NEW N-GLUCOSYLATED SUBSTITUTED ANILINES

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Abstract. The reaction of (+)-*D*-glucose **1** with 4-chloroaniline **6b** or 3,5-dibromoaniline **12** leads almost exclusively to the β-configuration of N-glucosylated anilines **7b** and **13**. Acetylated derivatives **8b, 14** and **15** were obtained by dissolving/suspending substances **7b** and **13** in Ac_2O/Py mixture. The acetylation of 2-(3,5-dibromophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol **13** is less selective than in the case of the 2-(4-chlorophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol **7b** and leads to compounds 2-(acetoxymethyl)-6-(3,5-dibromophenylamino)tetrahydro-2H-pyran-3,4,5-triyl triacetate **14** and 2-(acetoxymethyl)-6-(3,5-dibromophenylamino)-5-hydroxytetrahydro-2*H*-pyran-3,4-diyl diacetate **15** in a 2:1 ratio. The product **14** is formed with greater selectivity and in a higher yield (up to 80%) when the reaction is catalyzed by DMAP and stored for one week at +4°C.

Keywords: N-glucosylated anilines, (+)-*D*-glucose, 4-chloroaniline, 3,5-dibromoaniline, Convolutamydines A-E.

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Introduction

N-Glycosylated anilines represent an important product scaffold cluster by virtue of their bioactivity and as intermediates for generating further molecular complexity including natural compounds [1], for example some natural alkaloids. The vital roles played by sugars in biological systems continue to be unravelled. It is known that, various drugs, amino acids, sugars and many other chiral natural compounds show different influence on human organism, their biological properties being directly dependent on chirality. That is why the "structure-property" relationship should be studied very well. From the other side, properties are determined by the structure. It means, construction of chemically pure and defined molecule is an interesting and important goal in synthetic chemistry.

Langer *et al.* [1,2] has shortly offered the opinion that the preparation of analogues of N-glycosylated indolinones in high yields remains an important problem of carbohydrate and medicinal chemistry. This challenge also applies to the related problem of synthesis of N-linked alkaloids. For example, Kamano, Y. *et al.* [3], reported the isolation of the alkaloids - Convolutamydines A, B, and C from bryozoan *Amathia convoluta,* see Figure 1. In contrast to the pharmacologically inactive non-glycosylated indigo, N-glycosylated indigo demonstrate a considerable growth inhibitory activity toward various human tumor cell lines [4,5].

Our approaches to N-glucosylated indoline-2,3-dione **4** from (+)-*D*-glucose **1** and N-glucosylated 3-hydroxy-2-oxindole **5** are presented below. They show benefit from the rapid advances in mainstream carbohydrate chemistry, allowing for convenient integration in glucosylated Convolutamydine A-E and analogues of structure **5** preparation (see Figure 1).

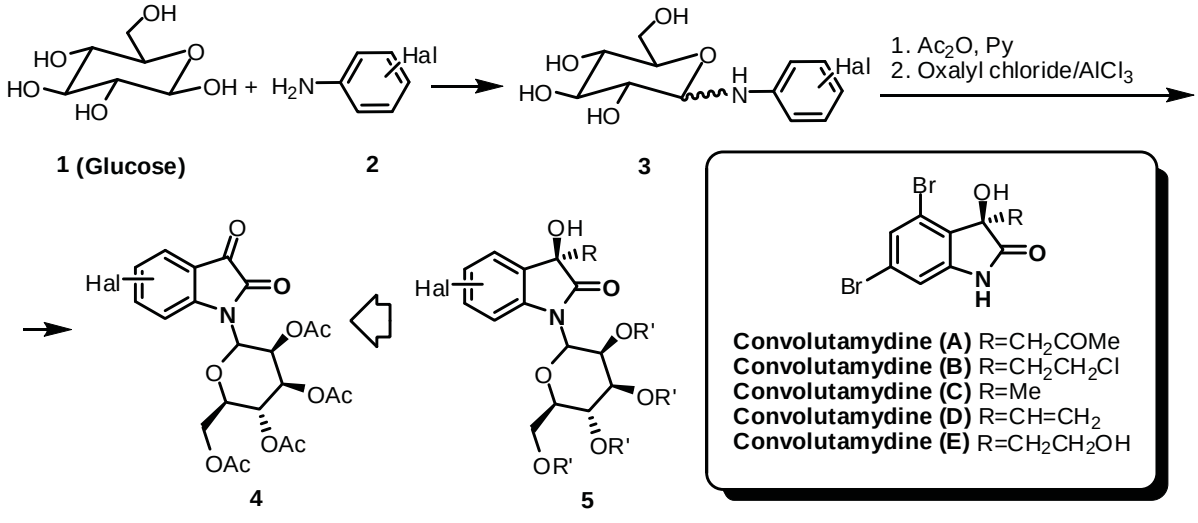
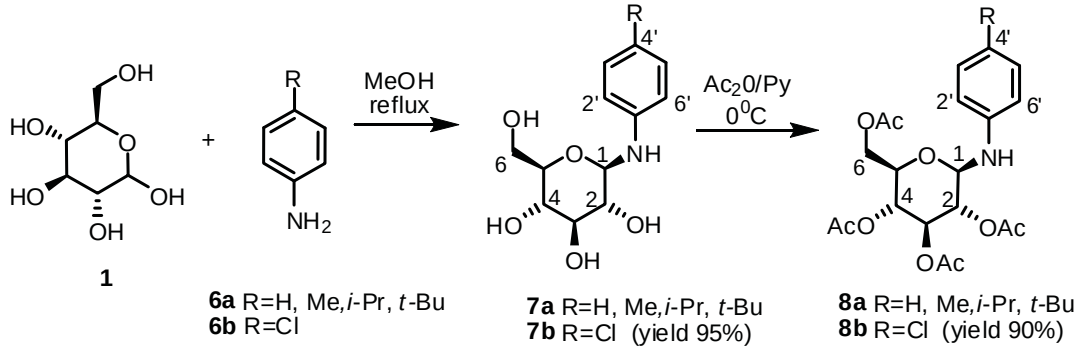


Figure 1. Synthesis of N-glucosylated indoline-2,3-dione (4).

The main purpose of the present research was to test the effectiveness of this approach for the synthesis of halogen phenylamino-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetates from the corresponding intermediates **3** (see Figure 1). It has already been demonstrated that such type compounds are suitable building units for the synthesis of a variety of non-halogenated isatin-N-glucosides [1,2]. We also report in this paper the preparation of 3,5-dibromoaniline **12.**

Results and discussion

It was reported [1], that similar aniline-*N*-glucosides **7a** were prepared from corresponding anilines (R=H, Me, *i*-Pr, *t*-Bu) and (+)-*D*-glucose **1.** The formed product **7a** was directly used for the next step (see Scheme 1). However, some of the derivatives of glycosides can be isolated as pure β-anomers **8a**, whereas the others contain a small amount of the corresponding α-anomer [1,2].



Scheme 1. Syntheses of N-glucosylated 4-substituted anilines 8a and 8b [1].

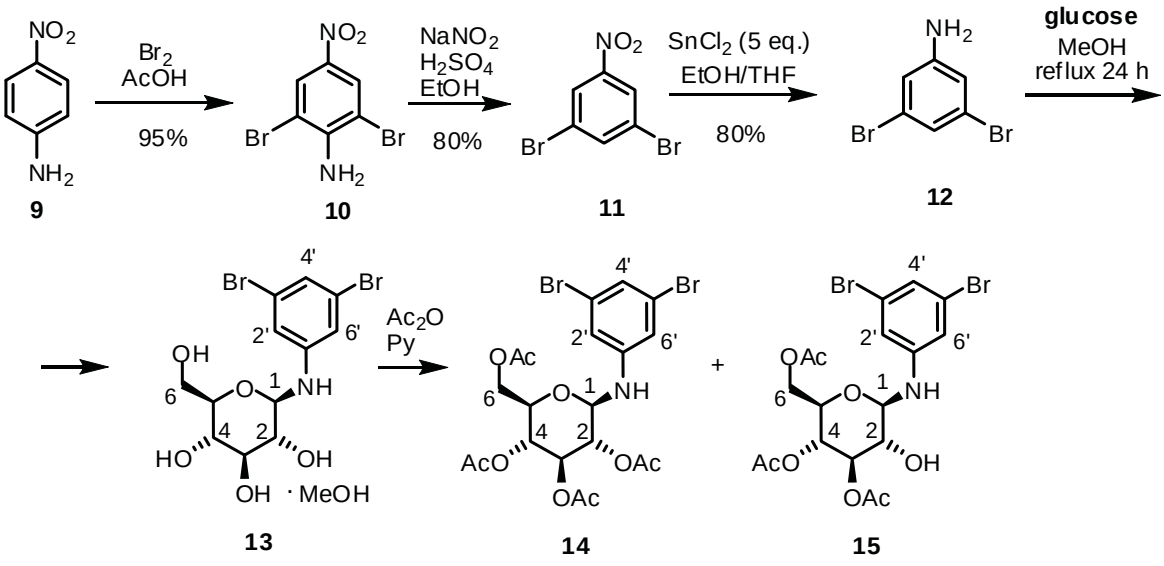
In the course of our studies the (2*R*,3*R*,4*S*,5*S*,6*R*)-2-(4-chlorophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol **7b** was prepared according to the reported method [1] primarily by reason of convenience: medium solubility of (+)-*D*-glucose **1** in MeOH and easy removal from the excess of aniline by filtration and washing with cool MeOH, provides ready access to the solid aminoglycoside, which is slightly soluble in MeOH. A mixture of (+)-*D*-glucose **1** and 4-chloroaniline **6b** was refluxed for 12 hours (see Scheme 1). TLC of the reaction mixture indicated the disappearance of the starting glucose **1** and an increase in the intensity of the neighbouring spot. On keeping the solution overnight in the refrigerator an adduct precipitated that was easily isolated by filtration, being then identified as compound **7b**. It had m.p. 154-156 ^{o}C and characteristic IR absorption bands ν_{OH} at 3271 and 3209 cm^{-1}, the primary (C-6) and secondary (C-2, C-3 and C-4) nature of the alcohol functions being confirmed by the ^{1}H NMR spectrum (triplet at δ_H 4.44-4.47 ppm with a splitting constant *J*=5.8 Hz and three doublets at δ_H 4.88-4.90, 4.92-4.9 and 5.00-5.02 ppm with splitting constants *J*=5.4 Hz, *J*=5.2 Hz and *J*=4.7 Hz. NH group shows doublet at δ_H 6.46-6.48 ppm. Moreover, ^{1}H NMR spectrum has resonances at δ_H 6.67-6.69 ppm (C-2'-H and C-6'-H, doublet, *J*=8.8 Hz) and δ_H 7.10-7.12 ppm (C-3'-H and C-5'-H, doublet, *J*= 8.8 Hz), indicating that compound **7b** is an anilide. Additionally, absorption in the low-field region of its ^{13}C NMR spectrum confirmed the presence of aromatic carbons at δ_C 115.06 ppm (C-2' and C-6'), 120.7 ppm (C-4'), 128.92 ppm (C-3' and C-5') ppm and 146.7 ppm (C-1').

In fact compound **7b** shows in the ^{1}H NMR spectrum a clear triplet at δ_H 4.30-4.34 ppm with the magnitude of a spin-spin coupling constant *J*=8 Hz and an important peak at 883.8 cm^{-1} in its IR-spectrum, which is characteristic for a β-anomer.

The reaction of compound 7**b** with (+)-*D*-glucose **1** was slow and only after 48 hrs provided a solid compound with m.p. 146-149^{0}C. The substance **8b** was obtained in 97% yield, being identified as (2*R*,3*R*,4*S*,5*S*,6*R*)-2-(4-chlorophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol **8b** on the basis of its NMR spectroscopic data. Thus, the ^{1}H NMR spectrum of it showed in the low-field region two singlets and a doublet at δ_H 1.95, 1.97, 2.00 ppm characterizing four acetate groups, according to the integral. The ^{1}H NMR spectrum of compound **8b** also contains two doublets of aromatic protons, centered at δ_H 6.75 ppm (2H, C-2'-H and C-6'-H, J=8.9 Hz) and δ_H 7.14 ppm (2H, C-3'-H and C-5'-H, J=8.8Hz) and a doublet of NH group at δ_H 6.71 ppm with the spin-spin coupling constant J=9.8 Hz. The ^{13}C NMR spectroscopic data totally confirm the structure **8b**, see experimental part. Thus, the preparation of (2*R*,3*R*,4*S*,5*S*,6*R*)-2-(4-chlorophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol **8b** *via* aniline-*N*-glucoside **7b** has been successfully reproduced by us.

It was found that the 4,6-dibromohydroxyoxindole nucleus exhibit a potent activity in the differentiation of HL-60 human promyelocytic cells [3,6]. Therefore, as a part of the program aimed at developing new N-glucosylated oxindoles, we supposed, that the 3,5-dibromoaniline **12** scaffold has potential to enhance the selectivity. As obvious precursor for the synthesis of glucosylated Convolutamydines A-E **5**, 3,5-dibromoaniline **12** was prepared by initial bromination of 4-nitroaniline **9,** followed by deamination of aniline **10,** to form 3,5-dibromonitrobenzene **11**. $SnCl_2$

reduction of the latter was found to proceed with difficultly, but when 5 equivalents of $SnCl_2$ were used, 3,5-dibromoaniline **12** has been produced in good yield (see Scheme 2 and experimental part).



Scheme 2. Synthesis of 3,5-dibromoaniline (12) and its N-glucosylated derivatives 13, 14 and 15.

As it can be seen from Scheme 2, the reaction of **12** with (+)-*D*-glucose **1** was slow and only after 24 hrs provided a solid compound with m.p. 169-170^{0}C. The substance obtained in 97% yield was identified as (2*R*,3*R*,4*S*,5*S*,6*R*)-2-(3,5-dibromophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol **13** on the basis of its ^{1}H NMR spectrum, which showed in the low-field region signals at δ_H 6.86 ppm and δ_H 6.95 ppm (aromatic), δ_H 6.89 ppm (NH), δ_H 4.52 ppm, δ_H 4.96 ppm, δ_H 4.97 ppm and δ_H 5.05 ppm (C-6-OH, C-4-OH, C-2-OH and C-3-OH, respectively). Moreover, in its ^{1}H spectrum the multiplets at δ_H 3.10-3.24 ppm (C-2-H, C-3-H, C-4-H, C-5-H, C-6-H) and triplet centred at δ_H 4.36 ppm (C-1-H) are present. The IR-spectrum showed a low intensity band at 892.4 cm^{-1} assigned to the C-1-H scissoring of the protons in the β-anomer. Similarly, the ^{1}H NMR spectrum indicated the presence of C-1-H (a triplet at δ_H 4.36 ppm with a splitting constant *J*=8 Hz. However, ^{1}H NMR spectrum shows two additional signals: a doublet attributable to three protons for methanol at δ_H 3.17 ppm and a quartet of one proton at δ_H 6.95 ppm (OH), shielded by an additional carbon, which appears in ^{13}C NMR spectrum at δ_C 49.10 ppm. Finally, the structure of the product is considered to be **13** on the basis of its elemental analysis, as well. The formation of intermolecular complex **13** has been rationalized by considering the participation of the hydroxyl, as well as NH groups in the addition of **12** with (+)-*D*-glucose in MeOH medium.

The acetylation reaction of **13** was performed with acetic anhydride in pyridine and lead to esters **14**. The reaction was very slow (one week) and after work-up two main products in the obtained mixture were then separated by column chromatography over silica gel.

As a result, pure **14** was isolated as the least polar product with m.p. 72-73^{0}C in 35% yield and its structure has been proved by NMR. The ^{1}H NMR spectrum of it contains singlets at δ_H 2.03 ppm, δ_H 2.06 ppp, δ_H 2.07 ppm, δ_H 2.11 ppm (AcO groups), a doublet centered at δ_H 5.04 ppm (*J*=9.7 Hz) (NH group), multiplets at δ_H 3.86, 4.68, 5.00, 5.04, 5.37 ppm (C2-H, C3-H, C4-H, C5-H, C6-H, correspondingly), multiplets at δ_H 4.14 and 4.23 ppm (CH_2), and doublet of aromatic protons at δ_H 6.74 ppm (2H, *J*=1.5, C-2'-H, C-6'-H) and δ_H 7.11 ppm (1H, t, *J*=1.5, C-4'-H).
Moreover, its formulation as an ester has been sustained by peaks in higher field ^{13}C NMR spectrum at δ_C 72.7 ppm (C-5), 68.9 (C-4), 72.5 (C-3), 71.0 (C-2), 83.6 (C-1) and 146.7 ppm (C-1') and peaks in lower field at δ_C 62.3 (C-6), 115.9 (C-2', C-6'), 123.2 (C-3', C-5') and 125.1 ppm (C-4'). This resonance pattern differs markedly from that observed for the initial compound **13**. The comparative examination also suggests that four acetyl group functions should have eight peaks as well. This is consistent with the observation of signals at δ_C 20.6 (CH_3), 20.65 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 170.7 (C=O), 169.6 (C=O), 169.9 (C=O) and 171.3 (C=O). The IR-spectrum showed bands at 1740 cm^{-1}, 1588 cm^{-1}, 3371 cm^{-1}, 915 cm^{-1} and 671 cm^{-1} assigned to the COO, aromatic, NH, β-glucopyranoside and C-Br, respectively.

Additionally, another product was isolated, which presumably corresponded to the structure **15**. According to NMR data, the isolated product is a mixture of compounds **14** and **15** in 2:1 ratio, which has been determined by integration of the signals belonging to the acetate groups. It could be easily identified according to ^{13}C NMR spectrum by the double set of signals: four C=O groups at δ_C 169.6, 169.9, 170.7 and 171.3 ppm for compound **14**, and three C=O groups for compound **15** at δ_C 169.1, 169.5 and 170.4 respectively. Similarly, double set of signals has been noted for pyranic (δ_C 60-83 ppm) and aromatic (δ_C 115-147 ppm) parts of molecules of the discussed derivative (see experimental

part). Thus, the ^{1}H NMR spectrum shows multiplets at δ_H 3.85-3.90, 4.01-4.30, 4.99-5.07, 5.16-5.20 and 5.30-5.46 ppm, which are characteristic for pyranic part (CH and OH), two doublets at δ_H 6.74 and 7.00 ppm and two triplets centered at δ_H 7.10 and 7.14 ppm (aromatic), four singlets at δ_H 2.04, 2.05, 2.06 and 2.07 ppm for compound **14**, a singlet at δ_H 2.03 ppm and a doublet centered at δ_H 2.08 ppm for compound **15,** respectively.

Catalysis by pyridine is of the nucleophilic type and it is known that 4-(N,N-dimethylamino)pyridine is a better catalyst when pyridine fails. Indeed, compound **13** readily undergoes reaction with acetic anhydride under analogues conditions in presence 4-(N,N-dimethylamino)pyridine to yield up to 90% compound **14**.

Conclusions

The present work demonstrates that interaction of 4-chloro- and 3,5-dibromo- substituted anilines with (+)-*D*-glucose affords N-glycosylated adducts **7b** and **13** as β-anomers. The position and steric course of further estherification are catalytically dependent. We confirmed that in the case of 4-chloro substituted aniline reaction with Ac_2O in Py occurs mainly to give tetra-acetate **8b**. On the contrary, reaction of 3,5-dibromo substituted aniline gives a mixture of adducts **14** and **15** in a 2:1 ratio with overall yield 65%. In the case when the reaction was catalysed by 4-(N,N-dimethylamino)pyridine only compound **14** was obtained in 80% overall yield. The structures of all new compounds **13, 14** and **15,** including configurations of anomeric carbon atoms, were characterized through IR and NMR spectroscopic methods.

Experimental

All used solvents were of reagent quality, and all commercial reagents were used without additional purification. Removal of all solvents was carried out under reduced pressure. Analytical TLC plates Silufol® UV-254 (Silpearl on aluminum foil, Czecho-Slovakia) were used and spots were detected under UV-lamp with wavelength 254 nm.

M. p.s (uncorrected) were determined on a Boetius apparatus.

IR spectra were recorded on a Spectrum 100 FT-IR spectrophotometer (Perkin - Elmer) using the universal ATR sampling accessory. ^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ and DMSO-d_6 2-% solution on a "Bruker-Avance III" (400.13 and 100.61 MHz) spectrometer.

General procedure for the synthesis of N-glucosylated anilines 7b and **13.**

To a solution of (+)-*D*-glucose **1** (2g, 0.011 mol) in 25 mL of absolute methanol corresponding aniline (**6b** or **12**) (0.013 mol) was added. The mixture was refluxed for 24 hours. After completion of the reaction (TLC control, solvent system 2% MeOH in CH_2Cl_2) the mixture was stored in refrigerator at sub-zero temperature so long, as white volume is being precipitated. The precipitate was filtered and washed with methanol and dried at room temperature.

(2*R*,3*R*,4*R*,5*R*,6*R*)-2-(4-chlorophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol 7b. White solid. Yield 95 %. M. p. 154-156^{o}C (MeOH). $[\alpha]_D^{20}$-41.0(c 0.068, DMSO). IR-spectra (ν/cm^{-1}): 3271.5, 3209.1 (OH), 1523.4 (NH), 883.8 (C-1-H), 683.3 (C-Cl). ^{1}H NMR (400 MHz, DMSO- d_6, δ, ppm, *J*/Hz): 3.07-3.27 (4H, m, C-2-H, C-3-H, C-4-H, C-5-H), 3.39-3.45 (1H, m, C-6-H), 3.61-3.66 (1H, m, C-6-H), 4.30-4.34 (1H, t, C-1-H, J=8), 4.44-4.47 (1H, t, C-6-OH, J=5.8), 4.88-4.90 (1H, d, C-4-OH, J=5.4), 4.92-4.93 (1H, d, C-2-OH, J=5.2), 5.00-5.02 (1H, d, C-3-OH, J=4.7), 6.46-6.48 (1H, d, NH, J=7.5), 6.67-6.69 (2H, d, C-2'-H, C-6'-H, J=8.8), 7.10-7.12 (2H, d, C-3'-H, C-5'-H, J=8.8). ^{13}C NMR (100.6 MHz, DMSO-d_6): 61.4 (C-6), 70.6 (C-4), 73.5 (C-2), 77.8 (C-3), 78.1 (C-5), 85.3 (C-1), 115.0 (C-2', C-6'), 120.7 (C-4'), 128.9 (C-3', C-5'), 146.8 (C-1'). Calculated, %: C 49.75; H 5.57; N 4.83. $C_{12}H_{16}ClNO_5$. Found, %: C 49.80; H 5.60; N 4.80.

(2*R*,3*R*,4*S*,5*S*,6*R*)-2-(3,5-dibromophenylamino)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol 13. White solid. Yield 97 %. M. p. 169-170^{o}C (MeOH). $[\alpha]_D^{20}$-62.40(c 0.05, DMSO). IR-spectra (ν/cm^{-1}): 3367.5, 3210.1, 3073.2 (OH); 1518.0 (NH); 892.4 (C-1-H), 668.2 (C-Br), 1574.5 (aromatics), 3367-3073 (OH). ^{1}H NMR (400 MHz, DMSO- d_6, δ, ppm, J/Hz): 3.40-3.65 (1H, ddd, J=11.8, 5.8, 1.9, C-6-H), 3.1 (2H, m, C-4-H, C-2-H), 3.24 (2H, m, C-5-H, C-3-H), 3.17 (3H, d, J=5.2, $\underline{CH_3}OH$), 4.18 (1H, q, J=5.2, $CH_3O\underline{H}$), 4.36 (1H, t, J=8, C-1-H), 4.52 (1H, t, J=5.8, C-6-OH), 4.96, (1H, d, J=1.7, C-4-OH), 4.97 (1H, d, J=1.4, C-2-OH), 5.05 (1H, d, J=4.8, C-3-OH), 6.89 (1H, d, J=7.8, NH), 6.86 (2H, d, J=7.6, C-2'-H, C-6'-H), 6.95 (1H, t, J=1.6, C-4'-H). ^{13}C NMR (100.6 MHz, DMSO-d_6): 49.1 (<u>Me</u>OH), 61.3 (C-6), 70.7 (C-4), 73.5 (C-2), 77.8 (C-3), 77.9 (C-5), 84.5 (C-1), 115.1 (C-2'and C-6'), 121.5 (C-4'), 123.1 (C-3'and C-5'), 150.7 (C-1'). Calculated, %: C 34.89; H 3.66; N 3.39. $C_{12}H_{15}Br_2NO_5$. Found, %: C 34.94; H 3.64; N 3.41.

Procedure for the synthesis of compound 8b.

The anilide **7b** is maximally dissolved in dry pyridine under stirring (for every hydroxyl group 1.8-2.0 eq. of pyridine are used) and cooled in an ice bath to 0^{0}C. Then, freshly distillated acetic anhydride is rapidly added (for every hydroxyl group 1.5-1.6 eq. of acetic anhydride are used). Stirring is continued at the same temperature until a homogeneous solution appeared (about 3 hours). The mixture was hold for 24-48 hours in a refrigerator without stirring. After completion of the

reaction (TLC control, system hexane-ethyl acetate 4:1), the mixture was poured into ice-water (1:2) and extracted with ethyl acetate (4x30 mL). The combined organic phases were washed with sodium hydrogen carbonate solution, water and brine, dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The resulting white solid mass was recrystallised from methanol. The bittern was evaporated and recrystallised again. The obtained product is white solid.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(4-chlorophenylamino)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate 8b. White solid. Yield 97 %. M. p. 146-149°C (MeOH). $[\alpha]_D^{20}$-48.6(c 0.076, $CHCl_3$). IR-spectra (ν/cm^{-1}): 1059.5, 1180.7 (C-O-C), 1511.7 (NH), 878.9 (C-1-H), 688.4 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.95 (3H, s, $\underline{CH_3}CO$), 1.95 (3H, s, $\underline{CH_3}CO$), 1.97 (3H, s, $\underline{CH_3}CO$), 2.00 (3H, s, $\underline{CH_3}CO$), 4.08-4.18 (2H, m, C-6), 4.88-4.93 (2H, ddd, C-5-H and C-4-H, J=0.9; 1.6; 2.1), 3.93-3.97 (1H, dd, C3-H, J=1.8; 1.8), 5.18-5.22 (1H, t, C-3-H, J=9.4), 5.32-5.36 (1H, t, C-1-H, J=9.5), 6.71 (1H, d, NH, J=9.8), 6.75 (2H, d, C-2'-H and C-6'-H, J=8.9), 7.14 (2H, d, C-3'-H and C-5'-H, J=8.8). ^{13}C NMR (100.6 MHz, DMSO-d_6): 20.8 (CH_3), 20.8 (CH_3), 20.9 (CH_3), 20.9 (CH_3), 62.3 (C-6), 68.7 (C-4), 71.2 (C-3), 71.5 (C-5), 73.7 (C-2), 82 (C-1), 115.8 (C-2' and C-6'), 122.0 (C-4'), 129 (C-3'and C-5'), 145.5 (C-1'), 169.6 (C=O), 169.8 (C=O), 170.1 (C=O), 170.4 (C=O). Calculated, %: C 52.46; H 5.28; N 3.06. $C_{20}H_{24}ClNO_9$. Found, %: C 52.52; H 5.30; N 3.10.

General procedure for the synthesis of compounds 14 and 15.
Method A: The anilide **13** is maximally dissolved in dry pyridine under stirring (for every hydroxyl group 1.8-2.0 eq. of pyridine are used) and cooled in an ice bath to 0°C. Then, freshly distillated acetic anhydride is rapidly added (for every hydroxyl group 1.5-1.6 eq. of acetic anhydride are used). Stirring is continued at the same temperature until a homogeneous solution appeared (about 3 hours). The mixture was hold for 24-48 hours in a refrigerator without stirring. After completion of the reaction (TLC control, system hexane-ethyl acetate 4:1), the mixture was poured into ice-water (1:2) and extracted with ethyl acetate (4x30 mL). The combined organic phases were washed with sodium hydrogen carbonate solution, water and brine, dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The resulting oily mass was purified by column chromatography on silica gel, using as eluent hexane-ethyl acetate (6:1 to 3:1).

Method B: The anilide **13** is maximally dissolved in dry pyridine under stirring. For every hydroxyl group is used 1.8-2.0 eq. of pyridine, and cooled in an ice bath to 0°C. Then, freshly distillated acetic anhydride is added rapidly. For every hydroxyl group is used 1.5-1.6 eq., of acetic anhydride. Then 10 mol% of DMAP (catalyst) was added and stirring was continued at the same temperature until a homogeneous solution (about 3 hours). The mixture was hold for one week in a refrigerator without stirring. After completion of the reaction (TLC control, system hexane-ethyl acetate 4:1), the mixture was poured into ice-water (1:2) and extracted with ethyl acetate (4x30 mL). The combined organic phases were washed with sodium hydrogen carbonate solution, water and brine, dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The resulting oily was purified by column chromatography on silica gel, using as eluent hexane-ethyl acetate (6:1 to 3:1).

(2*R*,3*R*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(3,5-dibromophenylamino)tetrahydro-2H-pyran-3,4,5-triyl triacetate 14. White solid. Yield 60 %. M. p. 72-73° C (MeOH). $[\alpha]_D^{16}$ -38.45 (c 0.098, $CHCl_3$). IR-spectra (ν/cm^{-1}): 1083.8, 1059.5 (C-O-C); 1517.3 (NH); 915.3 (C6-H), 671.2 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.03 (3H, s, $\underline{CH_3}CO$), 2.06 (3H, s, $\underline{CH_3}CO$), 2.07 (3H, s, $\underline{CH_3}CO$), 2.11 (3H, s, $\underline{CH_3}CO$), 3.86 (1H, ddd, J=10.1, 6.3, 2.1, C-5-H), 4.14 (1H, dd, J=12.1, 2.1, C-6-H), 4.23 (1H, dd, J=12.1, 6.3, C-6-H), 4.68 (1H, t, J=8.8, C-1-H), 5.00 (1H, t, J=9.1, C-2-H), 5.04 (1H, t, J=9.7, C-4-H), 5.04 (1H, d, J=8.8, NH), 5.37 (1H, т, J=9.1, C-3-H), 6.74 (2H, d, J=1.5, C-2'-H, C-6'-H), 7.11 (1H, т, J=1.5, C-4'-H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 20.6 (CH_3), 20.6 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 62.3 (C-6), 68.8 (C-4), 71 (C-2), 72.5 (C-3), 72.7 (C-5), 83.6 (C-1), 115.9 (C-2', C-6'), 125.1 (C-4'), 123.2 (C-3', C-5'), 146.7 (C-1'), 169.6 (C=O), 169.9 (C=O), 170.7 (C=O), 171.3 (C=O). Calculated, %: C 41.33; H 3.99; N 2.41. $C_{20}H_{23}Br_2NO_9$. Found, %: C 41.40; H 4.01; N 2.39.

Mixture of (2*R*,3*R*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(3,5-dibromophenylamino)tetrahydro-2H-pyran-3,4,5-triyl triacetate 14 and (2*R*,3*R*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-(3,5-dibromophenylamino)-5-hydroxytetrahydro-2*H*-pyran-3,4-diyl diacetate 15 in ratio 2:1. $[\alpha]_D^{16}$ -29.44 (c 0.119, $CHCl_3$). White solid. Yield 32 %.
Minor compound has been identified as **15**. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.03 (s, $\underline{CH_3}CO$), 2.04 (s, $\underline{CH_3}CO$), 2.05 (s, $\underline{CH_3}CO$), 2.06 (s, $\underline{CH_3}CO$), 2.07 (s, $\underline{CH_3}CO$), 2.08 (d, $\underline{CH_3}CO$), 3.85-3.90 (m), 4.04-4.10 (m), 4.11-4.13 (d, J=8), 4.15-4.16 (t, J=4), 4.21-4.30 (m), 5.01-5.02 (d, J=4.5), 5.03-5.04 (d, J=4.7), 5.16-5.20 (kv), 5.30-5.32 (d, J=5.2), 5.37 (t, J=9.4), 6.74 (2H, d, J=1.6). 6.99 (2H, d, J=1.6), 7.10 (2H, d, J=1.6), 7.14 (1H, d, J=1.6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 20.6-20.8 (CH_3), 61.9, 62.2, 66.4, 68.5, 68.7, 69.5, 70.2, 71.1, 72.6, 72.6, 79.7, 83.5, (C1-C6), 115.9, 116.1 (C-2', C-6'), 123.4, 123.4 (C-4'), 125.1, 125.4 (C-3', C-5'), 146.7 147.2 (C-1'), 169.1, 169.5, 169.6, 169.9, 170.4, 170.7, 171.3 (C=O).

2,6-Dibromo-4-nitroaniline 10

To a heated solution (up to 65^{0}C) of 4-nitroaniline **9** (11 g, 0.08 mol) in 100 mL of glacial acetic acid under stirring is added drop wise a solution of bromine (26 g, 0.16 mol) in 60 mL of glacial acetic acid for 2 hours. After dropping of all bromine, the mixture was stirred for another 1.5 hours at the same temperature. The mixture was allowed to cool up to room temperature, next it was poured into a mixture, consisting of 500 mL of water and 250 g of ice and hold for 1.5 hours. The precipitate was filtered and washed 3 times with water to remove residual of acetic acid and dried at 100^0 C, getting 22 g of product (melting at 199-200^{0}C). Yield 95%. Further recrystallization from ethylene glycol monomethyl gives yellow-green crystals (prisms). Yellow-green prisms. M. p. 201-202^0 C. IR-spectra (ν/cm^{-1}): 3417, 1564 (NH_2), 1525, 1389 (NO_2), 1599 (aromatics), 695 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.69 (2H, s, NH_2), 8.22 (2H, s, C3-H and C5-H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 105.77 (C-Br), 128.3 (C-3 and C-5), 136.9 (C-4), 149.5 (C-1). Calculated, %: C 24.35; H 1.36; N 9.47. $C_6H_4Br_2N_2O_2$. Found, %: C 24.42; H 1.34; N 9.51.

3,5-Dibromonitrobenzene 11

To a heated up to 70^{0}C solution of 2,6-dibromo-4-nitroaniline **10** (20 g, 0.067 mol) in 160 mL of ethanol, concentrated sulfuric acid (11 mL) is slowly added under stirring until the mixture become a homogeneous system. Next, sodium nitrite (10 g, 0.14 mol) is added in small portions, and the mixture is stirred at the same temperature about an hour, until precipitation. After that, the heating was stopped and the mixture was stirred before room temperature. Then 300 mL of water was added, the precipitate was filtered and washed 3 times with water to remove residual sodium nitrite. Further recrystallization from ethanol gives 14 g of product **11**. The product is an orange solid. Yield 80%. M. p. 110^0 C (EtOH). IR-spectra (ν/cm^{-1}): 1528, 1336 (NO_2), 650 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.98-7.99 (1H, t, J=1.6), 8.31 (2H, d, J=1.6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 123.47 (C-2 and C-6), 125.58 (C-Br), 140.05 (C-4), 149 (C-1). Calculated, %: C 25.65; H 1.08; N 4.99. $C_6H_3Br_2NO_2$ Found, %: C 25.72; H 1.06; N 5.02.

3,5-Dibromoaniline 12

To a solution of 3,5-dibromonitrobenzene **11** (10g, 0.035 mol) in a 1:1 mixture of ethanol and THF (200 mL) tin(II) chloride dihydrate (40g, 0.175 mol) was added portionwise under stirring. The mixture was stirred at room temperature for 20 hours. After reaction solvents were removed *in vacuo*, 250 mL of water was added into remained orange liquid and dry alkali is added under stirring. Stirring was continued for 2 hours in strongly alkaline medium (pH 11-12). Next, the mixture was poured into separatory funnel, extra 150 mL of water was added. The reaction was extracted with diethyl ether (4x40 mL), the combined organic phases were washed with water to remove residues of alkali, dried over anhydrous sodium sulfate and the solvent was removed. The resulting brown mass was purified by column chromatography on silica gel, using as eluent system petroleum ether-ethyl acetate (12:1). As a result, 7.5 g of product **12** have been obtained. Light brown solid. Yield 80 %. M. p. 55-56 ^{o}C. IR-spectra (ν/cm^{-1}): 3417, 1624 (NH_2), 1581 (aromatics), 670 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.78 (2H, s, NH_2), 6.75 (2H, d, C2-H, C6-H, J=1.5), 7.02 (1H, t, C4-H, J=1.5). ^{13}C NMR (100.6 MHz, $CDCl_3$): 116.51 (C-2 and C-6), 123.36 (C-Br), 123.70 (C-4), 148.64 (C-1). Calculated, %: C 28.72; H 2.01; N 5.58. $C_6H_5Br_2N$ Found, %: C 28.77; H 2.00; N 5.60.

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References

1. Kleeblatt, D.; Siyo, B.; Hein, M.; Iaroshenko, V.O.; Iqbal, J.; Villingera, A.; Langer, P. Synthesis of N,N'-diglycosylated isoindigos. Organic and Biomolecular Chemistry, 2013, 11, pp. 886-895.
2. Erben, F.; Kleeblatt, D.; Sonneck, M.; Hein, M.; Feist, H.; Fahrenwaldt, T.; Fischer, C.; Matin, A.; Iqbal, J.; Plötz, M.; Eberlee, J.; Langer, P. Synthesis and antiproliferative activity o selenoindirubins and selenoindirubin-N-glycosides. Organic and Biomolecular Chemistry, 2013, 11, pp. 3963-3978.
3. Kamano, Y.; Zhang, H.P.; Ichihara, Y.; Kizu, H.; Komiyama, K.; Pettit, G. R. Convolutamydine A, a novel bioactive hydroxyoxindole alkaloid from marine bryozoans *Amathia convoluta*. Tetrahedron Letters, 1995, 36, pp. 2783-2784.
4. Maskey, R.P.; Grün-Wollny, I.; Fiebig, H.H.; Laatsch, H. Akashins A, B, and C: Novel Chlorinated Indigoglycosides from *Streptomyces* sp. GW 48/1497. Angewandte Chemie International Edition, 2002, 41, pp. 597-599.
5. Maskey, R.P.; Grün-Wollny, I.; Laatsch, H. Isolation and structure elucidation of diazaquinomycin C from a terrestrial *Streptomyces* sp. and confirmation of the akashin structure. Natural Product Research, 2005, 19, pp. 137-142.
6. Zhang, H.P.; Kamano, Y.; Ichihara, Y.; Kizu, H.; Komiyama, K.; Itokawa, H.; Pettit, G.R. Isolation and structure of convolutamydines B -D from marine bryozoans *Amathia convoluta*. Tetrahedron, 1995, 51, pp. 5523-5528.