The First N-Glycosylated Indoxyls and Their Application to the Synthesis of Indirubin-*N*-glycosides (Purple Sugars)

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Abstract: The first indirubin-*N*-glycosides (purple sugars), containing a sugar moiety located at the amine-type nitrogen atom, are prepared by condensation of hitherto unknown indoxyl-*N*-glycosides with isatines.

Key words: carbohydrates, heterocycles, indirubin, indol

Glycosylated indoles are of great pharmacological importance. For example, it has been shown that the natural products staurosporine, K-252d, rebeccamycin, and the tjipanazoles exhibit a strong cancerostatic activity against various human cancer cell lines.[1,2] Indigo, indirubin, and isoindigo are prominent molecules containing a bisindole framework. Until recently, these compounds and their derivatives received attention mainly due to their pigment properties. Glycosylated derivatives of indigo, indirubin, and isoindigo are relatively rare. Recently, we reported[3] the synthesis of indigo-*N*-glycosides (blue sugars) which are present in the natural products akashine A, B, and C isolated from *Streptomyces sp. GW48/1497*.[4] The akashines show a remarkable cancerostatic activity against various human cancer cell lines. In contrast, parent indigo is pharmacologically inactive. Moreau and coworkers reported the synthesis of isoindigo-*N*-glycosides, which also possess a considerable antiproliferative activity and kinase inhibitory potency.[5]

In recent years, there has been a dramatically renewed interest in indirubin and its derivatives, due to the discovery of their great pharmacological relevance.[6] Indirubin is the active ingredient of the traditional Chinese medicinal recipe *Danggui Longhui Wan* which has been used for a long time for the treatment of myelocytic leukemia.[7] Meijer and coworkers have recently shown that indirubin derivatives selectively inhibit cyclin-dependent kinases (CDKs), which represent important components of cell cycles taking place in many human tumors, and thus have a great potential for the treatment of cancer.[8,9]

Indirubin derivatives contain an amine- and an amide-type nitrogen atom. Recently, we reported[10] the first synthesis of indirubin-*N*′-glycosides (red sugars) containing a carbohydrate moiety located at the amide-type nitrogen (Figure 1). The synthesis was carried out based on the condensation of isatin-*N*-glycosides with indoxyls. Indirubin-*N*′-glycosides were shown to exhibit a strong antiproliferative activity against various human cancer cell lines which is considerably higher than the activity of the simple aglycons.[11]

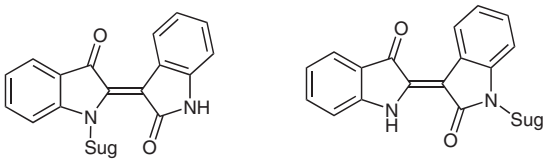

Figure 1 Structure of N- and N′-glycosylated indirubins (Sug = glycosyl moiety)

Herein, we report what is, to the best of our knowledge, the first synthesis of indirubin-*N*-glycosides (purple sugars). These molecules contain a sugar moiety attached to the amine-type nitrogen atom (Figure 1). Our synthetic strategy relies on the synthesis of hitherto unknown indoxyl-*N*-glycosides and their condensation with isatines.

It is expected that indirubin-*N*-glycosides will exhibit a considerable activity as CDK inhibitors. In fact, the hydrogen bonds in the ATP binding site should be considerably different for indirubin-*N*- and indirubin-*N*′-glycosides.

The synthesis of N-glycosylated indoxyl derivatives is carried out as follows (Scheme 1). The reaction of L-rhamnose **1**α/β with indoline afforded **2**α/β (β/α = ca. 4:1) which was transformed into the anomerically pure indol-*N*-glycoside **3**β by dehydrogenation (DDQ). Benzylation and methylation of **3**β afforded **4**β and **5**β, respectively. The iodination of simple indoles has been previously reported to give 3-iodoindoles in nearly quantitative yields.[12] To our satisfaction, the iodination of **4**β and **5**β proceeded smoothly to give **6**β and **7**β, respectively. Due to the basic reaction conditions (I_2, NaOH, DMF), ether rather than ester protective groups had to be employed. The reaction of 3-iodoindoles **6**β and **7**β with an excess of silver acetate under mild acidic conditions resulted in formation of the desired N-glycosylated indoxyl-3-acetates **8**β and **9**β as colorless crystals in good yields, respectively.[13] The glycosylated oxindoles **10**β and **11**β were isolated as byproducts (Figure 2).[14,15] The structures of all products were established by spectroscopic studies (2D NMR). The

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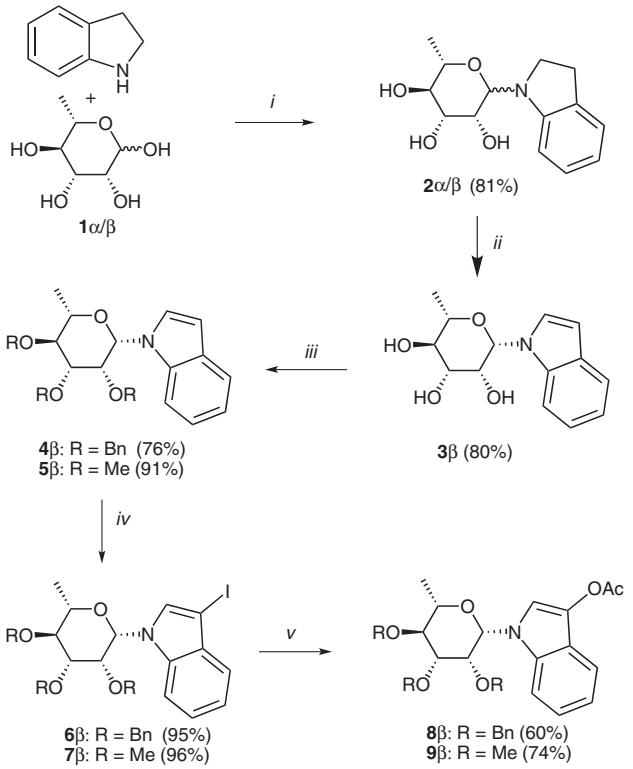


Scheme 1 Synthesis of glycosylated indoxyl-3-acetates **8**β and **9**β. *Reagents and conditions*: (*i*) EtOH, 20 °C, 12 h; (*ii*) DDQ, dioxane, 20 °C, 12 h; (*iii*) BnBr (for **4**β) or MeI (for **5**β), NaH, DMF, 0 °C to 4 °C, 12 h; (*iv*) I_2, NaOH, DMF, 20 °C, 1 h; (*v*) AgOAc, AcOH, 80 °C, 4 h.

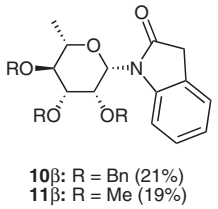
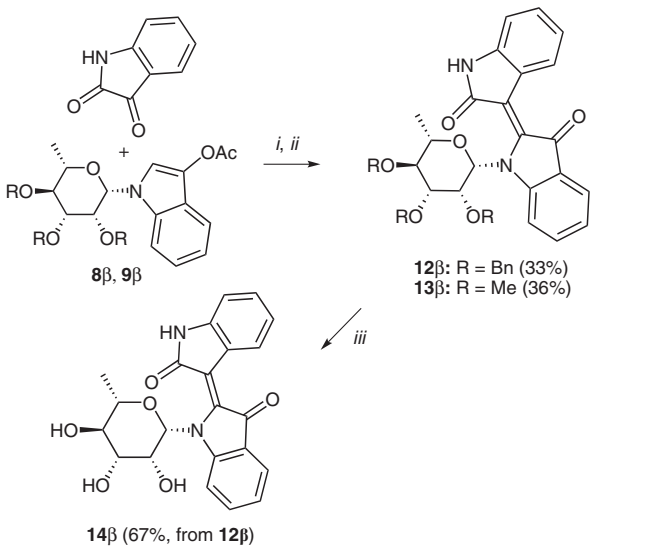


Figure 2 Structure of side products **10**β and **11**β

structures of **9**β and **11**β were independently confirmed by X-ray crystal structure analysis.[16]

It was hoped that the aldol-type condensation of isatine with indoxyl-*N*-glycosides, generated in situ by deacetylation of **8**β or **9**β, would result in the formation of the desired indirubin-*N*-glycosides. However, all attempts to realize this one-pot transformation, using standard conditions for the deacetylation (basic conditions: KO*t*-Bu, Na_2CO_3; acidic conditions: MeOH, HCl), resulted in the formation of complex mixtures. This can be explained by the unstable nature of the products under these conditions.

We have been finally successful by application of an alternative protocol for deacetylation and isolation of the free indoxyl-*N*-glycosides. The acetyl groups of **8**β and **9**β were selectively removed under slightly basic and reductive conditions (Na_2SO_3, dioxane, H_2O).[17,18] The deacetylation of the methyl-protected indoxyl-3-acetate **9**β was already complete after stirring for three hours at 80 °C.



Scheme 2 Synthesis of the deprotected indirubin-*N*-glycoside **14**β. *Reagents and conditions*: (*i*) Na_2SO_3, dioxane, H_2O (for **12**β: 110 °C, 2 d; for **13**β: 80 °C); (*ii*) piperidine, benzene, 80 °C, 2 h; (*iii*) BBr_3, CH_2Cl_2, –78 °C, 3.5 h.

The complete deacetylation of benzyl-protected indoxyl-3-acetate **8**β required stirring in a pressure tube at 110 °C for two days, presumably due to steric reasons. In both cases, the slightly yellow-colored free indoxyl-*N*-rhamnoside could be detected as a fluorescent spot by TLC under UV light. Because of the apparent instability of these compounds in the presence of air, the crude materials were directly used for the next reaction step after a short workup using thoroughly degassed solvents under an argon atmosphere. The reaction of the crude deacetylated indoxyl-*N*-rhamnosides with isatine, in the presence of a catalytic amount of piperidine[19–21] resulted in the formation of the desired products **12**β and **13**β, respectively, which were isolated as purple solids (Scheme 2).

All attempts to deprotect methylated derivative **13**β failed. In contrast, treatment of benzyl-protected derivative **12**β with BBr_3 resulted in formation of the desired deprotected indirubin-*N*-glycoside **14**β which was isolated as a purple solid in up to 67% yield.[22]

The structures of compounds **12**β, **13**β, and **14**β were elucidated by one-dimensional (^{1}H, ^{13}C, DEPT) and two-dimensional NMR studies (COSY, CORR, HMBC, NOESY). The *Z*-configuration of the double bond between the oxindole and the indoxyl moiety is supported by the downfield shift observed for protons H-4′ and H-2″ (due to their interaction with the carbonyl oxygen atoms). In addition, the *Z*-configured product represents the thermodynamically more stable isomer, and its formation can be expected under the thermodynamic reaction conditions employed.

In conclusion, the first indirubin-*N*-glycosides (purple sugars), containing a sugar moiety located at the amine-type nitrogen atom, have been prepared by condensation of hitherto unknown indoxyl-*N*-glycosides with isatines. The preparative scope of the synthetic strategy is currently

being studied. Preliminary results suggest that different isatins and N-glycoslyated indoxyls can be successfully employed.

Acknowledgement

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(13) **General Procedure for the Synthesis of N-Glycosylated Indoxyl-3-acetates**

Silver acetate (6.0 equiv) was added to a stirred solution of 3-iodoindolylrhamnoside in glacial acetic acid. The resulting suspension was stirred at 80 °C for 4 h. After the reaction was completed, the solution was allowed to cool to r.t., and ice water was subsequently added. The suspension was filtered, and the filtrate was extracted with EtOAc (3×). The combined organic layers were washed with an aq solution of $NaHCO_3$ and H_2O and dried over Na_2SO_4. The solution was filtered and the solvent of the filtrate was removed under reduced pressure. The residue was purified by column chromatography.

3-Acetoxy-1-(2′,3′,4′-tri-*O*-benzyl-β-L-rhamnopyranosyl) indole (8β)

Starting with **6**β (2.30 g, 3.5 mmol), AcOH (20 mL), and AgOAc (3.50 g, 21.0 mmol), **8**β was isolated after column chromatography (heptanes–EtOAc = 10:1 → 6:1) as colorless crystals (1.23 g, 60%), mp 125–126 °C; $[\alpha]_D^{22}$ –24.24 (*c* 1.13, $CHCl_3$); R_f = 0.36 (heptanes–EtOAc = 3:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.66 (s, 1 H, H-2); 7.54 (m, ^3J = 7.6 Hz, 1 H, Hetar), 7.39–7.10 (m, 18 H, Hetar, Ph), 5.55 (d, $^3J_{1',2'}$ = 0.9 Hz, 1 H, H-1′), 5.01, 4.73 (2 d, $^2J_{Ha,Hb}$ = 10.9 Hz, 2 H, CH_2Ph), 4.68 (s, 2 H, CH_2Ph), 4.41, 4.26 (2d, $^2J_{Ha,Hb}$ = 11.1 Hz, 2 H, CH_2Ph), 4.00 ('dd', $^3J_{1',2'}$ = 0.9 Hz, $^3J_{2',3'}$ = 2.4 Hz, 1 H, H-2′), 3.80–3.75 (m, $^3J_{2',3'}$ = 2.3 Hz, $^3J_{4',5'}$ = 9.3 Hz, 2 H, H-3′, H-4′), 3.67–3.55 (m, $^3J_{5',6'}$ = 6.1 Hz, $^3J_{4',5'}$ = 9.3 Hz, 1 H, H-5′), 2.36 [s, 3 H, C(O)CH_3], 1.42 (d, $^3J_{5',6'}$= 6.1 Hz, 1 H, H-6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.4 [*C*(O)CH_3], 138.3, 138.0, 137.7 (C_qPh), 132.2, 130.3 (C_q-Hetar), 128.4, 128.1, 128.0, 127.7, 127.5 (*C*HPh), 122.4 (CH-Hetar), 120.9 (C_q-Hetar), 120.0, 117.8 (2 s, 2 × CH-Hetar), 116.0 (C-2), 109.5 (CH-Hetar), 83.1, 82.9, 79.6, 76.0, (C-1′, C-2′, C-3′, C-4′), 75.5 (*C*H_2Ph), 75.0 (C-5′), 74.6, 72.3 (*C*H_2Ph), 20.9 [C(O)*C*H_3], 18.1 (C-6′). MS (EI, 70 eV): *m/z* (%) = 591 (82) [M$^+$], 549 (20) [benzylated indoxyl-*N*-rhamnoside$^+$], 133 (16) [indoxyl$^+$], 91 (100) [Bn$^+$]. HRMS (EI, 70 eV): *m/z* calcd for $C_{37}H_{37}NO_6$ [M$^+$]: 591.26154; found: 591.26148. In addition, **10**β (0.41 g, 21%) was isolated as a byproduct, R_f = 0.23 (heptane–EtOAc = 3:1).

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(18) **General Procedure for the Synthesis of Indirubin-*N*-glycosides**
To a stirred dioxane solution of N-glycosylated indoxyl-3-acetate was added an aq solution of Na_2CO_3. The reaction mixture was stirred at 80 °C. After the reaction was completed, a fluorescent spot was visible on the TLC plate. Ice-water was added, and the mixture was extracted by degassed EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and the solvent of the filtrate was removed under reduced pressure. The residue was dissolved in degassed benzene and isatine (2.0 equiv), and a catalytic amount of piperidine was added. The solution was stirred at 80 °C for 2 h. After completion of the reaction, the mixture was diluted with toluene. The solvent was removed under reduced pressure, and the residue was purified by column and thin-layer chromatography (Figure 3).
***N*-(2″,3″,4″-Tri-*O*-benzyl-β-L-rhamnopyranosyl) indirubin 12β**
Starting with **8**β (250 mg, 0.42 mmol), dioxane (5 mL), and Na_2SO_3 (160 mg, 1.27 mmol, dissolved in 5 mL of H_2O) and isatine (124 mg, 0.84 mmol), **12**β was isolated as a purple solid (95 mg, 33%). The purification was carried out by column chromatography (heptanes–EtOAc = 9:1 → 4:1) and TLC (heptanes–EtOAc = 3.5:1 → 1:1), mp 93–95 °C (heptanes–EtOAc); R_f = 0.37 (heptanes–EtOAc = 3:1). ^{1}H NMR (300 MHz, C_6D_6): δ = 8.78 (dd, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{4,5}$ = 7.7 Hz, 1 H, H-4′), 8.36 (d, $^3J_{6,7}$ = 8.4 Hz, 1 H, H-7), 7.82 (s, 1 H, NH), 7.60 (dd, $^4J_{4,6}$ = 1.2 Hz, $^3J_{4,5}$ = 7.4 Hz, 1 H, H-4), 7.52 (m, ^3J = 7.7 Hz, 2 H, Hetar), 7.39–6.84 (m, 15 H, Hetar), 6.63 (‘t’, ^3J = 7.3 Hz, 1 H, Hetar), 6.28 (‘d’, $^3J_{6',7'}$ = 7.4 Hz, 1 H, H-7′), 5.65 (d, $^3J_{2'',3''}$ = 2.2 Hz, 1 H, H-2″), 5.56 (s, 1 H, H-1″), 5.07, 4.59 (2 d, $^2J_{Ha,Hb}$ = 11.4 Hz, 2 H, CH_2Ph), 4.87, 4.62 (2 d, $^2J_{Ha,Hb}$ = 10.9 Hz, 2 H, CH_2Ph), 4.86, 4.79 (2d, $^2J_{Ha,Hb}$ = 11.9 Hz, 2 H, CH_2Ph), 3.95 (dd, $^3J_{2'',3''}$ = 2.4 Hz, $^3J_{3'',4''}$ = 9.5 Hz, 1 H, H-3″), 3.87 (‘t’, $^3J_{3'',4''}$ = $^3J_{4'',5''}$ = 9.2 Hz, 1 H, H-4″), 3.31 (dq, $^3J_{5'',6''}$ = 6.2 Hz, $^3J_{4'',5''}$ = 8.8 Hz, 1 H, H-5″), 1.23 (d, $^3J_{5'',6''}$ = 6.2 Hz, 3 H, H-6″). ^{13}C NMR (75 MHz, C_6D_6): δ = 187.4 (C-3), 169.2, 152.2, 144.0, 140.1 (4 s, C-2, C-2′, C-7a, C-7a′), 139.3, 139.1, 138.8 (3 s, 3 × C_qPh), 135.5 (C-6), 129.9 (C-6′), 129.1, 128.6, 128.6, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.1, 128.1, 128.1, 127.9, 127.8, 127.7 (15 s, 15 × *C*HPh), 126.9 (C-4′), 123.9 (C-4), 123.6, 122.6 (C-3a, C-3a′), 122.9 (C-5), 121.9 (C-5′), 120.4 (C-7), 112.0 (C-3′), 109.3 (C-7′), 89.9 (C-1″), 83.9 (C-3″), 79.8 (C-4″), 78.3 (C-2″), 75.4 (C-5″), 75.6, 75.3, 71.9 (3 s, 3 × *C*H_2Ph), 18.2 (C-6″). MS (EI, 70eV): *m/z* (%) = 678 (17) [M^+], 548 (9) [benzylated *N*-rhamnosyl indolone$^+$], 262 (83) [indirubin$^+$], 91 (100) [Bn^+]. HRMS (EI, 70 eV): *m/z* calcd for $C_{43}H_{38}N_2O_6$ [M^+]: 678.27244; found: 678.27242.

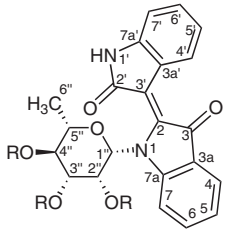

Figure 3

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(22) **Synthesis of *N*-β-L-Rhamnopyranosylindirubin (14β)**
To a cooled (–78 °C) CH_2Cl_2 solution (8 mL) of **12**β (90 mg, 0.133 mmol) was added BBr_3 (1 M solution in CH_2Cl_2, 2.0 mmol). After stirring for 3.5 h at –78 °C, an aq solution of $NaHCO_3$ was added at –78 °C. The mixture was allowed to warm to 20 °C and was extracted with EtOAc. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$–EtOH = 20:1 → 5:1) to give **14**β (36 mg, 67%) as a purple solid, mp 148–151 °C; R_f = 0.39 ($CHCl_3$–EtOH = 5:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 10.70 (s, 1 H, NH), 8.33 (d, $^3J_{4',5'}$ = 8.1 Hz, 1 H, H-4′), 8.20 (d, $^3J_{6,7}$ = 8.3 Hz, 1 H, H-7), 7.58 (d, $^3J_{4,5}$ = 7.6 Hz, 1 H, H-4), 7.53 (‘dt’, $^4J_{4,6}$ = 1.4 Hz, $^3J_{5,6}$ = $^3J_{6,7}$ = 7.9 Hz, 1 H, H-6), 7.22 (‘dt’, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{5',6'}$ = $^3J_{6',7'}$ = 7.6 Hz, 1 H, H-6′), 7.08 (t, $^3J_{4,5}$ = $^3J_{5,6}$ = 7.5 Hz, 1 H, H-5), 6.90 (‘t’, $^3J_{4',5'}$ = $^3J_{5',6'}$= 7.6 Hz, 1 H, H-5′), 6.84 (d, $^3J_{6',7'}$ = 7.7 Hz, 1 H, H-7′), 5.49 (s, H-1″), 5.23 (d, $^3J_{x,OH}$ = 5.0 Hz, 1 H, OH), 4.83 (s, 1 H, OH), 4.75 [s(br), 2 H, H-2″, OH], 3.50–3.25 (m, 3 H, H-3″, H-4″, H-5″), 1.17 (d, $^3J_{5'',6''}$ = 5.1 Hz, 3 H, H-6″). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 187.6 (C-3), 168.1 (C-2′), 153.0 (C-7a), 142.4 (C-2), 141.6 (C-7a′), 135.7 (C-6), 130.3 (C-6′), 125.6 (C-4′), 123.3 (C-4), 122.7, 121.5 (C-3a, C-3a′), 122.5 (C-5), 120.7 (C-5′), 120.5 (C-7), 112.8 (C-3′), 109.5 (C-7′), 89.6 (C-1″), 75.5, 73.7, 71.8 (C-3″, C-4″, C-5″), 71.4 (C-2″), 18.3 (C-6″). MS (EI, 70eV): *m/z* (%) = 408 (3) [M^+], 262 (100) [indirubin$^+$]. HRMS (EI, 70 eV): *m/z* calcd for $C_{22}H_{20}N_2O_6$ [M^+]: 408.13159; found: 408.13156.

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