate Chemistry* (S. Hanessian, Ed.), pp. 87–104. Dekker, New York 1997.
5. a) Wimmer Z., Rejzek M., Zarevúcka M., Kuldová J., Hrdý I., Němec V., Romaňuk M.: *J. Chem. Ecol.* **1997**, *23*, 605; b) Wimmer Z., Kuldová J., Hrdý I., Bennettová B.: *Insect Biochem. Mol. Biol.* **2006**, *36*, 442.
6. a) Turner W. J. in: *Enzymes in Action* (B. Zwanenburg, M. Mikolajczyk and P. Kielbasinski, Eds), pp. 71–94. Kluwer, Dordrecht 2000; b) Santaniello E., Ferraboschi P., Manzocchi A. in: *Enzymes in Action* (B. Zwanenburg, M. Mikolajczyk and P. Kielbasinski, Eds), pp. 95–115. Kluwer, Dordrecht 2000; c) Wong C.-H., Whitesides G. M. in: *Enzymes in Synthetic Organic Chemistry* (J. E. Baldwin and P. D. Magnus, Eds), pp. 131–194. Pergamon, New York 1994; d) Roberts S. M.: *J. Chem. Soc., Perkin Trans. 1* **2001**, 1475.
7. a) Novák J., Zarevúcka M., Wimmer Z., Tykva R.: *Biotechnol. Lett.* **2001**, *23*, 1517; b) Zarevúcka M., Sochůrková P., Wimmer Z., Šaman D.: *Biotechnol. Lett.* **2003**, *25*, 987; c) Zarevúcka M., Wimmer Z., Šaman D., Demnerová K., Macková M.: *Tetrahedron: Asymmetry* **2004**, *15*, 1325.
8. Šaman D., Wimmerová M., Wimmer Z.: *Collect. Czech. Chem. Commun.* **2006**, *71*, 1186.
9. Schmidt R. R., Jung K.-H. in: *Preparative Carbohydrate Chemistry* (S. Hanessian, Ed.), pp. 283–312. Dekker, New York 1997.
10. a) von Kienlin M., Moonen C. T. W., van der Toorn A., van Zijl C. M.: *J. Magn. Reson.* **1991**, *93*, 423; b) Willker W., Leibfritz D., Kerssebaum R., Bermel W.: *Magn. Reson. Chem.* **1993**, *31*, 287.
11. Rejzek M., Wimmer Z., Zarevúcka M., Šaman D., Pavlík M., Říčánková M.: *Tetrahedron: Asymmetry* **1994**, *5*, 1501.

12. Wimmer Z., Šaman D., Zarevúcka M., Wimmerová M.: *Tetrahedron: Asymmetry* **2005**, *16*, 2810.
13. Wimmer Z., Skouridou V., Zarevúcka M., Šaman D., Kolisis F. N.: *Tetrahedron: Asymmetry* **2004**, *15*, 3911.