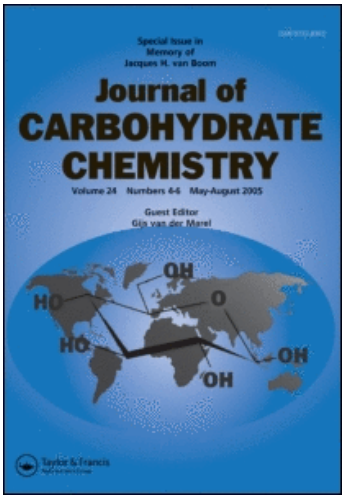


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Syntheses of β-Mannopyranosedes by Enzymatic Approaches

N. Taubken[a]; B. Sauerbrei[a]; J. Thiem[a]
[a] Institut für Organische Chemie, Universität Hamburg, Hamburg 13, Germany

To cite this Article Taubken, N. , Sauerbrei, B. and Thiem, J.(1993) 'Syntheses of β-Mannopyranosedes by Enzymatic Approaches', Journal of Carbohydrate Chemistry, 12: 4, 651 — 667
To link to this Article: DOI: 10.1080/07328309308019414
URL: http://dx.doi.org/10.1080/07328309308019414

J. CARBOHYDRATE CHEMISTRY, 12(4&5), 651-667 (1993)

SYNTHESES OF β–MANNOPYRANOSIDES BY ENZYMATIC APPROACHES[1]

N. Taubken, B. Sauerbrei, and J. Thiem*

Institut für Organische Chemie, Universität Hamburg
Martin-Luther-King-Platz 6, D-2000 Hamburg 13, Germany

Received November 17, 1992 - Final Form April 14, 1993

ABSTRACT

The transmannosylation activity of β–mannosidase from snail and β–galactosidase from *Aspergillus oryzae* was used for the synthesis of methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, 1-hexyl, cyclohexyl, and 1-octyl β–D-mannopyranosides (**3a-i**), respectively. The regioisomeric specificities and wide substrate acceptance of this galactosidase are demonstrated. Thus, 4-nitrophenyl 4-*O*-(α-D-glucopyranosyl)-β–D-glucopyranoside (**6**), 4-nitrophenyl 2-*O*-(β–D-glucopyranosyl)-β–D-glucopyranoside (**7**), 4-nitrophenyl 2-deoxy-2*N*-acetyl-6-*O*-(2-deoxy-2-*N*-acetyl-β–D-glucopyranosyl)-β–D-glucopyranoside (**8**), 4-nitrophenyl 3-*O*-(β–D-mannopyranosyl)-α-D-mannopyranoside (**9**), and 4-nitrophenyl 4-*O*-(β–D-mannopyranosyl)-β–D-mannopyranoside (**10**) were prepared by chemoenzymatic self-transfer reaction.

Introduction

Due to the axial 2-hydroxy group in mannopyranose, formation of β–mannosidically linked saccharides is particulary difficult. In 1930, *Haworth et al.* isolated small amounts of the methyl β–anomer from the isomeric mixture of a *Fischer* glycosylation by recrystallization of the peracetylated products.[2]

Over the years, various routes have been developed to increase the ratio of the physiologically interesting β–D-mannopyranosides. These can be summarized as two main strategies: either, starting from α-D-mannopyranosides, inversion at C1 leads to

the desired products or, alternatively, epimerization at C2 of β–D-glucopyranosides opens a way to the β–mannopyranose series.

Already in 1972, *Lindberg et al.* developed a procedure for oxidation and stereoselective reduction at C2,[3] which was used by *Jeanloz et al.* for the synthesis of the core trisaccharide of *N*-glycoproteins.[4,5] The epimerization strategy was used by *David et al.* via nucleophilic substitution of 2-*O*-(triflate)- or 2-*O*-(*N*-sulfuryl imidazole)-activated gluco-configurated precursors.[6,7] In order to avoid undesired by-products, *Kunz et al.* extended this approach and applied 3-*O*-(*N*-phenyl carbamoyl) glucosyl derivatives for an intramolecular substitution at C2.[8,9]

Anomerization of α-mannopyranosyl halides can be achieved under catalysis of silver or mercury salts.[10-12] *Paulsen et al.* introduced silver silicate into *Koenigs-Knorr* reactions as an effective catalyst for the formation of β–mannopyranosidic linkages of oligosaccharides in heterogeneous phases.[13] The corresponding mannopyranosyl triflate and tosylate were employed in the anomerization reaction by *Srivastava* and *Schuerch.*[14] Recently, two similar pathways for intramolecular substitution at C1 were described by *Hindsgaul et al.*[15] (Scheme 1: X = C) and *Stork et al.*[16] (X = Si) which led exclusively to the β–configurated mannopyranosides.

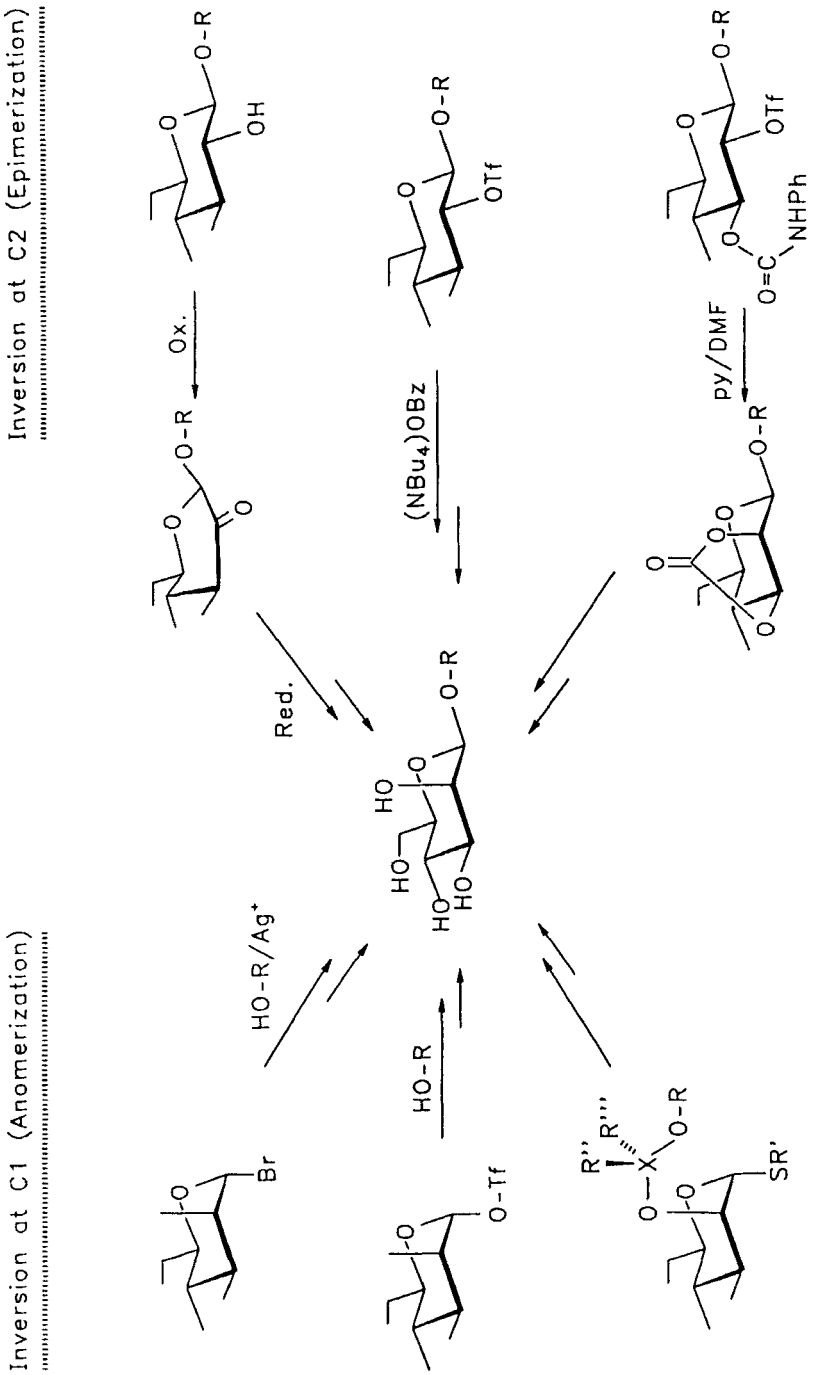
Aryl mannopyranosides can be obtained from the anomerically unblocked starting material mainly as the β–anomer under Mitsunobu-conditions.[17,18] Thus, 4-nitrophenyl β–D-mannopyranoside (**1**), which is a good substrate for β–mannosidases, can be synthesized easily in two steps and in 32 % overall yield.

All these strategies generally require a pretentious protection and deprotection of hydroxy groups as well as the separation of isomeric mixtures.

Recently, we proposed a chemoenzymatic approach to β–mannopyranosides, employing commercially available β–mannosidase [E.C. 3.2.1.25] from snail as a biocatalyst.[19] Various alkyl and hydroxyalkyl β–D-mannopyranosides could be obtained in yields up to 76 % depending on hydrophobicity and steric demands of the aglycon.

In contrast to other glycosidases, which have been utilized as quite powerful instruments in the synthesis of anomerically pure glycosides (compare e.g. [20-23]), the β–mannosidase has been used mostly as a tool in typically biochemical research, for partial degradation studies of glycoproteins and other heteropolysaccharides. One reason may be the high price compared to other glycosidases (e.g. the β–mannosidase is about 30,000 times as expensive as β–galactosidase from *Aspergillus oryzae*).

Whereas considerable work has been done in isolation and purification of β–mannosidases from various sources,[24-33] only *Kyosaka et al.* in 1986 described the use of β–mannosidase from guinea pig liver for preparative transmannosylations. Starting



SCHEME 1. Strategies towards β-D-Mannopyranosides.

with 4-chloro- or 4-nitrophenyl β–D-mannopyranoside, β(1-2)- and β(1-6)-linked 4-nitrophenol mannobiosides were obtained in 0.9 and 0.7 % or 1.3 and 3.7 % yield, respectively.[33] Recently, it was shown that even a crude β–mannosidase from *Aspergillus niger* can transfer a β–mannopyranosyl moiety to chitobiose from a β(1-4) linked mannotrisaccharide donor. The main product was identified as Manβ(1-4)GlcNAcβ(1-4)GlcNAc.[34]

Recently, some enzyme preparations from fungi and crude fiber hydrolyzing enzyme preparations, e.g. cellulases and hemicellulases, were screened for β–mannosidase activity.[35] Quite a number of them were also found to catalyze transmannosylations, at least on an analytical scale.

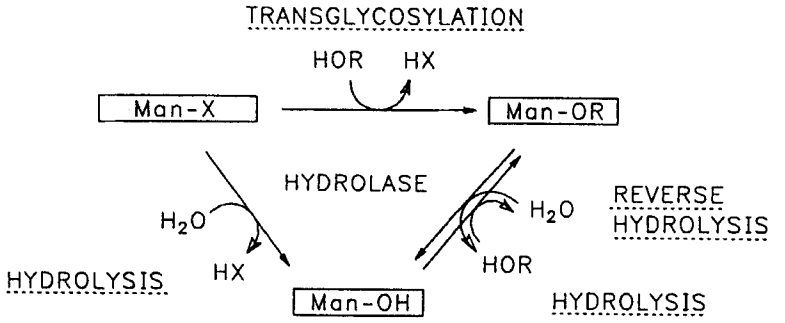
However, another commercially available hydrolase, which is less pretentious with regard to the donor molecule than the mannanase, seemed to us very promising for a favourable synthesis of β–mannopyranosides. In 1985 *Ooi et al.* reported about the unspecific glucosyl activity of β–galactosidase [E.C. 3.2.1.23] from *A. oryzae*.[36,37] Recently, we used this hydrolase for the synthesis of self-transfer products starting from 4-nitrophenyl β–D-glucopyranoside.[38] Our further studies showed that this enzyme preparation also has a side-activity for β–mannopyranosides, and this opened a less expensive route to β–D-mannopyranosides.[39]

In this communication we wish to report on the preparative chemoenzymatic synthesis of transfer and self transfer products of 4-nitrophenyl β–D-mannopyranosides using β–mannosidase from snail as well as β–galactosidase from *A. oryzae*, respectively. The comparatively low substrate specifity of the galactosidase will be demonstrated by self transfer products obtained from glucosyl and *N*-acetyl glucosaminyl donors.

Results and Discussion

Transglycosylation reactions are known to be catalyzed by glycosidases either under thermodynamic or kinetic control. As depicted in Scheme 2, the interdependence of both strategies employing "reverse hydrolysis" (thermodynamic) or "transglycosylation" (kinetic) conditions becomes evident. *Ajisaka et al.* studied the former method for the equilibrium controlled approach to several di- and trisaccharides using batch or continuous systems.[40-42] Donor molecules with better leaving groups than hydroxy at the anomeric center will give rise to kinetically controlled reactions. Several groups used the latter method, e.g. as a route to alkyl glycosides.[37,43-45]

As the main problem, the ratio of hydrolysis to transglycosylation has to be optimized. High concentrations of the alcoholic components or organic co-solvents



SCHEME 2. Transglycosylation and Hydrolysis by Use of Hydrolases.

may lower the content of water and thus should lead to a reduced hydrolysis. In contrast to *Ooi et al.*, who used solutions with 50 % acetonitrile for the synthesis of alkyl β–D-galactopyranosides with β–galactosidase,[37] we preferred incubation mixtures with up to 50 % alcohol because the β–mannosidase from snail was determined not to be very stable in typical organic co-solvents, such as acetonitrile or dimethylformamide.

Thus, simple incubation of 4-nitrophenyl β–D-mannopyranoside (**1**) with β–mannosidase [E.C. 3.2.1.25] from snail or β–galactosidase [E.C. 3.2.1.23] from *A. oryzae*, respectively, in a mixture of buffer and alcohol led to the corresponding alkyl β–D-mannopyranosides **3a-i**, which were isolated and characterized by ^1H and ^{13}C NMR. In case of more hydrophobic alcoholic components **2d-f**, **2h** and **i**, ethylene glycol dimethyl ether was added to reach sufficient miscibility of the two phases without remarkable loss of activity of the enzymes.

In general, alkyl glycosides seem to be rather poor substrates for hydrolases.[35] Nevertheless, in order to avoid further hydrolysis of the transfer products, the reaction should be stopped immediately after most of the starting material is converted.

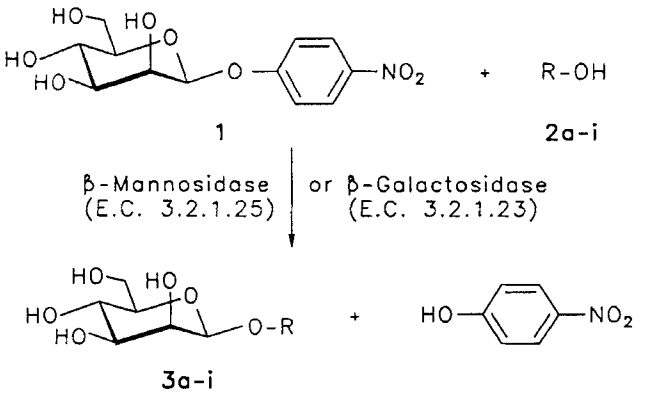
As shown in Table 1, the β–mannosidase transferred a mannosyl residue to hydrophilic alcohols in quite good yields. Generally, no α-products could be found in the NMR spectra and this confirmed the retaining capacity of this hydrolase.[33]

The increase of the concomitant hydrolysis with elongation of the carbon chain of the acceptor alcohol is demonstrated in Figure 1. The yields decrease from 75 to 2 %, in going from ethanol to 1-octanol. A corresponding effect was observed for secondary alcohols (see Figure 1b). Whereas 2-propanol was transferred in 16 % yield by β–mannosidase, cyclohexanol gave only 3 % of the transfer product.

TABLE 1. Yields of Alkyl β–Mannopyranosides **3a-i**.

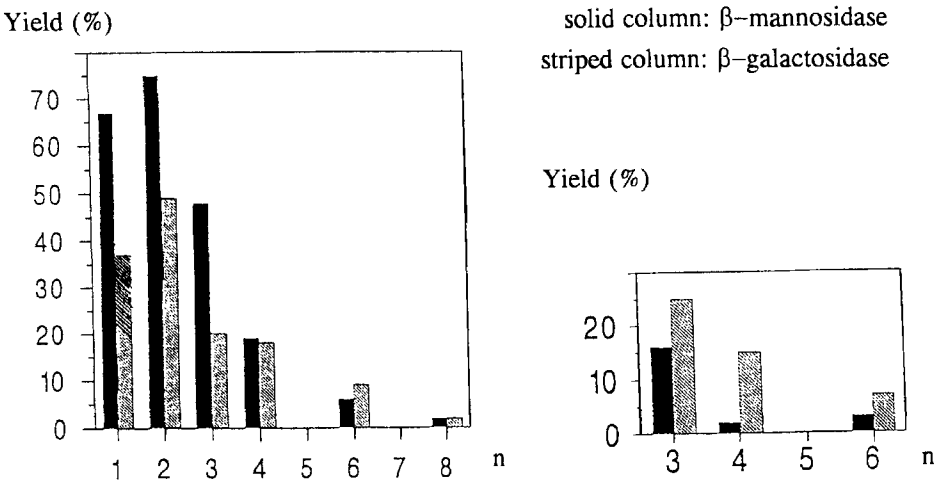
Product	Aglycon	Yield (%) with β–Mannosidase [E.C. 3.2.1.25]	Yield (%) with β–Galactosidase [E.C. 3.2.1.23]
3a	Methanol **(2a)**		
3b	Ethanol **(2b)**		
3c	1-Propanol **(2c)**		a
3d	1-Butanol **(2d)**		
3e	1-Hexanol **(2e)**		b
3f	1-Octanol **(2f)**		b
3g	2-Propanol **(2g)**		
3h	2-Butanol **(2h)**		
3i	c-Hexanol **(2i)**		b

a. In addition, 6 % of the anomeric glucosides were obtained (see text).
b. In addition, about 1 % **(3f)**/ 2 % **(3e)**/ 3 % **(3i)** of the β–glucosides were formed (see text).



SCHEME 3. Synthesis of Alkyl β–Mannopyranosides **3a-i**.

The reduced transfer of the methyl group to the mannosyl residues is assumed to depend on a faster denaturation of the β–mannosidase by this alcohol compared with others. Thus, only 30 % (v/v) of the alcoholic compound was used for the incubation mixture.



n: number of aglyconic carbon atoms.

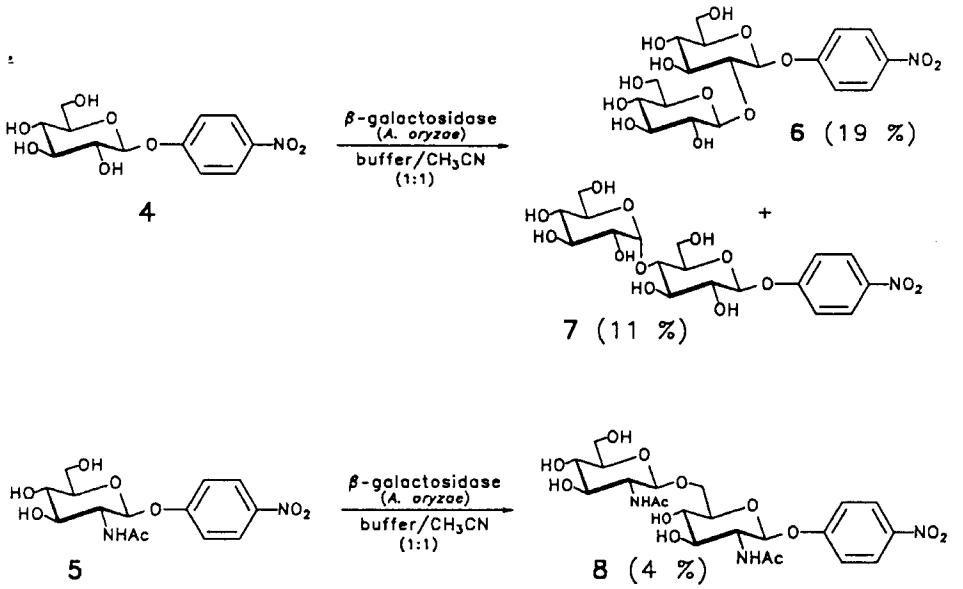
1a. Yields with Primary Alcohols **1b.** Yields with Secondary Alcohols

FIGURE 1. Synthesis of Alkyl β–D-Mannopyranosides.

The influence of steric effects on transmannosylation by β–mannosidase observed previously[19] could be confirmed for the two hexyl mannopyranosides: the secondary cyclohexyl alcohol is transferred even less than 1-hexanol (3 % vs. 6 % isolated products).

As shown in Table 1 and Figures 1a and 1b, corresponding results could be observed with β–galactosidase from *A. oryzae*. In cases of more hydrophobic aglycons, decreased amounts of transfer product were obtained. The yields of isolated transfer products **3a** and **3b** are about 30 % lower than for the β–mannosidase catalyzed reactions. However, in case of more hydrophobic or sterically more pretentious aglycons the portion of transmannosylations was similar or even better. Obviously, due to impurities of the enzyme preparation which display α-hydrolytic activities, small traces of the corresponding alkyl α-D-mannopyranosides were found in the ^{1}H NMR.

Due to the β–glucosidase activity of the enzyme preparation[37,38] traces of alkyl β–D-glucopyranosides were determined by ^{1}H NMR. Glucosyl donors are formed if the incubation times are long enough to cleave some of the standardizing starch of the enzyme preparation. For very slow reacting substrates, such as 1-octanol and cyclohexanol, respectively, the ratio of **3f** or **3i** to the corresponding β–glucosides became about 3:1 as measured by integration of the anomeric protons.

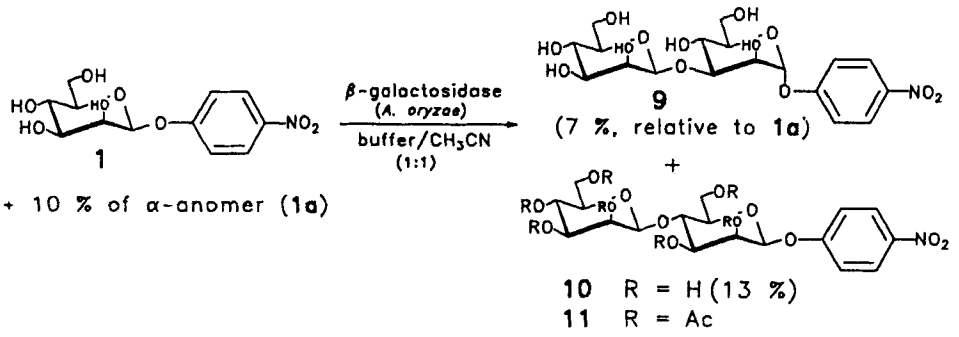


SCHEME 4. Syntheses of 4-Nitrophenyl Glucobiosides and Di-*N*-Acetyl Glucobiosaminide.

Astonishingly, incubation with 17 % (v/v) 1-propanol in buffer solution led to a 20 % yield of the desired β–product, and in addition to 6 % propyl α/β–D-glucopyranoside (about 1:1). Increased amounts of 1-propanol had to be avoided because the enzyme was quickly denaturated by this alcohol. Apparently, the α-hydrolytic activity of the enzyme preparation seemed to be more stable in the presence of 1-propanol. Until present, there is no real convincing explanation at hand for this increase in α-glycosidase activity. A similar observation could be described for the self transfer reaction of 4-nitrophenyl β–D-glucopyranoside (**4**) catalyzed by this galactosidase.[38] The formation of these impurities was not observed when ultrafiltration or immobilization of the enzyme preparation were applied.

In contrast to β–mannosidase, the β–galactosidase does not seem to have significant steric demands, as reflected by the ratio of transglycosylation to hydrolysis. Thus, 2-propyl β–D-mannopyranoside **3g** was obtained in 25 % isolated yield (vs 20 % **3c**; 16 % **3g** by β–mannosidase). Even the cyclohexyl derivative **3i** was obtained in 7 %, compared to 9 % of the primary *n*-hexyl derivative **3e**. This tendency may be important for the transmannosylation of sterically more demanding saccharides.

In the second part of these investigations, the synthesis of various nitrophenyl disaccharides was attempted utilizing the wide substrate range of β–galactosidase from



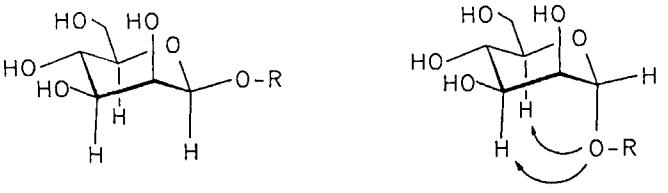
SCHEME 5. Synthesis of 4-Nitrophenyl Mannobiosides.

A. oryzae. As recently shown,[38] the self transfer reaction of 2-nitrophenyl β–galactopyranoside in 50 % acetonitrile catalyzed by this enzyme led to the formation of both the β(1-6)- and the β(1-3)-linked 2-nitrophenyl galactobiosides. In similar procedures 4-nitrophenyl β–D-glycosides of glucose (**4**), *N*-acetyl-glucosamine (**5**) and finally mannose (**1**), respectively, could be transformed into the corresponding nitrophenyl disaccharides and these could be separated from their regiomers by gel chromatography. Due to the lower activity of this β–galactosidase towards these compounds, the incubation times had to be considerably extended compared to that used with 2-nitrophenyl β–D-galactopyranoside.

In case of the glucoside **4** as substrate, the desired sophoroside **6** was obtained, but in addition the maltoside **7** was formed.[38] In analogy to the synthesis of alkyl mannopyranosides, incubation of **1** gave small amounts of α-D-mannobiosides. However, no such α-linked disaccharide side-products were observed in case of incubations of **4** and **5**. Most likely these compounds are formed by impurities with α-glycosidase activity.

Whereas **6** is β(1-2)-linked, the enzymatic conversion of **1** which included about 10 % of the α-anomer (**1a**) yielded 7 % of the β(1-3)- and 13 % of the β(1-4)-isomer (**9** and **10**). Amazingly, neither the glucosyl nor the mannosyl donors led to products showing the β(1–6) linkage. In contrast to that, **5** was transformed *only* to the β(1-6)-isomer **8**. Thus, the regioselectivity of the β–galactosidase strongly depends on the chosen donor substrate.

The different positions of β–mannosylation of compounds **1** and **1a** indicate that the regioselectivity is also decisively affected by the anomeric configuration of the acceptor. Corresponding results were obtained by *Nilsson*[46] with β–galactosidase from *E. coli*. Although β–galactosidase from *A. oryzae* is an enzyme with a wide acceptor



β–D-Mannopyranosides α-D-Mannopyranosides

FIGURE 2. The 1,3-Syn-diaxial Effect .

TABLE 2. ^{1}H NMR Data of Alkyl β–D-Mannopyranosides **3a-i**.

	Chemical Shift δ/ppm[a]							
Compoun	H1	H2	H3	H4	H5	H6A	H6B	H(aglycon)[b]
3a	4.63	4.04	3.70	3.62	3.43	3.98	3.79	3.56(3H)
3b	4.70	4.03	3.70	3.62	3.43	3.98	3.79	3.99(1H); 3.77(1H); 1.27(3H)
3c	4.72	4.03	3.69	3.62	3.41	3.98	3.78	3.89(1H); 3.66(1H); 1.65(2H); 0.94(3H)
3d	4.71	4.03	3.68	3.62	3.41	3.97	3.78	3.94(1H); 3.72(1H); 1.64(2H); 1.41(2H); 0.95(3H)
3e	4.71	4.03	3.69	3.62	3.41	3.97	3.78	3.93(1H); 3.71(1H); 1.66(2H); 1.36(6H); 0.92(3H)
3f	4.77	4.03	3.69	3.62	3.42	3.97	3.78	3.93(1H); 3.71(1H); 1.66(1H), 1.3 1.32(8H); 0.91(3H)
3g	4.83	3.99	3.69	3.63	3.44	3.98	3.78	4.18(1H); 1.28(3H); 1.24(3H)
3h[c]	4.82/4	3.98/4	3.70/3	3.62	3.41	3.97	3.78	1.21/1.26(3H); 3.94/3.93(1H); 1.66/1.55(2H); 0.95/0.93(3H)
3i	4.86	3.98	3.96	3.63	3.41	3.96	3.78	3.82(1H); 2.00(2H); 1.78(2H); 1.4 1.25(5H);1.59(1H of C4')

a. ^{1}H NMR spectra were recorded in D_2O with acetone as internal standard at 2.28 ppm. The coupling constants are very similar for all mannosyl residues of **3a-i**: $J_{1,2} < 1.0$ Hz; $J_{2,3} = 3.0 - 3.2$ Hz; $J_{3,4} = 9.4 - 10.0$ Hz; $J_{4,5} = 9.4 - 9.6$ Hz; $J_{5,6A} = 2.0 - 2.2$ Hz; $J_{5,6B} = 6.4 - 6.6$ Hz; $J_{6A,6B} = 12.0 - 12.2$ Hz.

b. The signals of the aglycon are given in increasing distance from the anomeric centre.

c. For aglyconic protons and H1 - H3 two signals were obtained, respectively, which belong to the compound with either (R)- or (S)-configuration at C2'.

TABLE 3. ^{1}H NMR Data of 4-Nitrophenyl Disaccharides **6 - 11.**

		Chemical Shift δ/ppm[a]								
Com-pound	Sugar Unit[b]	H1 ($J_{1,2}$	H2 ($J_{2,3}$	H3 ($J_{3,4}$	H4 ($J_{4,5}$	H5 ($J_{5,6}$	H6A ($J_{5,6}$	H6B ($J_{6A,6}$	Ac	Nitro phen
6	Glc	5.40	3.81	3.74	3.49	3.63	3.86	3.68		7.17
		(7.5)	(9.0)	(9.2)	(9.3)	(2.0)	(5.6)	(12.1)		8.19
	Glc'	4.75	3.22	3.44	3.32	3.27	3.27	3.26		
		(8.0)	(9.4)	(9.0)	(9.4)	(2.6)	(6.0)	(12.0)		
7	Glc	5.21	3.59	3.84	3.70	c	3.88	c		7.17
		(8.0)	(9.6)	(9.2)	(9.6)	n.d.	n.d.	(12.0)		8.19
	Glc'	5.37	3.52	3.63	3.35	3.65	3.78	c		
		(3.8)	(9.8)	(9.2)	(9.1)	(2.0)	(6.0)	(12.0)		
8	*N*AcGlc	5.19	3.88	3.56	3.39	3.72	4.12	3.65	1.92	7.07
		(8.1)	(10.3	(9.2)	(10.1	(1.7)	(6.4)	(11.2)		8.15
	*N*AcGlc'	4.41	3.60	n.d.	3.32	3.39	3.79	3.60	1.88	
		(8.6)	(10.2	(9.2)	(9.6)	(1.3)	(5.6)	(12.0)		
9	Man	5.68	4.18	4.13	3.91	d	3.87	d		7.20
		(1.6)	(3.2)	(9.5)	(9.0)	(2.0)	n.d.	(12.2)		8.18
	Man'	4.68	3.96	3.57	3.48	3.36	d	d		
		(<1)	(2.9)	(9.7)	(9.4)	(2.0)	(7.2)	(12.2)		
10	Man	5.40	4.18	e	e	e	e	e		7.09
		(<1)	(2.1)	n.d.	n.d.	n.d.	n.d.	n.d.		8.13
	Man'	4.65	3.96	3.54	3.45	3.33	e	e		
		(<1)	(2.9)	(9.7)	(9.5)	(2.1)	(6.6)	n.d.		
11	Man	5.25	5.58	5.20	3.98	3.80	4.34	4.23	1.93 -2	6.98
		(1.0)	(3.5)	(9.2)	(9.2)	(3.0)	(6.1)	(12.2)	(7s, 3	8.12
	Man'	4.69	5.37	4.98	5.16	3.59	4.09	4.24	each)	
		(<1)	(3.2)	(9.8)	(9.6)	(3.1)	(5.6)	(12.2)		

a. ^{1}H NMR spectra of **6 - 10** were recorded in D_2O with acetonitrile as internal standard at 1.98 ppm; **11** was measured in $CDCl_3$.

b. reducing terminus: Hex; nonreducing terminus: Hex'.

c. 3.67 - 3.76 (mc, 3H) d. 3.62 - 3.70 (mc, 4H)

e. 3.58 - 3.86 (mc, 7H) n.d.: not determined

TABLE 4. ^{13}C NMR Data of **3a-e, g-i, 6, 7** and **11**.

Compoun		Chemical Shift δ/ppm[a] C1	C2	C3	C4	C5	C6	C(aglycon)[b]
3a		97.82	67.08	69.73	63.66	73.02	57.83	53.68
3b		96.42	67.52	69.91	63.74	73.14	57.94	62.45; 11.12
3c		96.72	67.52	69.99	63.82	73.18	58.01	68.54; 19.06; 6.56
3d		96.70	67.51	69.98	63.80	73.16	57.98	66.69; 27.73; 15.43; 9.99
3e		96.68	67.52	69.98	63.79	73.16	57.98	67.00; 27.74; 25.47; 21.70; 18.83; 10.22
3g		94.65	68.06	70.07	63.79	73.14	57.99	19.17; 68.92(CH); 17.73
3h[c]		95.86	67.87	70.10	63.81	73.14	58.00	16.73/ 15.07; 74.86/73.56(CH);
		94.35	68.17					26.10/25.13(CH_2); 6.11/ 6.64
3i		94.47	68.12	70.06	63.74	73.12	57.96	74.78(CH); 29.73; 21.94; 20.64; 20.81; 28.30
6	Glc	95.97	79.23	73.23	66.96	73.96	58.49	159.45, 114.20 (2C), 124.17 (2C), 140.65
	Glc'	100.81	71.66	73.41	67.15	73.82	58.07	
7	Glc	97.29	70.64	73.87	74.39	72.93	58.48	159.87, 114.51 (2C), 124.12(2C), 140.64
	Glc'	97.66	69.68	70.85	67.35	70.76	58.46	
10	Man	94.68	68.14	70.41	72.75	74.07	58.64	113.95 (2C), 123.71 (2C), 145.95
	Man'	97.84	67.32	68.93	64.32	73.99	58.00	

a. ^{13}C NMR spectra were recorded in D_2O with acetone as internal standard at 27.14 ppm.

b,c. see Table 2.

range of various different β–glycosides, it is sensitive to the shape of the donor molecule as well as to the anomeric center of the acceptor.

Determination of the anomeric configuration of mannosides is rather difficult in comparison to other glycosides. Due to the 2-axial hydroxy group, the $J_{1,2}$ coupling constants for both anomers are rather similar. Even though the H1 signal of α-anomers are generally shifted further downfield, this as well as a negative optical rotation are no clean characteristics which prove the anomeric configuration.

The anomeric configuration of glycosides can be determined by C1-H1 coupling constants.[47,48] For **3b**, this value was determined by us to be 158.9 Hz, which is in

good agreement with a β–anomer. Tables 2 and 3 show the NMR data of the alkyl β–D-mannopyranosides **3a-i**. The chemical shifts of the proton signals are nearly identical. Only δ(H1) of β–mannopyranosides derived from secondary alcohols **2g-i** is shifted significantly downfield compared to signals of the primary alcohol glycosides **3a-f**. The ^{1}H NMR data of the mannobiosides **9** and **10** together with those of the other disaccharide compounds are compiled in Table 4. The positions of the H1' signals and the coupling constants below 1 Hz indicate β–configurations. Further proof is given by the absence of 1,3-syn-diaxial effects, which in the case of α-products should lead to a downfield shift of 0.2-0.3 ppm for the deshielded H3 and H5 signal, respectively.[49]

In conclusion, β–mannosylation using β–galactosidase form *A. oryzae* seems to be an interesting alternative to both classical or other chemoenzymatic approaches with regard to easy availability of the enzyme and simplicity of the procedures.

EXPERIMENTAL

General Procedures. Specific rotations were determined on a PERKIN-ELMER Polarimeter 241 in 1 dm cuvettes at 589 nm. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AMX-400 spectrometer (^{1}H: 400 MHz; ^{13}C: 100.67 MHz), UV data on a BECKMAN DU-62 spectrophotometer. 4-Nitrophenol was removed by irreversible attachment to ion exchange resin DOWEX 1 X2 (50 - 100 mesh; SERVA; column-size: 10 x 2 cm; eluent: water). Preparative flash chromatography was performed on cellulose (microcrystalline; MERCK; column-size: 10 x 1 cm; eluent: 1-butanol, saturated with water), gel permeation chromatography on BIO-GEL P-2 (200 - 400 mesh; BIO-RAD; column-size: 100 x 2 cm; eluent: water) or Sephadex G-10 (2.5 x 70 cm; water).

4-Nitrophenyl β–D-mannopyranoside (**1**) was prepared from 2,3:4,6-di-*O*-cyclohexylidene mannopyranose by treatment with 4-nitrophenol under conditions of a Mitsunobu reaction.[17,18] β–Mannosidase (E. C. 3.2.1.25; from snail acetone powder) and β–galactosidase (E. C. 3.2.1.23; from *A. oryzae*; grade XI) were obtained from SIGMA. Sodium citrate (0.1 M; pH 4.0; from MERCK) was used as buffer for incubations with β–mannosidase (buffer A), and potassium dihydrogen phosphate (0.1 M; pH 5.0; from MERCK) for those with β–galactosidase (buffer B).

TABLE 5. Incubation Times and Optical Rotations.

	Compounds								
Incubation Times	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**
with β–mannosidase	1 d	1 d	2 d	2 d	3 d	5 d	5d	5 d	3 d
with β–galactosidase	2 d	3 d	2 d	2 d	6 d	5 d	2d	3 d	10
Opt. Rotation $[\alpha]_D^{20}$	-54.3	-22.9	-25.	-26.2	n. d	n. d	-40.0	-35.1	n. d
Concentration (g/ 100 mL) in H_2O	0.4	0.6	0.1	0.2			0.2	0.2	

n. d.: not determined, due to impurities and small amounts of product

Preparation of Alkyl β–D-Mannopyranosides. A suspension of **1** (0.15 mmol; 45.6 mg) and β–mannosidase (2.5 U in 0.45 mL buffer A) or β–galactosidase (100 mg/ ca. 500 U in 0.30 mL buffer B), respectively, was treated with alcohol **2** (0.5 mL) in the corresponding buffer (total volume: 0.5 mL). In case of more hydrophobic compounds **2d-f**, **h**, and **i**, the buffer was mixed with ethylene glycol dimethyl ether (0.5 mL). The mixtures were incubated at 30 °C until about 95 % of **1** was converted (photometric determination of 4-nitrophenol at 400 nm; of **1** at 304 nm). The incubation times and optical rotations are listed in Table 5. The reaction was stopped by heating to 100 °C for 5 min, the mixture centrifuged, and the supernatant freed from 4-nitrophenol by an ion exchange column. Chromatography on cellulose and subsequently on BIO-GEL P-2 lead to yellow syrups **3a-i**.

Preparation of Nitrophenyl Disaccharides. To a mixture of 2 mL of acetonitrile and 1 mL of buffer B containing 1 mmol nitrophenyl glycoside, a suspension of 200 mg (1100 U) β–galactosidase in 1 mL of buffer B was added. The incubation was carried out at 20 °C with shaking for several days until conversion of 70 - 90 % of starting material and terminated by boiling for 5 min. In the meantime more enzyme had to be added. After centrifugation of the diluted mixtures the supernatants were concentrated *in vacuo* at 40 °C and passed through an anion exchange column. The residue was fractioned on Sephadex G-10, giving separations of regiomeric nitrophenyl disaccharides, and recovery of unconverted starting material. The products obtained were characterized by NMR spectroscopy and yields were determined according a donor/acceptor ratio of 1:1.

4-Nitrophenyl 2/4-*O*-(β/α-D-Glucopyranosyl)-β–D-glucopyranosides (6 and 4). 318 Mg (1.06 mmol) of 4-nitrophenyl β–D-glucopyranoside (**4**) were incubated with

208 mg (1167 U) β–galactosidase for 4 days. After 2 and 3 days, respectively, further 50 mg of enzyme were added.

4-Nitrophenyl 2-O-(β–D-Glucospyranosyl)-β–D-glucopyranoside (**6**) -
Yield: 46.5 mg (19 %); mp. 255 °C; $[\alpha]_D^{20}$ -25.7° (*c* 0.5 in H_2O).

4-Nitrophenyl 4-O-(α-D-Glucopyranosyl)-β–D-glucopyranoside (**7**) -
Yield: 26.9 mg (11 %); syrup; $[\alpha]_D^{20}$ -0.1° (*c* 0.3 in H_2O).

4-Nitrophenyl 6-*O*-(*N*-Acetyl β–D-glucosaminopyranosyl)-*N*-acetyl β–D-glucopyranoside (8). 260 Mg (0.76 mmol) of 4-nitrophenyl *N*-acetyl β–D-glucosaminopyranoside (**5**) were incubated with 197 mg β–galactosidase for 7 days in 2 mL of acetonitrile/buffer mixture. After 2 and 4 days, respectively, further 90 mg of enzyme were added. 68 Mg of **5** were recovered.

Yield: 6 mg (4 %); mp 205 - 207 °C.

4-Nitrophenyl 3/4-*O*-(β–D-Mannopyranosyl)-α/β–D-mannopyranosides (9 and **10).** To the solution of 180 mg (0.5 mmol) of 4-nitrophenyl-α/β–D-mannopyranoside (**1**) (α/β ratio 1:9) dissolved in 1.1 mL acetonitrile and 0.4 mL buffer, 100 mg (550 U) β–galactosidase suspended in 0.7 mL buffer were added. While incubating the mixture for 7 days, another 150 mg of enzyme were added gradually. 30 Mg of **1** were recovered by gel chromatography.

4-Nitrophenyl 3-O-(β–D-Mannopyranosyl)-α-D-mannopyranoside (**9**) -
Yield: 1.5 mg (7 %, in relation to the α-anomer **1a**).

4-Nitrophenyl 4-O-(β–D-Mannopyranosyl)-β-D-mannopyranoside (**10**) -
Yield: 14.5 mg (13 %, in relation to the β–anomer **1**); $[\alpha]_D^{20}$ -22.9° (*c* 0.3 in H_2O).

4-Nitrophenyl 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β–D-mannopyranosyl)-β–D-mannopyranoside (11). 5 mg of compound **10** were peracetylated according to standard procedures;[50]

Yield: 7.8 mg (95 %); syrup.

ACKNOWLEDGEMENT

Support of this work by the *Fonds der Chemischen Industrie,* the *Bundesministerium für Forschung und Technologie,* and a *Kekule-Stipendium* to N. T. is gratefully acknowledged.

REFERENCES AND NOTES

1. Presented in part at the *XVIth International Carbohydrate Symposium*, Paris, France, July 5-10, 1992.

2. H. G. Bott, W. N. Haworth, E. E. Hirst, *J. Chem. Soc.*, 2653 (1930).
3. G. Ekborg, B. Lindberg, J. Lönngren, *Acta Chem. Scand.*, **26**, 3287 (1972).
4. C. D. Warren, C. Augé, M. L. Laver, S. Suzuki, D. Power, R. W. Jeanloz, *Carbohydr. Res.*, **82**, 71 (1990);
5. C. Augé, C. D. Warren, R. W. Jeanloz, M. Kiso, L. Anderson, *Carbohydr. Res.*, **82**, 85 (1980).
6. S.David, A. Malleron, C. Dini, *Carbohydr. Res.*, **188**, 193 (1989).
7. J. Alais, S. David, *Carbohydr. Res.*, **201**, 69 (1990).
8. H. Kunz, W. Günther, *Angew. Chem., Int. Ed. Engl.*, **27**, 1087 (1988).
9. W. Günther, H. Kunz, *Carbohydr. Res.*, **228**, 217 (1992).
10. G. M. Bebault, G. G. S. Dutton, *Carbohydr. Res.*, **37**, 309 (1974).
11. M. A. E. Shabau, R. W. Jeanloz, *Carbohydr. Res.*, **52**, 115 (1976).
12. A. P. Kaplun, V. E. Kalugin, V. F. Shvets, R. P. Evstigneeva, *Tr. Mosk. Inst. Tonkoi Khim. Tekhnol.*, **5**, 57 (1974); *Chem. Abstr.* **86**, 90196w (1974).
13. H. Paulsen, O. Lockhoff, *Chem. Ber.*, **114**, 3102 (1981).
14. V. K. Srivastava, C. Schuerch, *J. Org. Chem.*, **46**, 1221 (1981).
15. F. Barresi, O. Hindsgaul, *J. Am. Chem. Soc.*, **113**, 9376 (1991).
16. G. Stork, G. Kim, *J. Am. Chem. Soc.*, **114**, 1087 (1992).
17. P. J. Garegg, T. Iversen, T. Norberg, *Carbohydr. Res.*, **73**, 313 (1979).
18. B. V. McCleary, *Methods Enzymol.*, **160** Biomass, 515 (1988).
19. N. Taubken, J. Thiem, *Synthesis*, 517 (1992).
20. W. M. Watkins, *Nature*, **181**, 117 (1958).
21. S. C. T. Svensson, J. Thiem, *Carbohydr. Res.*, **200**, 391 (1990).
22. J. Thiem, B. Sauerbrei, *Angew. Chem.*, **103**, 1521 (1991); *Angew. Chem. Int. Ed. Engl.*, **30**, 1503 (1991).
23. K. Sakai, R. Katsumi, H. Ohi, T. Usui, Y. Ishido, *J. Carbohydr. Chem.*, **11**, 553.(1992).
24. T. Sukeno, A. Tarentino, T. Plummer, F. Maley, *Biochemistry*, **11**, 1493 (1972).
25. Y. Li, Y. Lee, *J. Biol. Chem.*, **247**, 3677 (1972).
26. K. Sugahara, T. Okumura, I. Yamashina, *Biochem. Biophys. Acta*, **268**, 488 (1972).
27. C. Houston, S. Latimer, E. Mitchell, *Biochem. Biophys. Acta*, **370**, 276 (1974).
28. J. Montreuil, *Pure Appl. Chem.*, **42**, 431 (1975).
29. C. Wan, J. Muldry, Y. Li, Y. Lee, *J. Biol. Chem.*, **251**, 4384 (1976).

30. A. Elbein, S. Adya, Y. Lee, *J. Biol. Chem.*, **252**, 2026 (1978).

31. S. Bouquelet, G. Spik, J. Montreuil, *Biochem. Biophys. Acta,* **522**, 521 (1978).

32. B. V. McCleary, *Carbohydr. Res.*, **101**, 75 (1982).

33. S. Kyosaka, S. Murata, Y. Tsuda, M. Tanaka, *Chem. Pharm. Bull.*, **34**, 5140 (1986).

34. T. Usui, M. Suzuki, T. Satoh, H. Kawagishi, *Ann. Meeting Jap. Soc. Bioscience Biotechn. Agrochem.*, Tokyo, Japan, 1992, Abstract 31a8.

35. A. Holazo, H. Shinoyama, Y. Kamiyama, T. Yasui, *Bioscience Biotech. Biochem.*, **56**, 822 (1992).

36. Y. Ooi, T. Hashimoto, N. Mitsuo, T. Satoh, *Tetrahedron Lett.*, **25**, 2241 (1984).

37. Y. Ooi, T. Hashimoto, N. Mitsuo, T. Satoh, *Chem. Pharm. Bull.*, **33**, 1808 (1985).

38. B. Sauerbrei, J. Thiem, *Tetrahedron Lett.*, **33**, 201 (1992).

39. N. Taubken, J. Thiem, *XVIth International Carbohydrate Symposium,* Paris, France, July 5-10, 1992, Abstract A218.

40. K. Ajisaka, H. Fujimoto, *Carbohydr. Res.*, **199**, 227 (1990).

41. K. Ajisaka, H. Fujimoto, H. Nishida, *Carbohydr. Res.*, **180**, 35 (1988).

42. K. Ajisaka, H. Fujimoto, H. Nishida, *Biotechnol. Lett.*, **9**, 243 (1987).

43. H. Shinoyama, A. Ando, T. Fuji, T. Yasui, *Agric. Biol. Chem.*, **55**, 847 (1991).

44. D. H. G. Crout, D. A. MacManus, P. Critchley, *J. Chem. Soc., Perkins Trans. I,* 1865 (1990).

45. E. N. Vulfson, R. Patel, B. A. Law, *Biotechnol. Lett.*, **12**, 397 (1990).

46. K. G. I. Nilsson, *Carbohydr. Res.*, **180**, 53 (1988).

47. A. S. Perlin, B. Casu, *Tetrahedron Lett.*, 2921 (1969).

48. K. Bock, I. Lundt, C. Pedersen, *Tetrahedron Lett.*, 1037 (1972).

49. K. Bock, *Pure Appl. Chem.*, **55**, 605 (1983).

50. R. Behrend, P. Roth, *Liebigs Ann. Chem.*, **331**, 359 (1904).