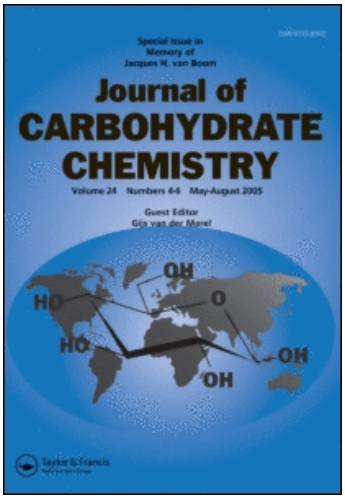

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An Exceptionally Simple Chemical Synthesis of *O*-Glycosylated d-Glucosamine Derivatives by Heyns Rearrangement of the Corresponding *O*-Glycosyl Fructoses

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An Exceptionally Simple Chemical Synthesis of *O*-Glycosylated D-Glucosamine Derivatives by Heyns Rearrangement of the Corresponding *O*-Glycosyl Fructoses[†]

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ABSTRACT

2-*N*-Acetyl-4-*O*-(β-D-galactopyranosyl)-D-glucosamine (*N*-acetyl-D-lactosamine), a very important building block of biologically relevant oligosaccharides such as sialyl Lewis^x, is easily accessible via the Heyns rearrangement of the corresponding *O*-glycosylated ketohexose, D-lactulose. This approach can also be extended to other glucosamine derivatives employing suitable *O*-glycosylated ketoses many of which are commercially available. For example, nigerosamine (3-*O*-α-D-glucopyranosyl-D-glucosamine) was prepared from turanose (3-*O*-α-D-glucopyranosyl-D-fructose). In combination with a recently introduced vinylogous amide type *N*-protecting group, [1,3-dimethyl-2, 4, 6 (1H, 3H, 5H)-trioxopyrimidine-5-ylidene] methyl (DTPM), this access is clearly superior to other routes and eminently suitable for scaling up.

[†]This paper is dedicated to Professor Dr. Kurt Heyns.

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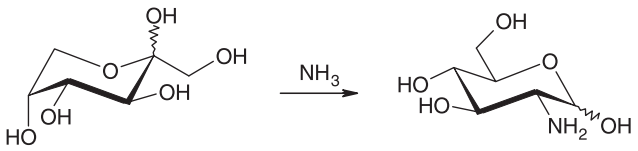
INTRODUCTION

The Heyns rearrangement, wherein ketoses react with suitable amines to form ketosylamines which subsequently isomerise to the corresponding 2-amino-2-deoxy-aldoses, was first discovered by Fischer[1,2] during his studies on the osazone formation of sugars and later further investigated by Heyns and Koch in the 1950s when they found that D-glucosamine **2** was formed in the reaction of D-fructose **1** with ammonia[3] (Scheme 1).

Subsequently, this reaction was extended by Carson[4–6] as well as the Heyns group[7] to a wide range of different primary and secondary amines[8] yielding the corresponding *N*-substituted glucosamine derivatives. In addition, amino acids[9–12] and other ketoses[13–16] have been employed in this reaction. Generally, despite many efforts,[17] yields rarely exceeded 20% because this reaction suffers from a variety of problems such as competition between hydrolysis and rearrangement of the initial condensation product, epimer formation at position *C*-2 of the rearrangement product, separation problems, Amadori rearrangement as a side reaction, disubstitution when employing primary amines and chemical instability of some products.

Because of overestimation of these difficulties there has been practically no synthetic application of this rearrangement. In context with a demand for multigram quantities of *N*-acetyl-D-lactosamine **3**, which was first discovered by Freudenberg when he investigated blood substances,[18,19] we became interested in this reaction. Compound **3** is a constituent of various glycoproteins and glycolipids and consequently plays a very important role in many biochemical processes.[20,21] Amongst others, it is, for example, the key intermediate in most syntheses of Lewis^X[22–25] **4** and sulfo-Le^x, which have been identified as ligands of selectins, carbohydrate recognising receptors on the surface of endothelial cells involved in cell adhesion phenomena[26–29] (Figure 1).

Not surprisingly, synthetic approaches to **3** have been investigated for many years and it has remained an eminently interesting target for synthetic chemists. Consequently, many different approaches have been reported, either by multistep chemical syntheses,[30–35] with the inherent need for protecting groups, or by enzymatic methods.[36–38] In addition, solid-phase approaches were reported.[23] The best chemical syntheses, concerning yields and scale-up potential starting from lactose and allowing access to **3** or derivatives thereof, require seven to nine steps with overall yields ranging from 10 to, at most, 30%.[39–44] Enzymatic approaches have employed D-galactosidases from various sources[36–38,45–51] or D-galactosyl transferases[52–55] as components of multi-enzyme systems which require co-factor recycling. Thus far, they have been limited to relatively small scale, and require know-how which is not generally available in synthetically oriented laboratories.



1 **2**

Scheme 1. The Heyns rearrangement of D-Fructose.

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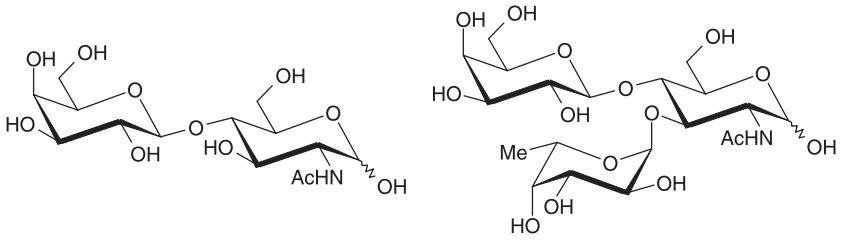


3 4

Figure 1. NAHc-Lactosamine (3) and Lewis^X (4).

Following the Heyns rearrangement protocol, we have recently communicated a short and efficient synthesis of selected *N*-substituted D-lactosamine derivatives starting from D-lactulose.[56] Due to limited space, this contribution could not address the general applicability to other suitable *O*-glycosylketoses, nor was it possible to include some experimental details.

Now we would like to exemplify the tremendous practicability of the Heyns rearrangement reporting two examples of wider interest, the syntheses of new *N*-protected derivatives of D-lactosamine such as **9** and D-nigerosamine **16** [3-*O*-(α-D-glucopyranosyl)-D-glucosamine], starting from the corresponding commercially available glycosylated ketoses, lactulose **5** and turanose (3-*O*-α-D-glucopyranosyl-D-fructose) **11**, respectively. The successful Heyns rearrangement of turanose is particularly interesting due to the fact that β-elimination of the substituent at *C*-3 is a feasible side-reaction potentially limiting the scope of this approach.

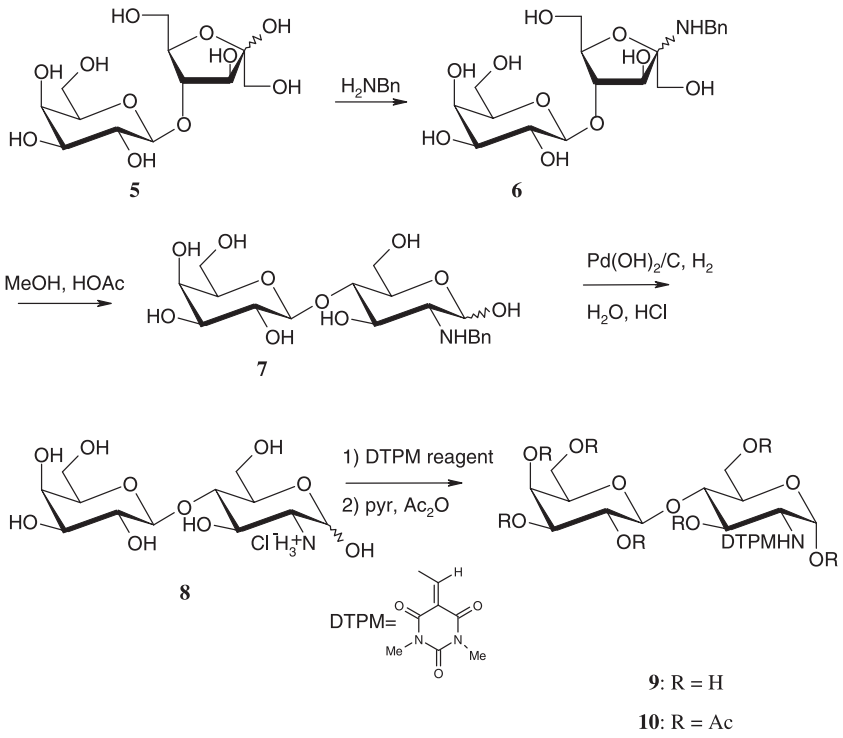
In the case of *N*-protected lactosamine, the Heyns rearrangement in combination with the *N*-protecting group [1,3-dimethyl-2, 4, 6 (1H, 3H, 5H)-trioxopyrimidine-5-ylidene]methyl (DTPM), a vinylogous amide type (23), was found to be eminently intriguing, allowing access to the product in a straightforward procedure without chromatography in an overall yield of 65 % starting from lactulose. This approach is, because of the simple preparative operations and easy handling, clearly suitable for the preparation of large amounts at low costs and in a short time.

RESULTS AND DISCUSSION

Lactulose **5** (4-*O*-β-D-galactopyranosyl-D-fructose), by reaction with benzylamine following the Heyns rearrangement pathway initially gives *N*-benzyllactulosylamine **6**, which is converted under acidic conditions to the rearrangement product *N*-benzyllactosamine **7**. Hydrogenolysis of **7** leads to lactosamine hydrochloride **8** which chemoselectively undergoes *N*-acetylation to yield *N*-acetyllactosamine **3** (38 – 45%). This reaction sequence can be performed in a one-pot procedure. Nevertheless, a final chromatographic purification step is necessary.[56]

We have now optimized the purification of the intermediates and, by employing a suitable *N*-protecting group, the corresponding lactosamine derivative precipitates from the reaction mixture in the final step of the synthesis. Following this route, lactulose **5**

reacted with commercial grade benzylamine to give the corresponding ketosyl amine **6**, which could be obtained together with unreacted lactulose and side products by precipitation from diethyl ether. The rearrangement was conducted in methanol in the presence of glacial acetic acid (10:1) at room temperature within two hours. The desired *N*-benzyllactosamine **7** as well as unreacted lactulose **5** and side products were obtained as a crude solid by treatment of the reaction mixture with diethyl ether. Hydrogenolysis of the *N*-benzyl group was performed in water at pH 1–2 employing Pearlman´s catalyst (20 %) to yield a mixture of lactosamine hydrochloride **8** and unreacted **5**. Fortunately, side products from previous steps do not survive these reaction conditions, which allows for an ''indirect'' partial purification of the mixture. Finally, *N*-protection takes place in methanol employing triethylamine and 1,3-dimethyl-5-[(dimethylamino)methylene] 2,4,6 (1H, 3H, 5H)-trioxopyrimidine (DTPM-reagent).[57] This protecting group is stable during most reaction conditions commonly used in carbohydrate chemistry such as acetylation, Zemplén conditions, alkylation, hydrogenolysis, acetal formation, silylation as well as glycosylation and can be easily removed with ammonia, hydrazine or primary amines at room temperature in a few minutes. Employing this protecting group, the desired lactosamine derivative **9** precipitates from the reaction mixture, whereas unreacted **5** remains in solution. Following this method, **9** could be obtained as a white powder in an overall yield of 65% from lactulose **5**. Per-*O*-acetylation in pyridine with acetic anhydride allowed,



Scheme 2. Synthesis of lactosamine via Heyns rearrangement.

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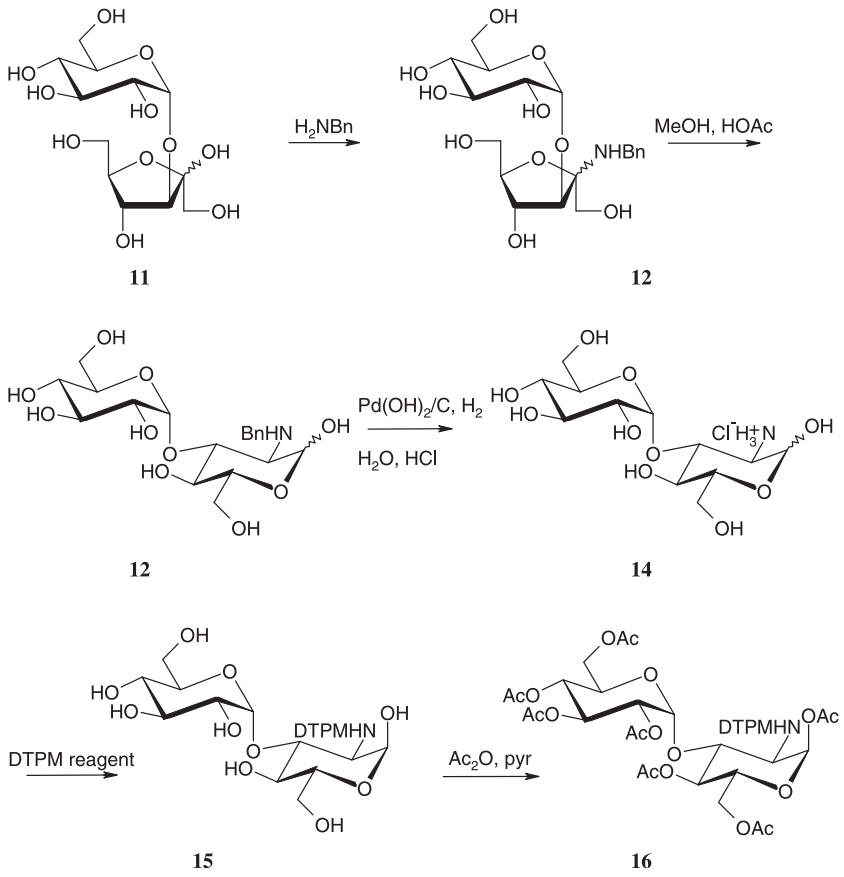
after recrystallisation from diisopropyl ether, access to the fully protected derivative **10** in almost quantitative yield, ready for further transformations. Interestingly, formation of the epimer at C-2, the D-*manno* configured 2-amino-2-deoxysugar, was not observed under the particular conditions employed (Scheme 2).

Likewise, turanose **11** (3-*O*-α-D-glucopyranosyl-D-fructose), an easily available and cheap constituent of melezitose, a highly crystalline non-reducing trisaccharide found in various types of honey,[58,59] could also be shown to serve as a substrate for the Heyns rearrangement, despite the fact that the glucosyl residue at position *O*-3 can give rise to a highly undesired β-elimination during the reaction. Nevertheless, it was possible to obtain the Heyns rearrangement product, nigerosamine **14**. Yields are somewhat lower than in the case of the lactulose-to-lactosamine conversion. In this synthesis, turanose **11** was stirred with benzylamine for 3 days until TLC showed the corresponding ketosylamine **12** as the main product. The reaction mixture was diluted with methanol and stirred into ether to give a precipitate containing a mixture of **12**, unreacted starting material as well as side products. For the rearrangement reaction, this mixture was



Scheme 3. Synthesis of nigerosamine via Heyns rearrangement.

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dissolved in methanol/glacial acetic acid, stirred at room temperature for one to two hours and added to an excess of diethyl ether, to give a precipitate of *N*-benzylnigerosamine **12**, turanose and side products. Hydrogenolysis afforded the free amine **14** as the hydrochloride together with the starting material. Its treatment with the DTPM-reagent gave the *N*-protected nigerosamine **15**. Unfortunately, due to the presence of pyranoid and furanoid tautomers at the reducing end as well as side products, this product failed to precipitate. Consequently, per-*O*-acetylation followed by conventional column chromatography was necessary to yield pure nigerosamine derivative **16** (Scheme 3).

CONCLUSION

In summary, starting from commercially available lactulose it was possible to synthesise the DTPM-protected lactosamine derivative **9** via the Heyns rearrangement in four steps without chromatography and in overall yields of 65%. The vinylogous amide type protecting group employed allows for easy purification by crystallization, leading to free sugar **9** which is ready for further transformations. Following this approach, the corresponding nigerosamine **16** was prepared from turanose. The entire sequence is simple and relies on cheap and commercially available reagents only. Other glycosylated ketoses reacted accordingly,[a] but the observed equilibrium of furanoid and pyranoid conformations as well as side product formation demanded purification by column chromatography.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian INOVA 500 operating at 499.925 MHz. ^{13}C NMR spectra were recorded at 75.47 or 50.29 MHz on a BRUKER MSL 300 or on a Varian Gemini 200. Residual non-deuterated solvent was used as internal standard. Signals of protecting groups were found in the expected regions and are not listed explicitly. Melting points were measured on a Büchi 530 apparatus and are uncorrected. Mass spectra were recorded on an HP 1100 series MSD, Hewlett Packard. Samples were dissolved in acetonitrile or acetonitrile/water mixtures. The scan mode for positive ions (mass range 100–1000 D) was employed varying the fragmentation voltage from 50 to 250 V with best molecular peaks observed at 150 V. Analytical

[a]Maltulose and palatinose could be converted accordingly. In case of maltulose, only the monohydrate was commercially available. The presence of water led to lower yields, shifting the equilibrium of the condensation reaction, the first step of the Heyns rearrangement. This was reached at around 60% conversion of maltulose into the corresponding ketosylamine. Palatinose is an excellent substrate for the Heyns reaction, which is driven by the ring expansion from the furanoid fructose to the pyranoid glucosamine derivative. Both glycosylated glucosamine derivatives were found to be mixtures of pyranoid and furanoid tautomers creating the need for column chromatography of the *N*-protected derivatives which dod not precipitate well under the conditions employed.

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TLC was performed on precoated aluminum plates silica gel 60 F254 (Merck 5554), detected with UV light (254nm), as well as staining with 5% vanillin/sulfuric acid or ceric ammonium molybdate (100g ammonium molybdate/4g cerium sulfate in 1L 10% H_2SO_4) and heating on a hotplate. For column chromatography, silica gel 60 (230–400 mesh, Merck 9385) was employed.

GENERAL METHODS

Heyns rearrangement of glycosylated ketoses with benzylamine: Benzylamine (7.8 equiv) was added at 0°C to the respective ketose. The reaction mixture was allowed to reach room temperature and was subsequently stirred at 40°C until TLC ($MeOH/CHCl_3/NH_4OH$ 2:1:1) showed the ketosylamine as the main product. Methanol (same volume as benzylamine) was added and this mixture was stirred into diethyl ether and kept at 0°C for 3 h. The resulting precipitate consisting of the product and unreacted starting material as well as side products was collected by filtration and dried under reduced pressure.

The crude product was dissolved in methanol/glacial acetic acid (8:1), and stirred at room temperature for 1–2 hours, slowly added to excess ether, and kept at 0°C for 5 h. The precipitate thus obtained containing the glycosylated *N*-benzylglucosamine, unreacted starting material and side products was collected by filtration and dried under reduced pressure.

Hydrogenolysis: The above crude material was dissolved in deionized water, the pH value was brought to 1 by dropwise addition of concd HCL, Pearlman's catalyst [$Pd(OH_2)/C$, 20%, (5 % by weight)] was added and this reaction mixture was kept on a Parr apparatus at 3 bar until TLC ($MeOH/CHCl_3/NH_4OH$ 2:1:1) showed completed removal of the *N*-benzyl group. The reaction could be performed under ambient pressure but requiring reaction times of up to 5 days.

The catalyst was filtered off, the solution was concentrated under reduced pressure and the residue was treated with benzene/ethanol (1:1) followed by removal of the solvent under reduced pressure. The so obtained yellowish residue contained the glycosylated glucosamine hydrochloride and unreacted starting material.

DTPM protection: The hydrochloride of the free aminosugar was dissolved in a small amount of methanol, triethylamine (2 equiv) and DTPM reagent (1.1 equiv in methanol) were added and the reaction mixture was kept at room temperature for 2 h. The solid product formed was collected by filtration and washed with methanol twice.

***N*-Benzyllactosamine (7).** Following the general procedures for the Heyns rearrangement, in a typical experiment lactulose **5** (50 g, 146.1 mmol) was reacted with benzylamine (125mL, 1144.3 mmol) and stirred at 40°C for 3 days, until TLC showed the condensation product as the main component in the mixture. Methanol (125 mL) was added and this solution was slowly stirred into ether (5000 mL). The resulting precipitate was collected by filtration and dried under reduced pressure. A crude product (71.1 g) containing *N*-benzyllactulosylamine (**6**) and side products were obtained.

This mixture was dissolved in methanol (250 mL) containing glacial acetic acid (30 mL), kept at room temperature for two h and this solution was stirred into ether (4500 mL). A precipitate containing *N*-benzyllactosamine **7**, lactulose **5** and side products (77.9 g in total) was obtained.

Lactosamine hydrochloride (8). The crude material (30.6 g) from the rearrangement reaction was dissolved in deionized water (150 mL), acidified dropwise with concd HCl (7.6 mL) to pH 1, $Pd(OH)_2/C$ (1.5 g) was added and following the general procedures, a mixture of **8** with lactulose **5** could be obtained (26.5 g).

***N*-[1,3-Dimethyl-2,4,6 (1H, 3H, 5H)-trioxopyrimidine-5-ylidene]methyl lactosamine (9).** The mixture obtained after hydrogenolysis (6.8 g) was dissolved in methanol (70 mL), Et_3N (3.7 mL, 26.7 mmol) and DTPM reagent (4.5 g, 21.3 mmol) in methanol (20 mL) were added and the reaction mixture was stirred at ambient temperature for 2 h. The white, amorphous precipitate formed was collected by filtration and washed with methanol twice to give pure **9** (4.6 g, 65% from **5**). Recrystallization from CH_3CN/H_2O gave an analytical sample, mp 265–267°C; $[\alpha]^{20}_{D}$+67.7 (*c* 0.68, DMSO); ^{13}C NMR (DMSO-d_6) δ 164.64, 162.56, 160.09, 152.01 (DTPM), 104.35 (C-1′), 90.41, 90.29 (C-1, DTPM), 81.03 (C-4′), 76.08 (C-5′), 73.61 (C-3′), 71.08, 70.67, 70.14 (3 C, C-2′, C-3, C-5), 68.66 (C-4), 63.88, 60.99, 60.72 (3 C, C-2, C-6, C-6′), 27.83, 27.21 (DTPM). MS: (150V): *m/z* : 508.48 [M^+-H].

Anal. Calcd for $C_{19}H_{29}O_{13}N_3$. $^1/_2$ H_2O (516.61): C, 44.17; H, 5.86. Found: C, 43.91; H, 5.75.

1,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-*N*-[1,3-dimethyl-2,4,6 (1H, 3H, 5H)-trioxopyrimidine-5-ylidene]methyl lactosamine (10). To a 10% solution of **9** (10.5 g, 20.7 mmol) in pyridine, acetic anhydride (50 mL, 528.9 mmol) was added dropwise at 0°C, a catalytic amount of dimethylaminopyridine was added and the reaction kept at room temperature for 16 h. The solvent was removed under reduced pressure, the residue dissolved in CH_2Cl_2 and the solution was consecutively washed with 6% HCl and sat aqueous $NaHCO_3$, then dried over $MgSO_4$. The crude product was precipitated from diisopropyl ether, collected by filtration and dried over P_2O_5 to give 15.6g (94 %) of **10**. Recrystallization from $CHCl_3$/cyclohexane gave an analytical sample, mp 145–147°C; $[\alpha]^{20}_{D}$+64.8 (*c* 0.66, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.16 (dd, 1H, NHDTPM), 8.06 (d, 1H, HC=CDTPM), 6.18 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.37 (t, 1 , $J_{2,3=3,4}$ 9.8 Hz, H-3), 5.33 (d, 1H, H-4′), 5.08 (dd, 1H, H-2′), 4.93 (m, 1 H, $J_{2',3'}$ 10.3 Hz, $J_{3',4'}$ 3.0 Hz, H-3′), 4.46 (d, 1 H, $J_{1',2'}$ 7,9 Hz, H-1′), 4.39 (m, 1 H, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.14 – 4.04 (m, 3H, H-6b, H-6′a, H-6′b), 3.97 (m, 1H, H-5), 3.87 – 3.79 (m, 2H, H-2, H-4), 3.66 – 3.61 (ddd, 1 H, $J_{5',6'a=4',5'}$ 9.7 Hz, $J_{5',6'b}$ 3.5 Hz, H-5′), 3.2 (2s, 6H, 2 NCH_3); ^{13}C NMR ($CDCl_3$) δ 170.09, 169.98, 169.89, 169.84, 169.38, 168.86, 168.53 (acetyl), 164.45, 162.41, 158.41, 151.68 (DTPM), 100.86 (C-1′), 92.41 (DTPM), 89.82 (C-1), 74.86 (C-4), 70.68, 70.60, 70.52 (3 C, C-2, C-5, C-3′), 70.09 (C-3), 68.89 (C-2′), 66.40 (C-4′), 61.36, 61.26, 60.63 (3 C, C-5′, C-6, C-6′), 27.67, 27.05 (DTPM), 20.63, 20.60, 20.51, 20.46, 20.29 (acetyl). MS (150V): *m/z* : 802.7 [M^+-H];

Anal. Calcd for $C_{33}H_{43}O_{20}N_3$ (801.8): C, 49.44; H, 5.41. Found: C, 49.27; H, 5.48.

***N*-Benzylnigerosamine (13).** Following the general procedure for the Heyns rearrangement in a typical experiment, turanose **11** (3 g, 8.8 mmol) was reacted with

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benzylamine (7.5 mL, 68.7 mmol) and the mixture stirred at 40°C for 4 days, when TLC showed the condensation product as the main component in the mixture. Methanol (7.5 mL) was added and this solution then stirred into ether (400 mL). The precipitate was collected by filtration and dried. A slightly yellow material (3.0 g) containing *N*-benzylturanosyl amine **12** and side products was obtained. This crude material (2.9 g) was dissolved in MeOH/HOAc (14/2 mL), kept at room temperature for 90 min and this solution was stirred into 900 mL of ether. A solid (2.7 g) containing *N*-benzyl-nigerosamine **13,** turanose **11** and side products was collected.

Nigerosamine hydrochloride (14). The rearrangement mixture (4.14 g) were dissolved in 50 mL deionised water, acidified dropwise with concd HCl (0.5 mL), 10% by weight of $Pd(OH)_2/C$ (20 wt.%) was added and, following the general procedures, 3.84 g of a mixture of **14** and turanose **11** were obtained.

1,4,6,2′,3′,4′,6′-Hepta-*O*-acetyl-*N*-(1,3-dimethyl-2,4,6 (1H, 3H, 5H)-trioxopyrimidine-5-ylidene)methyl nigerosamine (16). The above mixture (3.27 g) was dissolved in methanol (30 mL). Et_3N (1.8 mL, 13 mmol) and DTPM reagent (2.7 g, 12.8 mmol) in methanol (5 mL) were added and the reaction mixture was stirred at room temperature for 2 h. For purification and identification, the compounds were per-*O*-acetylated. The residue (7.81 g) was dissolved in pyridine (30 mL, 371 mmol), acetic anhydride (15 mL, 158.5 mmol), and a catalytic ammount of DMDP was added, and the reaction was stirred at room temperature for 3 h. Methanol was added, the volume was reduced to 50% under reduced pressure and the resulting solution was diluted with CH_2Cl_2, washed with 6% aqueous HCl, satd $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography employing ethyl acetate/cyclohexane 3:1 gave a mixture of furanoid and pyranoid tautomers of per-*O*-Ac-NHDTPM nigerosamine (**16**) in an overall yield of 15% from turanose. Recrystallization from $CHCl_3$/cyclohexane gave an analytical sample of **16** (pyranose form), $[\alpha]^{20}_D$+126.9 (*c* 0.63, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.18 (m, 1H, NHDTPM), 8.27 (d, 1 H, J 13.68 Hz, HC=CDTPM), 6.25 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.36 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 5.26 (m, 1H, H-3′), 5.20 (m, 1H, H-3), 4.98 (m, 1 H, $J_{3',4'}$ 9.8 Hz, H-4′), 4.80 (dd, 1 H, H-2′), 4.26 (m, 1 H, $J_{3,4}$ 10.3 Hz, $J_{4,5}$ 9.3 Hz, H-4), 4.19 (dd, 1 H, $J_{5,6a}$ 4 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.06 (dd, 1 H, $J_{5',6'a}$ 2.4 Hz, $J_{6'a,6'b}$ 12.7 Hz, H-6′a), 4.01 – 3.29 (m, 3H, H-6b, H-5, H-6′b), 3.83 (ddd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 3.61 (ddd, 1H, H-5′); ^{13}C NMR ($CDCl_3$) δ 170.90, 170.78, 170.69, 169.75, 169.53, 169.40, 168.66 (acetyl), 165.37, 162.11, 159.74, 152.04 (DTPM), 95.73 (C-1′), 93.01 (DTPM), 90.67 (C-1), 72.94 (C-4), 70.41 (C-5), 70.27, 70.17 (2 C, C-2′, C-3′), 69.22 (C-3), 68.21 (C-5′), 67.81 (C-4′), 62.57 (C-2), 61.40 (C-6), 61.15 (C-6′), 28.18, 27.47 (DTPM), 20.99, 20.92, 20.80, 20.76, 20.45 (acetyl). MS (150V): *m/z* : 802.7 [M^+-H].

Anal. Calcd for $C_{33}H_{43}O_{20}N_3$ (801.8): C, 49.44; H, 5.41. Found: C, 49.30; H, 5.47.

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