175. Synthesis of 2-Acetamido-2-deoxy-D-gluconhydroximolactone- and Chitobionhydroximolactone-Derived *N*-Phenylcarbamates, Potential Inhibitors of *β*-*N*-Acetylglucosaminidase

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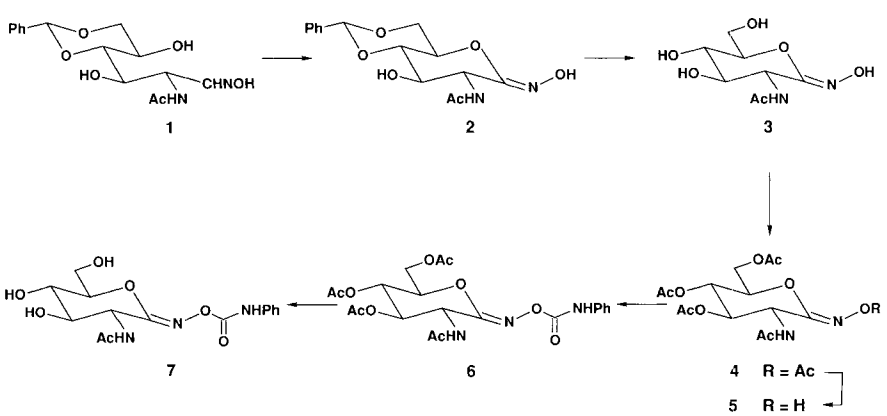
The *N*-phenylcarbamate **7**, derived from 2-acetamido-2-deoxy-D-gluconhydroximo-1,5-lactone (**3**) and the analogous *N*-phenylcarbamate **14**, derived from chitobionhydroximo-1,5-lactone (**10**) have been prepared as potential inhibitors of *β*-*N*-acetylglucosaminidases. The unambiguous synthesis of the hydroximo-1,5-lactone **3** involves oxidation of the oxime **1**, followed by deprotection with Na/NH_3.

Introduction and Plan. – We have described the synthesis of the *N*-phenylcarbamate derived from gluconhydroximo-1,5-lactone [1] and the strong inhibition of emulsin by this compound [2], which also inhibits phosphofructokinase b [3].

Consequently, the *N*-phenylcarbamate **7** (*Scheme 1*), derived from the 2-acetamido-2-deoxygluconhydroximolactone **4** [1] and its analogue **14** derived from chitobionhydroximolactone **10** (*cf. Scheme 2*) became of interest as potential inhibitors of *β*-*N*-acetylglucosaminidase (*EC 3.2.1.30*). The amorphous hydroximo-1,5-lactone **3** had been obtained by oxidation (MnO_2) of *N*-acetyl-D-glucosamine oxime, together with the isomeric, crystalline hydroximo-1,4-lactone [1]. Of these products, the latter is easily obtained pure, while isolation of the former is cumbersome and not easily reproduced.

Scheme 1

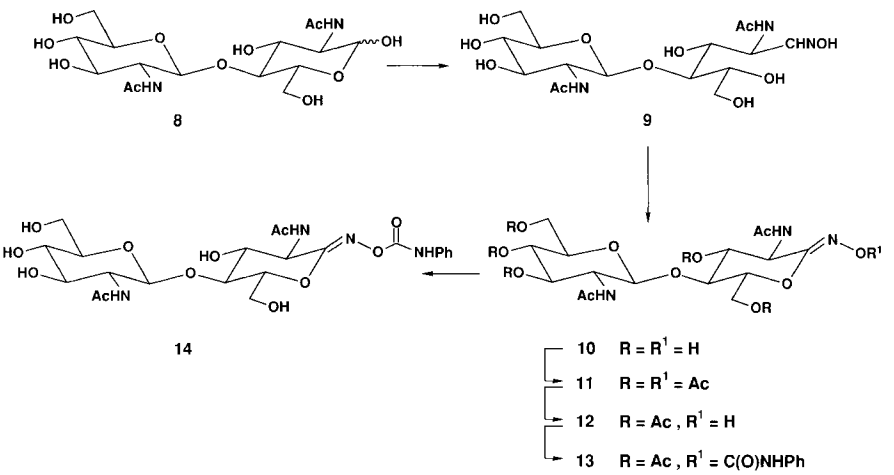


We now report on a more convenient, if somewhat longer procedure for the preparation of the hydroximolactone **3**, on the preparation of the *N*-phenylcarbamate **7** from **3** *via* the selectively protected tetraacetyl derivative **5** [1], and on the analogous preparation of the *N*-phenylcarbamate **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 debenzylation 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 Ac_2O/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; H_2O was distilled twice. Activated MnO_2 was prepared following [5]. TLC: *Merck* precoated silica gel *60* F_{254} plates; detection by spraying with a 0.025M I_2 soln. in 10% aq. H_2SO_4, 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/H_2O, *B* = $CHCl_3$/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% $CHCl_3$ 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 MnO_2 [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 (CH_2Cl_2/MeOH 10:1) afforded **1** (1.4 g, 35%) and **2** (1.98 g, 50%), which was crystallized from AcOEt/MeOH. R_f (CH_2Cl_2/MeOH 8:1) 0.4. M.p. 229–231° (dec.) $[\alpha]_D^{25} = +48.2$ ($c = 0.2$, MeOH). IR (KBr): 3360*s* (br.), 3095*w*, 3060*w*, 2870*w*, 1710*w*, 1660*s* (sh), 1645*s* (br.), 1565*m* (sh), 1550*m*, 1470*w*, 1450*m*, 1430*w*, 1390*m*, 1380*m*, 1330*w*, 1315*m*, 1250*m*, 1210*w*, 1150*w*, 1135*m*, 1120*m*, 1095*s*, 1045*m*, 1030*m*, 1000*m*, 970*m*, 945*m*, 925*w*, 895*w*, 875*w*, 790*w*, 760*m*, 750*w*, 700*m*. ^{1}H-NMR (400 MHz, (D_6) 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), PhC*H*); 4.36–4.34 (*m*, 2 H); 3.85–3.7 (*m*, 4 H); 1.85 (*s*, CH_3). ^{13}C-NMR (50 MHz, (D_6)DMSO): 169.1 (*s*, CH_3CO); 150.25 (*s*, C(1)); 137.6 (*s*, arom. C); 129.2 (*d*, arom. C); 128.3 (2*d*, arom. C); 126.5 (2*d*, 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*, CH_3). CI-MS (NH_3): 340 (54, $[M + 1 + NH_3]^+$), 323.3 (25, $[M + 1]^+$). Anal. calc. for $C_{15}H_{18}N_2O_6$ (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 NH_3 (45 ml), until the blue color persisted for more than 1 min, and TLC showed the disappearance of **2**. Evaporation of NH_3 left a residue which was redissolved in MeOH and purified by FC (neutral alumina, AcOEt/MeOH/H_2O 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 μl, 2.64 mmol) and Et_3N (950 μl, 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 ($CHCl_3$): 3424*w*, 3380*w*, 3320*w* (br.), 3030*w*, 3000*w*, 2960*w*, 1750*s* (br.), 1680*m*, 1395*m* (sh), 1370*m*, 1320*w* (sh), 1310*m*, 1210*m*, 1030*m*, 1020*m*, 990*w*, 895*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.82 (*s*, PhN*H*); 7.45–7.28 (*m*, 4 arom. H); 7.14 (*d*, $J = 7.8$, AcN*H*); 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*, CH_3); 2.05 (*s*, CH_3); 2.04 (*s*, CH_3); 2.0 (*s*, CH_3). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.5 (*s*, CO); 170.3 (*s*, CO); 170.2 (*s*, CO); 169.1 (*s*, CO); 155.2 (*s*, *C*ONHPh); 151.8 (*s*, C(1)); 136.8 (*s*, arom. C); 129.2 (2*d*); 124.3 (*d*); 119.2 (2*d*); 77.2 (*d*); 71.3 (*d*); 67.1 (*d*); 61.2 (*t*, C(6)); 49.6 (*d*, C(2)); 22.8 (*q*, CH_3); 20.62 (*q*, CH_3); 20.57 (*q*, CH_3); 20.5 (*q*, CH_3).

Sat. NH_3/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): 3470*m* (sh), 3420*s*, 3310*s*, 3060*w*, 2950*w*, 2930*w*, 2900*w*, 2880*w*, 1745*s*, 1705*m*, 1660*m*, 1645*s*, 1605*m*, 1600*m* (sh), 1553*m*, 1530*s*, 1495*w*, 1450*m*, 1415*w*, 1380*m*, 1330*w*, 1310*m*, 1290*w* (sh), 1260*w*, 1205*m*, 1295*m*, 1160*w*, 1130*w*, 1005*m*, 1080*m*, 1070*m*, 1040*m*, 1020*m*, 1000*w*, 960*w*, 930*w*, 900*w*, 880*w*, 860*w*, 770*w*, 750*m*, 690*m*, 640*m*, 620*m*. ^{1}H-NMR (400 MHz, CD_3OD): 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*, CH_3). ^{13}C-NMR (50 MHz, (D_6)DMSO): 169.3 (*s*, CH_3CO); 158.2 (*s*, *C*ONHPh); 151.8 (*s*, C(1)); 138.6 (*s*); 128.8 (2*d*); 122.9 (*d*); 118.7 (2*d*); 82.4 (*d*); 72.3 (*d*); 68.6 (*d*); 60 (*t*, C(6)); 51 (*d*, C(2)); 22.8 (*q*, CH_3). Anal. calc. for $C_{15}H_{19}N_3O_7$ (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. Ac_2O (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. $[\alpha]_D = -0.8$ ($c = 1.09$, H_2O, final value). IR (KBr): 3370 (br.), 3100 (sh), 2970*m*, 2930*m*, 2890 (br.), 1740*s*, 1550 (br.), 1420 (br.), 1374*s*, 1315*s*, 1230 (sh), 1150 (sh), 1100 (sh), 1077*s*, 1039 (br.), 940*m*, 875*m*. FAB-MS: 440 ($[M + 1]^+$).

Chitobionhydroximo-1,5-lactone (**10**). Activated MnO_2 [5] (800 mg) was added to a stirred soln. of **9** (980 mg, 2.2 mmol) in a mixture of KH_2PO_4 (1.03 g) and NaOH (0.18 g) in H_2O (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.). $[\alpha]_D = +40.4$ ($c = 1.04$, H_2O). IR (KBr): 3500 (sh), 3400 (br.), 3290 (sh), 3265*s*, 2920*m*, 2875*m*, 1660*s*, 1570*m*, 1550*m*, 1430*m*, 1400 (sh), 1372*s*, 1343*m*, 1307*s*, 1260*m*, 1250*s*, 1210*m*, 1160*m*, 1112*s*, 1090*s*, 1070*s*, 1050*s*, 1028*s*, 1010*s*, 995*s*, 940*s*, 875*m*, 752*m*. ^{13}C-NMR (50.4 MHz, D_2O): 175.1 (*s*, CH_3CO); 174.8 (*s*, CH_3CO); 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 $C_{16}H_{27}N_3O_{11}$ (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, Ac_2O (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 ($CHCl_3$/1M $NaHCO_3$). Addition of CH_2Cl_2, Et_2O, 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.). $[\alpha]_D = -20.1$ ($c = 1.12$, $CHCl_3$). IR 3450 (sh), 3422*w*, 3300 (br.), 3030 (sh), 3000*m*, 2960 (sh), 1748*s*, 1680*s*, 1651*m*, 1648*m*, 1635 (sh), 1508*s*, 1425 (br.), 1368*s*, 1290 (sh), 1220 (br.), 1160 (sh), 1115 (br.), 1070 (sh), 1045*s*, 1000*m*. ^{13}C-NMR (50.4 MHz, $CDCl_3$): 170.9, 170.9, 170.6, 170.5, 170.2, 169.5, 169.2, 168.1 (8*s*, CH_3CO); 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 (8*q*, CH_3CO).

To an ice-cold soln. of **11** (592 mg, 0.86 mmol) in MeOH (30 ml) was added dropwise over 50 min a $MeNH_2$ 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 CH_2Cl_2/Et_2O 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: 3660*w*, 3570*m*, 3425*m*, 3360 (br.), 3210 (sh), 3020 (sh), 3000*m*, 2970 (sh), 1742*s*, 1670*s*, 1510*s*, 1430*m*, 1370*s*, 1290*m*, 1220*s*, 1260 (br.), 1110 (sh), 1070 (sh), 1045 (sh), 1015 (sh), 945*m*.

Et_3N (112 µl) and phenyl isocyanate (305 mg) were added to a stirred soln. of **12** (260 mg, 0.40 mmol) in CH_2Cl_2 (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 CH_2Cl_2/EtO. R_f (*B* 9:1) 0.56. M.p. 129–131° (dec.). IR: 3680*m*, 3460*m*, 3380*m*, 3340 (br.), 3020 (sh), 3000*m*, 1750*s*, 1677*s*, 1600 (br.), 1520*s*, 1445*s*, 1370*s*, 1320 (sh), 1310*m*, 1295*m*, 1120*m*, 1075*m*, 1048*s*, 1015 (sh), 995*m*.

MeOH sat. with NH_3 (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°. $[\alpha]_D = +28.1$ ($c = 0.7$; H_2O). IR (KBr): 3460*s*, 3300*s*, 3080 (sh), 2915*m*, 2840*m*, 2825 (sh), 1755*s*, 1740*s*, 1715 (br.), 1655*s*, 1640*s*, 1601*s*, 1560 (sh), 1550 (br.), 1525*s*, 1445*m*, 1370*m*, 1320*m*, 1300*m*, 1255 (br.), 1210*m*, 1160 (br.), 1105*m*, 1072*s*, 1035 (sh), 1020*m*. ^{1}H-NMR (400 MHz, D_2O): 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*, CH_3CO); 2.08 (*s*, CH_3CO). ^{13}C-NMR (100.6 MHz, D_2O): 175.1, 174.9 (2*s*, CH_3CON); 158.5 (*s*); 154.9 (*s*); 129.8, 125.6, 121.5 (3*d*, 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 (2*t*, C(6), C(6′)); 56.1 (*d*), 50.9 (*d*); 22.5, 22.55 (2*q*, CH_3CO). FAB-MS: 558 ($[M + 1]^+$). Anal. calc. for $C_{23}H_{32}N_4O_{12}$ (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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