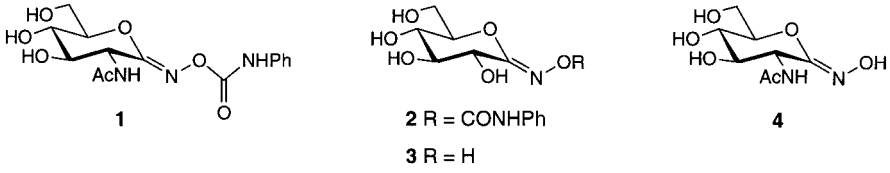
An Improved Synthesis of 2-Acetamido-2-deoxy-D-gluconohydroximolactone (PUGNAc), A Strong Inhibitor of β-*N*-Acetylglucosaminidases

by **Halasyam Mohan** and **Andrea Vasella***

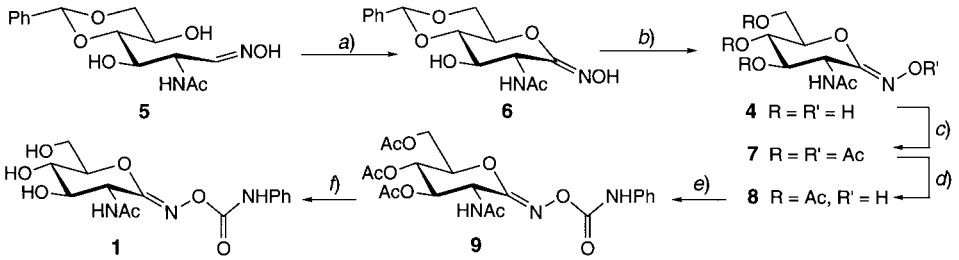
Laboratorium für Organische Chemie E41, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

O-(2-Acetamido-3,4,6-tri-*O*-acetyl-D-glucopyranosylidene)amino *N*-phenylcarbamate (**1**), an established inhibitor of β-*N*-acetylglucosaminidases, has been prepared by an improved six-step synthesis from *N*-acetyl-D-glucosamine.

Introduction. – The phenylcarbamate **1** (PUGNAc for Phenyl Urethane of GlcNAc) [1], derived from *N*-acetylgluconic acid hydroximolactone, is a strong inhibitor of glucosaminidases from different sources [2–4]. Similarly to the phenylcarbamate **2**, which is a stronger inhibitor than **3** [5], **1** is a much stronger inhibitor than the parent oximolactone **4** (*Scheme 1*) and has proven a useful tool in a range of biological investigations [6–23].



Scheme 1

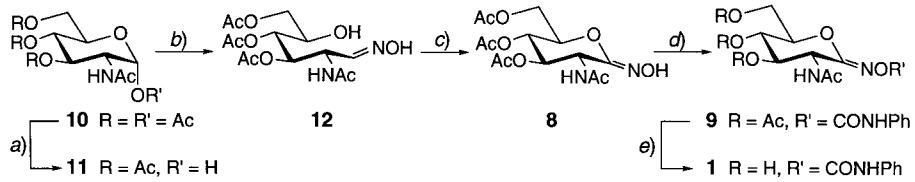


a) MnO_2, MeOH, 50°, 12 h; 35% **5**, 50% **6**. *b*) Na/NH_3, −35°, MeOH; 86%. *c*) Ac_2O, C_5H_5N, 0° to r.t.; 96%. *d*) $MeNH_2$, EtOH, 0°; 71%. *e*) PhNCO, Et_3N, THF, 3 h; 94%. *f*) NH_3/MeOH, 0°, 4 h; 61%.

The reported synthesis of **1** [1], shown in *Scheme 1*, involves two critical steps, MnO_2 oxidation of the oximes **5** (prepared from *N*-Acetyl-D-glucosamine in two steps [1][24]) to the hydroximo-1,5-lactone **6**, and debenzylidenation of **6** with Na/NH_3 to the hydroximolactone **4**. The reported yields for these steps were 50% for the MnO_2

oxidation on a 4-g scale, and 86% for the debenzylidenation on a 2-g scale. While the yield of oxidation was improved by prolonged reaction, yields for debenzylidenation were consistently lower. Scale-up of both transformations to produce multigram quantities of the inhibitor led to markedly lower yields and involved inconvenient workup procedures. Considering the useful applications of the inhibitor, we have developed the improved, much more convenient synthesis shown in *Scheme 2*.

Scheme 2



a) $(NH_4)_2CO_3$, THF/MeOH, 24°, 24 h; 74% (from 100 g of **10**). *b*) $NH_2OH \cdot HCl$, C_5H_5N/MeOH, reflux, 2 h; 75% (from 65.5 g of **11**). *c*) DBU, NCS/CH_2Cl_2. −40° to r.t., then 3 h; 59% (from 7 g of (*E*/*Z*)-**12**). *d*) PhNCO, Et_3N, CH_2Cl_2, r.t., 3 h; 70% (from 5 g of **9**). *e*) Aq. NH_3, −10° to r.t., 3 h; 73% (from 5 g of **9**).

Results and Discussion. – Cleavage of the anomeric AcO group of the known *N*-acetyl-D-glucosamine-derived tetra-*O*-acetate **10** [25][26] was examined with $PhCH_2NH_2$ [27], NH_3 [28], hydrazine hydrate [29], MeONa in MeOH [30], or $(NH_4)_2CO_3$ [31]. On a gram scale, the last-mentioned method [31] gave the best result in our hands. On a 20-g scale, we obtained 80% of the triacetate **11** after purification by a short flash chromatography. Performing the reaction on a scale above 25 g of **10** led to by-products resulting from cleavage of additional AcO groups. TLC revealed the triacetate **11** (R_f (AcOEt) 0.35) tailed by a minor spot (R_f (AcOEt) 0.31). Purification of **11** by flash column chromatography was not sastisfactory. Therefore, the crude product resulting from the monodeacetylation of **10** on a scale of 100 g was only partially purified by rapid filtration through a short pad of silica gel. The resulting triacetate **11**, which still contained minor amounts of the polar impurity, was transformed into the oximes **12** by treatment with $NH_2OH \cdot HCl$ and pyridine in MeOH under reflux. As detailed in the *Exper. Part*, a 5:1 mixture of the (*E*/*Z*)-oximes **12** was conveniently obtained as a white solid, and oxidised with NCS/DBU [32] in CH_2Cl_2 at −40° to the hydroximolactone **8**. It proved critical to monitor the temperature and maintain it at −40° during the addition of NCS. Addition of NCS at lower temperature resulted in incomplete conversion to **8**, while addition at higher temperatures (> −35°) led to the 1,4-lactone oxime. The oximes **12** did not react with MnO_2 under sonication at temperatures of up to 75°. Crude **8**, purified by flash chromatography, was obtained in 59% yield and in over 99% purity (HPLC) on a scale of up to 10 g. Treatment of **8** with PhNCO in THF at 24° followed by flash chromatography yielded 70% of the phenyl carbamate **9**, which was de-*O*-acetylated with saturated aqueous NH_3 to afford PUGNAc (**1**) in 73% yield after crystallization from MeOH/Et_2O. This sample was characterized by physical and spectroscopic properties identical to those reported for **1** [1].

Experimental Part

General. All solvents were distilled before use; THF was freshly distilled over K/benzophenone. Et_3N was freshly distilled and stored over KOH under N_2. All reagents were obtained from *Fluka.* NCS was crystallised from benzene and stored in a container wrapped with Al foil at $-10°$. DBU was stored over freshly activated 4-Å molecular sieves under N_2. Reactions were run under N_2 atmosphere. Tetraacetate **10** was prepared according to [25][26][33]. TLC: *Merck* Al-precoated silica gel *60* F_{254} plates; detection by spraying with a 0.25M I_2 soln. in 10% aq. H_2SO_4, followed by heating at *ca.* 200°, or treatment with a soln. of 5% $(NH_4)_6Mo_7O_{26}\cdot 4\,H_2O$, 0.1% $Ce(SO_4)_2\cdot H_2O$, in 10% H_2SO_4 soln. Flash chromatography (FC): silica gel *60* (40–63 μm). Anal. HPLC analyses were run on silica gel, *Spherisorb SW5*, column dimensions: 4 × 250 mm, detection by RID, t_R values in min, unless otherwise mentioned. M.p.: uncorrected. Optical rotations: 1-dm cell, 589 nm. FT-IR Spectra: absorption in cm^{-1}. NMR Spectra: chemical shifts in ppm relative to TMS (^1H, ^{13}C); *J* values in Hz. FAB-MS in 3-nitrobenzyl alcohol (NOBA) matrix.

2-Acetamido-3,4,6-tri-O-*acetyl-2-deoxy*-α-D-*glucopyranose* (**11**) [34]. A magnetically stirred soln. of **10** (100 g, 257 mmol) in anh. THF/MeOH 1:2 (771 ml) was treated with one batch of $(NH_4)_2CO_3$ (96.21 g, 1 mol) and stirred for 24 h. After evaporation, the residue was dissolved in H_2O (100 ml) and extracted with AcOEt (10 × 150 ml). The combined org. layers were washed with brine (2 × 50 ml), dried (Na_2SO_4), and evaporated at 30°. Filtration through a short pad of silica gel (250 g, AcOEt), evaporation, and drying at 0.01 mm Hg over KOH for 24 h gave crude **11** (66.05 g), which was used for next step. FC (AcOEt) of a small sample afforded pure **11** as a solid, which shows identical spectroscopic data as those reported. M.p. 86° (sintering at 62°) ([34]: 84–87°). R_f (AcOEt) 0.35. IR: see [34]. ^1H-NMR: see [27][28]. ^{13}C-NMR: see [34].

*(*E*)- and (*Z*)-2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy*-D-*glucose Oxime* (**12**). A soln. of crude **11** (65.5 g, *ca.* 188.7 mmol) in MeOH (1132 ml) was treated with $NH_2OH\cdot HCl$ (19.8 g, 284.8 mmol) and C_5H_5N (29.84 ml, 484.7 mmol), stirred at reflux for 2 h (→ brown red soln.), and evaporated. C_5H_5N was removed by azeotropic distillation with toluene (3 × 100 ml) at 20 mm Hg/30°. The residue was dissolved in H_2O (75 ml) and extracted with AcOEt (10 × 150 ml). The combined org. layers were washed with brine (2 × 50 ml), dried (Na_2SO_4), and evaporated at 30°. The foamy residue was dissolved in MeOH (30 ml), treated with Et_2O (400 ml), and kept at −10° for 12 h. The white precipitate was suction-filtered (sintered funnel *G3*), washed with Et_2O (3 × 50 ml), and dried at 0.01 mm Hg over KOH for 24 h, affording crude (*E*)-**12**/(*Z*)-**12** 5:1 (51.1 g, *ca.* 75%), which was used directly for the next step. TLC displayed two close spots at R_f (AcOEt/MeOH 97:3) 0.35 (intense) and 0.30 (weak). FC (AcOEt/MeOH 97:3) of a small sample afforded pure (*E*)-**12**/(*Z*)-**12** *ca.* 5:1. Anal. HPLC: t_R (AcOEt, flow rate 2 ml/min) 3.48 ((*E*)-isomer), 5.30 ((*Z*)-isomer). IR (KBr): 3359*s* (br.), 3092*m*, 2966*w*, 2879*w*, 1748*s*, 1708*s*, 1656*s*, 1548*s*, 1434*m*, 1376*s*, 1225*s* (br.), 1075*m*, 1045*s*, 971*w*, 948*m*, 911*w*, 865*w*, 758*w*, 715*w*, 636*w*, 605*m*, 527*w*, 492*w*, 455*w*. ^1H-NMR (300 MHz, CD_3OD; (*E*)/(*Z*) 5:1): 7.29 (*d*, *J* = 5.6, 0.84 H), 6.58 (*d*, *J* = 6.2, 0.16 H) (H−C(1)); 5.63 (br. *t*, *J* = 5.1, 0.16 H), 5.55 (*dd*, *J* = 3.4, 6.7, 0.84 H) (H−C(3)); 5.45 (br. *t*, *J* = 5.8, 0.16 H), 4.88 (*t*, *J* = 5.9, 0.84 H) (H−C(2); 5.11 (*dd*, *J* = 3.7, 7.5, H−C(4)); 4.09–4.00 (*m*, 2 H−C(6)); 4.00–3.84 (*m*, H−C(5)); 2.08 (*s*, 2 Ac); 2.04, 1.97 (2*s*, 2 Ac). ^{13}C-NMR (75 MHz, CD_3OD; (*E*)/(*Z*) 5:1): signals of (*E*)-**12**: 164.08, 163.77, 162.89, 162.83 (4*s*, 4 C=O); 137.91 (*s*, C(1)); 63.28, 63.02 (2*d*, C(3), C(4)); 59.72 (*t*, C(6)); 57.04 (*d*, C(5)); 41.67 (*d*, C(2)); 13.42, 11.67, 11.59, 11.48 (4*q*, 4 Me); signals of (*Z*)-**12**: 164.14 (*s*, C=O); 138.48 (*s*, C(1)); 64.07, 62.88 (2*d*, C(3), C(4)); 56.89 (*d*, C(5)); 37.46 (*d*, C(2)). FAB-MS: 725 (9, $[2M+1]^+$), 386 (7), 385 (35, $[M+Na]^+$), 364 (19), 363 (100, $[M+1]^+$), 347 (9), 346 (10), 345 (23), 330 (24), 321 (13), 303 (22), 288 (9), 183 (8), 141 (22), 132 (17), 123 (20).

*(*Z*)-2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy*-D-*gluconohydroximo-1,5-lactone* (**8**) [35]. A magnetically stirred suspension of crude (*E*)-**12**/(*Z*)-**12** 5:1 (7 g, *ca.* 19.3 mmol) in anh. CH_2Cl_2 (69 ml) was cooled to −40°, treated dropwise with DBU (3.17 ml, 21.26 mmol) over a period of 10 min, stirred for 5 min, treated portionwise (*ca.* 0.5 g) with NCS (2.84 g, 21.3 mmol) over a period of 15 min at −35 to −40°, allowed to warm to r.t., and stirred for 3 h. The clear soln. was treated with H_2O (25 ml) and extracted with AcOEt (10 × 75 ml). The combined org. layers were washed with brine (2 × 25 ml), dried (Na_2SO_4), and evaporated at 30°. FC (AcOEt/MeOH 97:3) and drying at 0.01 mm Hg over KOH for 24 h afforded pure **8** (3.6 g, 59%) as a foam. R_f (AcOEt/MeOH 97:3) 0.36. Anal. HPLC: t_R (hexane/Et_2O/dioxane 2:1:1, flow rate 2 ml/min) 8.42. IR ($CHCl_3$): 3659*w*, 3410*m*, 3390*m*, 3339*m* (br.), 3007*m* (sh), 2949*w*, 2892*w*, 1742*s*, 1668*s*, 1518*m*, 1431*w*, 1373*s*, 1248*s* (br.), 1181*s* (br.), 1152*w*, 1046*s*, 973*w*, 938*w*, 883*w*, 641*w*, 603*w*, 532*w*. ^1H-NMR (400 MHz, $CDCl_3$): see [35]. ^{13}C-NMR (75 MHz, $CDCl_3$): 171.9, 169.2 (2*s*, 2 C=O); 170.7 (*s*, 2 C=O); 151.1 (*s*, C(1)); 77.0 (*d*, C(5)); 73.0 (*d*); 67.3 (*d*); 61.6 (*t*, C(6)); 48.9 (*d*, C(2)); 22.5, 20.6, 20.5, 20.3 (4*q*, 4 Me). FAB-MS: 743 (8, $[2M+Na]^+$), 722 (6), 721 (24, $[2M+1]^+$), 384 (5), 383 (33, $[M+Na]^+$), 363 (4), 362 (21), 361 (100, $[M+1]^+$), 360 (12, M^+), 301 (7), 259 (7), 181 (25).

O-*(2-Acetamido-3,4,6-tri*-O-*acetyl*-D-*glucopyranosylidene)amino* N-*Phenylcarbamate* (**9**) [1]. A soln. of **8** (5 g, 13.88 mmol) in anh. THF (69.4 ml) was treated with PhNCO (3.03 ml, 27.76 mmol) and Et_3N (9.67 ml, 69.4 mmol, 9.67 ml) and magnetically stirred for 3 h. After evaporation, the residue was adsorbed on silica gel (20 g). FC (hexane/AcOEt/MeOH 23:75:2) gave **9** (4.65 g, 70%) as a foam. R_f (hexane/AcOEt/MeOH 23:75:2) 0.30. Anal. HPLC: t_R (hexane/Et_2O 5:2, flow rate 2 ml/min) 5.32. IR ($CHCl_3$): 3420*m* (sh), 3308*w* (br.), 3016*w* (br.), 2397*w*, 1754*s*, 1686*m*, 1661*m*, 1602*m* (sh.), 1524*s*, 1444*m* (sh), 1370*m*, 1311*w*, 1248*s* (br.), 1181*s* (br.), 1108*w*, 1079*w*, 1036*m*, 1023*m*, 1000*w*, 947*w*, 899*w*, 598*w*, 519*w*. ^{1}H- ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$): see [1]. FAB-MS: 959 (10, $[2M+1]^+$), 502 (10, $[M+Na]^+$), 482 (8), 481 (29), 480 (100, $[M+1]^+$), 479 (17, M^+), 421 (10), 420 (34), 361 (14), 359 (41), 344 (8), 343 (7), 338 (7), 326 (13), 317 (7), 300 (15), 284 (12), 259 (14), 258 (11), 241 (16) 240 (8), 199 (10), 181 (27).

O-*(2-Acetamido-2-deoxy*-D-*glucopyranosylidene)amino* N-*Phenylcarbamate* (**1**) [1]. A cooled (−15°) soln. of **9** (5 g, 10.44 mmol) in MeOH (104 ml) was treated dropwise with 14.7M NH_3 (12.78 ml, 187.92 mmol) and stirred for 3 h. After evaporation, the residue was dissolved in MeOH (10 ml), treated with Et_2O (100 ml) and kept at −10° for 12 h. The supernatant solvent was decanted, and the solids were washed with cold Et_2O (3 × 5 ml), dried at 0.01 mm Hg over KOH for 24 h to afford **1** (2.25 g). Evaporation of the mother liquor and FC (AcOEt/MeOH 9:1) gave further **1** (0.45 g; total yield: 73%). R_f (dioxane/hexane/MeOH 14:5:1) 0.21; (AcOEt/MeOH/H_2O 85:10:0.5) 0.43. M.p. 172° ([10]: 171–174°]. $[\alpha]_D^{25} = +65.3$ ($c = 0.2$, MeOH) ([10]: +64.8 ($c = 0.2$, MeOH)). Anal. HPLC: t_R (hexane/dioxane/MeOH 7:7:0.5, flow rate 2 ml/min) 6.40, t_R (column: *Lichrospher 100 RP-18*, dimensions: 4 × 250 mm, MeOH/H_2O 1:4, flow rate 1 ml/min) 26.7. IR (KBr): 3456*s* (br.), 3305*s* (br.), 3078*m*, 2933*m*, 2878*m*, 1739*s*, 1719*s*, 1656*s*, 1600*s*, 1550*s*, 1533*s*, 1500*m*, 1444*s*, 1410*m*, 1371*s*, 1315*s*, 1304*m*, 1259*w*, 1209*s*, 1209*s*, 1158*m*, 1108*s*, 1074*s*, 1040*s*, 1029*s*, 990*m*, 961*w*, 934*w*, 906*w*, 872*w*, 838*w*, 754*s*, 692*s*, 647*w*, 620*s*, 597*m*, 558*m*, 507*m*. ^{1}H- ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$): see [1]. FAB-MS: 707 (9, $[2M]^+$), 376 (19), 355 (20), 354 (100, M^+), 353 (14), 308 (14), 307 (57), 290 (11), 289 (26), 234 (14), 175 (15).

REFERENCES

[1] D. Beer, J. L. Maloisel, D. M. Rast, A. Vasella, *Helv. Chim. Acta* **1990**, *73*, 1918.
[2] M. Horsch, L. Hoesch, A. Vasella, D. M. Rast, *Eur. J. Biochem.* **1991**, *197*, 815.
[3] M. Horsch, L. Hoesch, G. W. Fleet, D. M. Rast, *J. Enzyme Inhib.* **1993**, *7*, 47.
[4] M. Horsch, C. Mayer, D. M. Rast, *Eur. J. Biochem.* **1996**, *237*, 476.
[5] D. Beer, A. Vasella, *Helv. Chim. Acta* **1986**, *69*, 267.
[6] A. Godknecht, T. D. Honegger, *Dev. Biol.* **1991**, *143*, 398.
[7] D. J. Miller, X. H. Gong, G. Decker, B. D. Shur, *J. Cell Biol.* **1993**, *123*, 1431.
[8] D. J. Miller, X. H. Gong, B. D. Shur, *Development* **1993**, *118*, 1279.
[9] A. J. Godknecht, T. G. Honegger, *Dev. Growth Differ.* **1995**, *37*, 183.
[10] F. Cattaneo, M. E. Pasini, M. E. Perotti, *Mol. Reprod. Dev.* **1997**, *48*, 276.
[11] D. L. Y. Dong, G. W. Hart, *J. Biol. Chem.* **1994**, *269*, 19321.
[12] J. Rodriguez, J. L. Copapatino, F. Reyes, M. I. Perezleblic, *Lett. Appl. Microbiol.* **1994**, *19*, 217.
[13] N. D. Jordan, M. S. Barber, *Plant Sci.* **1995**, *107*, 41.
[14] M. Takada, N. Yonezawa, M. Yoshizawa, S. Noguchi, Y. Hatanaka, T. Nagai, K. Kikuchi, H. Aoki, M. Nakano, *Biol. Reprod.* **1994**, *50*, 860.
[15] K. Matsuura, H. Sawada, H. Yokosawa, *Biochem. Biophys. Res. Commun.* **1995**, *213*, 311.
[16] P. V. Miranda, A. Brandelli, J. G. Tezon, *Int. J. Androl.* **1995**, *18*, 263.
[17] A. Hodge, G. W. Gooday, I. J. Alexander, *Phytochemistry* **1996**, *41*, 77.
[18] U. Sennhauser, C. Mayer, D. M. Rast, *Adv. Chitin Sci.* **1996**, *1*, 114.
[19] I. Tews, K. S. Wilson, C. E. Vorgias, *Adv. Chitin Sci.* **1996**, *1*, 26.
[20] M. J. G. Fernandes, S. Yew, D. Leclerc, B. Henrissat, C. E. Vorgias, R. A. Gravel, P. Hechtman, F. Kaplan, *J. Biol. Chem.* **1997**, *272*, 814.
[21] T. Pusztahelyi, I. Pocsi, A. Szentirmai, *Biotechnol. Appl. Biochem.* **1997**, *25*, 87.
[22] R. S. Haltiwanger, K. Grove, G. A. Philipsberg, *J. Biol. Chem.* **1998**, *273*, 3611.
[23] E. Sandor, T. Pusztahelyi, L. Karaffa, Z. Karanyi, I. Pocsi, S. Biro, A. Szentirmai, I. Pocsi, *FEMS Microbiol. Lett.* **1998**, *164*, 231.
[24] W. Roth, W. Pigman, *J. Am. Chem. Soc.* **1960**, *82*, 4608.
[25] F. Micheel, F.-P. v. d. Kamp, H. Wulff, *Ber. Dtsch. Chem. Ges.* **1955**, *88*, 2011.
[26] D. Chaplin, D. H. G. Crout, S. Bornemann, D. W. Hutchinson, R. Khan, *J. Chem. Soc., Perkin Trans. 1* **1992**, 235.

[27] M. M. Sim, K. Hirosato, C. Huey, *J. Am. Chem. Soc.* **1993**, *115*, 2260.
[28] J. Fiandor, M. T. Garcia-Lopez, F. G. D. L. Heras, *Synthesis* **1985**, *12*, 1121.
[29] K. Riaz, P. A. Konowicz, L. Gardossi, M. Matulova, S. Gennarao, *Aust. J. Chem.* **1996**, *49*, 293.
[30] S. S. Pertel, V. Y. Chirva, *Chem. Nat. Comp. (Engl. Transl.)* **1994**, *30*, 160.
[31] M. Masatomo, *Carbohydr. Res.* **1989**, *191*, 150.
[32] A. Vasella, C. Witzig, C. Waldraff, P. Uhlmann, K. Briner, B. Bernet, L. Panza, R. Husi, *Helv. Chim. Acta* **1993**, *76*, 2847.
[33] D. Horton, *J. Org. Chem.* **1964**, *29*, 1776.
[34] B. Paul, R. L. Bernacki, W. Korytnyk, *Carbohydr. Res.* **1980**, *80*, 99.
[35] D. Beer, A. Vasella, *Helv. Chim. Acta* **1985**, *68*, 2254.

Received November 26, 1999