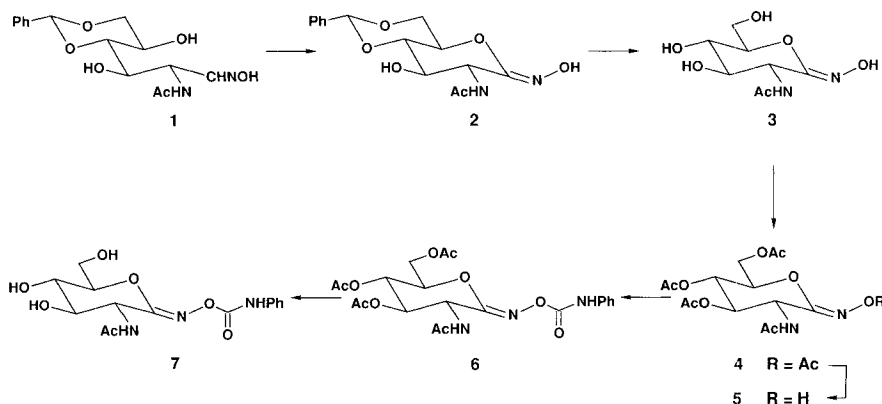


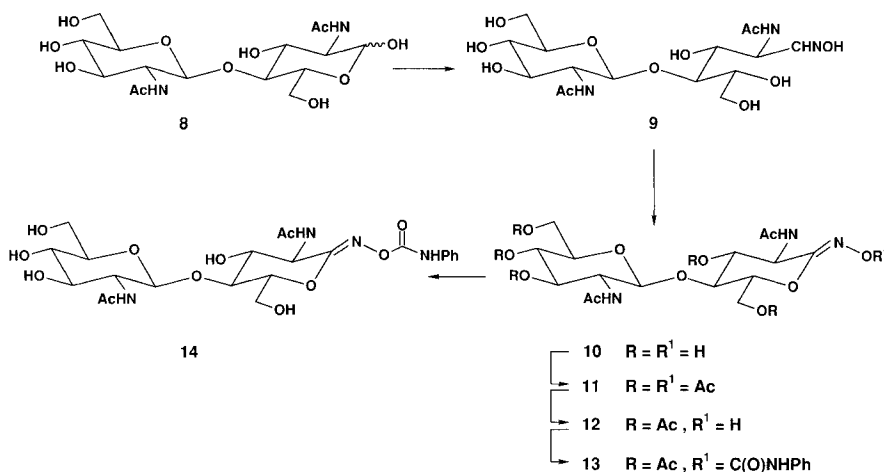


We now report on a more convenient, if somewhat longer procedure for the preparation of the hydroximolactone **3**, on the preparation of the *N*-phenylcarbamate **7** from **3** via the selectively protected tetraacetyl derivative **5** [1], and on the analogous preparation of the *N*-phenylcarbamate **14** from chitobionhydroximolactone (**10**).

Results. – The partially protected oxime **1** (Scheme 1), available from *N*-acetyl-D-glucosamine in two steps and in a yield of 81% [1] [4], was oxidized with MnO_2 [5]. The crystalline hydroximo-1,5-lactone **2** was obtained in a yield of 50%, together with 35% of starting material. While catalytic debenzoylation of 2,3,4,6-Tetra-*O*-benzyl-D-gluconhydroximo-1,5-lactone had led to decomposition [6], debenzylidenation of **2** with Na/NH_3 , followed by chromatography on neutral alumina, afforded **3** in 86% yield. The pentaacetyl derivative **4**, obtained according to [1] was partially deacetylated (MeNH_2 in MeOH) to **5** (68% from **3**). This hydroximolactone reacted smoothly with phenyl isocyanate, and the resulting carbamate **6** was isolated in a yield of 94%. Transacetylation gave the desired, crystalline *N*-phenylcarbamate **7** (61%). It proved stable for many months when stored in the refrigerator.

To obtain the analogous *N*-phenylcarbamate, derived from chitobiose (**8**), we oxidized the oxime **9** (Scheme 2) with MnO_2 in the presence of a phosphate buffer at pH 7.

Scheme 2



The hydroximolactone **10** was crystallized from MeOH (69%) and acetylated in $\text{Ac}_2\text{O}/\text{pyridine}$ to yield 83% of the hexaacetate **11**. Selective deacetylation (MeNH_2 in MeOH) gave the pentaacetyl derivative **12** (43%), and treatment of **12** with phenyl isocyanate afforded the protected *N*-phenylcarbamate **13** (76%). Transacetylation of **13** with NH_3 in MeOH gave 46% of the carbamate **14**. All these compounds, derived from chitobiose crystallize easily and have relatively high melting points.

The carbamate **7** is a strong inhibitor of β -*N*-acetylglucosaminidase. Its biochemical properties and those of the hydroximolactone **3** will be reported separately [7].

We thank *Sandoz Ltd.*, Basel, and the *Swiss National Science Foundation* for generous support.

Experimental Part

General. Cf. [8]. All solvents were distilled before use; H₂O was distilled twice. Activated MnO₂ was prepared following [5]. TLC: *Merck* precoated silica gel 60 *F*₂₅₄ plates; detection by spraying with a 0.025M I₂ soln. in 10% aq. H₂SO₄, followed by heating at ca. 200°. Column chromatography: silica gel *Merck* 60; flash chromatography (FC): 40–63 μ ; medium-pressure chromatography (MPLC) [9]: 15–40 μ . Solvents mixtures: *A* = AcOEt/MeOH/H₂O, *B* = CHCl₃/EtOH. M.p.: not corrected. Optical rotations: *Perkin-Elmer-241* polarimeter, 1-dm cell, measured at 365, 436, 546, 578, and 589 nm; the specific rotation at 589 nm was determined using a regression curve. IR: 3% CHCl₃ solns., unless stated otherwise.

2-Acetamido-4,6-O-benzylidene-2-deoxy-D-gluconhydroximo-1,5-lactone (2). To a stirred soln. of **1** [**1**] (4 g, 12.3 mmol) in MeOH (150 ml) at 50° were added 2 equiv. of activated MnO₂ [5] (2.139 g, 24.6 mmol). The mixture was stirred overnight at 50° and filtered through *Celite*. Concentration of the filtrate to 40 ml followed by adsorption on silica gel and FC (CH₂Cl₂/MeOH 10:1) afforded **1** (1.4 g, 35%) and **2** (1.98 g, 50%), which was crystallized from AcOEt/MeOH. *R*_f (CH₂Cl₂/MeOH 8:1) 0.4. M.p. 229–231° (dec.). $[\alpha]_D^{25} = +48.2$ (*c* = 0.2, MeOH). IR (KBr): 3360s (br.), 3095w, 3060w, 2870w, 1710w, 1660s (sh), 1645s (br.), 1565m (sh), 1550m, 1470w, 1450m, 1430w, 1390m, 1380m, 1330w, 1315m, 1250m, 1210w, 1150w, 1135m, 1120m, 1095s, 1045m, 1030m, 1000m, 970m, 945m, 925w, 895w, 875w, 790w, 760m, 750w, 700m. ¹H-NMR (400 MHz, (D₆)DMSO): 10.08 (s, NOH); 8.26 (*d*, *J* = 8.4, NH); 7.46–7.43 (*m*, 2 arom. H); 7.38–7.36 (*m*, 3 arom. H); 5.68 (s, HO–C(3), PhCH); 4.36–4.34 (*m*, 2 H); 3.85–3.7 (*m*, 4 H); 1.85 (s, CH₃). ¹³C-NMR (50 MHz, (D₆)DMSO): 169.1 (s, CH₃CO); 150.25 (s, C(1)); 137.6 (s, arom. C); 129.2 (*d*, arom. C); 128.3 (*2d*, arom. C); 126.5 (*2d*, arom. C); 100.9 (*d*, PhCH); 80.4 (*d*, C(4)); 71.3 (*d*); 68.4 (*d*), 67.6 (*t*, C(6)); 52.5 (*d*, C(2)); 23 (*q*, CH₃). CI-MS (NH₃): 340 (54, [M + 1 + NH₃]⁺), 323.3 (25, [M + 1]⁺). Anal. calc. for C₁₅H₁₈N₂O₆ (322.32): C 55.90, H 5.63, N 8.69; found: C 55.77, H 5.85, N 8.49.

2-Acetamido-2-deoxy-D-gluconhydroximo-1,5-lactone (3). Five equiv. of Na (759 mg, 33 mmol) were added in portions at –35° to a soln. of **2** (2 g, 6.6 mmol) in NH₃ (45 ml), until the blue color persisted for more than 1 min, and TLC showed the disappearance of **2**. Evaporation of NH₃ left a residue which was redissolved in MeOH and purified by FC (neutral alumina, AcOEt/MeOH/H₂O 7:2:1): **3** (1.25 g, 86%). Data of **3**: in agreement with those in [1].

O-(2-Acetamido-2-deoxy-D-glucopyranosylidene)amino N-Phenylcarbamate (7). Phenyl isocyanate (290 μ l, 2.64 mmol) and Et₃N (950 μ l, 6.6 mmol) were added a stirred soln. of **5** (480 mg, 1.32 mmol) [**1**] in THF (15 ml). After 3 h, the solvents were evaporated and the residue purified by FC (silica gel, AcOEt/hexane 3:2): **O-(2-acetamido-3,4,6-tri-O-acetyl-D-glucopyranosylidene)amino N-phenylcarbamate (6**, 600 mg, 94%) as a foam. *R*_f (AcOEt/hexane 5:2) 0.32. IR (CHCl₃): 3424w, 3380w, 3320w (br.), 3030w, 3000w, 2960w, 1750s (br.), 1680m, 1395m (sh), 1370m, 1320w (sh), 1310m, 1210m, 1030m, 1020m, 990w, 895w. ¹H-NMR (200 MHz, CDCl₃): 7.82 (s, PhNH); 7.45–7.28 (*m*, 4 arom. H); 7.14 (*d*, *J* = 7.8, AcNH); 7.11 (*m*, arom. H); 5.45 (*dd* app. as *t*, *J* = 8.8, H–C(3)); 5.33 (*dd* app. as *t*, *J* = 8.7, H–C(4)); 4.85 (*dd* app. as *t*, *J* = 8.4, H–C(2)); 4.54 (*ddd* app. as *dt*, *J* = 2.8, 8.6, H–C(5)); 4.42 (*dd*, *J* = 3.2, 12.9, H–C(6)); 4.3 (*dd*, *J* = 2.5, 12.9, H–C(6)); 2.12 (s, CH₃); 2.05 (s, CH₃); 2.0 (s, CH₃). ¹³C-NMR (50 MHz, CDCl₃): 170.5 (s, CO); 170.3 (s, CO); 170.2 (s, CO); 169.1 (s, CO); 155.2 (s, CONHPh); 151.8 (s, C(1)); 136.8 (s, arom. C); 129.2 (*2d*); 124.3 (*d*); 119.2 (*2d*); 77.2 (*d*); 71.3 (*d*); 67.1 (*d*); 61.2 (*t*, C(6)); 49.6 (*d*, C(2)); 22.8 (*q*, CH₃); 20.62 (*q*, CH₃); 20.57 (*q*, CH₃); 20.5 (*q*, CH₃).

Sat. NH₃/MeOH (ca. 4M, 2 ml) was added to an ice-cold soln. of **6** (300 mg, 0.626 mmol) in MeOH (15 ml). After 4 h, the soln. was concentrated to 5 ml and purified by FC (AcOEt/MeOH 10:1) to afford **7** (135 mg, 61%) which was crystallized from AcOEt/MeOH/hexane. *R*_f (AcOEt/MeOH 10:1) 0.21. M.p. 171–174° (dec.). $[\alpha]_D^{25} = +64.8$ (*c* = 0.2, MeOH). IR (KBr): 3470m (sh), 3420s, 3310s, 3060w, 2950w, 2930w, 2900w, 2880w, 1745s, 1705m, 1660m, 1645s, 1605m, 1600m (sh), 1553m, 1530s, 1495w, 1450m, 1415w, 1380m, 1330w, 1310m, 1290w (sh), 1260w, 1205m, 1295m, 1160w, 1130w, 1005m, 1080m, 1070m, 1040m, 1020m, 1000w, 960w, 930w, 900w, 880w, 860w, 770w, 750m, 690m, 640m, 620m. ¹H-NMR (400 MHz, CD₃OD): 7.44 (*m*, 2 arom. H); 7.29 (*m*, 2 arom. H); 7.05 (*m*, H arom.); 4.58 (*m*, X of ABX, H–C(2)); 3.95 (*dd*, *J* = 2.2, 12.8, H–C(6)); 3.94 (*m*, H–C(5)); 3.85 (*dd*, *J* = 4.6, 12.8, H–C(6)); 3.75 (*m*, AB of ABX, H–C(3), H–C(4)); 2.06 (s, CH₃). ¹³C-NMR (50 MHz, (D₆)DMSO): 169.3 (s, CH₃CO); 158.2 (s, CONHPh); 151.8 (s, C(1)); 138.6 (s); 128.8 (*2d*); 122.9 (*d*); 118.7 (*2d*); 82.4 (*d*); 72.3 (*d*); 68.6 (*d*); 60 (*t*, C(6)); 51 (*d*, C(2)); 22.8 (*q*, CH₃). Anal. calc. for C₁₅H₁₉N₃O₇ (353.33): C 50.99, H 5.42, N 11.89; found: C 51.08, H 5.66, N 11.6.

Chitobiose (= 2-Acetamido-4-O-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-2-deoxy-D-glucopyranose; **8**). To a soln. (pH ~ 3) of a dried, crude hydrolysis product (2.84 g)¹⁾ of chitin in MeOH was added 2.3 g of NaOAc. Ac₂O (14 ml) was added dropwise over 30 min to the neutral mixture at r.t. The mixture was concentrated *i.v.* MPLC (300 g; A 63:25:12) of the residue afforded 1.6 g of a colorless syrup, which crystallized spontaneously *i.v.*

¹⁾ We thank Dr. H. Braunschweiger, Sandoz AG, Basel, for a generous gift of the crude hydrolysate.

Recrystallization in EtOH afforded an anal. pure sample, whose physical properties were identical with those of a commercial sample of chitobiose (*Sigma*, ref.-No. *D-1523*).

Chitobiose Oxime (9). A hot soln. of NaOEt, prepared from Na (27.6 mg, 1.2 mmol) in EtOH (6 ml), was added to a vigorously stirred soln. of powdered hydroxylammonium chloride (90.3 mg, 1.3 mmol) in EtOH (11 ml). The mixture was cooled in an ice-bath and filtered. Then, **8** (424 mg, 1 mmol) was added with stirring (pH ~ 7) at 50°. After disappearance of **8** (TLC, *A* 65:23:12), the mixture was concentrated *i. v.* Recrystallization of the residue (434 mg, 97%) in hot EtOH gave a sample of **9** for analysis. R_f (*A* 65:23:12) 0.24. $[\alpha]_D = -0.8$ ($c = 1.09$, H₂O, final value). IR (KBr): 3370 (br.), 3100 (sh), 2970m, 2930m, 2890 (br.), 1740s, 1550 (br.), 1420 (br.), 1374s, 1315s, 1230 (sh), 1150 (sh), 1100 (sh), 1077s, 1039 (br.), 940m, 875m. FAB-MS: 440 ($[M + 1]^+$).

Chitobionhydroximo-1,5-lactone (10). Activated MnO₂ [5] (800 mg) was added to a stirred soln. of **9** (980 mg, 2.2 mmol) in a mixture of KH₂PO₄ (1.03 g) and NaOH (0.18 g) in H₂O (60 ml; pH 7). The mixture was stirred at 40° for 5 h, until TLC (*A* 65:23:12) indicated the disappearance of **9**. The mixture was filtered through a pad of *Celite* and the filtrate was concentrated *i. v.* MPLC (80 g, *A* 65:23:12) of the residue gave a colorless syrup (673 mg, 69%), which crystallized spontaneously *i. v.* and was recrystallized in MeOH. R_f (*A* 7:2:1) 0.16. M.p. 244–246° (dec.). $[\alpha]_D = +40.4$ ($c = 1.04$, H₂O). IR (KBr): 3500 (sh), 3400 (br.), 3290 (sh), 3265s, 2920m, 2875m, 1660s, 1570m, 1550m, 1430m, 1400 (sh), 1372s, 1343m, 1307s, 1260m, 1250s, 1210m, 1160m, 1112s, 1090s, 1070s, 1050s, 1028s, 1010s, 995s, 940s, 875m, 752m. ¹³C-NMR (50.4 MHz, D₂O): 175.1 (s, CH₃CO); 174.8 (s, CH₃CO); 153.2 (s, C(1)); 101.7 (d, C(1')); 79.8 (d); 77.6 (d); 76.4 (d); 73.9 (d); 71.6 (d); 70.2 (d); 61.1 (t); 60.2 (t); 56.0 (d); 50.9 (d); 22.6 (q); 22.5 (q). FAB-MS: 438 ($[M + 1]^+$). Anal. calc. for C₁₆H₂₇N₃O₁₁ (437.402): C 43.94, H 6.22, N 9.61; found: C 44.17, H 6.16, N 9.37.

O-(Chitobiosylidene)amino N-Phenylcarbamate (14). Under cooling, Ac₂O (15 ml) was added to a stirred suspension of **10** (656 mg, 1.5 mmol) in pyridine (50 ml). The mixture was warmed to 40° and stirred for 3.5 h, until TLC showed the disappearance of **10**. The mixture was concentrated under high vacuum and the residue worked up as usual (CHCl₃/1M NaHCO₃). Addition of CH₂Cl₂, Et₂O, and hexane to the remaining syrup gave the *1-N-3,3',4',6,6'-hexa-O-acetylchitobionhydroximo-1,5-lactone (11)* as colorless crystals (858 mg, 83%). R_f (*B* 9:1) 0.41. M.p. 198–200° (dec.). $[\alpha]_D = -20.1$ ($c = 1.12$, CHCl₃). IR 3450 (sh), 3422w, 3300 (br.), 3030 (sh), 3000m, 2960 (sh), 1748s, 1680s, 1651m, 1648m, 1635 (sh), 1508s, 1425 (br.), 1368s, 1290 (sh), 1220 (br.), 1160 (sh), 1115 (br.), 1070 (sh), 1045s, 1000m. ¹³C-NMR (50.4 MHz, CDCl₃): 170.9, 170.9, 170.6, 170.5, 170.2, 169.5, 169.2, 168.1 (8s, CH₃CO); 156.7 (s, C(1)); 100.5 (d, C(1')); 78.1 (d); 73.0 (d); 72.1 (d); 71.9 (d); 70.6 (d); 68.2 (d); 62.3 (t); 61.7 (t); 54.8 (d); 47.7 (d); 23.2, 22.9, 20.7, 20.6, 20.6, 20.5, 19.3 (8q, CH₃CO).

To an ice-cold soln. of **11** (592 mg, 0.86 mmol) in MeOH (30 ml) was added dropwise over 50 min a MeNH₂ soln. (0.08M in EtOH, 10.7 ml). The mixture was immediately concentrated at r. t. and purified by FC (50 g, *B* 9:1): colorless crystals (239 mg, 43%) which were recrystallized in CH₂Cl₂/Et₂O to give *3,3',4',6,6'-penta-O-acetylchitobionhydroximo-1,5-lactone (12)*. R_f (*B* 9:1) 0.26. M.p. 192–193°. IR: 3660w, 3570m, 3425m, 3360 (br.), 3210 (sh), 3020 (sh), 3000m, 2970 (sh), 1742s, 1670s, 1510s, 1430m, 1370s, 1290m, 1220s, 1260 (br.), 1110 (sh), 1070 (sh), 1045 (sh), 1015 (sh), 945m.

Et₃N (112 μl) and phenyl isocyanate (305 mg) were added to a stirred soln. of **12** (260 mg, 0.40 mmol) in CH₂Cl₂ (10 ml) and THF (10 ml). After 2 min, TLC (*B* 9:1) showed complete reaction. The mixture was evaporated and purified by FC (35 g, *B* 95:5), yielding *O-(3',4',6,6'-penta-O-acetylchitobiosylidene)amino N-phenylcarbamate (13)* as colorless crystals (235 mg, 76%), which were recrystallized in CH₂Cl₂/Et₂O. R_f (*B* 9:1) 0.56. M.p. 129–131° (dec.). IR: 3680m, 3460m, 3380m, 3340 (br.), 3020 (sh), 3000m, 1750s, 1677s, 1600 (br.), 1520s, 1445s, 1370s, 1320 (sh), 1310m, 1295m, 1120m, 1075m, 1048s, 1015 (sh), 995m.

MeOH sat. with NH₃ (15 ml, ca. 4M) was added to an ice-cold soln. of **13** (198 mg, 0.26 mmol) in MeOH (30 ml). After 3 h, the mixture was taken to dryness. The remaining syrup was treated with MeOH (2 ml) to give 44 mg (31%) of crystals. Evaporation of the mother liquor and FC (9 g, *A* 78:15:7) gave another 21 mg (15%) of **14**. R_f (*A* 7:2:1) 0.34. M.p. 218–220°. $[\alpha]_D = +28.1$ ($c = 0.7$; H₂O). IR (KBr): 3460s, 3300s, 3080 (sh), 2915m, 2840m, 2825 (sh), 1755s, 1740s, 1715 (br.), 1655s, 1640s, 1601s, 1560 (sh), 1550 (br.), 1525s, 1445m, 1370m, 1320m, 1300m, 1255 (br.), 1210m, 1160 (br.), 1105m, 1072s, 1035 (sh), 1020m. ¹H-NMR (400 MHz, D₂O): 7.47–7.42 (m, 4 arom. H); 7.25 (1 arom. H); 4.80–4.66 (m, DHO); 4.25 (br., NH); 4.13–3.92 (m, 5 H); 3.85–3.73 (m, 4 H); 3.63–3.43 (m, 3 H); 2.12 (s, CH₃CO); 2.08 (s, CH₃CO). ¹³C-NMR (100.6 MHz, D₂O): 175.1, 174.9 (2s, CH₃CON); 158.5 (s); 154.9 (s); 129.8, 125.6, 121.5 (3d, 5 arom. C); 101.7 (d, C(1')); 81.1 (d); 79.9 (d); 77.2 (d); 76.5 (d); 73.9 (d); 71.1 (d); 61.1, 60.3 (2t, C(6), C(6')); 56.1 (d), 50.9 (d); 22.5, 22.55 (2q, CH₃CO). FAB-MS: 558 ($[M + 1]^+$). Anal. calc. for C₂₃H₃₂N₄O₁₂ (556.525): C 49.64, H 5.80, N 10.07; found: C 49.72, H 5.75, N 10.34.

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