Glycosylation of Mycotoxins

Susanne Grabley,[a] Manfred Gareis,[b] Winfried Böckers,[c] Joachim Thiem*[d]

[a] Hoechst AG, Hauptlaboratorium G 830, Postfach 800320, D-6230 Frankfurt 80, Germany
[b] Institut für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin, Tierärztliche Fakultät der Ludwig-Maximilians-Universität München, Veterinärstraße 13, D-8000 München 22, Germany
[c] Organisch-Chemisches Institut, Westfälische Wilhelms-Universität, Orléansring 23, D-4400 Münster, Germany
[d] Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, Germany

Received 27 December 1991

Glycoconjugates of some mycotoxins were prepared by the phase-transfer catalyzed glycosylation procedure. With zearalenone a regio- and stereospecific glucosylation gave the natural derivative which proved to be a "masked mycotoxin". In the case of ochratoxin A glucosylation occurred exclusively at the amino acid part to give an unusual sugar ester component.

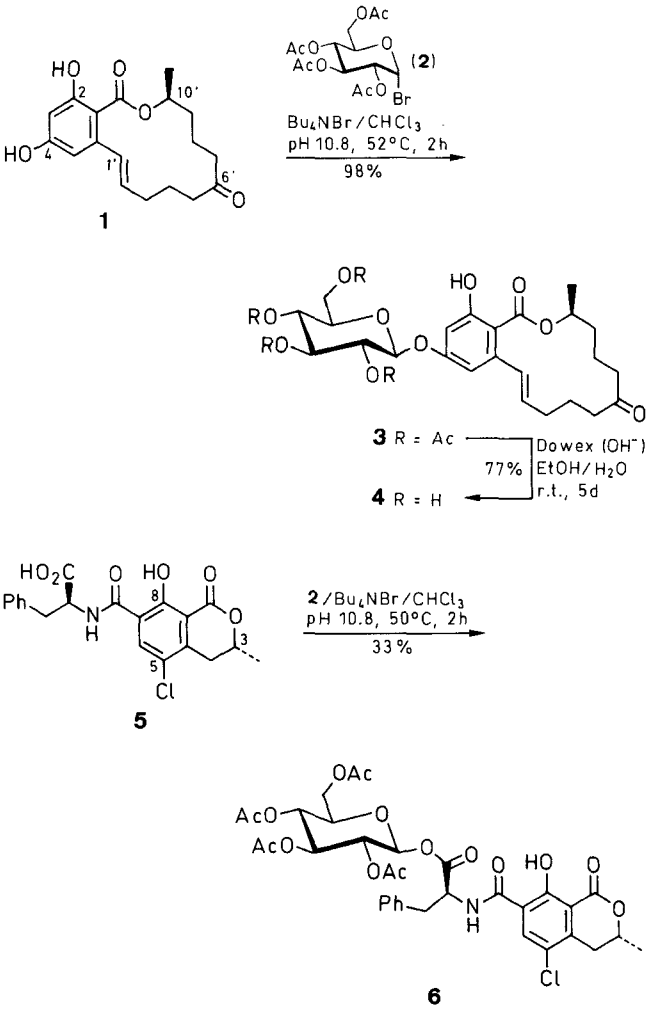
The potent mycotoxin zearalenone (**1**)[1] is produced by a number of *Fusarium* species[2] which frequently invade and grow on cereals in the field or during storage. Such grains used as animal feed may result in heavy damage owing to estrogenic and anabolic activity[3] of zearalenone (**1**).

Initially attempts were made to glucosylate zearalenone (**1**) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (α-acetobromoglucose, **2**) according to the Koenigs–Knorr[4] type reactions using silver carbonate, silver oxide or silver triflate;[5] however this did not meet with success. Similarly, the early apporaches of Fischer et al.[6] and others,[7] which could be successfully applied in the glycosylation of phenols,[9] failed here. As a quite efficient variation, we succeeded in the phase-transfer catalyzed glycosylation, previously described for a series of aryl glycosides of gluco and galacto configuration.[10]

Thus, the protected zearalenone β-glucoside **3** could be obtained easily in crystalline form and in virtually quantitative yield. An exclusive formation of the β-glucoside was expected and is evident from the *trans* diaxial coupling $J_{1'',2''} = 7.9$ Hz. The regioselective attachment to the positon 4 of the aglucone can be deduced from the only signal for the remaining OH group at C-2 observed as a singlet at $\delta = 11.98$ in the region of chelated phenolic groups. By mild deacetylation with anionic ion exchange resin the unprotected zearalenone β-D-glucopyranoside (**4**) could be obtained crystalline in approximately 80% yield. Previously **4** was obtained as the microbial conversion product of zearalenone (**1**) by the mycelium of *Rhizopus* species, but chemically only partially characterized.[11] The material obtained crystalline here proved identical with respect to the NMR data described in Ref. 11. In recent studies it was demonstrated that **4** was cleaved during digestion in pigs,[12] and thus may be considered a "masked mycotoxin" possibly involved in mycotoxicoses.

The major toxic principle isolated from *Aspergillus ochraceus* was shown to be ochratoxin A (**5**),[13,14] the toxicity of which is directed to liver and kidneys. Chemically this component consists of a 7-carboxylated 3,4-dihydro-3-methylisocoumarin moiety which *N*-acylates L-β-phenylalanine. As above, it was of interest to glycosylate ochratoxin A with regard to potentially active conjugates.

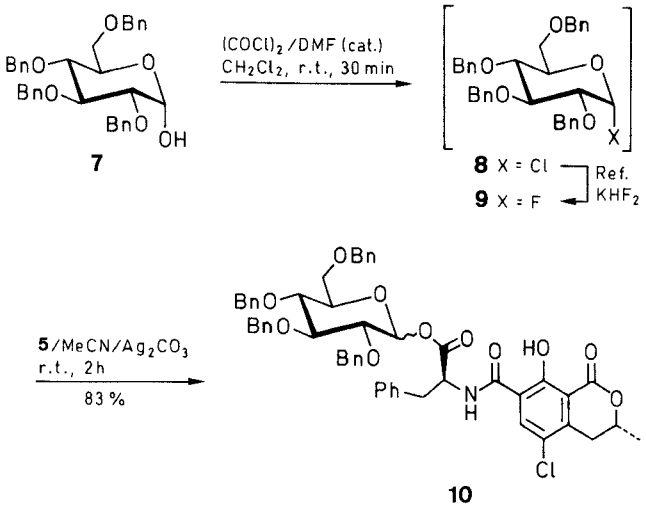
In contrast to the anticipation under phase-transfer conditions for glucosylation of the phenolic 8-OH group of **5** with α-acetotromoglucose **2** a regioselective glycosylation of the phenylalanine carboxy groups occurred to give the peracylated β-D-glucopyranose ester **6** in approximately 70% yield.



Again the trans coupling $J_{1',2'} = 7.8$ Hz gives evidence for the exclusive β-attachment, and at $\delta = 12.65$ the 8-OH is found as a singlet (in **5** 8-OH, $\delta = 12.75$), which indicates the chelation to both 10-lactone carbonyl and the 9-carboxy carbonyl groups. Obviously, the comparatively low nucleophilicity of the phenylalanine carboxyl group still exceeds that of a chelated phenolic group and thus a sugar ester is formed.

Until now all methods attempted did not result in a selective deacetylation of **6** to give the unblocked anome-

rically bound glucose ester of ochratoxin A (**5**). Therefore, alternatives were tested starting from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**7**).[15] Compound **7** was treated with oxalyl chloride in dimethylformamide[16] to form the α-chloride **8**.[17] Reaction of ochratoxin A (**5**) with **8** in acetonitrile in the presence of silver carbonate gave the glycoside **10** as an α/β-anomer mixture (ratio ca. 1:1) in 83% yield.



Alternatively **8** was converted to the corresponding α-fluoride **9**[18,19] (for another convenient route to **9**, cf. Ref. 20) and subjected to glucosylation with ochratoxin A (**5**) in the presence of a catalytic amount of titanium(IV) fluoride[21] or boron trifluoroetherate. However, in both cases neither anomeric acylation with ochratoxin A (**5**) to give **10** nor a glucosylation of the phenolic 8-OH of **5** could be observed. Attempts for a simple hydrogenolytic cleavage of the benzyl groups in the sugar moiety of **10** did not yet meet with success. Other procedures will be reported in due course.

TLC: aluminum foils, silica gel GFT_{254} (Merck); detection: UV absorption and/or spraying with conc. H_2SO_4 and heating to 120 °C. Column chromatography: silica gel 60 (Merck). Melting points (not corrected): Reichert heating microscope. Optical rotations: Perkin-Elmer 241 in 1 dm cuvettes at 20 °C and 589 nm. NMR: Bruker WM 300; ^1H: 300 MHz, ^{13}C: 75.5 MHz; TMS as internal standard; *J* values are given in Hz.

(S)-Zearalenone (**1**):^1H NMR (CD_3OD): δ = 5.97 (d, 1 H, H-3), 6.12 (d, 1 H, H-5), 6.73 (dd, 1 H, H-1'), 5.45 (ddd, 1 H, H-2'), 2.05 (m, 1 H, H-3a'), 1.90 (m, 1 H, H-3b'), 1.80 (m, 1 H, H-4a'), 1.30 (m, 1 H, H-4b'), 2.60 (ddd, 1 H, H-5a'), 1.99 (m, 1 H, H-5b'), 2.40 (ddd, 1 H, H-7a'), 1.85 (m, 1 H, H-7b'), 1.45–1.58 (m, 2 H, H-8a', b'), 1.31–1.41 (m, 2 H, H-9a', b'), 4.74 (ddq, 1 H, H-10), 1.11 (d, 3 H, CH_3); $J_{3,5}$ = 2.5, $J_{1',2'}$ = 15.4, $J_{1',3a'}$ = 1.5, $J_{2',3a'}$ = 10.1, $J_{2',3b'}$ = 4.0, $J_{4a',5a'}$ = 11.9, $J_{4b',5a'}$ = 2.5, $J_{5a',5b'}$ = 18.8, $J_{7a',7b'}$ = 12.3, $J_{7a',8a'}$ = 5.6, $J_{7a',8b'}$ = 4.2, $J_{9a',10'}$ = 11.8, $J_{9b',10'}$ = 12.1, $J_{10',11'}$ = 6.1.

^{13}C NMR (CD_3OD): δ = 104.1 (C-1), 166.3 (C-2), 102.9 (C-3), 163.9 (C-4), 109.5 (C-5), 144.8 (C-6), 134.5 (C-1'), 133.1 (C-2'), 43.9 (C-3'), 23.3 (C-4'), 35.9 (C-5'), 213.8 (C-6'), 37.5 (C-7'), 22.0 (C-8'), 32.1 (C-9'), 74.4 (C-10'), 21.0 (C-11'), 172.7 (C-12').

N-{[(R)-5-Chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl]carbonyl}-L-β-phenylalanine (Ochratoxin A, **5**):
^1H NMR ($CDCl_3$): δ = 4.73 (ddd, 1 H, H-3), 3.27 (dd, 1 H, H-4a), 2.84 (dd, 1 H, H-4b), 8.40 (s, 1 H, H-6), 12.75 (s, 1 H, OH-8), 8.48 (d, 1 H, NH), 5.00 (ddd, 1 H, H-13), 3.33 (dd, 1 H, H-14a), 3.20 (dd, 1 H, H-14b), 7.16–7.33 (m, 5 H, H-16-20), 1.58 (d, 3 H, CH_3); $J_{3,4a}$ = 3.5, $J_{3,4b}$ = 11.7, $J_{3,21}$ = 6.3, $J_{4a,4b}$ = 17.5, $J_{13,NH}$ = 6.6, $J_{13,14a}$ = 5.4, $J_{13,14b}$ = 7.2, $J_{14a,14b}$ = 14.2.

(*S*)-Zearalenone 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (3):
(*S*)-Zearalenone (**1**; 75 mg, 0.24 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**; 270 mg, 0.66 mmol), and tetra-n-butylammonium bromide (80 mg, 0.25 mmol) were dissolved in $CHCl_3$ (20 mL). Borate buffer (pH 10.8, 20 mL) was added, the mixture heated to 52 °C and the pH kept constant by automatic titration with 0.1 N NaOH (total consumption 5 mL). After 2 h no further change of pH was observed, the organic layer separated, dried ($MgSO_4$), evaporated to dryness and the residue purified by flash chromatography (EtOAc/toluene, 1:3); yield: 150 mg (98%); mp 200–220 °C; $[\alpha]_D^{20}$ −74° (*c* = 0.98, $CHCl_3$).

^1H NMR ($CDCl_3$): δ = 11.98 (s, 1 H, OH-2), 6.44 (d, 1 H, H-3), 6.50 (d, 1 H, H-5), 6.97 (dd, 1 H, H-1'), 5.65 (ddd, 1 H, H-2'), 1.90–2.40 (m, 5 H, H-3a',b', 4a',b', 5b'), 2.79 (ddd, 1 H, H-5a'), 2.56 (ddd, 1 H, H-7a'), 1.40–1.80 (m, 5 H, H-7b', 8a',b', 9a',b'), 4.99 (m, 1 H, H-10'), 1.37 (d, 3 H, CH_3), 5.25 (d, H-1''), 5.09–5.31 (m, H-2''-4''), 3.88 (ddd, 1 H, H-5''), 4.24 (dd, 1 H, H-6a''), 4.14 (dd, 1 H, H-6b''), 2.00–2.10 (4 s, 3 H each, $COCH_3$); $J_{3,5}$ = 2.5, $J_{1',2'}$ = 15.4, $J_{1',3a'}$ = 1.5, $J_{2',3a}$ = 10.1, $J_{2',3b'}$ = 4.0, $J_{4a',5a'}$ = 11.9, $J_{4b',5a'}$ = 2.5, $J_{5a',5b'}$ = 18.8, $J_{7a',7b'}$ = 12.3, $J_{7a',8a'}$ = 5.6, $J_{7a',8b'}$ = 4.2, $J_{10',11'}$ = 6.1, $J_{1'',2''}$ = 7.9, $J_{4'',5''}$ = 10.0, $J_{5'',6a''}$ = 5.8, $J_{5'',6b''}$ = 2.3, $J_{6a'',6b''}$ = 12.3.

^{13}C NMR ($CDCl_3$): δ = 105.6 (C-1), 165.2 (C-2), 102.6 (C-3), 160.6 (C-4), 109.5 (C-5), 143.5 (C-6), 132.8 (C-1',2'), 42.9 (C-3'), 22.1 (C-4'), 34.6 (C-5'), 210.8 (C-6'), 36.6 (C-7'), 21.0 (C-8'), 31.0 (C-9'), 73.8 (C-10'), 20.7 (C-11'), 171.1 (C-12'), 97.7 (C-1''), 71.0, 72.3, 68.2, 72.6 (C-2''-5''), 61.9 (C-6''), 20.5, 20.6, 20.7 ($COCH^3$), 169.2, 169.4, 170.2, 170.6 ($COCH_3$).

MS: *m/z* (%) = 648 (2.6, M^+), 331 (76), 319 (3), 271 (36), 211 (28), 109 (100).

(*S*)-Zearalenone 4-*O*-β-D-glucopyranoside (4):
A solution of **3** (150 mg, 0.23 mmol) in EtOH (20 mL) and water (20 mL) was stirred with ion exchange resin (Dowex 1 × 2, OH^-, 200–300 mesh, ca. 100 mg) for 5 d. After filtration, the resin was rinsed with sufficient EtOH/water (1:1) and then with aq 0.2% NH_4Cl solution. Following evaporation, the residue was extracted with $CHCl_3$ and evaporated; yield: 85.1 mg (77%); mp 180–182 °C, $[\alpha]_D^{20}$ −65° (*c* = 0.42, MeOH).

^1H NMR (CD_3OD): δ = 6.27 (d, 1 H, H-3), 6.43 (d, 1 H, H-5), 6.70 (d, 1 H, H-1'), 5.59 (ddd, 1 H, H-2'), 1.70–2.00 (m, 6 H, H-3a',b', 4a', b', 5b',7b'), 2.54 (ddd, 1 H, H-5a'), 2.39 (ddd, 1 H, H-7a'), 1.20–1.50 (m, 4 H, H-8a', b', 9a',b'), 4.79 (m, 1 H, H-10'), 1.10 (d, 3 H, CH_3), 4.71 (d, 1 H, H-1''), 3.08–3.28 (m, 4 H, H-2''-5''), 3.66 (dd, 1 H, H-6a''), 3.42 (dd, 1 H, H-6b''); $J_{3,5}$ = 2.5, $J_{1',2'}$ = 15.4, $J_{2',3a'}$ = 10.1, $J_{2',3b'}$ = 4.0, $J_{4a',5a'}$ = 11.9, $J_{4b',5a'}$ = 2.5, $J_{5a',5b'}$ = 18.8, $J_{7a',7b'}$ = 12.3, $J_{7a',8a'}$ = 5.6, $J_{7a',8b'}$ = 4.2, $J_{10',11'}$ = 6.1, $J_{1'',2''}$ = 7.5, $J_{5'',6a''}$ = 5.8, $J_{5'',6b''}$ = 2.2, $J_{6b'',6b''}$ = 12.1.

***N*-{[(*R*)-5-Chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl]carbonyl}-L-β-phenylalanine (2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) Ester (6):**
To a solution of ochratoxin A (**5**; 10.5 mg, 0.026 mmol), glycosyl bromide **2** (23.2 mg, 0.056 mmol) and Bu_4NBr (16.5 mg, 0.051 mmol) in $CHCl_3$ (2.5 mL), was added borate buffer (pH 10.8, 2.5 mL) and the mixture warmed to 50 °C for 2 h. Further treatment and workup were carried out as described for **3**, and the crude material was purified by preparative TLC (silica gel, 0.5 mm, disopropyl ether/EtOAc, 1:1); yield: 6.3 mg (33%).

^1H NMR ($CDCl_3$): δ = 4.73 (ddd, 1 H, H-3), 3.27 (dd, 1 H, H-4a), 2.82 (dd, 1 H, H-4b), 8.38 (s, 1 H, H-6), 12.65 (s, 1 H, OH-8), 8.38 (d, 1 H, NH), 4.97 (ddd, 1 H, H-13), 3.23 (m, 2 H, H-14a, 14b), 7.12–7.31 (m, 5 H, H-16-20), 1.56 (d, 3 H, CH_3), 5.77 (d, 1 H, H-1'), 5.10–5.30 (m, 3 H, H-2'-4'), 3.84 (ddd, 1 H, H-5'), 4.28 (dd, 1 H, H-6a'), 4.14 (dd, 1 H, H-6b'), 1.99–2.11 (4 s, each 3 H, $COCH_3$);

$J_{3,4a} = 3.5$, $J_{3,4b} = 11.7$, $J_{3,CH_3} = 6.3$, $J_{4a,4b} = 17.5$, $J_{13,NH} = 6.6$, $J_{13,14a} = 5.4$, $J_{13,14b} = 7.2$, $J_{1',2'} = 7.8$, $J_{4',5'} = 9.9$, $J_{5',6a'} = 4.4$, $J_{5',6b'} = 2.2$, $J_{6a',6b'} = 12.4$.

N-{[(*R*)-5-Chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl]carbonyl}-L-β-phenylalanine (2,3,4,6-Tetra-*O*-benzyl-α/β-glucopyranosyl) Ester (10):

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (**7**; 28.2 mg, 0.052 mmol) dissolved in anhydrous CH_2Cl_2 (1 mL) was treated with oxalyl chloride (0.005 mL, 0.062 mmol) in the presence of anhydrous DMF (1 drop) for 30 min at r.t.. The mixture was evaporated to dryness, the 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride **(8)** formed was taken up in anhydrous EtOAc (1 mL), filtered over silica gel, and used in situ for the glycosylation.

A mixture of ochratoxin A (7 mg, 0.017 mmol) in anhydrous MeCN (1 mL) and Ag_2CO_3 (ca. 20 mg) was treated dropwise with the solution of **8** in EtOAc under stirring at r.t. for 2 h. Following filtration and evaporation the residue was purified by preparative TLC (silica gel, 1 mm, hexane/EtOAc, 2:1); yield: 13 mg (83%); **10α:10β** ≈ 1:1 (^{1}H NMR).

^{1}H NMR ($CDCl_3$): δ = 3.19/3.20* (dd, 1 H, H-4a), 2.82/2.76* (dd, 1 H, H-4b), 8.40 (s, 1 H, H-6), 12.70 (s, 1 H, OH-8), 8.44 (d, 1 H, NH), 5.02/5.03* (ddd, 1 H, H-13), 3.30 (dd, 1 H, H-14a), 3.14 (dd, 1 H, H-14b), 1.57* (d, 3 H, CH_3), 6.42 (d, 0.5 H, H-1'α), 5.69 (d, 0.5 H, H-1'β), 3.50–4.00 (m, 6 H, H-2'-5',6a',b'), 4.10–4.90 (m, 9 H, H-3 and 4 × $OCH_2C_6H_5$), 7.10–7.40 (m, 25 H_{arom}); $J_{3,4a} = 3.5$, $J_{3,4b} = 11.7$, $J_{3,CH_3} = 6.2$, $J_{4a,4b} = 17.5$, $J_{13,NH} = 6.6$, $J_{13,14a} = 5.4$, $J_{13,14b} = 7.2$, $J_{14a,14b} = 14.2$, $J_{1',2'}$ (α) = 3.4, $J_{1',2'}$ β) = 8.0 (* refers to to **10α/10β** mixture).

Support of this work by the Hoechst AG, Frankfurt, and the Fonds der Chemischen Industrie is gratefully acknowledged.

(1) Urry, W.H.; Wehrmeister, H.L.; Hodge, E.B.; Hidy, P.H. *Tetrahedron Lett.* **1966**, 3109.
(2) Hacking, A.; Rosser, W.R.; Dervish, M.T. *Ann. Appl. Biol.* **1976**, *84*, 7.
(3) Pathre, S.V.; Misocha, C.J. *Adv. Chem. Ser.* **1976**, *149*, 178.
(4) Koenigs, W.; Knorr, E. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 957.
(5) Bochkov, A.F.; Zaikov, G.E. *Chemistry of the O-Glycosidic Bond*; Pergamon Press: Oxford, 1977.
(6) Fischer, E.; Raske, K. *Ber. Dtsch. Chem. Ges.* **1909**, *42*, 1415.
(7) Mann, R.M. *J. Am. Chem. Soc.* **1934**, *56*, 1631.
(8) Noller, C.R.; Rockwell, W.C. *J. Am. Chem. Soc.* **1938**, *60*, 2076.
(9) Krohn, K.; Thiem, J. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1186.
(10) Dess, D.; Kleine, H.S.; Weinberg, D.V.; Kaufman, R.J.; Sidhu, R.S. *Synthesis* **1981**, 883.
(11) Kamimura, H. *Appl. Environ. Microbiol.* **1986**, *52*, 515.
(12) Gareis, M.; Bauer, J.; Thiem, J.; Plank, G.; Grabley, S.; Gedek, B. *J. Vet. Med. B.* **1990**, *37*, 236.
(13) Scott, De B. *Mycopathol. Mycol. Appl.* **1965**, *25*, 213.
(14) van der Merwe, K.J.; Steyn, P.S.; Fourie, L.; Scott, De B.; Theron, J.J. *Nature (London)* **1965**, *205*, 1112.
(15) Schmidt, O.Th.; Auer, T.; Schmadel, H. *Chem. Ber.* **1960**, *93*, 556.
(16) Wissner, A.; Grudzinskas, C.V. *J. Org. Chem.* **1978**, *43*, 3972.
(17) Grob, V.D.; Squires, T.G.; Vercelotti, J.R. *Carbohydr. Res.* **1969**, *10*, 595.
(18) Kreuzer, M. *Ph. D. Dissertation*, University of Münster, 1986.
(19) Thiem, J.; Kreuzer, M.; Fritsche-Lang, W.; Deger, H.-M. Ger. Offen. DE 3528654 (1987); *Chem. Abstr.* **1987**, *106*, 156814.
(20) Thiem, J.; Wiesner, M. *Synthesis* **1988**, 124.
(21) Kreuzer, M.; Thiem, J. *Carbohydr. Res.* **1986**, *149*, 347.
(22) Kunz, H.; Sager, W. *Helv. Chim. Acta* **1985**, *68*, 283.