LITERATURE CITED

1. M. Ya. Myagi, E. T. Lippmaa, S. L. Ioffe, V. A. Tartakovskii, A. S. Shashkov, B. N. Khasapov, and L. M. Makarenkova, Izv. Akad. Nauk SSSR, Ser. Khim., 463 (1974).
2. S. L. Ioffe, L. M. Makarenkova, and V. A. Tartakovskii, Izv. Akad. Nauk SSSR, Ser. Khim., 463 (1974).
3. E. J. Corey and A. Venkateswarlu, J. Am. Chem. Soc., 94, 6190 (1972).
4. B. Unterhalt and D. Thamer, Archiv. Pharm., 307, 731 (1974).
5. G. N. R. Smart and G. F. Wright, Can. J. Res. (B) 26, 292 (1948).

PREPARATION OF MODIFIED SUBSTRATES OF N-ACETYL-β-D-GLUCOSAMINIDASE

V. V. Kolesnikov, M. L. Shul'man, and A. Ya. Khorlin

UDC 542.91:547.455

This paper describes the synthesis of phenyl- and p-nitrophenyl-N-acetyl-β-D-glucosaminides. We used these compounds previously to study the active centers of N-acetyl-β-D-glucosaminidase B_1'' obtained from epididymis of sharks [1]. The overall synthesis of the title compounds is outlined in schemes 1 and 2. The reaction of benzyl-2-acetamido-2-desoxy-α-D-glucopyranoside (I) with tosyl chloride, followed by an exchange of the tosyl group in (II) for iodine gives benzyl-2-acetamido-2,6-didesoxy-6-iodo-α-D-glucopyranoside (III). It was found that hydrogenation of (III) using Pd in the presence of CH_3COONa leads to the removal of iodine but the benzyl group at 1-C is retained. The corresponding benzyl glycoside (IV) is obtained in nearly 100% yield. The benzyl group can be removed by hydrogenation using fresh Pd-catalyst and, as a result, 2-acetamido-2,6-didesoxy-D-glucopyranose (V) is obtained. Similarly, benzyl-2-acetamido-3,6-di-O-acetyl-2-desoxy-α-D-glucopyranoside (VI) gives 2-acetamido-2,4-didesoxy-D-xylohexopyranose (IX).

It is known that 3-chlorodesoxy derivatives cannot be obtained by reacting SO_2Cl_2 with alkyl-4,6-benzylidine-α-glucosides due to 1,3-diaxial interaction between the axial substituent at 1-C and the chloride that attacks 3-C in the axial direction [2, 3]. For this reason, 3-desoxy derivatives (XIV) are prepared from the benzylidine derivative of phenyl-N-acetyl-β-D-glucopyranoside that reacts with SO_2Cl_2 [3] to give the corresponding 3-chloro derivative (XI). The subsequent removal of the benzylidine blocking group with CH_3COOH, followed by dehydrochlorination with $i\text{-}C_3H_7ONa$ gives (XIII). The NMR and IR spectra confirmed that (XIII) is phenyl-2-acetamido-2-desoxy-β-D-erythrohex-2-enopyranoside. Hydrogenation of (XIII) gives phenyl-2-acetamido-2,3-didesoxy-β-D-ribohexopyranoside (XIV).

The reaction of SO_2Cl_2 with benzyl-2-acetamido-3,4-di-O-acetyl-2-desoxy-α-D-glucopyranoside (XV), followed by treatment with CH_3ONa and by subsequent removal of the benzyl blocking group in (XVI) gives 2-acetamido-2,6-didesoxy-6-chloro-D-glucopyranose (XVII) in a good yield.

The nitrophenyl analogues (XXI)-(XXVI) of N-acetyl-β-D-glucopyranoside are obtained by reacting corresponding chlorides with sodium nitrophenolate in DMF [4] (see the table). The partially methylated N-acetyl-β-D-glucosaminides (XXIX) and (XXX) are obtained by reacting diazomethane with the compounds (XXVII) and (XXVIII), respectively.

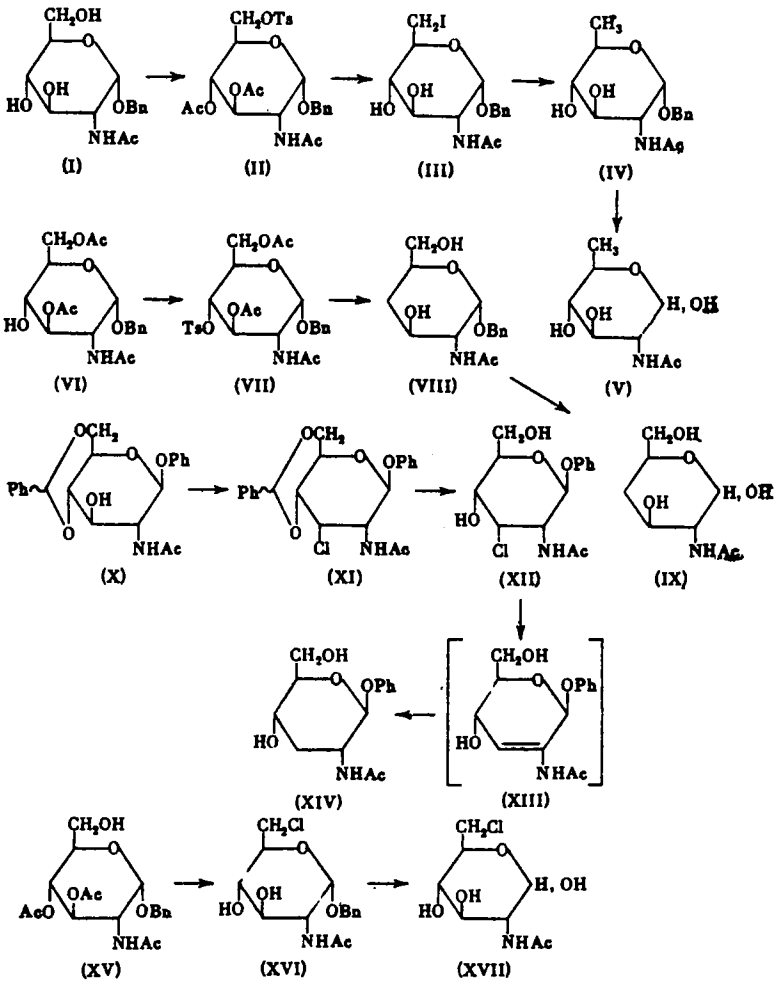
EXPERIMENTAL

The solvents were removed under vacuum at 45°C. TLC analyses (silica gel Chemapol CSSR, 5-40 μ, 10% gypsum) were carried out using $CHCl_3$ — MeOH, 50:1 (A), 9:1 (B), or ether — MeOH, 98:2 (C) solvent

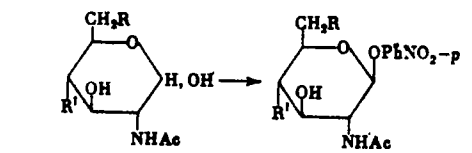
M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow.

Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 10, pp. 2331-2336, October, 1976. Original article submitted September 3, 1975.

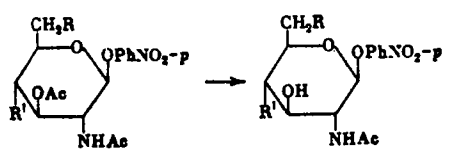
Scheme 1



Scheme 2



R = H, R′ = OH (V); R = H, R′ = OH (XXI);
R = OH, R′ = H (IX); R = OH, R′ = H (XXII);
R = Cl, R′ = OH (XVII); R = Cl, R′ = OH (XXIII);
R = Br, R′ = OH (XVIII); R = Br, R′ = OH (XXIV);
R = F, R′ = OH (XIX); R = F, R′ = OH (XXV);
R = R′ = OH (XX) R = R′ = OH (XXVI)



R = OH, R′ = OAc (XXVII); R = OMe, R′ = OH (XXIX);
R = OAc, R′ = OH (XXVIII) R = OH, R′ = OMe (XXX)

systems. The separated compounds were identified by heating with conc. H_2SO_4. The preparative chromatography was carried out using silica gel (Chemapol, CSSR) 100-250 μ. The melting points (corrected) were determined using Boetius apparatus and the values of optical rotation were determined on a Perkin–Elmer 14 IM

TABLE 1. Physical Constants of p-Nitrophenyl- Desoxy and Halodesoxy Derivatives of N-Acetyl-β-D-glucosaminide

Compound	mp, °C	$[\alpha]_D^{20-23}$ (C 0,4—0,7, MeOH)	Empirical formula	Found, %				Calculated, %			
				C	H	Hal	N	C	H	Hal	N
p-Nitrophenyl-2-acetamido-2,6-β-D-glucopyranoside (XXI)	214—215 (decomp.)	—13	—	—	—	—	—	—	—	—	—
p-Nitrophenyl-2-acetamido-2,4-didesoxy-β-xylohexopyranoside (XXII)	179—180 (decomp.)	—11	$C_{14}H_{18}O_7O_2$	51,42	5,24	—	8,45	51,53	5,52	—	8,59
p-Nitrophenyl-2-acetamido-2,6-didesoxy-6-chloro-β-D-glucopyranoside (XXII)	218—219 (decomp.)	—25	$C_{14}H_{17}ClO_7N_2$	46,55	4,61	9,79	7,65	46,60	4,72	9,85	7,77
p-Nitrophenyl-2-acetamido-6-bromo-2,6-didesoxy-β-D-glucopyranoside (XXIV)	198—199 (decomp.)	—50	$C_{14}H_{17}BrO_7N_2$	41,25	4,01	19,54	6,99	41,38	4,19	19,95	6,90
p-Nitrophenyl-2-acetamido-2,6-didesoxy-6-fluoro-β-D-glucopyranoside (XXV)	215—216 (decomp.)	—13,9	$C_{14}H_{17}FO_7N_2$	49,28	4,75	4,60	8,28	49,12	4,97	4,97	8,19

polarimeter. IR spectra were recorded on a UR-20 spectrometer. NMR spectra were measured in CD_3CN on a Varian XL-100 spectrometer at 100 MHz using TMS as an internal standard.

Benzyl-2-acetamido-3,4-di-O-acetyl-2-didesoxy-6-O-tosyl-α-D-glucopyranoside (II). Compound (I) (3 g) [5] and tosyl chloride (2 g) were dissolved in abs. pyridine (50 ml) and the mixture was left standing overnight at 5°C. $(CH_3CO_3)_2O$ (10 ml) was added and after 3-4 h the mixture was poured onto ice (100 g). The aqueous layer was extracted with $CHCl_3$ (3 × 50 ml), the chloroform extract was washed with water, 10% $NaHCO_3$, and water and then dried over $MgSO_4$. $CHCl_3$ was evaporated and the residue was crystallized from MeOH — ether mixture to give 3.5 g (55%) of (II), mp 125-127°C, $[\alpha]_{D20}$ + 69° (C 0.8 $CHCl_3$). Found: C 56.61; H 5.49; N 2.49; S 5.71%. $C_{26}H_{31}O_{10}NS$. Calculated: C 56.83; H 5.65; N 2.55; S 5.83.

Benzyl-2-acetamido-2,6-didesoxy-6-iodo-α-D-glucopyranoside (III). A mixture of (II) (3.4 g) and KI (2.5 g) in DMF was refluxed for 30 min (the progress of the reaction checked by TLC, system A), then cooled to about 20°C and poured into water; the aqueous layer was extracted with C_6H_6 — $CHCl_3$ 1:1 mixture (3 × 50 ml), the organic layer was successively washed with water, 2% $Na_2S_2O_3$, and water, dried over $MgSO_4$ and evaporated. The residue was treated with 0.01 N MeONa in abs. MeOH to give 1.6 g (62%) of (III), mp 196-197°C (from MeOH — ether, with decomposition), $[\alpha]_{D22}$ + 14.6° (C 0.7 MeOH). Found: C 42.58; H 4.86; I 29.88; N 3.29%. $C_{15}H_{20}IO_5N$. Calculated: C 42.76; H 4.75; I 30.17; N 3.32%.

Benzyl-2-acetamido-2,6-didesoxy-α-D-glucopyranoside (IV). Compound (III) (1.5 g) was hydrogenated in MeOH (10 ml) in the presence of 20% Pd/C (1.5 g) and $AcONa \cdot 3H_2O$ (4.5 g) for 16-20 h. The catalyst was then filtered off, the filtrate was evaporated to dryness and the residue, was chromatographed on silica gel using the system C (80 ml) to give (IV) in 90% yield (0.9 g), mp 200-201°C (from alcohol — ether), $[\alpha]_{D23}$ + 18.9° (C 1.6, MeOH). Found: C 61.20; H 7.08; N 4.80%. $C_{15}H_{21}O_5N$. Calculated: C 61.02; H 7.12; N 4.75%.

2-Acetamido-2,6-didesoxy-D-glucopyranose (V). A quantity (0.86 g) of (IV) was hydrogenated in MeOH (10 ml) in the presence of fresh 20% Pd/C (1.5 g) for 16 h to give 0.6 g of (V) (73%), mp 209-210°C (from alcohol), $[\alpha]_{D20}$ + 26° (C 0.8, H_2O); (cf. [3]).

Benzyl-2-acetamido-3,6-di-O-acetyl-2-desoxy-4-O-tosyl-α-D-glucopyranoside (VII). A solution of (VI) (9.5 g) [6] and tosyl chloride (24 g) in pyridine (50 ml) was kept for 3 days at about 20°C; the solvent was then evaporated, the residue was extracted with $CHCl_3$, the chloroform layer was washed with water, 10% $NaHCO_3$, water and then dried over $MgSO_4$ before evaporating to dryness. The yield of (VII) was 11 g (83%), mp 114-115°C (from ether), $[\alpha]_{D23}$ + 56° (C 1.0, $CHCl_3$). Found: C 56.50; H 5.55; N 2.35; S 5.75%. $C_{26}H_{31}O_{10}NS$. Calculated: C 56.83; H 5.65; N 2.55; S 5.83%.

Benzyl-2-acetamido-2,4-didesoxy-α-D-xylohexopyranoside (VIII). A solution of (VII) (10.9 g) and KI (9 g) in DMF (50 ml) was refluxed for 30 min and the reaction mixture was worked up as for (III). The product was deacetylated with 0.01 N MeONa in abs. MeOH; the resulting solution was then neutralized using the H^+ form of KU-2 cationic-exchange resin, and then hydrogenated on 20%-Pd/C (3.0 g) in the presence of $AcONa \cdot 3H_2O$ (4 g) for 16-20 h to give 3.2 g (54%) of (VIII), mp 168-169°C; (cf. [3]).

2-Acetamido-2,4-didesoxy-D-xylohexopyranose (IX). A quantity (3.1 g) of (VIII) were hydrogenated in the presence of a freshly prepared Pd/C (2.5 g) as for (IV). A quantity (1.8 g) (84%) of a viscous liquid (IX) were obtained, $[\alpha]_{D20}$ + 80° (C 0.6, H_2O); (cf. [3]).

Phenyl-2-acetamido-4,6-O-benzylidine-2,3-didesoxy-3-chloro-β-D-allopyranoside (XI). This compound was prepared according to [3] in 42% yield, mp 183-184°C (from CH_3CN, with decomposition), $[\alpha]_{D20}$ — 25° (C 0.6, $CHCl_3$).

Phenyl-2-acetamido-2,3-didesoxy-3-chloro-β-D-allopyranoside (XII). A quantity (3 g) of (XI) in 80% AcOH (70 ml) were refluxed for 10-15 min (checked by TLC, system B), the solution was then evaporated to dryness and the residue was crystallized from an alcohol — ether mixture. Further recrystallization from alcohol gave 1 g (43%) of (XII), mp 163-164°C, $[\alpha]_D^{20}$ — 56° (C 0.7, MeOH). Found: C 53.09; H 5.79; Cl, 11.00; N 4.26%. $C_{14}H_{18}ClO_5N$. Calculated: C 53.25; H 5.71; Cl 11.25; N 4.44%.

Phenyl-2-acetamido-2,3-didesoxy-β-D-ribohexopyranoside (XIV). To a mixture of Na (0.2 g) in $i\text{-}C_3H_7OH$ (5 ml) was added 0.4 g of (XII) at 55-60°C. The mixture was cooled, the resulting solid was dissolved in MeOH (5 ml) and acidified with 1% AcOH to pH 7-8. The volatile components were evaporated under vacuum, the residue was dissolved in the solvent system B and then passed through charcoal and silica gel. The filtrate was evaporated to give 0.13 g (28%) of phenyl-2-acetamido-2-desoxy-β-D-erythrohex-2-enopyranoside (XIII) as a viscous liquid. NMR spectrum (δ, ppm): 6.52 d(H^3), J = 4 Hz; 5.85 s(H^1); 4.20 t(H^4). IR spectrum (ν, cm^{-1}): 1665 and 1550 (amide I and II). 0.1 g of (XIII) was hydrogenated with 1 g of 20% Pd/C for 3

days. The catalyst was filtered off, the filtrate was evaporated and chromatographed on silica gel using the solvent system B. 0.04 g (40%) of (XIV) was obtained, mp 176-177°C (from alcohol), $[\alpha]_{D22}$ −32°, (C 0.4 MeOH); (cf. [3]).

Benzyl-2-acetamido-2,5-didesoxy-6-chloro-α-D-glucopyranoside (XVI). To a solution of (XV) (1.7 g) [5] in pyridine (10 ml) at −40°C was added SO_2Cl_2 (0.6 ml) and the mixture was kept at −20°C overnight. Pyridine was evaporated under vacuum, the residue was extracted with $CHCl_3$, the chloroform layer was washed with water, 10% $NaHCO_3$, water and then dried over $MgSO_4$. $CHCl_3$ was evaporated, the residue was deacetylated to give 1 g (71%) of (XVI), mp 189-190°C (from alcohol), $[\alpha]_{D20}$ + 175° (C 0.5, MeOH); (cf. [6]).

2-Acetamido-2,6-didesoxy-6-chloro-D-glucopyranose (XVII). A quantity (0.95 g) of (XVI) in MeOH (10 ml) was hydrogenated in the presence of 2.0 g of freshly prepared 20% Pd/C for 16-20 h to give 0.55 g (80%) of (XVII), mp 169-170°C (from alcohol), $[\alpha]_{D20}$ + 75° (C 0.9, H_2O, 7 min); (cf. [6]).

Preparation of p-Nitrophenyl Derivatives of N-Acetyl-β-D-glucoseaminide (XXI)-(XXVI). A suspension of 0.5-1 g of carefully dried N-acetyl-glucosamine in 10 ml of AcCl was saturated with dry HCl at −10°C for 30-40 min. The mixture was kept in a sealed vessel at about 20°C overnight, AcCl was then removed by an azeotropic distillation with abs. C_6H_6 under vacuum and the residue was dried at 1 mm for 2 h [4]. The residue was then dissolved in DMF (5-15 ml), 1.5 mole excess of sodium p-nitrophenolate was added and the mixture was kept at 5-10°C overnight. The reaction mixture was then poured onto an ice — water mixture and precipitated solid was filtered off, washed with water and dried. The product was deacetylated with 0.01 N MeONa in abs. MeOH and recrystallized from alcohol to give the corresponding nitrophenyl derivative of N-acetyl-β-D-glucoseaminide (see Table 1).

p-Nitrophenyl-2-acetamido-3,4-di-O-acetyl-2-desoxy-β-D-glucopyranoside (XXVII). A solution of p-nitrophenyl-N-acetyl-β-D-glucopyranoside (XXVI) [4] (6.7 g) in pyridine (50 ml) was reacted with Ph_3CCl (8.4 g) at 100°C until all (XXVI) disappeared from the reaction mixture (5-10 min, checked by TLC using the system C). The mixture was cooled, Ac_2O (10 ml) was added and the mixture was left standing overnight. The trityl blocking group was removed with 80% AcOH (50 ml) using the method [5]; 3.5 g (41%) of (XXVII) were obtained, mp 210-211°C (from alcohol), $[\alpha]_{D22}$ − 97° (C 0.4 MeOH). Found: C 50.68; H 5.15; N 6.82%. $C_{18}H_{22}O_{15}N_2$. Calculated: C 50.70; H 5.16; N 6.57%.

p-Nitrophenyl-2-acetamido-3,6-di-O-acetyl-2-desoxy-β-D-glucopyranoside (XXVIII). To a solution of (XXVI) (6.8 g) in abs. pyridine (25 ml) at −40°C was added, with efficient stirring, a solution of AcCl (3.1 ml) in abs. toluene (10 ml) during 30 min [7]. The mixture was then kept with stirring at the same temperature for another 1.5 h and for a further 1 h at ≈20°C. The resulting mixture was filtered, the solid was washed with toluene, the filtrate was evaporated and the residue was chromatographed on silica gel and eluted gradually with petroleum ether, ether, and the system C. A quantity (2.5 g) (30%) of (XXVIII) were obtained, mp 190-192°C (from alcohol, with decomposition), $[\alpha]_{D22}$ − 37° (C 0.9, MeOH). Found: C 50.75; H 5.03; N 6.39%. $C_{18}H_{22}O_{10}N_2$. Calculated: C 50.70; H 5.16; N 6.57%.

p-Nitrophenyl-2-acetamido-2-desoxy-6-O-methyl-β-D-glucopyranoside (XXIX). This compound was prepared from (XXVII) by methylation with diazomethane in the presence of BF_3 ester and CH_3CN according to [8], followed by deacetylation with MeONa in MeOH. The yield of (XXIX) was 30-40%, mp 182-183°C (from alcohol, with decomposition), $[\alpha]_{D22}$ − 36° (C 0.4 MeOH). Found: C 50.20; H 5.40; N 7.41%. $C_{15}H_{20}O_8N_2$. Calculated: C 50.56; H 5.62; N 7.87%.

p-Nitrophenyl-2-acetamido-2-desoxy-4-O-methyl-β-D-glucopyranoside (XXX). This compound was obtained from (XXVIII) using the same method as for (XXIX), mp 211-212°C (from alcohol), $[\alpha]_{D22}$ − 14° (C 0.4, MeOH). Found: C 50.33; H 5.57; N 7.70%. $C_{15}H_{20}O_8N_2$. Calculated: C 50.56; H 5.62; N 7.87%.

CONCLUSIONS

A number of desoxy derivatives of N-acetylglucosamine were prepared. These compounds serve as modified substrates for studying the catalytic properties of the active center in N-acetyl-β-D-glucosamidase.

LITERATURE CITED

1. V. V. Kolesnikov, A. Ya. Khorlin, and M. L. Shulman, Bioorg. Khim., 2, 82 (1976).
2. H. J. Jennings and J. K. N. Jones, Can. J. Chem., 43, 2372 (1965).
3. H. Arita, K. Fukukawa, and Y. Matsushima, Bull. Chem. Soc. Jap., 45, 3614 (1972).
4. S. E. Zurabyan, T. P. Volosyuk, and A. Ya. Khorlin, Izv. Akad. Nauk SSSR, Ser. Khim., 1612 (1968).

5. M. L. Shul'man, G. V. Abramova, V. N. Piskaeva, and A. Ya. Khorlin, Izv. Akad. Nauk SSSR, Ser. Khim., 630 (1971).
6. M. L. Shul'man, V. N. Eldikov, and A. Ya. Khorlin, Izv. Akad. Nauk SSSR, Ser. Khim., 412 (1973).
7. S. E. Zurabyan, E. N. Lopantseva, and A. Ya. Khorlin, Dokl. Akad. Nauk SSSR, 210, 1216 (1973).
8. I. O. Mastronardi, S. M. Flematti, J. O. Deferrari, and E. G. Gros, Carbohyd. Res., 3, 177 (1966).