Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 6907–6909

First synthesis of indirubin N-glycosides (red sugars)

Stefanie Libnow,[a] Martin Hein,[a,*] Dirk Michalik[b] and Peter Langer[a,b,*]

[a]*Institut für Chemie, Universität Rostock, Albert-Einstein-Str. 3a, D-18059 Rostock, Germany*
[b]*Leibniz-Institut für Katalyse e.V. an der Universität Rostock, Albert-Einstein-Str. 29a, D-18059 Rostock, Germany*

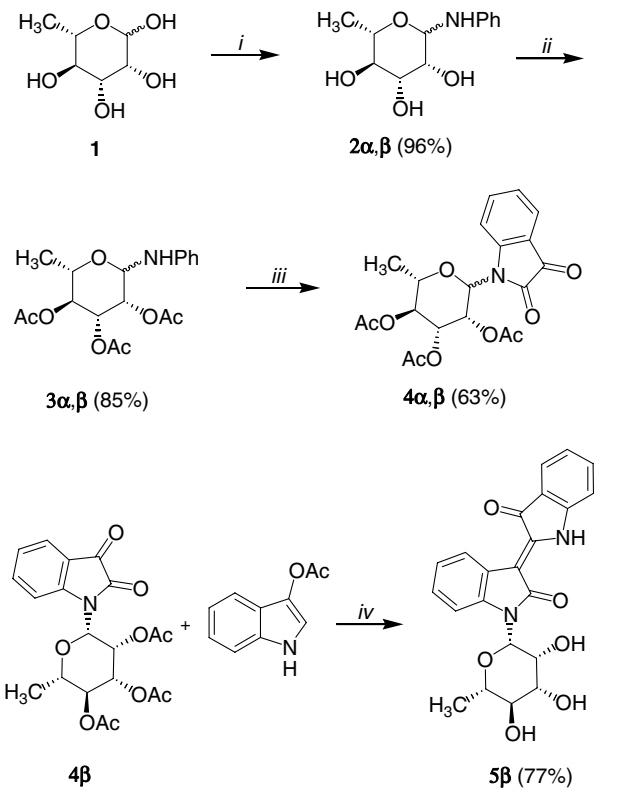
Received 30 May 2006; revised 3 July 2006; accepted 6 July 2006
Available online 4 August 2006

Abstract—The first indirubin N-glycosides were prepared by reaction of isatine N-glycosides with indoxyl acetate under basic conditions.
© 2006 Elsevier Ltd. All rights reserved.

Glycosylated indole derivatives are of considerable pharmacological relevance (e.g., cancerostatic staurosporine, K-252d, rebeccamycin and the tjipanazoles).[1,2] Recently, we have reported[3] the first synthesis of indigo N-glycosides (blue sugars), which represent analogues of the naturally occurring akashins.[4] In contrast to pharmacologically inactive parent indigo, the akashins show a considerable activity against various human tumour cell lines. Recently, the first isoindigo glycosides were prepared, which exhibit antiproliferative activity.[5] Indirubin, the most important isomer of indigo, and several of its derivatives exhibit considerable antiproliferative activity.[6,7] Herein, we report what are, to the best of our knowledge, the first syntheses of indirubin N-glycosides.

N-(2,3,4-Tri-*O*-acetyl-α,β-L-rhamnopyranosyl)isatine (**4α,β**) was prepared from L-rhamnose.[8–12] Simple indirubins have been previously prepared by reaction of a methanolic solution of indoxyl acetate with isatines.[13] The reaction of the pure beta anomer **4β** with indoxyl acetate resulted in the formation of the desired deprotected *N*-(β-L-rhamnopyranosyl)indirubin **5β** in up to 77% yield (Scheme 1).[14] During the optimisation 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.

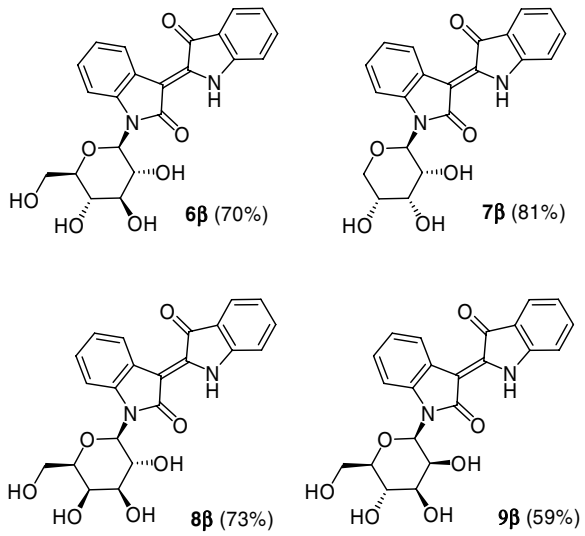
Following this procedure, the anomerically pure indirubin glycosides of D-glucose (product **6β**), D-ribose (product **7β**), D-galactose (**8β**), and D-mannose (**9β**) were successfully prepared in good yields starting from the



Scheme 1. Synthesis of *N*-(β-L-rhamnopyranosyl)indirubin **5β**. Reagents and conditions: (i) $PhNH_2$, EtOH, rt, 12 h; (ii) Ac_2O, pyridine, 0→4 °C, 8–12 h; (iii) oxalyl chloride, $AlCl_3$, 55 °C, 1.5 h; (iv) Na_2CO_3, MeOH, rt, 4 h.

Keywords: *N*-Glycosyl anilines; *N*-Glycosyl isatines; Indirubin; N-Heterocycles; N-Glycosides.
* Corresponding authors. Tel.: +49 381 4986410; fax: +49 381 4986412; e-mail: peter.langer@uni-rostock.de

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.07.024



Scheme 2. Synthesis of *N*-(β-D-glucopyranosyl)indirubin **6β**, *N*-(β-D-ribopyranosyl)indirubin **7β**, *N*-(β-D-galactopyranosyl)indirubin **8β** and *N*-(β-D-mannopyranosyl) indirubin **9β**; the yields, based on the corresponding *N*-β-glycosyl isatine, are given in brackets.

corresponding *N*-glycosyl isatines (Scheme 2). In case of L-rhamnose, both anomeric glycosyl isatines could be isolated after cyclisation of **3α,β** with oxalyl chloride. In contrast, only traces of α-anomers were detected for all other sugars. In these cases, isomerically enriched β-anomers (β/α >5:1) of the corresponding acetylated *N*-glycosyl anilines were employed in the cyclisation reaction. However, the β/α-ratio seems to slightly vary during the course of the reaction; otherwise, higher amounts of the α-anomeric *N*-glycosyl isatines would have been detected after the reaction. The determination of the anomeric configuration of the products was carried out by two-dimensional NMR spectroscopy (^{1}H,^{1}H COSY, ^{1}H,^{1}H NOESY, ^{13}C,^{1}H COR, HMBC).

In conclusion, we have reported the first syntheses of deprotected N-glycosides of indirubin (red sugars).

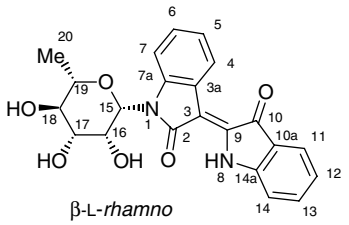
Acknowledgements

Financial support by the state of Mecklenburg-Vorpommern (scholarship for S.L.) is gratefully acknowledged.

References and notes

1. Review: Gribble, G.; Berthel, S. In *Studies in Natural Products Chemistry*; Elsevier Science: New York, 1993; Vol. 12, pp 365–409.
2. Isolation of staurosporin: (a) Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1977**, *30*, 275–282; synthesis: (b) Link, J. T.; Raghavan, S.; Gallant, M.; Danishefsky, S. J.; Chou, T. C.; Ballas, L. M. *J. Am. Chem. Soc.* **1996**, *118*, 2825–2842; pharmacological activity: (c) Yamashita, Y.; Fujii, N.; Murakata, C.; Ashizawa, T.; Okabe, M.; Nakano, H. *Biochemistry* **1992**, *31*, 12069–12075.
3. (a) Hein, M.; Michalik, D.; Langer, P. *Synthesis* **2005**, *20*, 3531–3534; (b) Hein, M.; Nguyen, T. B. P.; Michalik, D.; Görls, H.; Lalk, M.; Langer, P. *Tetrahedron Lett.* **2006**, *47*, 5741.
4. Maskey, R. P.; Grün-Wollny, I.; Fiebig, H. H.; Laatsch, H. *Angew. Chem.* **2002**, *114*, 623–625; *Angew. Chem., Int. Ed.* **2002**, *41*, 597–599.
5. (a) Sassatelli, M.; Saab, E.; Anizon, F.; Prudhomme, M.; Moreau, P. *Tetrahedron Lett.* **2004**, *45*, 4827–4830; (b) Sassatelli, M.; Bouchikhi, F.; Messaoudi, S.; Anizon, F.; Debiton, E.; Barthomeuf, C.; Prudhomme, M.; Moreau, P. *Eur. J. Med. Chem.* **2006**, *41*, 88–100.
6. Moon, M. J.; Lee, S. K.; Lee, J.-W.; Song, W. K.; Kim, S. W.; Kim, J. I.; Cho, C.; Choi, S. J.; Kim, Y.-C. *Bioorg. Med. Chem.* **2006**, *14*, 237–246.
7. Indirubin, the red shade of indigo. In *Life in Progress*; Meijer, L., Guyard, N., Skaltsounis, L. A., Eisenbrand, G., Ed.; Station Biologique, Roscoff, in press.
8. Chavis, C.; De Gourcy, C.; Dumont, F.; Imbach, J.-L. *Carbohydr. Res.* **1983**, *113*, 1–20.
9. Douglas, J. G.; Honeyman, J. *J. Chem. Soc.* **1955**, 3674–3680.
10. Preobrazhenskaya, M. N.; Yartseva, I. V.; Ektova, L. V. *Nucleic Acid Chem.* **1978**, *2*, 725–727.
11. Yartseva, I. V.; Ektova, L. V.; Preobrazhenskaya, M. N. *Bioorg. Khim.* **1975**, *1*, 189–194.
12. Synthesis of isatine glycosides: To a stirred solution of the acetylated rhamnosyl aniline in oxalyl chloride (about 10 equiv), an equivalent amount of anhydrous aluminium 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 stirred solution. The mixture was extracted with EtOAc (3×). The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate and with water, dried (sodium sulfate) and filtered. The filtrate was concentrated in vacuo and the residue was purified by column chromatography to give **4α,β**. Starting with **3α,β** (1.24 g, 3.39 mmol, β/α = 2.5:1), **4β** (320 mg, 23%) and **4α,β** (570 mg, 40%, β/α = 3:1) were isolated after column chromatography (heptane/EtOAc = 3:1).
The syntheses of *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)isatine,[10] *N*-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)isatine,[11] *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)isatine and *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)isatine were carried out following the procedure as described above. In contrast, isomerically enriched β-anomers (β/α >5:1) were employed as starting materials. Starting with *N*-(2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranosyl)aniline (1.00 g, 2.36 mmol), *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)isatine was isolated (0.34 g, 30%). *N*-(2,3,4-Tri-*O*-acetyl-α,β-D-ribopyranosyl)aniline (0.75 g, 2.13 mmol) was transformed into *N*-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)isatine (0.18 g, 21%). Starting with *N*-(2,3,4,6-tetra-*O*-acetyl-α,β-D-galactopyranosyl)aniline (0.80 g, 1.89 mmol), *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)isatine was isolated (0.47 g, 52%). *N*-(2,3,4,6-Tetra-*O*-acetyl-α,β-D-mannopyranosyl)aniline (1.00 g, 2.36 mmol) was transformed into *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)isatine (0.24 g, 21%). All products were purified by column chromatography (heptane/EtOAc = 2:1).
13. (a) Russell, G. A.; Kaupp, G. *J. Am. Chem. Soc.* **1969**, *91*, 3851–3859; (b) Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A.-L.; Myrianthopoulos, V.; Mikros, E.; Tarricone, A.; Musacchio, A.; Roe, S. M.; Pearl, L.; Leost, M.; Greengard, P.; Meijer, L. *J. Med. Chem.* **2004**, *47*, 935–946.
14. Synthesis of indirubin glycosides: To a MeOH solution of the acetylated glycosyl isatine, indoxyl acetate (1.0 equiv)

and sodium carbonate (4.0 equiv) were added under argon atmosphere. The mixture was stirred for 1.5–4 h at 20 °C. The yellow to orange colour of the solution changed from red to violet. The mixture was neutralised with IR 120 (H^+), filtered and the filtrate was concentrated under reduced pressure. Column chromatography of the residue gave the indirubin glycosides as red to violet solids: Starting with **4β** (300 mg, 0.715 mmol) (reaction time: 4 h), **5β** (224 mg, 77%) was isolated by column chromatography ($CHCl_3$/MeOH, 20:1→10:1). ^{1}H NMR (500.13 MHz, DMSO) δ: 10.08 (s, 1H, NH); 8.80 (dd, 1H, $^3J_{4,5} = 7.9$ Hz, $^4J_{4,6} = 1.0$ Hz, H-4); 7.67 (d, 1H, $^3J_{11,12} = 7.6$ Hz, H-11); 7.64 (d, 1H, $^3J_{6,7} = 7.9$ Hz, H-7); 7.60 (dt, 1H, $^3J_{13,14} = 7.9$ Hz, $^3J_{12,13} = 7.6$ Hz, $^4J_{11,13} = 1.0$ Hz, H-13); 7.42 (d, 1H, $^3J_{13,14} = 7.9$ Hz, H-14); 7.23 (dt, 1H, $^3J_{6,7} = 7.9$ Hz, $^3J_{5,6} = 7.5$ Hz, $^4J_{4,6} = 1.0$ Hz, H-6); 7.06–7.02 (m, 2H, H-5,12); 5.62 (s (br), 1H, H-15); 5.12 (d, 1H, $^3J_{16,OH} = 5.0$ Hz, $OH_{(16)}$); 4.96 (d, 1H, $^3J_{18,OH} = 5.0$ Hz, $OH_{(18)}$); 4.85 (d, 1H, $^3J_{17,OH} = 6.0$ Hz, $OH_{(17)}$); 3.86 (m, 1H, H-16); 3.50 (m, 1H, H-17); 3.41–3.35 (m, 2H, H-18,19); 1.27 (d, 3H, $^3J_{19,20} = 5.8$ Hz, $Me_{(20)}$); ^{13}C NMR (125.8 MHz, DMSO) δ: 188.7 (C-10); 168.5 (C-2); 152.4 (C-14a); 140.9 (C-7a); 138.8 (C-9); 137.3 (C-13); 128.5 (C-6); 124.6 (C-11); 123.7 (C-4); 121.6, 121.5 (C-5,12); 120.7 (C-3a); 119.2 (C-10a); 114.9 (C-7); 113.6 (C-14); 105.5 (C-3); 82.3 (C-15); 75.5 (C-18); 73.3 (C-17); 72.0 (C-16); 71.5 (C-19); 18.3 (C-20). HRMS (ESI): *m*/*z* calcd for $C_{22}H_{20}N_2NaO_6$ ($[M+Na]^+$): 431.1214; found: 431.1216.



Compound **6β**: *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)isatine (160 mg, 0.335 mmol) was converted according to the above mentioned procedure (reaction duration: 4 h). Column chromatography ($CHCl_3$–MeOH, 20:1→10:1) yielded **6β** (100 mg, 70%). Compound **7β**: *N*-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)isatine (120 mg, 0.296 mmol) was converted according to the above mentioned procedure (reaction duration: 1.5 h). Column chromatography ($CHCl_3$–MeOH, 20:1→10:1) yielded **7β** (95 mg, 81%). Compound **8β**: *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)isatine (200 mg, 0.419 mmol) was converted according to the above mentioned procedure (reaction duration: 3 h). Column chromatography ($CHCl_3$–MeOH, 20:1→10:1) yielded **8β** (130 mg, 73%). Compound **9β**: *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)isatine (190 mg, 0.398 mmol) was converted according to the above mentioned procedure (reaction duration: 3 h). Column chromatography ($CHCl_3$–MeOH, 20:1→10:1) yielded **9β** (100 mg, 59%).