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Synthesis of indirubin-*N*′-glycosides and their anti-proliferative activity against human cancer cell lines

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Abstract—The first indirubin-*N*′-glycosides were prepared based on reactions of isatin-*N*′-glycosides with indoxyls. The products show a significant anti-proliferative activity against various human cancer cell lines. Good results were observed for an indirubin-*N*′-mannoside which was shown to have medium to high anti-proliferative activity against all investigated cell lines. The highest activities and selectivities against the MCF-7 breast cancer cell line were observed for indirubin-*N*′-rhamnosides.

1. Introduction

In recent years, there has been a dramatic renewal of the interest in indirubin—the red isomer of the well-known dye indigo—due to the discovery of its great pharmacological potential.[1] The pharmacological activity of indirubin has been known for a long time. In fact, it is the active ingredient of the traditional Chinese medicinal recipe *Danggui Longhui Wan* for the treatment of myelocytic leukemia.[2] Recently, Meijer and coworkers showed 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.[3,4] The anti-proliferative effect of indirubin on human cancer cells is based on the inhibition of genes or proteins which regulate cell cycle progression.[5] The inhibition of GSK-3β and CDK5 by indirubin derivatives, important for treatment of Alzheimer's disease, has also been studied.[6,7] Indirubin-3′-monoxime was shown to influence $p27^{Kip1}$ transcription and to inhibit C-Jun NH2-terminal kinase, which plays an important role in neuronal apoptosis.[8–10] In addition, it was reported that indirubin-3′-monoxime can selectively stop centrosome duplication in tumor cells without affecting normal cells.[4] The anti-proliferative activity of substituted indirubin derivatives against various cancer cell lines and CDK2 inhibitory activity have recently been studied.[11] Some years ago, Laatsch et al. reported the isolation of *N*-glycosides of indigo.[12] In contrast to pharmacologically inactive indigo, the indigo-*N*-glycosides (akashines) show a significant antiproliferative activity against various human cancer cell lines. Owing to the strong activity of the akashines, we set up a program directed towards the synthesis of *N*-glycosides of indigo (blue sugars)[13] and of indirubin (red sugars).[14] Herein, we report full details of the synthesis of indirubin-*N*′-glycosides. With respect to our preliminary communication in this field,[14] we studied the scope of our synthetic strategy and disclose, for the first time, the anti-proliferative activity of various indirubin-*N*′-glycosides on human cancer cells in vitro, which is considerably higher than the activity of the corresponding non-glycosylated indirubins.

2. Results and discussion

2.1. Chemistry

Indirubins are available by reaction of a methanol solution of indoxyl acetate with isatines under oxygen-free

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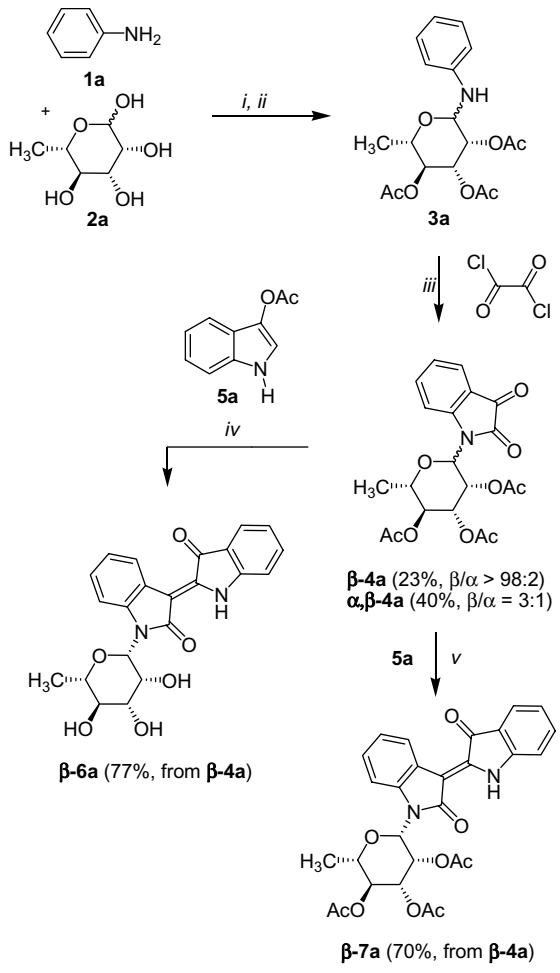
atmosphere. This transformation could be successfully employed for the synthesis of indirubin-*N*-glycosides. The starting materials, *N*-glycosyl isatines **4a**–**e**, were prepared from rhamnose, glucose, galactose and mannose in three steps (Scheme 1). The reaction of the free sugars with different anilines and subsequent acetylation afforded the *N*-glycosyl anilines **3a**–**e**.[15] The $AlCl_3$-mediated cyclization[16] of **3a**–**e** with oxalyl chloride afforded the *N*-glycosyl isatines **4a**–**e**. The isatin-*N*-rhamnosides were formed as separable mixtures of anomers **α-4a** and **β-4a**. The major isomer, **β-4a**, could be readily isolated in anomerically pure form in good yield. The α-anomer could be isolated by chromatographic separation of the combined anomerically enriched fractions of several reaction batches. Isatine-*N*-rhamnoside **4b** was prepared in good yield as a mixture of anomers (β/α > 3.5:1). Recrystallization gave the pure β-anomer **β-4b**. The reaction of the latter with **5a** afforded the indirubin-*N*-rhamnoside **β-6b**. The cyclization of oxalyl chloride with *N*-glycosyl anilines **3c**–**e**, employed as isomerically enriched β-anomers (β/α > 5:1), gave isatine-*N*-glucoside **β-4c**, isatine-*N*-galactoside **β-4d**, and isatine-*N*-mannoside **β-4e**. All products were isolated as the pure β-anomers. Only traces of α-anomers were formed in these reactions. The configurations of all isatine-*N*-glycosides were established by spectroscopic methods. The molecular structures of rhamnosyl derivatives **β-4a** and **β-4b** were independently confirmed by X-ray structure analysis (Figs. 3 and 4).



Scheme 1. Synthesis of *N*-glycosyl indirubines. Reagents and conditions: (i) EtOH, rt, 12 h; (ii) Ac_2O, pyridine, 0–4 °C, 12 h; (iii) $AlCl_3$, 55 °C, 1.5 h; (iv) Na_2CO_3, MeOH, 20 °C, 4 h; (v) 1—Na_2CO_3, MeOH, 20 °C, 4 h; 2—Ac_2O/pyridine = 1:1, 0 °C, 12 h.

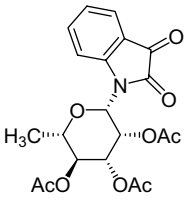
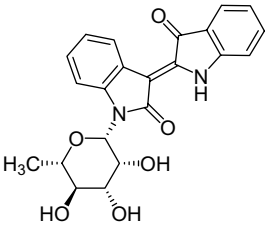
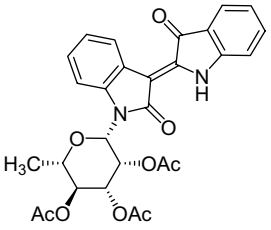
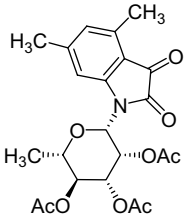
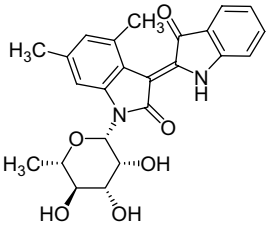
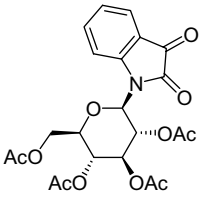
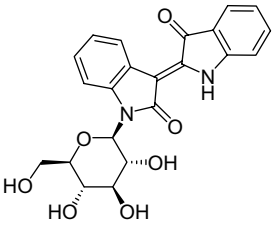
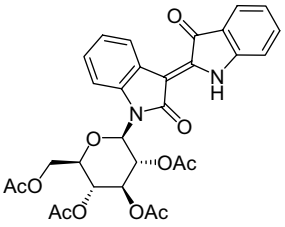
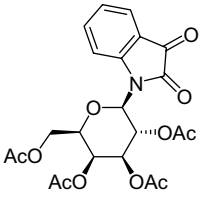
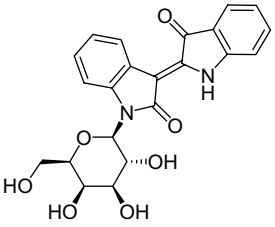
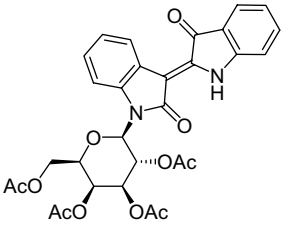
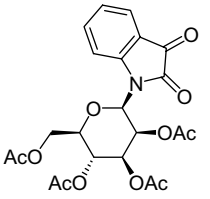
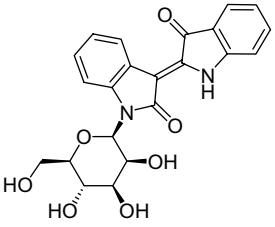
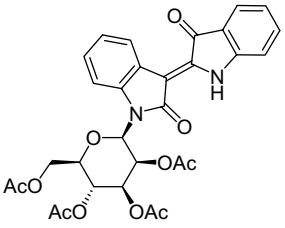
The Na_2CO_3-mediated reaction of indoxyl acetate (**5a**) with isatine-*N*-glycoside **β-4a** (MeOH, 20 °C, 4 h) afforded the deprotected *N*′-(β-L-rhamnopyranosyl)indirubin **β-6a** in 77% yield (Scheme 1, Table 1). During the optimization of this reaction, the use of an excess of sodium carbonate proved to be important to achieve a complete cleavage of the acetyl protective groups of the sugar moiety. For the sake of a convenient structural elucidation, **β-6a** was transformed, by treatment with acetic anhydride/pyridine, into its tetra-*O*-acetate **β-7a**. The reaction of **5a** with **β-4b** gave the deprotected *N*′-(β-L-rhamnopyranosyl)indirubin **β-6b**. The condensation of **5a** with **β-4c**, **β-4d**, and **β-4e** gave rise to the formation of *N*′-(β-D-gluco-pyranosyl)indirubin (**β-6c**), *N*′-(β-D-galactopyranosyl)-indirubin (**β-6d**), and *N*′-(β-D-mannopyranosyl)indirubin (**β-6e**) which were transformed into their acetylated derivatives **β-7c**, **β-7d**, and **β-7e**, respectively.

The Na_2CO_3-mediated reaction of isatine-*N*-rhamnoside **β-4a** with chlorinated indoxyl acetate **5b**[17] and subsequent acetylation of the crude product afforded the tetra-*O*-acetylated indirubin-*N*′-rhamnoside **β-7f** in 63% yield (Scheme 2). The acetylation was necessary, as a mixture of partially deprotected products was formed during the condensation. Stirring of a methanol solution of **β-7f** in the presence of a catalytic amount of KO^{t}Bu (0.06 equiv) gave the deprotected rhamnoside **β-6f** in 72% yield.

The reaction of isatine-*N*-rhamnoside **α-4a** with indoxyl acetates **5a** and **5b** and subsequent acetylation gave the tetra-*O*-acetylated indirubin-*N*′-rhamnosides **α-7a** and **α-7f**, respectively (Scheme 3). The KO^{t}Bu-catalyzed deacetylation of the latter afforded the deprotected rhamnosides **α-6a** and **α-6f**. The rhamnosylated indirubin-3-oxime **β-9a** was prepared by reaction of *O*-acetylated indirubin-*N*′-glycoside **β-7a** with hydroxylamine hydrochloride (to give **β-8a**) and subsequent deprotection (Scheme 4). The glycosylated oxime **β-9a** was prepared in order to compare its anti-proliferative activity with that of the aglycon (*vide infra*). Crystals of the *O*-acetylated indirubin-3-oxime derivative **β-8a** were suitable for X-ray structural analysis (Fig. 5).

The configuration and conformation of the products was studied in detail by NMR spectroscopy. The assignment of the signals was established by DEPT and two-dimensional $^1H,^1H$ COSY and $^1H,^{13}C$ correlation spectra (HETCOR, HSQC). In addition, $^1H,^1H$ NOESY and $^1H,^{13}C$ HMBC spectra were recorded for compounds **β-4e**, **β-6c**, **β-7a**, and **β-7c**. For example, in the HMBC spectrum of **β-6c** the following correlations are found: NH with C-2, C-3, C-3a, and C-7a; H-4′ with C-3′, C-6′, and C-7a′; H-6′ with C-7a′; H-4 with C-6 and C-7a; H-6 with C-7a. In the NOESY spectrum of **β-6c**, NOE cross peaks are observed for protons H-5

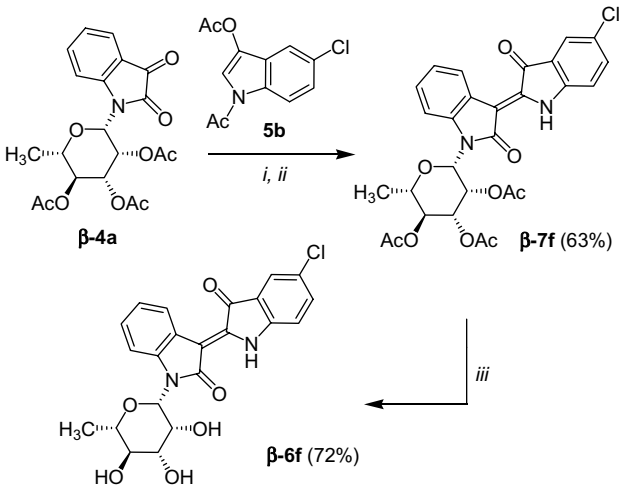
Table 1. Synthesis of *N*′-glycosyl indirubines from *N*-glycosyl anilines

Series	% (**4**)[a]	% (**6**)[a]	% (**7**)[a]
L-Rhamno			
	β-4a (23%, β/α > 98:2) **α,β-4a** (40%, β/α = 3:1)	**β-6a** (77%, β/α > 98:2)	**β-7a** (70% based on **β-4a**, β/α > 98:2)
L-Rhamno			
	α,β-4b (76%, β/α = 3.5:1)	**β-6b** (62%, β/α > 98:2)	
D-Gluco			
	β-4c (30%, β/α > 98:2)[16]	**β-6c** (70%, β/α > 98:2)	**β-7c** (75% based on **β-6c**, β/α > 98:2)
D-Galacto			
	β-4d (52%, β/α > 98:2)	**β-6d** (73%, β/α > 98:2)	**β-7d** (70% based on **β-6d**, β/α > 98:2)
D-Manno			
	β-4e (21%, β/α > 98:2)	**β-6e** (59%, β/α > 98:2)	**β-7e** (60% based on **β-6e**, β/α > 98:2)

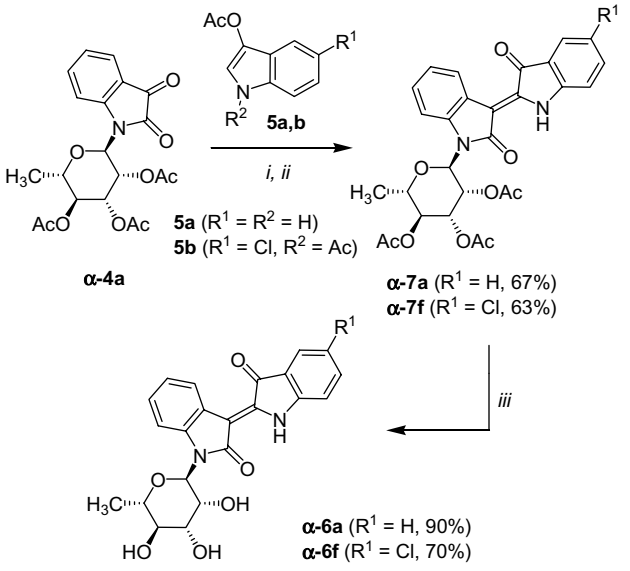
[a] Yields of isolated products.

with H-4 and H-6; H-5′ with H-4′ and H-6′; H-7′ with H-1″ and H-4″; H-5″ with H-1″, H-3″ and H-6″. These findings confirm the assignments given for the ^{1}H and ^{13}C NMR signals of the indirubin and the sugar moiety. Furthermore, the stereochemistry of the pyranosyl ring was proven by the NOE's found in the NOESY spectrum for the protons H-1″, H-3″, and H-5″ (Fig. 1).

The β-configuration at carbon atom C-1″ is also confirmed by the coupling constant $^{3}J_{1'',2''} = 9.5$ Hz for H-1″ and H-2″ indicating a bis-axial position of these protons. Based on the coupling constants $^{3}J_{1'',2''}$ ($^{3}J_{1',2'}$ for **β-4d**) ~9 Hz the D-*gluco* and D-*galacto* pyranosides can be considered as β-anomers with a $^{4}C_{1}$ conformation. In case of the D-*manno* derivatives **β-4e**, **β-6e**, and **β-7e**, exhibiting smaller coupling constants ($^{3}J < 1.5$ Hz), the anomeric configuration could not be assigned based on the analysis of the coupling constants. However, the β-configuration could be unambigiously assigned based on NOESY measurements carried out for **β-4e**. The cor-



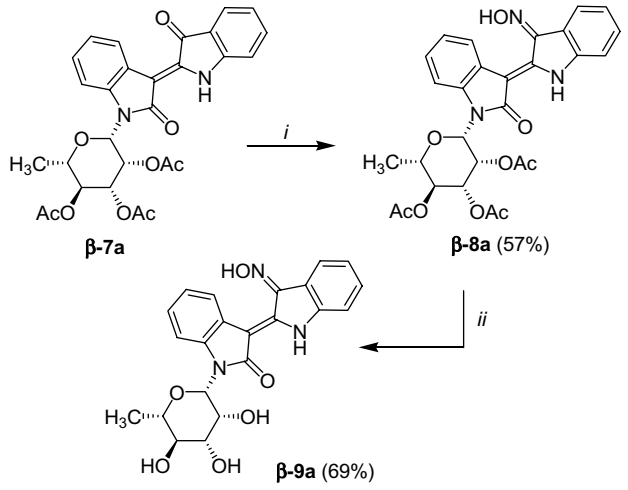
Scheme 2. Synthesis of the indirubin-*N*′-rhamnoside **β-6f**. Reagents and conditions: (i) Na_2CO_3, MeOH, 20 °C, 2 h; (ii) Ac_2O, pyridine, 0 °C, 12 h; (iii) KO′Bu (cat.), MeOH, 20 °C, 12 h.



Scheme 3. Synthesis of **α-6a** and **α-6f**. Reagents and conditions: (i) Na_2CO_3, MeOH, 20 °C, 2 h; (ii) Ac_2O, pyridine, 0–4 °C, 12 h; (iii) KO′Bu, MeOH, 20 °C, 12 h.

relations of proton H-1′ with H-3′ and H-5′ prove an axial position of these protons.

Those L-*rhamno* derivatives, which show small vicinal coupling constants [i.e., $^3J_{1'',2''} < 1.6$ Hz (for **β-6a**, **6b**, **β-6f**, **β-7a**, **7b**, **β-7f**), and $^3J_{1',2'} = 1.5$ Hz (for **β-4a**, **β-4b**)], have to be assigned to β-anomeric structures with $^1C_4$ conformation. The $\beta - {}^1C_4$ conformation of **β-7a** was independently confirmed by a NOESY experiment in which relevant NOE correlations were observed for protons H-1″, H-3″, and H-5″ proving their axial position (Fig. 1). Those L-rhamnosides, which exhibit larger vicinal coupling constants [i.e., $^3J_{1'',2''} = 8.5$ Hz (**α-6a**, **α-6f**), $^3J_{1'',2''} = 4.6$–4.7 Hz (**α-7a**, **α-7f**), and $^3J_{1',2'} = 5.7$ Hz (**α- 4a**)], have to be assigned to the α-anomers. The coupling constants $^3J_{1'',2''} = 8.5$ Hz of the deprotected



Scheme 4. Synthesis of the glycosylated indirubin-3-monoxim **β-9a**, Reagents and conditions: (i) $H_2NOH{\cdot}HCl$, pyridine, 90 °C, 7 h, (ii): KO′Bu, MeOH, 20 °C, 12 h.

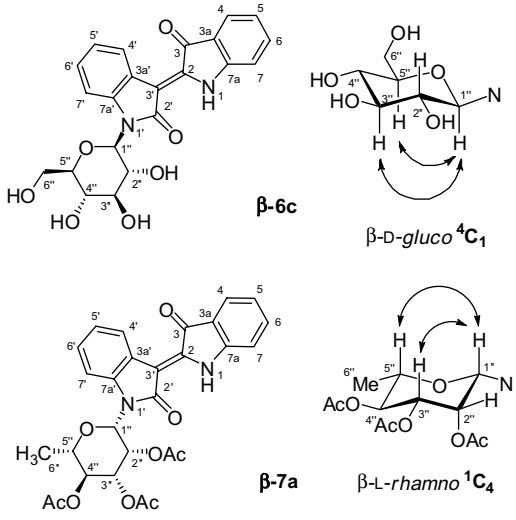


Figure 1. Numbering of the atoms for NMR assignment and relevant NOE correlations for **β-6c** and **β-7a**.

rhamnosides **α-6a** and **α-6f** clearly indicate a bis-axial position of protons H-1″ and H-2″ confirming a $^4C_1$ conformation (Fig. 2). In contrast, the protected derivatives **α-4a**, **α-7a**, and **α-7f** seem to prefer a twisted boat conformation. This can be concluded from the smaller coupling constants $^3J_{1'',2''}$($^3J_{1',2'}$ for **α-4a**) = 4.6–5.7 Hz as well as from the increase of the coupling constants

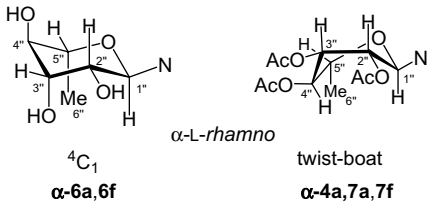


Figure 2. Configurations and conformations of acetylated and deacetylated isatine- and indirubin-*N*′-β-L-rhamnopyranosides.

$^3J_{3'',4''} \sim {}^3J_{4'',5''}$ ($^3J_{3',4'} \sim {}^3J_{4',5'}$ for **α-4a**) $\cong$ 7 Hz compared to the corresponding deprotected indirubin-*N*′-rhamnosides. The $^1C_4$ conformation should be excluded, because coupling constants $^3J_{3'',4''}$ and $^3J_{4'',5''}$ of at least 9 Hz would have been expected (corresponding to a full axial arrangement of these protons similar to the $\beta - {}^1C_4$ sugars discussed above).

The molecular structures of **β-4a**, **β-4b**, and **β-8a** were independently confirmed by X-ray crystal structure analyses (Figs. 3–5).[18]

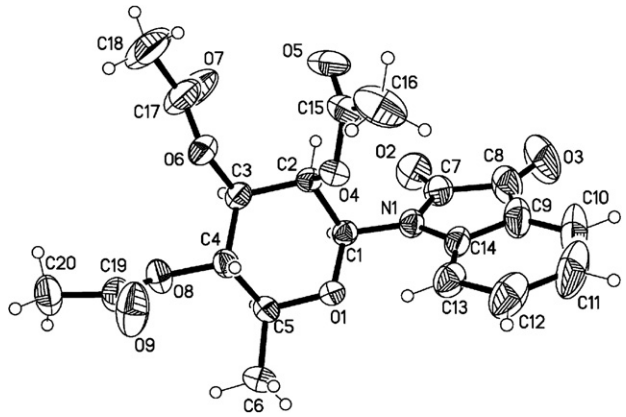


Figure 3. ORTEP plot of **β-4a** (50% probability of the thermal ellipsoids).

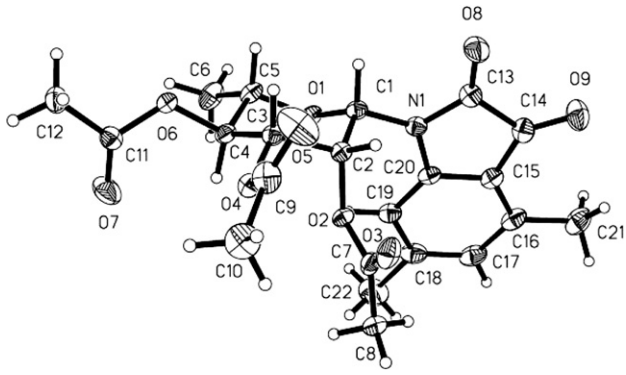


Figure 4. ORTEP plot of **β-4b** (30% probability of the thermal ellipsoids).

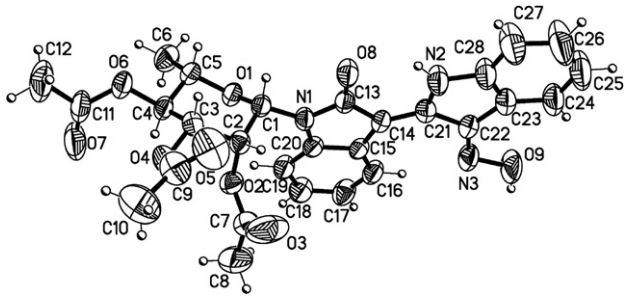


Figure 5. ORTEP plot of **β-8a** (50% probability of the thermal ellipsoids).

2.2. Biological evaluation

The anti-proliferative activity of indirubin-*N*′-glycosides **6a**–**f** and **9a** against four adherent human cancer cell lines [bladder (5637), small cell lung (A-427), esophageal (Kyse-70), and breast (MCF-7)] was studied (Table 2). The shapes of the dose–response curves were rather broad over the five concentrations tested, in contrast to the dose–response curves of many antitumor agents. The IC_{50} values obtained for indirubin-*N*′-glycosides **6a**–**f** and **9a** are summarized in Table 1. Rhamnosides **α-6a**, **β-6a** and **β-6b** were insoluble in the culture medium at concentrations above 12.5 and 25 μM, respectively; thus, the growth inhibition could only be examined in the MCF-7 cell line, where **α-6a** and **β-6b** proved to be very potent. In the other three cell lines, less than 20% growth inhibition was observed at concentrations of 12.5 μM. The IC_{50} values of rhamnosides **α-6a** and **β-6a** (tested against MCF-7 cells) are comparable to those observed for etoposide.

The rhamnosides **α-6f**, **β-6f**, and **β-6b**, containing a substituted indirubin moiety, show a lower activity compared to those rhamnosides containing a non-substituted indirubin moiety. Noteworthy, rhamnoside **α-6f** shows a better selectivity for MCF-7 cancer cells. Rhamnoside **β-6f**, containing a chlorinated indirubin moiety, possesses a higher, but less selective anti-proliferative activity against all cell lines. The galactoside **β-6d** exhibits the lowest activity followed by the oxime **β-9a** which shows the lowest selectivity of all compounds tested.

The activities of compounds **6c**–**e** were comparable with chlorambucil. Comparing the activities of glycosides **6c-e** against all cell lines, mannoside **β-6e** was found to be most potent, followed by glucoside **β-6c**. Galactoside **β-6d** required a two to fivefold higher concentration for a 50% inhibition of cell growth. These experiments clearly demonstrate that the sugar moiety has an important influence on the anti-proliferative activity of indirubin-*N*′-glycosides. Notably, the anti-proliferative activity of indirubin-*N*′-glycosides **6a**–**f** and **β-9a** is considerably higher than the activity recently reported[11] for non-glycosylated indirubins. In particular, the activity of rhamnosides **α-6a** and **β-6a** against the human breast cancer cell line MCF-7 is much higher (by the factor 10 to 100) than the activity of all non-glycosylated indirubins tested before.[11] Notably, only nitro derivatives of non-glycosylated indirubins show an activity which is in the same range.

The experiments suggest that the deoxy-sugar moiety present in rhamnosides **α-6a** and **β-6a** is the reason for their higher activity compared to *gluco*, *galacto*, and *manno* configured glycosides **β-6c**, **β-6d**, and **β-6e**. In contrast, the configuration of the anomeric carbon atom of the rhamnoside did not show a major influence. A substitution in the aromatic ring system considerably lowers the cytotoxic activity of the tested compounds. This could be caused by a lower binding affinity to CDK2 which is described as the main reason for the cancerostatic properties of the indirubins.[11] The reason

Table 2. Average IC_{50} values (μM ± SD, results are from three to six independent determinations)

Compound	Cell lines			
	5637	A-427	KYSE-70	MCF-7
β-6a	>12.5[a]	>12.5[a]	> 12.5[a]	0.76 ± 0.16
α-6a	>25[a]	>25[a]	> 25[a]	0.67 ± 0.08
β-6f	8.23 ± 1.47	n.d.[b]	5.94 ± 2.29	7.98 ± 1.97
α-6f	12.87 ± 0.7	n.d.	15.28 ± 1.28	6.96 ± 0.46
β-6b	>12.5[a]	n.d.	>12.5[a]	6.16 ± 2.33
β-6c	14.36 ± 2.46	11.84 ± 2.75	28.92 ± 5.52	15.67 ± 2.48
β-6d	24.94 ± 2.26	13.16 ± 2.93	41.45 ± 5.38	25.75 ± 4.27
β-6e	10.63 ± 1.67	5.55 ± 0.67	11.83 ± 2.46	4.22 ± 1.07
β-9a	15.53 ± 2.44	n.d.	15.40 ± 2.03	17.03 ± 2.57
Etoposide[c]	0.54 ± 0.30	0.13 ± 0.10	0.94 ± 0.23	0.50 ± 0.19
Cisplatin[c]	0.35 ± 0.10	1.96 ± 0.54	0.63 ± 0.14	1.38 ± 0.29
5-Aza-cytidine[c]	1.73 ± 1.20	0.63 ± 0.05	1.59 ± 1.93	6.78 ± 5.21
Chlor-ambucil[c]	6.55 ± 3.46	9.50 ± 3.47	38.4 ± 1.7	18.4 ± 5.5

[a] An IC_{50} value could not be determined because **α-6a** , **β-6a** and **β-6b** were insoluble above concentrations of 12.5 and 25 μM, respectively.
[b] Not determined.
[c] From Ref. 19.

for the better selectivity of the rhamnoside **α-6f** compared to compound **β-6f** remains unclear at present. Notably, a strong inhibition of the growth of tumor cells was reported[12] for akashine A, B, and C which represent naturally occurring *N*-glycosides of indigo.

An interesting result was the highly selective anti-proliferative activity of compounds **α-6a** and **β-6a** in the MCF-7 breast cancer cell line. The poor solubility of **α-6a** and **β-6b** did not allow the determination of IC_{50} values for the other three cell lines under investigation. However, less than 20% cell growth inhibition was observed even at the highest concentrations tested (i.e., 12.5 and 25 μM, respectively). The growth of MCF-7 breast cancer cells is sensitive to estrogens and antiestrogens and this cell line is known to possess a functional estrogen receptor. Therefore, compounds **α-6a** and **β-6b** may act by interfering with this pathway. In fact, the planar, conjugated structure of the indirubin moiety is an integral part of some synthetic estrogens and antiestrogens.[20] However, further studies are needed to support this hypothesis. Moon et al. suggested[11] that the formation of a hydrogen bond between the backbone oxygen of Leu83 in CDK2 and the secondary amine function of the indirubine moiety might play an important role during cell growth inhibition. This is in agreement with our studies, since the presence of the sugar residue in indirubin-*N*′-glycosides **6a-f** and **9a** should not avoid the formation of such a hydrogen bond. The lower cytotoxic activity and selectivity of rhamnosyl-oxime **β-9a**, compared to rhamnosides **α-6a** and **β-6a**, suggests that the carbonyl group plays an important role in the biochemical mechanism of cytotoxic activity.

Future studies are directed towards the influence of other substituents present in the indirubin moiety and of other (more rare) sugar moieties on the anti-proliferative activity. The effects of such substituents should give more insight in the pharmacological activity of glycosylated indirubins and their mode of action.

3. Conclusions

In conclusion, we have reported the synthesis of the first indirubin-*N*′-glycosides based on reactions of isatine-*N*-glycosides with indoxyl acetates. Our strategy allows the synthesis of unsubstituted indirubin-*N*′-glycosides as well as derivatives containing different substitution patterns at both indol moieties of the indirubin. The products show a significant anti-proliferative activity against various human cancer cell lines. Good results were observed for an indirubin-*N*′-mannoside, which was shown to have medium to high anti-proliferative activity against all investigated cell lines. The highest activity and selectivity against the MCF-7 breast cancer cell line were observed for the anomeric indirubin-*N*′-rhamnosides. The anti-proliferative activity of the indirubin-*N*′-glycosides was shown to be higher than the activity of the corresponding aglycons in most of the cases.

4. Experimental

4.1. General

^{1}H NMR spectra (250.13, 300.13, and 500.13 MHz) and ^{13}C NMR spectra (62.9, 75.5, and 125.8 MHz) were recorded on Bruker spectrometers AV II 250, AV III 300, and AV 500 in $CDCl_3$, DMSO-d_6, and C_6D_6 as solvents. The calibration of spectra was carried out on solvent signals ($CDCl_3$: δ (^{1}H) = 7.25, δ (^{13}C) = 77.0; DMSO-d_6: δ (^{1}H) = 2.50, δ (^{13}C) = 39.7; C_6D_6: δ(^{1}H) = 7.16, δ(^{13}C) = 128.0). Infrared spectra were recorded on a FTIR spectrometer. Mass spectrometric data (MS) were obtained by electron ionization (EI, 70 eV), chemical ionization (CI, isobutane) or electrospray ionization (ESI). Melting points are uncorrected. Analytical thin layer chromatography was performed on 0.20 mm 60 A silica gel plates. Column chromatography was performed on 60 A silica gel (60–200 mesh). Crystallographic data were collected on a Bruker–Nonius X8Apex-CCD diffractometer with MoK_α radiation

($\lambda = 0.71073$ Å). The structures were solved by direct methods using SHELXS-97 and refined against F^2 on all data by full matrix least-squares with SHELXL-97.[21] All non-hydrogen atoms were refined anisotropically, all hydrogen atoms were refined in the model at geometrically calculated positions and refined using a riding model.

4.2. Biological screening

The four adherent human cancer cell lines used for determination of cytotoxic activity [bladder (5637), small cell lung (A-427), esophageal (Kyse-70), and breast (MCF-7)] were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ Braunschweig). Cells were grown in medium containing 90% RPMI 1640 medium and 10% FCS, and supplemented with penicillin G/streptomycin; 1000-fold stock solutions of the compounds for testing were prepared in DMSO and serially diluted in DMSO to concentrations 500-fold of the desired concentrations (giving series of 5 dilutions). The concentration of substances **α-6a**, **β- 6a**, and **6b** ranged in the area of 1.5–100 mM (giving concentration ranges of 1.5–100 μM on the test plates). Twofold dilutions were done for these compounds. For the testing of compounds **α-6a** and **β-6a** with MCF-7 cells fourfold dilutions were carried out; the substances were used in a concentration range between 0.024 and 6.25 μmol/l on the microtiter plates. The cells of all cell lines were plated out 24 h prior to testing in 96-well microtiter plates at a density of 1000 cells/well in 100 μL of the medium.[19] After 24 h, one untreated plate of each cell line was removed and served later as 'C,0' control. The dilution series of the test compounds were 500-fold diluted into the culture medium to give concentrations twofold of the final test concentration; 100 μL of this treated medium was added to each well, giving a final DMSO concentration of 0.1% in all wells. Two substances at five concentrations per substance and one row (i.e., eight wells) per concentration were tested on each plate. Each plate contained two rows of control wells with cells only treated with medium containing 0.1% of DMSO. The cells were continuously exposed to all compounds for a period of 96 h. Growth inhibition of the cells was measured using the Crystal Violet assay.[19] After 96 h of incubation with the substance, the medium was discarded from the wells and replaced for 20 min by a glutaraldehyde buffer solution (1%) to fix the cells. After discarding the fixing solution the cells were stored under PBS at 4 °C until staining. Cells were treated with 100 μL of staining solution (0.02% aqueous solution of Crystal Violet) for 30 min. The excess of dye was removed and the cells were washed for 30 min with water. The cell-bound dye was dissolved in ethanol/water (70%) and the optical density was subsequently measured at $\tilde{\nu} = 570$ nm using an Anthos 2010 plate reader. To construct the dose–response curves the corrected T/C values were calculated as follows: $(T/C)_{\mathrm{corr}}$ (%) = $(OD_T - OD_{C,0})/(OD_C - OD_{C,0}) \times 100$, with OD_T as the mean optical density of the treated cells after staining, OD_C as the mean optical density of the controls and $OD_{C,0}$ as the mean optical density at the time the substances were added. The IC_{50} values were estimated by a linear least-squares regression of the T/C_{corr} values versus the logarithm of the substance concentration and extrapolating to values of 50%. Only test concentrations yielding T/C_{corr} values between 10% and 90% were used in the calculations.

4.3. General procedure for the synthesis of isatine-*N*-glycosides

To a stirred solution of the acetylated glycosyl aniline in oxalyl chloride (about 10 equiv) an equivalent of anhydrous aluminum chloride was added. The mixture was stirred for 1.5 h at 55 °C (TLC control). After cooling to 0 °C, ice water was added to the solution with stirring. The yellow sugar precipitated. The mixture was extracted three times with EtOAc, the combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate and with water and dried (Na_2SO_4). The solution was filtered, the filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography (silica gel).

N-(2′,3′,4′-Tri-*O* -acetyl-β-L- rhamnopyranosyl)isatine (**β-4a**) and *N*-(2′,3′,4′-tri-*O* -acetyl-α-L-rhamnopyranosyl)isatine (**α-4a**): Starting with a mixture of the acetylated rhamnosyl anilines **α/β-3a** (1.24 g, 3.4 mmol, β/α = 2.5:1), **β-4a** (320 mg, 23%) and a mixed fraction of **α-4a** and **β-4a** (570 mg, 40%, β/α = 3:1) were isolated after column chromatography (*n*-heptane/EtOAc = 3:1). A mixture of enriched α-anomer gave an analytical sample of pure *N*-(2′,3′,4′-tri-*O*-acetyl-α-L- rhamnopyranosyl)isatine (**α-4a**).

4.4. Data of β-4a

Yellow solid. Mp 94–95 °C; $[\alpha]_D^{23}$ +152.00 ($c = 0.82$, $CHCl_3$); $R_f = 0.54$ (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (250 MHz, C_6D_6): $\delta = 7.41$ (ddd, $^5J_{4,7} = 0.6$ Hz, $^4J_{5,7} = 1.0$ Hz, $^3J_{6,7} = 8.2$ Hz, 1 H, H-7); 7.19 (ddd, $^5J_{4,7} = 0.6$ Hz, $^4J_{4,6} = 1.5$ Hz, $^3J_{4,5} = 7.5$ Hz, 1H, H-4); 6.95 (ddd, $^4J_{4,6} = 1.5$ Hz, $^3J_{5,6} = 7.6$ Hz, $^3J_{6,7} = 8.2$ Hz, 1H, H-6); 6.48 (d′t′, $^4J_{5,7} = 1.0$ Hz, $^3J_{4,5} = 7.5$ Hz, $^3J_{5,6} = 7.6$ Hz, 1H, H-5); 5.84 (dd, $^3J_{1',2'} = 1.5$ Hz, $^3J_{2',3'} = 3.0$ Hz, 1H, H-2′); 5.82 (d, $^3J_{1',2'} = 1.5$ Hz, 1H, H-1′); 5.46–5.32 (m, 2H, H-3′, H-4′); 3.31–3.19 (m, 1H, H-5′); 1.77, 1.72, 1.44 (3s, 9H, 3xC(O)CH_3); 1.17 (d, $^3J_{5',6'} = 6.2$ Hz, 3H, H-6′). ^{13}C NMR (75 MHz, C_6D_6): $\delta = 181.8$ (C-3); 169.7, 169.5, 169.4 (3x*C*(O)CH_3); 157.2 (C-2); 149.6 (C-7a); 136.9, 124.7, 123.4 (C-4, C-5, C-6); 118.6 (C-3a); 115.6 (C-7); 81.1 (C-1′); 74.0, 71.0, 70.9, 70.5 (C-2′, C-3′, C-4′, C-5′); 20.3, 20.3, 20.3 (3xC(O)*C*H_3); 17.7 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 419 (7) [M^+], 273 (42) [M^+-isatine], 153 (42) [M^+-isatine-2HOAc], 146 (10) [M^+-sugar]. HRMS (EI, 70 eV): calcd for $C_{20}H_{21}NO_9$ ([M^+]): 419.12108; found: 419.12245. Anal. calcd for $C_{20}H_{21}NO_9$ (419.38): C, 57.28; H, 5.05; N, 3.34. Found: C, 57.68; H, 5.39; N, 3.07.

4.5. Data of α-4a

Yellow solid. Mp 66–67 °C; $[\alpha]_D^{23}$ −92.19 (*c* 0.61, $CHCl_3$); $R_f = 0.52$ (*n* -heptane/EtOAc = 1:3). ^{1}H NMR

(250 MHz, C_6D_6): δ = 7.21 (dd, $^4J_{4,6}$ = 1.5 Hz, $^3J_{4,5}$ = 7.5 Hz, 1H, H-4); 7.03 (br d, $^3J_{6,7}$ = 8.0 Hz, 1H, H-7); 6.91 (d′t′, $^4J_{4,6}$ = 1.5 Hz, $^3J_{5,6}$ = 7.3 Hz, $^3J_{6,7}$ = 8.0 Hz, 1H, H-6); 6.48 (d′t′, $^3J_{5,7}$ = 1.0 Hz, $^3J_{5,6}$ = 7.3 Hz, $^3J_{4,5}$ = 7.5 Hz, 1H, H-5); 6.28 (dd, $^3J_{2',3'}$ = 3.7 Hz, $^3J_{1',2'}$ = 5.7 Hz, 1H, H-2′); 6.01 (dd, $^3J_{2',3'}$ = 3.7 Hz, $^3J_{3',4'}$ = 6.7 Hz, 1H, H-3′); 5.60 (d, $^3J_{1',2'}$ = 5.7 Hz, 1H, H-1′); 5.16 (dd, $^3J_{4',5'}$ = 5.7 Hz, $^3J_{3',4'}$ = 6.7 Hz, 1H, H-4′); 3.78 (dq, $^3J_{4',5'}$ = 5.7 Hz, $^3J_{5',6'}$ = 6.7 Hz, 1H, H-5′); 1.67, 1.65, 1.61 (3s, 9H, 3xC(O)CH_3); 1.14 (d, $^3J_{5',6'}$ = 6.7 Hz, 3H, H-6′). ^{13}C NMR (75 MHz, C_6D_6): δ = 181.7 (C-3); 169.4, 169.3, 168.9 (3x*C*(O)CH_3); 157.7 (C-2); 149.7 (C-7a); 137.6, 125.0, 124.0 (C-4, C-5, C-6); 118.6 (C-3a); 113.1 (C-7); 78.0 (C-1′); 71.8, 71.3, 69.4, 66.9 (C-2′, C-3′, C-4′, C-5′); 20.3, 20.2, 20.1 (3xC(O)*C*H_3); 16.5 (C-6′). MS (CI, isobutane): *m*/*z* (%)=420 (13) [M+H]$^+$, 273 (100) [M$^+$-isatine]. HRMS (CI, isobutane): calcd for $C_{20}H_{21}NO_9$ ([M$^+$]): 419.12108; found: 419.12130. Anal. calcd for $C_{20}H_{21}NO_9$ (419.38): C, 57.28; H, 5.05; N, 3.34. Found: C, 57.68; H, 5.39; N, 3.07.

4.6. 4,6-Dimethyl-*N*-(2′,3′,4′-tri-*O*-acetyl-β-L-rhamnopyranosyl)isatine (β-4b)

Starting with 3,5-dimethyl-*N*-(2′,3′,4′-tri-*O*-acetyl-β/α-L-rhamnopyranosyl)aniline (**α/β-3b**) (1.00 g, 2.5 mmol, β/α = 2:1), 4,6-dimethyl-*N*-(2′,3′,4′-tri-*O*-acetyl-β/α-L-rhamnopyranosyl)isatine (**α/β-4b**) was isolated as a yellow solid (860 mg, 76%, β/α = 3.5:1). Recrystallization (*n*-heptane/ EtOAc) gave the pure anomer **β-4b**. Mp 191–193 °C; $[\alpha]_D^{22}$ +201.87 (*c* 0.78, $CHCl_3$); R_f = 0.58 (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.14, 6.68 (2br s, 2H, H-5, H-7); 5.82 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′); 5.54 (dd, $^3J_{1',2'}$ = 1.5 Hz, $^3J_{2',3'}$ = 2.8 Hz, 1H, H-2′); 5.26–5.15 (m, $^3J_{2',3'}$ = 2.8 Hz, $^3J_{4',5'}$ = 9.4 Hz, 2H, H-3′, H-4′); 3.80–3.66 (m, $^3J_{4',5'}$ = 9.4 Hz, $^3J_{5',6'}$ = 6.2 Hz, 1H, H-5′); 2.49, 2.37 (2s, 6H, 2xCH_3); 2.09, 1.97, 1.89 (3s, 9H, 3xC(O)CH_3); 1.34 (d, $^3J_{5',6'}$ = 6.2 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 181.4 (C-3); 170.1, 169.6, 169.4 (3x*C*(O)CH_3); 157.7 (C-2); 149.4, 149.0, 141.0 (C-4, C-6, C-7a); 126.8, 113.6 (C-5, C-7); 111.3 (C-3a); 80.2 (C-1′); 74.2, 70.5, 70.4, 70.0 (C-2′, C-3′, C-4′, C-5′); 22.9, 18.2 (2xCH_3); 20.8, 20.8, 20.5 (3xC(O)*C*H_3); 17.6 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 447 (11) [M$^+$], 273 (54) [M$^+$-dimethylisatine], 174 (39) [dimethylisatine], 153 (100) [M$^+$-dimethylisatine-2HOAc]. Anal. calcd for $C_{22}H_{25}NO_9$ (447.44): C, 59.06; H, 5.63; N, 3.13. Found: C, 59.18; H, 5.88; N, 3.01.

4.7. *N*-(2′,3′,4′,6′′-Tetra-*O*-acetyl-β-D-galactopyranosyl)isatine (β-4d)

Starting with *N*-(2′,3′,4′,6′-tetra-*O*-acetyl- α,β-D-galactopyranosyl)aniline (**α/β-3d**) (0.80 g, 1.9 mmol), *N*-(2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-galactopyranosyl)isatine β-D-galactopyranosyl)isatine (**β-4d**) was isolated as a yellow solid (0.47 g, 52%). Mp 78–80 °C; $[\alpha]_D^{22}$ -76.76 (*c* 1.02, $CHCl_3$); R_f = 0.45 (*n*-heptane/EtOAc = 1:3); ^{1}H NMR (250 MHz, C_6D_6): δ = 7.44 (′d′, $^3J_{6,7}$ = 8.0 Hz, 1H, H-7); 7.23 (dd,$^4J_{4,6}$ = 1.5 Hz, $^3J_{4,5}$ = 7.6 Hz, 1H, H-4); 7.05 (d′t′, $^4J_{4,6}$ = 1.5 Hz, $^3J_{5,6}$ = 7.8 Hz, $^3J_{6,7}$ = 8.0 Hz, 1H, H-6); 6.52 (d′t′, $^4J_{5,7}$ = 0.8 Hz, $^3J_{4,5}$ = 7.6 Hz, $^3J_{5,6}$ = 7.8 Hz, 1H, H-5); 5.91 (dd, $^3J_{1',2'}$ = 9.3 Hz, $^3J_{2',3'}$ = 10.0 Hz, 1H, H-2′); 5.67 (d, $^3J_{1',2'}$ = 9.3 Hz, 1H, H-1′); 5.49 (dd, $^3J_{4',5'}$ = 1.0 Hz, $^3J_{3',4'}$ = 3.2 Hz, 1H, H-4′); 5.23 (dd, $^3J_{3',4'}$ = 3.2 Hz, $^3J_{2',3'}$ = 10.0 Hz, 1H, H-3′); 4.05 (dd, $^3J_{5',6a'}$ = 6.2 Hz, $^2J_{6\ a',6b'}$ = 11.4 Hz, 1H, H-6a′); 3.95 (dd, $^3J_{5',6b'}$ = 6.8 Hz, $^2J_{6a',6b'}$ = 11.4 Hz, 1H, H-6b′); 3.28 (d′t′, $^3J_{4',5'}$ = 1.0 Hz, $^3J_{5',6a'}$ = 6.2 Hz, $^3J_{5',6b'}$ = 6.8 Hz, 1H, H-5′); 1.69, 1.65, 1.59, 1.46 (4s, 12H, 4xC(O)CH_3). ^{13}C NMR (75 MHz, C_6D_6): δ = 181.8 (C-3); 169.7, 169.7, 169.5, 169.5 (4xC(O)*C*H_3); 157.7 (C-2); 148.7 (C-7a); 137.6 (C-6); 125.3 (C-4); 124.0 (C-5); 118.5 (C-3a); 113.5 (C-7); 80.5 (C-1′); 73.2 (C-5′); 71.4 (C-3′); 67.5 (C-4′); 65.8 (C-2′); 61.3 (C-6′); 20.1, 20.1, 20.0, 19.7 (4xC(O)*C*H_3). MS (EI, 70 eV): *m*/*z* (%) = 477 (8) [M$^+$], 331 (100) [M$^+$ -isatine], 169 (100) [M$^+$-isatine-HOAc-Ac$_2$O]. HRMS (EI, 70 eV): calcd for $C_{22}H_{23}NO_{11}$ ([M$^+$]): 477.12656; found: 477.12687. Anal. calcd for $C_{22}H_{23}NO_{11}$ (477.42): C, 55.35; H, 4.86; N, 2.93. Found: C, 55.28; H, 5.04; N, 2.71.

4.8. *N*-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-mannopyranosyl)isatine (β-4e)

N-(2′,3′,4′,6′-tetra-*O*-acetyl- α,β-D-mannopyranosyl)aniline (**α/β-3e**) (1.00 g, 2.4 mmol) was transformed into the yellow colored *N*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-mannopyranosyl)isatine (**β-4e**) (0.24 g, 21%). Mp 82–83 °C; $[\alpha]_D^{22}$ +126.36 (*c* 0.3, $CHCl_3$); R_f = 0.43 (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.58 (m, 1H, H-4); 7.55–7.51 (m, 2H, H-6, H-7); 7.18 (m, 1H, H-5); 5.88 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′); 5.58 (dd, $^3J_{1',2'}$ = 1.5 Hz, $^3J_{2',3'}$ = 3.5 Hz, 1H, H-2′); 5.39 (′t′, $^3J_{3',4'}$=$^3J_{4',5'}$ = 10.0 Hz, 1H, H-4′); 5.28 (dd, $^3J_{2',3'}$ = 3.5 Hz, $^3J_{3',4'}$ = 10.0 Hz, 1H, H-3′); 4.31 (dd, $^3J_{5',6a'}$ = 5.4 Hz, $^2J_{6a',6b'}$ = 12.5 Hz, 1H, H-6a′); 4.20 (dd, $^3J_{5',6b'}$ = 2.5 Hz, $^2J_{6a',6b'}$ = 12.5 Hz, 1H, H-6b′); 3.89 (ddd, $^3J_{5',6b'}$ = 2.5 Hz, $^3J_{5',6a'}$ = 5.3 Hz, $^3J_{4',5'}$ = 10.0 Hz, 1H, H-5′); 2.10, 2.08, 1.97, 1.87 (4s, 12H, 4xC(O)CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 181.6 (C-3); 170.4, 169.7, 169.5, 169.5 (4x*C*(O)CH_3); 157.0 (C-2); 149.0 (C-7a); 137.7 (C-6); 125.2 (C-4); 124.2 (C-5); 118.0 (C-3a); 115.6 (C-7); 80.5 (C-1′); 75.6 (C-5′); 70.4 (C-3′); 70.0 (C-2′); 65.1 (C-4′); 62.1 (C-6′); 20.7, 20.7, 20.6, 20.4 (4xC(O)*C*H_3). MS (EI, 70 eV): *m*/*z* (%) = 477 (12) [M$^+$], 331 (72) [M$^+$-isatine], 169 (100) [M$^+$-isatine-HOAc-Ac$_2$O], 146 (13) [M$^+$-sugar].HRMS (EI, 70 eV): calcd for $C_{22}H_{23}NO_{11}$ ([M$^+$]): 477.12656; found: 477.12599. Anal.: calcd for $C_{22}H_{23}NO_{11}$ (477.42): C, 55.35; H, 4.86; N, 2.93. Found: C, 55.52; H, 5.17; N, 2.73.

4.9. General procedure 1 for the synthesis of the *O*-acetylated indirubin-*N*′-glycosides

To a stirred degassed MeOH solution of the acetylated glycosyl isatine **4**, the corresponding indoxyl acetate **5** (1.0 equiv) and sodium bicarbonate (2.0–3.0 equiv) were added (under Argon atmosphere). The mixture was stirred for 2 h at 20 °C during which time the yellow to orange color of the solution changed to red to violet. The mixture was neutralized with IR 120 (H$^+$), filtered and

the filtrate was concentrated under reduced pressure. The crude product was acetylated with acetic anhydride/pyridine (v:v = 1:1) and stirred overnight at 0 °C. To the solution was added ice water with stirring. The mixture was extracted three times with EtOAc. The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate and with water and dried (Na_2SO_4). The solution was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (*n*-heptane/EtOAc = 2:1).

4.10. *N*′-(2″,3″,4″-Tri-*O*-acetyl-β-L-rhamnopyranosyl)-indirubin (β-7a)

Starting with **β-4a** (200 mg, 0.48 mmol), **β-7a** was isolated as a red solid (178 mg, 70%). Mp 160–161 °C; R_f = 0.56 (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.42 (s, 1H, NH); 8.81 (dd, $^4J_{4',6'} = 1.0$ Hz, $^3J_{4',5'} = 7.9$ Hz, 1H, H-4′); 7.65 (d, $^3J_{4,5}$ = 7.6 Hz, 1H, H-4); 7.55 (d, $^3J_{6',7'} = 7.9$ Hz, 1H, H-7′); 7.45 (d′t′, $^4J_{4,6}$ = 1.0 Hz, $^3J_{5,6}$ = 7.6 Hz, $^3J_{6,7}$ = 7.9 Hz, 1H, H-6); 7.22 (d′t′, $^4J_{4',6'} = 1.0$ Hz, $^3J_{5',6'} = 7.5$ Hz, $^3J_{6',7'} =$ 7.9 Hz, 1H, H-6′); 7.06 (d′t′, $^4J_{5',7'} = 1.0$ Hz, $^3J_{5',6'} = 7.5$ Hz, $^3J_{4',5'} = 7.9$, 1H, H-5′); 6.98 (‘t’, $^3J_{4,5} = {}^3J_{5,6}$ = 7.6 Hz, 1H, H-5); 6.91 (d, $^3J_{6,7}$ = 7.9 Hz, 1H, H-7); 5.98 (d, $^3J_{1'',2''} = 1.6$ Hz, 1H, H-1″); 5.65 (dd, $^3J_{1'',2''} = 1.6$ Hz, $^3J_{2'',3''} = 3.5$ Hz, 1H, H-2″); 5.33 (dd, $^3J_{2'',3''} = 3.5$ Hz, $^3J_{3'',4''} = 10.0$ Hz, 1H, H-3″); 5.26 (‘t’, $^3J_{4'',5''} = 9.5$ Hz, $^3J_{3'',4''} = 10.0$ Hz, 1H, H-4″); 3.82 (dq, $^3J_{5'',6''} = 6.3$ Hz, $^3J_{4'',5''} = 9.5$ Hz, 1H, H-5″); 2.10, 1.98, 1.81 (3s, 9H, 3xC(O)CH_3); 1.38 (d, $^3J_{5'',6''} = 6.3$ Hz, 3H, H-6″). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 187.9 (C-3); 170.0, 169.8, 169.8 (3x*C*(O)CH_3); 169.5 (C-2′); 151.4 (C-7a); 139.7, 139.2 (C-2, C-7a′); 136.9 (C-6); 128.5 (C-6′); 125.1 (C-4); 125.0 (C-4′); 122.7 (C-5′); 121.7 (C-5); 121.4 (C-3a′); 119.9 (C-3a); 113.2 (C-7′); 112.0 (C-7); 105.5 (C-3′); 80.4 (C-1″); 74.0 (C-5″); 70.7, 70.4, 70.3 (C-2″, C-3″, C-4″); 20.8, 20.8, 20.6 (3xC(O)*C*H_3); 17.7 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 534 (96) [M^+], 273 (21) [M^+-indirubin], 262 (100) [indirubin]. HRMS (EI, 70 eV): calcd for $C_{28}H_{26}N_2O_9$ ([M^+]): 534.16328; found: 534.16316.

4.11. *N*′-(2″,3″,4″-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-indirubin (α-7a)

Starting with **α-4a** (120 mg, 0.286 mmol), **α-7a** was isolated by column chromatography (*n*-heptane/ EtOAc= 2:1) -heptane/EtOAc = 2:1) as a red solid (102 mg, 67%). Mp 105–106 °C; R_f = 0.54 (*n*-heptane/ EtOAc=1:3). -heptane/EtOAc = 1:3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 10.56 (s, 1H, NH); 8.94 (‘d‘, $^3J_{4',5'} = 7.7$ Hz, 1H, H-4′); 7.73 (‘d‘, $^3J_{4,5}$ = 7.6 Hz, 1H, H-4); 7.50 (ddd, $^4J_{4,6}$ = 1.3 Hz, ^3J = 7.5 Hz, ^3J =8.0 Hz, 1H, H-6); 7.31 (ddd, $^4J_{4'6'} = 1.3$ Hz, ^3J =7.3 Hz, ^3J = 8.0 Hz, 1H, H-6′); 7.24–7.14 (m, ^4J = 1.3 Hz, ^3J = 7.3 Hz, $^3J_{4',5'} = 7.7$ Hz, 2H, H-5′, H-7′); 7.06–6.95 (m, ^4J = 0.8 Hz, $^3J_{4,5}$ = 7.6 Hz, 2H, H-5, H-7); 6.19 (dd, $^3J_{2'',3''}$ =4.0 Hz, $^3J_{1'',2''} = 4.7$ Hz, 1H, H-2″); 5.95 (dd, $^3J_{2'',3''} = 4.0$ Hz, $^3J_{3'',4''} = 7.3$ Hz, 1H, H-3″); 5.71 (d, $^3J_{1'',2''} = 4.8$ Hz, 1H, H-1″); 5.06 (‘t’, $^3J_{3'',4''} = {}^3J_{4'',5''} = 6.9$ Hz, 1H, H-4″); 3.99 (‘quintet‘, $^3J_{4'',5''} = {}^3J_{5'',6''} = 6.7$ Hz, 1H, H-5″); 2.13, 2.11, 2.07 (3s, 9H, 3xC(O)CH_3); 1.34 (d, $^3J_{5'',6''} = 6.7$ Hz, 3H, H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.1 (C-3); 170.7, 169.9, 169.7 (3x*C*(O)CH_3); 169.7 (C-2′); 151.4 (C-7a); 139.8, 139.7 (C-2, C-7a′); 137.0 (C-6); 129.2 (C-6′); 125.3 (C-4); 125.3 (C-4′); 123.4, 121.9 (C-5, C-5′); 121.5, 119.9 (C-3a, C-3a′); 112.0, 110.5 (C-7, C-7′); 105.9 (C-3′); 78.3 (C-1″); 71.7 (C-4″); 70.3 (C-5″); 69.6 (C-3″); 67.3 (C-2″); 20.9, 20.8, 20.7 (3xC(O)*C*H_3); 16.7 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 534 (74) [M^+], 273 (13) [M^+-indirubin], 262 (100) [indirubin], 234 (12) [indirubin-(C=O)]; HRMS (EI, 70 eV): calcd for $C_{28}H_{26}N_2O_9$ ([M^+]): 534.16328; found: 534.16329.

4.12. 5-Chloro-1′-(2″,3″,4″-tri-*O*-acetyl-β-L-rhamnopyranosyl)indirubin (β-7f)

Starting with *N*-(2′,3′,4′-tri-*O*-acetyl-β-L-rhamnopyranosyl)isatine (**β-4a**) (150 mg, 0.36 mmol), **β-7f** was isolated (130 mg, 63%) as a red solid. Mp 159–161 °C; R_f = 0.64 (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 10.46 (s, 1H, NH); 8.82 (dd, $^4J_{4',6'} = 1.3$ Hz, $^3J_{4',5'} = 7.9$ Hz, 1H, H-4′); 7.65 (d, $^4J_{4,6}$ = 2.2 Hz, 1H, H-4); 7.55 (d, $^3J_{6',7'} = 8.0$ Hz, 1H, H-7′); 7.44 (dd, $^4J_{4,6}$ = 2.2 Hz, $^3J_{6,7}$ = 8.5 Hz, 1H, H-6); 7.26 (d′t′, $^4J_{4',6'} = 1.3$ Hz, $^3J_{5',6'} = 7.6$ Hz, $^3J_{6',7'} = 8.0$ Hz, 1H, H-6′); 7.09 (d′t′, $^4J_{5',7'} = 1.1$ Hz, $^3J_{5',6'} = 7.6$ Hz, $^3J_{4',5'} =$ 7.9 Hz, 1H, H-5′); 6.93 (d, $^3J_{6,7}$ = 8.5 Hz, 1H, H-7); 5.96 (d, $^3J_{1'',2''} = 1.5$ Hz, 1H, H-1″); 5.61 (dd, $^3J_{1'',2''}$ =1.5 Hz, $^3J_{2'',3''} = 3.2$ Hz, 1H, H-2″); 5.30 (dd, $^3J_{2'',3''} = 3.2$ Hz, $^3J_{3'',4''} = 10.2$ Hz, 1H, H-3″); 5.24 (dd, $^3J_{4'',5''} = 9.2$ Hz, $^3J_{3'',4''} = 10.2$ Hz, 1H, H-4″); 3.79 (dq, $^3J_{5'',6''} = 6.2$ Hz, $^3J_{4'',5''} = 9.2$ Hz, 1H, H-5″); 2.10, 1.98, 1.80 (3s, 9H, 3xC(O)CH_3); 1.38 (d, $^3J_{5'',6''} = 6.2$ Hz, 3H, H-6″). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 186.8 (C-3); 170.0, 170.0, 169.9 (3x*C*(O)CH_3); 169.5 (C-2′); 149.6 (C-7a); 139.5, 139.3 (C-2, C-7a′); 136.5 (C-6); 129.1 (C-6′); 127.3 (C-5); 125.2, 124.9 (C-4′, C-4); 122.9 (C-5′); 121.1, 120.9 (C-3a, C-3a′); 113.4, 113.2 (C-7, C-7′); 106.5 (C-3′); 80.4 (C-1″); 74.1 (C-5″); 70.6, 70.3, 70.2 (C-2″, C-3″, C-4″); 20.8, 20.8, 20.6 (3xC(O)*C*H_3); 17.7 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 570 (19) [M^+; ^{37}Cl]; 568 (51) [M^+; ^{35}Cl]; 298 (23) [5-chloroindirubin; ^{37}Cl]; 296 (65) [5-chloroindirubin; ^{35}Cl]; 273 (19) [M^+- 5-chloroindirubin]; 153 (100) [M^+-aglycon-2HOAc]. HRMS (EI, 70 eV): calcd for $C_{28}H_{25}ClN_2O_9$ ([M^+]): 568.12431; found: 568.12338.

4.13. 5-Chloro-1′-(2″,3″,4″-tri-*O*-acetyl-α-L-rhamnopyranosyl)indirubin (α-7f)

Starting with *N*-(2′,3′,4′-tri-*O*acetyl-α-L-rhamnopyranosyl)isatine (**α-4a**) (300 mg, 0.72 mmol), **α-7f** was isolated (256 mg, 63%) as a red solid. Mp 93–95 °C; R_f = 0.6 (*n*-heptane/EtOAc=1:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.57 (s, 1H, NH); 8.91 (dd, $^4J_{4',6'} =$ 1.2 Hz, $^3J_{4',5'} = 7.8$ Hz, 1H, H-4′); 7.69 (‘d’, $^4J_{4,6}$ = 2.1 Hz, 1H, H-4); 7.46 (dd, $^4J_{4,6}$ = 2.1 Hz, $^3J_{6,7}$ = 8.5 Hz, 1H, H-6); 7.36–7.30 (m, 1H, H-6′); 7.21 (‘d’, $^3J_{6',7'} = 7.8$ Hz, 1H, H-7′); 7.19 (d′t′, $^4J_{5',7'} = 1.2$ Hz, $^3J_{5',6'} = 7.6$ Hz, $^3J_{6',7'} = 7.8$ Hz, 1H, H-5′); 6.95 (d, $^3J_{6,7}$ = 8.5 Hz, 1H, H-7); 6.18 (‘t‘, $^3J_{1'',2''} = 4.6$ Hz, $^3J_{2'',3''} = 4.0$ Hz, 1H, H-2″); 5.93 (dd, $^3J_{2'',3''} = 4.0$ Hz, $^3J_{3'',4''} = 7.3$ Hz, 1H, H-3″); 5.69 (d, $^3J_{1'',2''} = 4.6$ Hz, 1H, H-1″); 5.06 (‘t’, $^3J_{4'',5''} = 7.0$ Hz, $^3J_{3'',4''} = 7.3$ Hz, 1H, H-4″); 3.97 (‘quin-

tet', $^3J_{5'',6''} = 6.6$ Hz, $^3J_{4'',5''} = 7.0$ Hz, 1H, H-5″); 2.13, 2.11, 2.08 (3s, 9H, 3xC(O)CH_3); 1.34 (d, $^3J_{5'',6''} = 6.6$ Hz, 3H, H-6″). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 186.9 (C-3); 170.6, 169.9, 169.7, 169.7 (3x*C*(O)CH_3, C-2′); 149.7, 140.0, 139.4 (C-7a, C-7a′, C-2); 136.6 (C-6); 129.7 (C-6′); 127.4 (C-5); 125.5, 125.0, 123.6 (C-4, C-4′, C-5′); 121.3, 120.9 (C-3a, C-3a′); 113.1, 110.6 (C-7, C-7′); 106.8 (C-3′); 78.5 (C-1″); 71.7 (C-4″); 70.2 (C-5″); 69.6 (C-3″); 67.3 (C-2″); 20.9, 20.8, 20.7 (3xC(O)*C*H_3); 16.8 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 570 (12) [M^+; ^{37}Cl]; 568 (31) [M^+; ^{35}Cl]; 298 (18) [5-chloroindirubin; ^{37}Cl]; 296 (51) [5-chloroindirubin; ^{35}Cl]; 273 (23) [M^+-5-chloroindirubin]; 153 (63) [M^+-aglycon-2HOAc]. HRMS (EI, 70 eV): calcd for $C_{28}H_{25}ClN_2O_9$ ([M^+]): 568.12431; found: 568.12427.

4.14. General procedure 2 for the synthesis of the *O*-acetylated indirubin-*N*′-glycosides

To a stirred solution of a deprotected indirubin-*N*-glycoside **6** in pyridine was added acetic anhydride at 0 °C. The mixture was stirred overnight at 0 °C. To the solution was added ice water with stirring. The mixture was extracted three times with EtOAc. The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate and with water. The solution was dried (Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (*n*-heptane/EtOAc = 2:1).

4.15. *N*′-(2″,3″,4″,6″-Tetra-*O*-acetyl-β-D-glucopyranosyl)indirubin (β-7c)

Starting with **β-6c** (100 mg, 0.236 mmol), **β-7c** was isolated as a red solid (105 mg, 75%). Mp 176 °C (polymorphism), 294-295 °C; R_f = 0.46 (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.46 (br, 1H, NH); 8.92 (dd, $^4J_{4',6'} = 1.2$ Hz, $^3J_{4',5'} = 7.9$ Hz, 1H, H-4′); 7.73 (br d, $^3J_{4,5} = 7.6$ Hz, 1H, H-4); 7.51 (d′t′, $^4J_{4,6} = 1.0$ Hz, $^3J_{5,6} = {}^3J_{6,7} = 7.9$ Hz, 1H, H-6); 7.32 (d′t′, $^4J_{4',6'} = 1.2$ Hz, $^3J_{5',6'} = {}^3J_{6',7'} = 7.9$ Hz, 1H, H-6′); 7.22 (br d, $^3J_{6',7'} = 7.9$ Hz, 1H, H-7′); 7.17 (d′t′, $^4J_{5',7'} = 1.0$ Hz, $^3J_{4',5'} = {}^3J_{5',6'} = 7.9$ Hz, 1H, H-5′); 7.02 ('t', $^3J_{4,5} = {}^3J_{5,6} = 7.6$ Hz, 1H, H-5); 6.99 (d, $^3J_{6,7} = 7.9$ Hz, 1H, H-7); 5.78 (br, 2H, H-1″, H-2″); 5.43 ('t', $^3J_{2'',3''} = {}^3J_{3'',4''} = 9.5$ Hz, 1H, H-3″); 5.30 ('t', $^3J_{3'',4''} = 9.5$ Hz, $^3J_{4'',5''} = 10.0$, 1H, H-4″); 4.26 (dd, $^3J_{5'',6a''} = 4.5$ Hz, $^2J_{6a'',6b''} = 12.5$ Hz, 1H, H-6a″); 4.21 (dd, $^3J_{5'',6b''} = 2.5$ Hz, $^2J_{6a'',6b''} = 12.5$ Hz, 1H, H-6b″); 3.97 (ddd, $^3J_{5'',6a''} = 4.5$ Hz, $^3J_{4'',5''} = 10.0$, $^3J_{5'',6b''} = 2.5$ Hz, 1H, H-5″); 2.08, 2.08, 2.01, 1.83 (s, 12H, 4xC(O)CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 188.0 (C-3); 170.5, 170.4, 170.1, 169.5, 168.9 (4x*C*(O)CH_3, C-2′); 151.3 (C-7a); 139.9 (C-2); 138.1 (C-7a′); 137.0 (C-6); 129.2 (C-6′); 125.6 (C-4′); 125.4 (C-4); 123.4 (C-5′); 122.0 (C-5); 121.3 (C-3a′); 120.0 (C-3a); 112.0 (C-7); 110.9 (br, C-7′); 105.4 (C-3′); 79.5 (C-1″); 74.7 (C-5″); 73.5 (C-3″); 68.0 (C-4″); 67.9 (C-2″); 61.9 (C-6″); 20.7, 20.6, 20.6, 20.3 (4xC(O)*C*H_3). MS (EI, 70 eV): *m*/*z* (%) = 592 (94) [M^+], 331 (9) [M^+-indirubin], 262 (100) [indirubin]. HRMS (EI, 70 eV): calcd for $C_{30}H_{28}N_2O_{11}$ ([M^+]): 592.16876; found: 592.16834.

4.16. *N*′-(2″,3″,4″,6″-Tetra-*O*-acetyl-β-D-galactopyranosyl)indirubin (β-7d)

Starting with **β-6d** (50 mg, 0.118 mmol), **β-7d** was isolated (49 mg, 70%) as a red solid. Mp 249 °C (polymorphism), 268–269 °C; R_f = 0.48 (*n* -heptane/EtOAc = 1:3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 10.46 (s, 1H, NH); 8.93 (br d, $^3J_{4',5'} = 7.8$ Hz, 1H, H-4′); 7.74 (br d, $^3J_{4,5} = 7.6$ Hz, 1H, H-4); 7.51 (d′t′, $^4J_{4,6} = 1.1$ Hz, $^3J_{5,6} = 7.6$ Hz, $^3J_{6,7} = 8.0$ Hz, 1H, H-6); 7.42–7.28 (m, $^4J_{4',6'} = 1.0$ Hz, $^3J_{5',6'} = 7.8$ Hz, $^3J_{6',7'} = 8.0$ Hz, 2H, H-6′, H-7′); 7.18 ('t', $^3J_{4',5'} = 7.8$ Hz , 1H, H-5′); 7.07–6.97 (m, $^3J_{5,6} = 7.6$ Hz, $^3J_{6,7} = 8.0$ Hz, 2H, H-5, H-7); 5.95–5.75 (br, 2H, H-1″, H-2″); 5.57 (d, $^3J_{3'',4''} = 3.2$ Hz, 1H, H-4″); 5.26 (dd, $^3J_{3'',4''} = 3.2$ Hz, $^3J_{2'',3''} = 10.0$ Hz, 1H, H-3″); 4.28–4.08 (m, $^3J = 4.5$ Hz, $^3J = 9.8$ Hz, $^2J_{6a'',6b''} = 12.6$ Hz, 3H, H-5″, H-6a″, H-6b″); 2.30, 2.04, 2.00, 1.86 (4s, 12H, 4xC(O)CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 187.9 (C-3); 170.4, 170.3, 170.0, 169.9 (4s, 4x*C*(O)CH_3); 169.1 (C-2′); 151.3 (C-7a); 139.8, 138.3 (C-7a′, C-2); 137.0, 129.2, 125.6, 125.4, 123.3, 122.0 (C-4, C-4′, C-5, C-5′, C-6, C-6′); 121.2, 120.0 (C-3a, C-3a′); 111.9, 111.2 (C-7, C-7′), 105.6 (C-3′); 79.6 (C-1″); 73.2, 71.6, 67.3, 65.6 (C-2″, C-3″, C-4″, C-5″); 61.5 (C-6″); 20.8, 20.7, 20.6, 20.4 (4s, 4xC(O)C*H*$_3$). MS (EI, 70 eV): *m*/*z* (%) = 592 (86) [M^+], 331 (17) [M^+-indirubin], 262 (100) [indirubin]. HRMS (EI, 70 eV): calcd for $C_{30}H_{28}N_2O_{11}$ ([M^+]): 592.16876; found: 592.16879.

4.17. *N*′-(2″,3″,4″,6″-Tetra-*O*-acetyl-β-D-mannopyranosyl)indirubin (β-7e)

Starting with 1′-β-D-mannopyranosylindirubin (**β-6e**) (60 mg, 0.141 mmol), **β-7e** was yielded as a red solid (49 mg, 60%). Mp 132–134 °C; R_f = 0.46 (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 10.42 (br, 1H, NH); 8.83 (dd, $^4J_{4',6'} = 1.0$ Hz, $^3J_{4',5'} = 7.9$ Hz, 1H, H-4′); 7.68 (d, $^3J_{4,5} = 7.6$ Hz, 1H, H-4); 7.54–7.44 (m, $^3J_{6,7} = {}^3J_{6',7'} = 7.9$ Hz, 2H, H-6, H-7′); 7.20 (d′t′, $^4J_{4',6'} = 1.2$ Hz, $^3J_{6',7'} = 7.9$ Hz, 1H, H-6′); 7.06 (d′t′, $^4J_{5',7'} = 1.1$ Hz, $^3J_{4',5'} = {}^3J_{5',6'} = 7.9$ Hz, 1H, H-5′); 7.00 ('t', $^3J_{4,5} = {}^3J_{5,6} = 7.6$ Hz, 1H, H-5); 6.93 (d, $^3J_{6,7} = 7.9$ Hz, 1H, H-7); 6.03 (d, $^3J_{1'',2''} = 1.4$ Hz, 1H, H-1″); 5.65 (dd, $^3J_{1'',2''} = 1.4$ Hz, $^3J_{2'',3''} = 3.1$ Hz, 1H, H-2″); 5.46 ('t', $^3J_{4'',5''} = 9.5$ Hz, $^3J_{3'',4''} = 10.0$ Hz, 1H, H-4″); 5.38 (dd, $^3J_{2'',3''} = 3.1$ Hz, $^3J_{3'',4''} = 10.0$ Hz, 1H, H-3″); 4.27 (dd, $^3J_{5'',6a''} = 2.8$ Hz, $^2J_{6a'',6b''} = 12.5$ Hz, 1H, H-6a″); 4.33 (dd, $^3J_{5'',6b''} = 4.7$ Hz, $^2J_{6a'',6b''} = 12.5$ Hz, 1H, H-6b″); 3.98 (ddd, $^3J_{5'',6a''} = 2.8$ Hz, $^3J_{5'',6b''} = 4.7$ Hz, $^3J_{4'',5''} = 9.5$ Hz, 1H, H-5″); 2.11, 2.10, 1.99, 1.81 (4s, 12H, 4xC(O)CH_3); ^{13}C NMR (63 MHz, $CDCl_3$): δ = 188.0 (C-3); 170.6, 169.8, 169.8, 169.7, 169.5 (5s, 4x*C*(O)CH_3, C-2′); 151.4 (C-7a); 140.0, 139.0 (C-2, C-7a′); 137.0 (C-6); 128.5 (C-6′); 125.3 (C-4); 125.0 (C-4′); 122.8 (C-5′); 121.9 (C-5); 121.4, 119.9 (C-3a′, C-3a); 113.2, 112.0 (C-7, C-7′); 105.4 (C-3′); 80.4 (C-1″); 75.5 (C-5″); 70.7 (C-3″); 70.1 (C-2″); 65.4 (C-4″); 62.3 (C-6″); 20.7, 20.7, 20.7, 20.5 (4s, 4xC(O)*C*H_3). MS (EI, 70 eV): *m*/*z* (%) = 592 (57) [M^+], 331 (7) [M^+-indirubin], 262 (100) [indirubin], 169 (91) [M^+-indirubin-HOAc-Ac_2O], 133 (12) [indoxyl]. HRMS (EI, 70 eV): calcd for $C_{30}H_{28}N_2O_{11}$ ([M^+]): 592.16876; found: 592.16850.

4.18. General procedure 1 for the synthesis of indirubin-*N*′-glycosides

To a stirred, degassed methanol solution of glycosyl isatine **4**, indoxyl acetate **5** (1.0 equiv) and sodium carbonate (4.0 equiv) were added under Argon atmosphere. The mixture was stirred for1.5–4 h at 20 °C during which time the yellow to orange color of the solution changed to red to violet. The mixture was neutralized with IR 120 (H^+), filtered and the filtrate was concentrated under reduced pressure. Column chromatography of the residue gave the desired indirubin glycosides as red to violet solids.

4.19. *N*′-β-L-Rhamnopyranosylindirubin (β-6a)

Starting with **β-4a** (300 mg, 0.72 mmol) (reaction time: 4 h), **β-6a** (224 mg, 77%) was isolated by column chromatography ($CHCl_3$/MeOH = 20:1) as a red solid. Mp 287–288 °C; R_f = 0.76 ($CHCl_3$/MeOH = 5:1). ^{1}H NMR (500 MHz, DMSO): δ = 10.08 (s, 1H, NH); 8.80 (dd, $^4J_{4',6'}$ = 1.0 Hz, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-4′); 7.67 (d, $^3J_{4,5}$ = 7.6 Hz, 1H, H-4); 7.64 (d, $^3J_{6',7'}$ = 7.9 Hz, 1H, H-7′); 7.60 (d′t′, $^4J_{4,6}$ = 1.0 Hz, $^3J_{5,6}$ = 7.6 Hz, $^3J_{6,7}$ = 7.9 Hz, 1H, H-6); 7.42 (d, $^3J_{6,7}$ = 7.9 Hz, 1H, H-7); 7.23 (d′t′, $^4J_{4',6'}$ = 1.0Hz, $^3J_{5',6'}$ = 7.5 Hz, $^3J_{6',7'}$ = 7.9 Hz, 1H, H-6′); 7.06-7.02 (m, 2H, H-5, H-5′); 5.62 (s, 1H, H-1″); 5.12 (d, $^3J_{2'',OH}$ = 5.0 Hz, 1H, $OH_{(2'')}$); 4.96 (d, $^3J_{4'',OH}$ = 5.0 Hz, 1H, $OH_{(4'')}$); 4.85 (d, $^3J_{3'',OH}$ = 6.0 Hz, 1H, $OH_{(3'')}$); 3.86 (m, 1H, H-2″); 3.50 (m, 1H, H-3″); 3.41-3.35 (m, 2H, H-4″, H-5″); 1.27 (d, $^3J_{5'',6''}$ = 5.8 Hz, 3H, H-6″). ^{13}C NMR (126 MHz, DMSO): δ = 188.6 (C-3); 168.5 (C-2′); 152.4 (C-7a); 140.9 (C-7a′); 138.8 (C-2); 137.3 (C-6); 128.5 (C-6′); 124.6 (C-4); 123.7 (C-4′); 121.6, 121.5 (C-5,5′); 120.7 (C-3a′); 119.2 (C-3a); 114.9 (C-7′); 113.6 (C-7); 105.5 (C-3′); 82.3 (C-1″); 75.5 (C-4″); 73.3 (C-3″); 72.0 (C-2″); 71.5 (C-5″), 18.2 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 408 (13) [M^+], 262 (100) [indirubin], 234 (35) [indirubin-(C=O)], 147 (22) [M^+-indirubin]. HRMS (ESI): calcd for $C_{22}H_{20}N_2NaO_6$ ([M+Na]$^+$) 431.12136; found 431.12162.

4.20. 4′,6′-Dimethyl-1′-β-L-rhamnopyranosylindirubin (β-6b)

Starting with 4,6-dimethyl-1-β-L-rhamnopyranosylisatine (**β-4b**) (250 mg, 0.56 mmol), **β-6b** was isolated (150 mg, 62%) by column chromatography ($CHCl_3$/EtOH = 30:1 → 20:1) as a red solid. Mp 210–213 °C; R_f = 0.79 ($CHCl_3$/EtOH 5:1). ^{1}H NMR (300 MHz, DMSO): δ = 11.07 (s, 1H, NH); 7.65 (d, $^3J_{4,5}$ = 7.5 Hz, 1H, H-4); 7.58 (d′t′, $^4J_{4,6}$ = 1.2 Hz, $^3J_{5,6}$ = 7.5 Hz,$^3J_{6,7}$ = 8.0 Hz, 1H, H-6); 7.37 (d, $^3J_{6,7}$ = 8.0 Hz, 1H, H-7); 7.31 (s, 1H, H-7′); 7.03 (‘t’, $^3J_{5,6}$ = 7.5 Hz, 1H, H-5); 6.72 (s, 1H, H-5′); 5.58 (s, 1H, H-1″); 5.16 (d, $^3J_{2'',OH}$ = 4.8 Hz, 1H, $OH_{(2'')}$); 4.96 (d, $^3J_{4'',OH}$ = 4.9 Hz, 1H, $OH_{(4'')}$); 4.80 (d, $^3J_{3'',OH}$ = 6.0 Hz, 1H, $OH_{(3'')}$); 3.84 (m, 1H, H-2″); 3.51–3.44 (m, 1H, H-3″); 3.35–3.25 (m, 2H, H-4″, H-5″); 2.29, 2.08 (2s, 6H, 2xCH_3); 1.26 (d, $^3J_{5'',6''}$ = 5.3 Hz, 3H, H-6″). ^{13}C NMR (75 MHz, DMSO): δ = 186.7 (C-3); 169.5 (C-2′); 151.4, 141.9, 137.6 (C-2, C-7a′, C-7a); 136.7 (C-6); 136.5, 135.5 (C-4′, C-6′); 124.9 (C-5′); 124.3 (C-4); 121.5 (C-5); 119.4, 118.3 (C-3a, C-3a′); 113.3 (C-7); 112.7 (C-7′); 106.0 (C-3′); 82.3 (C-1″); 75.6, 71.7 (C-4″, C-5″); 73.5 (C-3″); 72.0 (C-2″); 22.9, 22.0 (2xCH_3); 18.3 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 436 (12) [M^+], 290 (79) [aglyconH], 273 (59) [M^+-aglycon], 161 (57) [4,6-dimethyloxindol]. HRMS (EI, 70 eV): calcd for $C_{24}H_{24}N_2O_6$ ([M^+]): 436.16289; found: 436.16259.

4.21. *N*′-β-D-Glucopyranosylindirubin (β-6c)

Starting with **β-4c** (160 mg, 0.335 mmol) (reaction time: 4 h), **β-6c** was isolated (100 mg, 70%) by column chromatography ($CHCl_3$/MeOH=20:1) as a red solid. Mp=221–222 °C; R_f = 0.5 ($CHCl_3$/MeOH = 5:1). ^{1}H NMR (500 MHz, DMSO): δ = 11.11 (s, 1H, NH); 8.88 (dd, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-4′); 7.67 (′d′, $^3J_{4,5}$ = 7.5 Hz, 1H, H-4); 7.60 (ddd, $^4J_{4,6}$ = 1.0 Hz, $^3J_{5,6}$ = 7.5 Hz, $^3J_{6,7}$ = 8.0 Hz, 1H, H-6); 7.43 (d, $^3J_{6,7}$ = 8.0 Hz, 1H, H-7); 7.32 (d′t′, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{5',6'}$= $^3J_{6',7'}$ = 7.9 Hz, 1H, H-6′); 7.26 (′d′, $^3J_{6',7'}$ = 7.9 Hz, 1H, H-7′); 7.11 (d′t′, $^4J_{5',7'}$ = 1.0 Hz, $^3J_{4',5'}$=$^3J_{5',6'}$ = 7.9 Hz, 1H, H-5′); 7.04 (‘t’, $^3J_{4,5}$ = $^3J_{5,6}$ = 7.5 Hz, 1H, H-5); 5.33 (d, $^3J_{1'',2''}$ = 9.5 Hz, 1H, H-1″); 4.57-4.03 (br m, 4H, 4xOH); 3.94 (br, 1H, H-2″); 3.75 (dd, $^3J_{5'',6a''}$ = 1.8 Hz, $^2J_{6a'',6b''}$ = 12.0 Hz, 1H, H-6a″); 3.49 (dd, $^3J_{5'',6b''}$ = 6.2 Hz, $^2J_{6a'',6b''}$ = 12.0 Hz, 1H, H-6b″); 3.37 (ddd, $^3J_{5'',6a''}$ = 1.8 Hz, $^3J_{5'',6b''}$ = 6.2 Hz, $^3J_{4'',5''}$ = 9.3 Hz, 1H, H-5″); 3.35 (‘t’, $^3J_{2'',3''}$=$^3J_{3'',4''}$ = 8.8 Hz, 1H, H-3″); 3.28 (‘t’, $^3J_{3'',4''}$ = 8.8 Hz, $^3J_{4'',5''}$ = 9.3 Hz, 1H, H-4″). ^{13}C NMR (126 MHz, DMSO): δ = 188.7 (C-3); 169.1 (C-2′); 152.6 (C-7a); 139.7 (C-7a′); 138.9 (C-2); 137.4 (C-6); 129.1 (C-6′); 124.7 (C-4); 124.6 (C-4′); 122.0 (C-5′); 121.7 (C-5); 121.2 (C-3a′); 119.2 (C-3a); 113.7 (C-7); 111.6 (C-7′); 105.7 (C-3′); 82.0 (C-1″); 80.2 (C-5″); 77.6 (C-3″); 70.1 (C-4″); 68.9 (C-2″); 61.3 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 424 (30) [M^+], 262 (100) [indirubin]. HRMS (EI, 70 eV): calcd for $C_{22}H_{20}N_2O_7$([M^+]): 424.12650; found: 424.12741.

4.22. *N*′-β-D-Galactopyranosylindirubin (β-6d)

Starting with **β-4d** (200 mg, 0.419 mmol) (reaction time: 3 h), **β-6d** was isolated (130 mg, 73%) by column chromatography ($CHCl_3$/MeOH = 20:1) as a red solid. Mp 275–276 °C; R_f 0.46 ($CHCl_3$/MeOH = 5:1). ^{1}H NMR (250 MHz, DMSO): δ = 11.09 (s, 1H, NH); 8.87 (dd, $^4J_{4',6'}$ = 1.0 Hz, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-4′); 7.67 (′d′, $^3J_{4,5}$ = 7.5 Hz, 1H, H-4); 7.60 (ddd, $^4J_{4,6}$ = 1.1 Hz, $^3J_{5,6}$ = 7.5 Hz, $^3J_{6,7}$ = 8.0 Hz, 1H, H-6); 7.48 (′d′, $^3J_{6',7'}$ = 8.0Hz, 1H, H-7′); 7.43 (′d′, $^3J_{6,7}$ = 8.0 Hz, 1H, H-7); 7.30 (d′t′, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{5',6'}$ = 7.8 Hz, $^3J_{6',7'}$ = 8.0 Hz, 1H, H-6′); 7.14–7.00 (m, 2H, H-5, H-5′); 5.35 (d, $^3J_{1'',2''}$ = 9.1 Hz, 1H, H-1″); 5.19–4.69 (br m, 4H, 4xOH); 4.18 (‘t’, $^3J_{1'',2''}$=$^3J_{2'',3''}$ = 9.1 Hz, 1H, H-2″); 3.81 (d, $^3J_{3'',4''}$ = 2.8 Hz, 1H, H-4″); 3.63-3.50 (m, 4H, H-3″, H-5″, H-6a″, H-6b″). ^{13}C NMR (63 MHz, DMSO): δ = 188.7 (C-3); 168.9 (C-2′); 152.6 (C-7a); 139.7, 138.9 (C-7a′, C-2); 137.4 (C-6); 129.0 (C-6′); 124.7 (C-4); 124.5 (C-4′); 122.0 (C-5′); 121.7 (C-5); 121.2, 119.3 (C-3a, C-3a′); 113.8 (C-7); 112.2 (C-7′); 106.0 (C-3′); 82.1 (C-1″); 78.0, 74.2 (C-3″, C-5″); 68.5 (C-4″); 66.3 (C-2″); 61.0 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 424 (8) [M^+], 262 (100) [indirubin], 234 (35)

[M^+-indirubin-(C=O)]. HRMS (EI): calcd for $C_{22}H_{20}N_2O_7$ ([M^+]): 424.12650; found: 424.12566.

4.23. *N*′-β-D-Mannopyranosylindirubin (β-6e)

Starting with **β-4e** (190 mg, 0.398 mmol) (reaction time: 3 h), **β-6e** was isolated (100 mg, 59%) by column chromatography ($CHCl_3$/MeOH = 20:1) as a red solid. Mp 137–138 °C; R_f = 0.55 ($CHCl_3$/MeOH = 5:1). ^{1}H NMR (250 MHz, DMSO): δ = 11.09 (s, 1H, NH); 8.80 (dd, $^4J_{4',6'}$ = 1.0 Hz, $^3J_{4',5'}$ = 7.8 Hz, 1H, H-4′); 7.68 (′d′, $^3J_{6',7'}$ = 7.9 Hz, 1H, H-7′); 7.67 (′d′, $^3J_{4,5}$ = 7.5 Hz, 1H, H-4); 7.59 (ddd, $^4J_{4,6}$ = 1.2 Hz, $^3J_{5,6}$ = 7.5 Hz, $^3J_{6,7}$ = 8.0 Hz, 1H, H-6); 7.42 (′d′, $^3J_{6,7}$ = 8.0 Hz, 1H, H-7); 7.22 (d′t′, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{5',6'}$ = 7.8 Hz, $^3J_{6',7'}$ = 8.0 Hz, 1H, H-6′); 7.05–7.00 (m, 2H, H-5, H-5′); 5.63 (d, $^3J_{1'',2''}$ = 1.0 Hz, 1H, H-1″); 5.13 (d, ^3J = 4.8 Hz, 1H, $OH_{(2'')}$); 4.94 (d, ^3J = 4.5 Hz, 1H), 4.89 (d, ^3J = 4.5 Hz, 1H) ($OH_{(3'')}$, $OH_{(4'')}$); 4.59 (t, ^3J = 5.5 Hz, 1H, $OH_{(6'')}$); 3.88–3.85 (m, 1H, H-2″); 3.82–3.78 (m, 1H, H-6a″); 3.59–3.50 (m, 3H, H-3″, H-4″, H-6b″); 3.40-3.28 (m, 1H, H-5″). ^{13}C NMR (63 MHz, DMSO): δ = 188.6 (C-3); 168.6 (C-2′); 152.5 (C-7a); 141.1 (C-7a′); 138.9 (C-2); 137.4, 128.7 (C-6, C-6′); 124.7, 123.7 (C-4, C-4′); 121.7, 121.6 (C-5, C-5′); 120.8, 119.3 (C-3a, C-3a′); 115.4, 113.7 (C-7, C-7′); 105.7 (C-3′); 82.4 (C-1″); 81.1 (C-5″); 73.7, 66.6 (C-3″, C-4″); 72.0 (C-2″); 61.5 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 424 (24) [M^+], 262 (100) [indirubin], 234 (28) [M^+-indirubin-(C=O)]. HRMS (EI, 70 eV): calcd for $C_{22}H_{20}N_2O_7$ ([M^+]): 424.12650; found: 424.12557.

4.24. General procedure 2 for the synthesis of indirubin-*N*′-glycosides

To a methanol solution of acetylated indirubin-*N*-glycoside **7** was added KO^{t}Bu (0.02 equiv per acetyl group). The mixture was stirred for 10–12 h at 20 °C. The mixture was neutralized by addition of IR 120 (H^+) and was subsequently filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography to give the desired indirubine glycoside as a red to violet solid.

4.25. *N*′-α-L-Rhamnopyranosylindirubin (α-6a)

Starting with **α-7a** (70 mg, 0.13 mmol), **α-6a** was isolated (48 mg, 90%) as a red solid by column chromatography ($CHCl_3$ /MeOH = 30:1 → 20:1). Mp 287–288 °C; R_f = 0.74 ($CHCl_3$/MeOH = 5:1). ^{1}H NMR (250 MHz, DMSO): δ = 11.10 (s, 1H, NH); 8.87 (′d′, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-4′); 7.67 (d, $^3J_{4,5}$ = 7.5 Hz, 1 H, H-4); 7.59 (d′t′, $^4J_{4,6}$ = 1.2 Hz, $^3J_{5,6}$ = 7.5 Hz, $^3J_{6,7}$ = 7.9 Hz, 1H, H-6); 7.41 (′d′, $^3J_{6,7}$ = 7.9 Hz, 1H, H-7); 7.38 (dd, $^4J_{5',7'}$ = 1.2 Hz, $^3J_{6',7'}$ = 8.0 Hz, 1H, H-7′); 7.31 (ddd, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{5',6'}$ = 7.6 Hz, $^3J_{6',7'}$ = 7.9 Hz, 1H, H-6′); 7.10 (d′t′,$^4J_{5',7'}$ = 1.2 Hz , $^3J_{5',6'}$ = 7.6 Hz, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-5′); 7.03 (d′t′,$^4J_{5,7}$ = 0.8 Hz, $^3J_{4,5}$ = $^3J_{5,6}$ = 7.5 Hz, 1H, H-5); 5.83 (d, $^3J_{1'',2''}$ = 8.5 Hz, 1H, H-1″); 5.16 (d, $^3J_{4'',OH}$ = 3.8 Hz, 1H, $OH_{(4'')}$); 5.03 (d, $^3J_{3'',OH}$ = 4.0 Hz, 1H, $OH_{(3'')}$); 4.90 (d, $^3J_{2'',OH}$ = 6.3 Hz, 1H, $OH_{(2'')}$); 4.52 (ddd, $^3J_{2'',3''}$ =3.5 Hz, $^3J_{2'',OH}$ = 6.3Hz, $^3J_{1'',2''}$ = 8.5 Hz, 1H, H-2″); 4.00 (′q′, $^3J_{2'',3''}$ = 3.5 Hz, $^3J_{3'',4''}$ = 3.8 Hz, $^3J_{3'',OH}$ = 4.0Hz, 1H, H-3″); 3.96 (dq, $^3J_{4'',5''}$ = 2.8Hz, $^3J_{5'',6''}$ = 7.0 Hz, 1H, H-5″); 3.61 (d′t′, $^3J_{4'',5''}$ = 2.8 Hz, $^3J_{4'',OH}$=$^3J_{3'',4''}$ = 3.8 Hz, 1H, H-4″); 1.37 (d, $^3J_{5'',6''}$ = 7.0 Hz, 3H, H-6″). ^{13}C NMR (75 MHz, DMSO): δ = 188.7 (C-3); 169.4 (C-2′); 152.6 (C-7a); 140.4 (C-7a′); 138.9 (C-2); 137.4 (C-6); 129.1 (C-6′); 124.7, 124.6 (C-4, C-4′); 122.0, 121.7 (C-5, C-5′); 121.3, 119.3 (C-3a, C-3a′); 113.7 (C-7); 111.6 (C-7′); 106.0 (C-3′); 76.1 (C-1″); 74.6 (C-5″); 72.4 (C-3″); 72.4 (C-4″); 63.7 (C-2″); 16.9 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 408 (26) [M^+], 262 (100) [indirubin], 234 (24) [indirubin-(C=O)]. HRMS (EI, 70 eV): calcd for $C_{22}H_{20}N_2O_6$ ([M^+]): 408.13159; found: 408.13061.

4.26. 5-Chloro-1′-β-L-rhamnopyranosylindirubin (β-6f)

Starting with **β-7f** (162 mg, 0.28 mmol), **β-6f** was isolated (91 mg, 72%) by column chromatography ($CHCl_3$/EtOH = 20:1 → 10:1) as a red solid. Mp 285–286 °C; R_f = 0.52 ($CHCl_3$ /EtOH = 5:1). ^{1}H NMR (250 MHz, DMSO): δ = 11.17 (s, 1H, NH); 8.78 (′d′, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-4′); 7.69–7.60 (m, 3H, H-4, H-6, H-7′); 7.45 (′d′, $^3J_{6,7}$ = 8.5 Hz, 1H, H-7); 7.24 (d′t′, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{5',6'}$ = 7.5 Hz, $^3J_{6',7'}$ = 7.9 Hz 1H, H-6′); 7.03 (d′t′, $^4J_{5',7'}$ = 1.0 Hz, $^3J_{5',6'}$ = 7.5 Hz, $^3J_{6',7'}$ = 7.9 Hz, 1H, H-5′); 5.62 (br s, 1H, H-1″); 5.14 (d, ^3J = 4.8 Hz, 1H, OH); 4.99 (d, ^3J = 4.8 Hz, 1H, OH); 4.89 (d, ^3J = 6.0 Hz, 1H, OH); 3.88–3.83 (m, 1H, H-2″); 3.52-3.35 (m, $^3J_{5'',6''}$ = 5.3 Hz, 3H, H-3″, H-4″, H-5″); 1.27 (d, $^3J_{5'',6''}$ = 5.3 Hz, 3H, H-6″). ^{13}C NMR (63 MHz, DMSO): δ = 187.5 (C-3); 168.4 (C-2′); 151.1 (C-7a); 141.3 (C-7a′); 138.6 (C-2); 136.7 (C-6); 129.0 (C-6′); 125.7 (C-5); 123.9 (C-4); 123.9 (C-4′); 121.6 (C-5′); 120.7, 120.5 (C-3a′, C-3a); 115.4, 115.1 (C-7, C-7′); 106.5 (C-3′); 82.4 (C-1″); 75.6 (C-4″); 73.4, 72.1 (C-2″, C-3″); 71.6 (C-5″); 18.3 (C-6″). MS (EI, 70 eV): *m*/*z* (%) = 444 (6) [M^+, ^{37}Cl]; 442 (17) [M^+, ^{35}Cl]; 298 (35) [5- chloroindirubin, ^{37}Cl]; 296 (100) [5- chloroindirubin, ^{35}Cl]; 209 (9) [5-chloroindirubin-C $_6H_4$N+2H]; 207 (36) [5-chloroindirubin-C $_6H_4$ N + 2H]; 129 (27) [4-chloroaniline, ^{37}Cl]; 127 (7) [4-chloroaniline, ^{35}Cl]; 93 (13) [aniline]. HRMS (EI, 70 eV): calcd for $C_{22}H_{19}ClN_2O_6$ ([M^+]): 442.09079; found: 442.09120.

4.27. 5-Chloro-1′-α-L-rhamnopyranosylindirubin (α-6f)

Starting with **α-7f** (110 mg, 0.19 mmol), **α-6f** was isolated (60 mg, 70%) by column chromatography ($CHCl_3$/EtOH = 20:1 → 10:1) as a red solid. Mp=247-250 °C; R_f = 0.49 ($CHCl_3$ /EtOH = 5:1). ^{1}H NMR (300 MHz, DMSO): δ = 11.20 (s, 1H, NH); 8.84 (′d′, $^3J_{4',5'}$ = 7.8 Hz, 1H, H-4′); 7.68 (d, $^4J_{4,6}$ = 2.0 Hz, 1H, H-4); 7.63 (dd, $^4J_{4,6}$ = 2.0 Hz, $^3J_{6,7}$ = 8.5 Hz, 1H, H-6); 7.45 (d, $^3J_{6,7}$ = 8.5 Hz, 1H, H-7); 7.40-7.29 (m, 2H, H-6′, H-7′); 7.11 (d′t′,$^3J_{5',6'}$ = 7.2 Hz, $^3J_{4',5'}$ = 7.8 Hz , 1H, H-5′); 5.82 (d, $^3J_{1'',2''}$ = 8.5 Hz, 1H, H-1″); 5.15 (d, $^3J_{4'',OH}$ = 3.8 Hz, 1H, $OH_{(4'')}$); 5.03 (d, $^3J_{2'',OH}$ = 4.0 Hz, 1H, $OH_{(3'')}$); 4.93 (d, $^3J_{2'',OH}$ = 6.3 Hz, 1H, $OH_{(2'')}$); 4.51 (ddd, $^3J_{2'',3''}$ = 3.4 Hz, $^3J_{2'',OH}$ = 6.3Hz, $^3J_{1'',2''}$ = 8.5 Hz, 1H, H-2″); 4.02-3.92 (m, 2H, H, H-3″, H-5″); 3.60 (m, 1H, H-4″); 1.37 (d, $^3J_{5'',6''}$ = 7.0 Hz, 3H, H-6″). ^{13}C NMR (75 MHz, DMSO): δ = 187.6 (C-3); 169.2 (C-2′);

151.2 (C-7a); 140.6, 138.6 (C-2, C-7a′); 136.7 (C-6); 129.5 (C-6′); 124.7, 123.9 (C-4, C-4′); 122.1 (C-5′); 125.7 (C-5); 121.1, 120.5 (C-3a′, C-3a); 115.5, 111.7 (C-7, C-7′); 107.0 (C-3′); 76.1 (C-1″); 74.6 (C-5″); 72.4 (C-3″); 72.4 (C-4″); 63.7 (C-2″); 16.9 (C-6″). MS (CI, isobutane): *m/z* (%)=445 (41) [M^++H; ^{37}Cl]; 443 (77) [M^++H; ^{35}Cl]. HRMS (CI, isobutane): calcd for $C_{22}H_{19}ClN_2O_6$ ([M^+]): 443.10044; found: 443.10063.

4.28. 1′-(2″,3″,4″-Tri-*O*-acetyl-β-L-rhamnopyranosyl)indirubin-3-monoxim (β-8a)

To a pyridine solution of **β-7a** hydroxylamine hydrochloride (2.0 equiv) was added. The mixture was stirred for 7 h at 90 °C. The solvent was removed under reduced pressure and the residue was purified by column chromatography (heptane/EtOAc = 5:1 → 1:1). Starting with **β-7a** (200 mg, 0.37 mmol), **β-8a** was isolated as a red solid(118 mg, 57%). Mp 94–96 °C; R_f = 0.52 (*n*-heptane/EtOAc = 1:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.43 (s, 1H, NH); 9.53 (br s, 1H, OH); 8.43 (dd, $^4J_{4',6'}$ = 1.1 Hz, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-4′); 8.21 (′d′, $^3J_{4,5}$ = 7.7 Hz, 1H, H-4); 7.57 (′d′ $^3J_{6',7'}$ = 7.9 Hz, 1H, H-7′); 7.29–7.07 (2 dt, 2H, H-5′, H-6′); 6.98–6.82 (m, 2H, H-5, H-6); 6.83 (′d′, $^3J_{6,7}$ = 7.9 Hz, 1H, H-7); 6.06 (d, $^3J_{1'',2''}$ = 1.5 Hz, 1H, H-1″); 5.67 (dd, $^3J_{1'',2''}$ = 1.5 Hz, $^3J_{2'',3''}$ = 3.2 Hz, 1H, H-2″); 5.39–5.22 (m, 2H, H-3″, H-4″); 3.80 (dq, $^3J_{5'',6''}$ = 6.2 Hz, $^3J_{4'',5''}$ = 9.0 Hz, 1H, H-5″); 2.11, 2.00, 1.85 (3s, 9H, 3xC(O)CH_3); 1.38 (d, $^3J_{5'',6''}$ = 6.2 Hz, 3H, H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.6, 170.1, 169.8 (3x*C*(O)CH_3); 169.2 (C-2′); 152.6, 146.6, 144.4, 136.8 (C-2, C-3, C-7a, C-7a′); 132.2, 129.2, 125.3, 123.2 (C-4, C-4′, C-6, C-6′); 122.5, 116.9 (C-3a, C-3a′); 121.9, 121.7 (C-5, C-5′); 112.5, 110.3 (C-7, C-7′); 98.1 (C-3′); 80.5 (C-1″); 74.0, 70.8, 70.7, 70.5 (C-2″, C-3″, C-4″, C-5″); 20.9, 20.8, 20.6 (3xC(O) *C*H_3); 17.7 (C-6″). MS (EI, 70 eV): *m/z* (%)=549 (7) [M^+]; 277 (7) [indirubin-3-monoxim]; 153 (11) [M^+-aglycon-2HOAc]; 135 (30). HRMS (EI, 70 eV): calcd for $C_{28}H_{27}N_3O_9$ ([M^+]): 549.17418; found: 549.17541.

4.29.1′-β-L-Rhamnopyranosylindirubin-3-monoxim (β-9a)

The synthesis of **β-9a** was carried out following the procedure as given for the preparation of **α-6a**. Starting with **β-8a** (100 mg, 0.18 mmol), **β-9a** was isolated (53 mg, 69%) by column chromatography ($CHCl_3$/MeOH = 20:1 → 10:1) as a red solid. Mp 295–297 °C; R_f = 0.7 ($CHCl_3$ /MeOH = 5:1). ^{1}H NMR (250 MHz, DMSO)[22]: δ = 11.77 (s, 1H, NH); 8.71 (dd, $^4J_{4',6'}$ = 1.2 Hz, $^3J_{4',5'}$ = 7.9 Hz, 1H, H-4′); 8.25 (d, $^3J_{4,5}$ = 7.6 Hz, 1H, H-4); 7.62 (dd, $^4J_{5',7'}$ = 1.0 Hz, $^3J_{6',7'}$ = 8.0 Hz, 1H, H-7′); 7.42-7.39 (m, 2H), 7.14-6.92 (m, 3H) (H-5, H-5′, H-6, H-6′, H-7); 5.66 (s, 1H, H-1″); 5.20 (d, ^3J = 5.2 Hz, 1H, OH); 4.97 (br s, 1H, OH); 4.85 (br s, 1H, OH); 3.89 (br s, 1H, H-2″); 3.60-3.40 (m, 3H, H-3″, H-4″, H-5″); 1.27 (d, $^3J_{5'',6''}$ = 5.5 Hz, 3H, H-6″). ^{13}C NMR (63 MHz, DMSO): δ = 168.5 (C-2′); 151.5, 146.1, 144.7, 138.4 (C-2, C-3, C-7a, C-7a′); 132.2, 128.1, 125.3, 122.0 (C-4, C-4′, C-6, C-6′); 122.2, 116.7 (C-3a, C-3a′); 122.0, 120.8 (C-5, C-5′); 114.0, 111.8 (C-7, C-7′); 97.8 (C-3′); 82.4 (C-1″); 75.6, 73.7, 72.2, 71.8 (C-2″, C-3″, C-4″, C-5″); 18.3 (C-6″). MS (CI, Isobutane): *m/z* (%) = 424 (100) [M^++H]. HRMS (EI, 70 eV): calcd. for $C_{22}H_{21}N_3O_6$ ([M^+]): 423.14249; found: 423.14240.

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