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Total synthesis of indole-3-acetonitrile-4-methoxy-2-*C*- β -D-glucopyranoside. Proposal for structural revision of the natural product†

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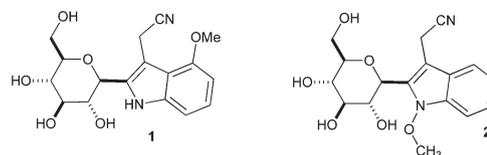
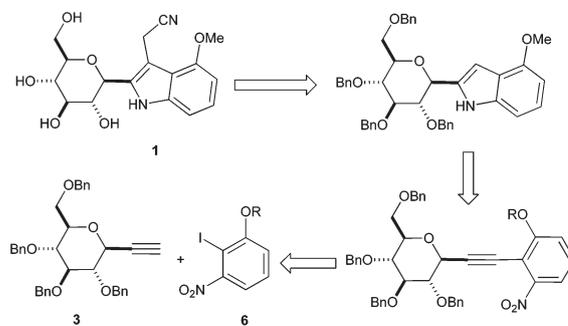
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Indole-3-acetonitrile-4-methoxy-2-*C*- β -D-glucopyranoside (**1**), a novel *C*-glycoside from *Isatis indigotica* with important cytotoxic activity, has been prepared in ten steps from ethynyl- β -*C*-glycoside **3** and 2-iodo-3-nitrophenyl acetate **6**. Key steps in the synthesis include a Sonogashira coupling and a CuI-mediated indole formation. NMR spectroscopic data for synthetic **1** differs from that reported for the natural product. A revised structure for the natural product, containing an alternate carbohydrate substituent, is proposed.

C-aryl glycosides are a class of natural products that exhibit a range of important biological properties.¹ Numerous members of this family display potent antitumor, antiviral, and antibiotic activities,² and there is ample experimental evidence that *C*-aryl glycosides bind duplex DNA.³

Two novel alkaloids recently isolated from the roots of the plant *Isatis indigotica* possess an indole-*C*-glycoside core.⁴ Indole-3-acetonitrile-4-methoxy-2-*C*- β -D-glucopyranoside (**1**, Fig. 1) displays cytotoxic activity against human myeloid leukemia HL60 cells (IC₅₀ = 1.3 mM) and human liver cancer HepG2 cells (IC₅₀ = 2.1 mM). The structural isomer of **1**, *N*-methoxy-indole-3-acetonitrile-2-*C*- β -D-glucopyranoside (**2**), shows cytotoxic activity against both HL60 cells (IC₅₀ = 5.1 mM) and human myeloid leukemia Mata cells (IC₅₀ = 12.1 mM). In view of its promising biological profile, and with the ultimate aim of exploring the DNA-binding properties of indole-*C*-glycosides, we decided to undertake a total synthesis of **1**.

We envisioned that the crucial linkage between the indole and glycoside moieties could be fashioned from protected alkynyl-*C*-glycoside **3** and 2-iodo-3-nitrophenol **6** through a Sonogashira coupling, nitro group reduction, and intramolecular amine-alkyne cyclization sequence (Fig. 2). Subsequent installation of the acetonitrile moiety under standard conditions and deprotection was envisaged to provide the natural product.

Fig. 1 Indole *C*-glycosides from *Isatis indigotica*.Fig. 2 Retrosynthetic analysis of **1**.

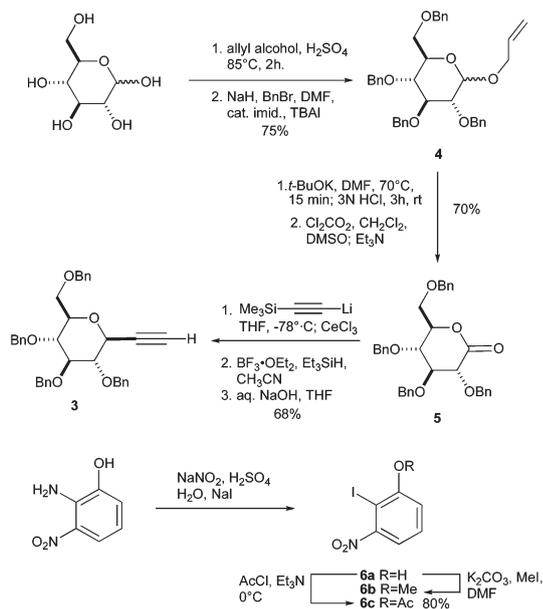
The assembly of the alkynyl-*C*-glycoside coupling partner **3**, possessing the requisite β -stereochemistry at the anomeric carbon, required efficient access to 2,3,4,6-tetra-*O*-benzyl gluconolactone **5** (Scheme 1).

Allylation of dextrose under acidic conditions,⁵ followed by exhaustive benzylation provided allyl glycoside **4** as a mixture of *C*-1 anomers in 75% yield. Removal of the allyl ether by standard base-mediated olefin isomerization and enol ether hydrolysis⁶ furnished 2,3,4,6-tetra-*O*-benzyl glucose, which upon Swern oxidation⁷ provided lactone **5**. Following literature precedent,^{8,9} reaction of **5** with lithium (trimethylsilyl)acetylide in the presence of CeCl₃ led to an intermediate lactol which was immediately reduced with BF₃·OEt₂-Et₃SiH to provide the silyl-protected alkynyl glycoside. Subsequent treatment with aqueous NaOH gave rise to alkyne **3**.⁹

The synthesis of the aryl iodide coupling partner commenced from commercially available 2-amino-3-nitrophenol; diazotization¹⁰ in the presence of NaI furnished iodide **6a**, which could be methylated (K₂CO₃, CH₃I, DMF) or acylated (AcCl, Et₃N, DCM, 0 °C) to give **6b** or **6c**, respectively.

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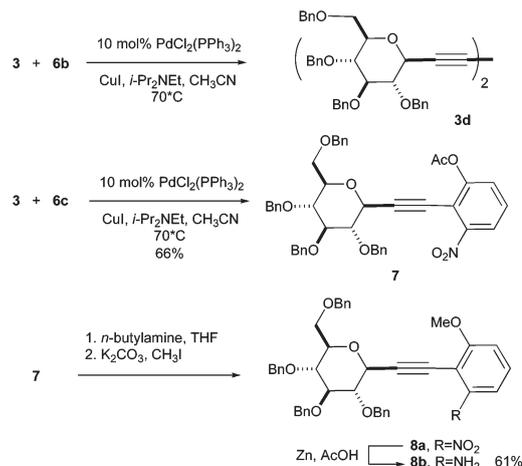
Scheme 1 Preparation of coupling fragments **3** and **6**.

Initial attempts at coupling methyl ether **6b** with alkyne **3** under standard Sonogashira conditions¹¹ met with limited success (Scheme 2). The major product isolated in these reactions was invariably symmetrical diyne **3d**. We hypothesized that, due to the electron-donating character of the methoxy substituent, oxidative addition of the palladium catalyst to **6b** was slow relative to alkyne dimerization. Gratifyingly, employing acetoxyaryl iodide **6c** in the cross-coupling reaction cleanly gave rise to alkyne **7** in 66% yield with minimal formation of dimer **3d**. Aminolysis of the acetate ester, followed by hydroxyl methylation and reduction of the nitro group provided aniline **8b** in 61% yield over three steps.

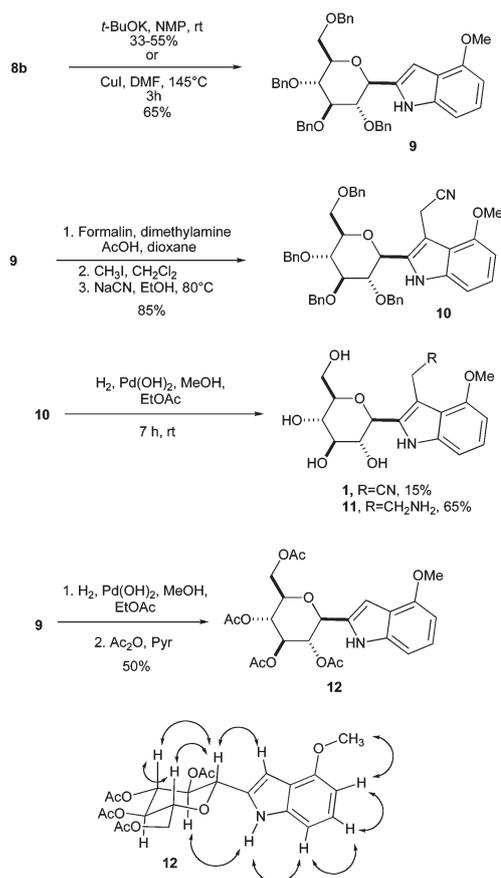
Next, a base-mediated indolization was attempted using Knochel's *t*-BuOK–NMP system (Scheme 3).¹² Treatment of **8b** with potassium *tert*-butoxide in NMP for 15 minutes furnished variable yields of indole *C*-glycoside **9** in the range of 33–55%. Prolonged exposure to the reaction conditions resulted in significant substrate decomposition. A superior protocol for producing **9** reproducibly involved exposure of **8b** to excess CuI (2–5 equivalents) at 145 °C in DMF for 2 hours.¹³ These conditions allowed indole **9** to be secured in 65% yield.

With **9** in hand, we next tested installation of the acetonitrile moiety *via* a three-step protocol.¹⁴ Treatment of **9** with formalin, diethylamine, and acetic acid overnight, isolation and subjection of the crude amine to methyl iodide in CH₂Cl₂, followed by refluxing the resulting quaternary ammonium salt with sodium cyanide in ethanol (80 °C), gave rise to nitrile **10** in 85% overall yield. However, attempted deprotection of the benzyl ether protecting groups by hydrogenolysis over Pearlman's catalyst¹⁵ led to the formation of significant quantities of amine **11** in addition to desired nitrile **1**. Hydrogenolysis of **9** instead, followed by alcohol acylation provided peracetate **12**, the three-dimensional structure of which was confirmed by NOESY spectroscopy.

An alternative, higher-yielding route to **1** was secured *via* persilyl derivative **13** (Scheme 4), prepared in 67% yield from **9** (H₂, Pd(OH)₂; TBS-Cl, imid, DMF, 60 °C). Acetonitrile

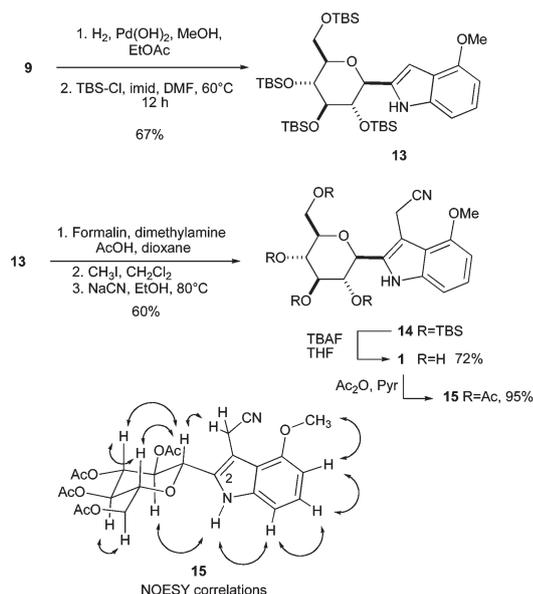


Scheme 2 Sonogashira fragment coupling.



Scheme 3 *C*-glycosyl indole synthesis and attempted deprotection; NOESY correlations for **12**.

installation under the aforementioned conditions gave rise to nitrile **14** in 60% overall yield. Removal of the silyl ether protecting groups was accomplished in 72% yield by exposure of **14** to TBAF (5 equiv) in THF for 9 hours, providing synthetic **1** as an amorphous white solid. The ¹H and ¹³C NMR data for synthetic **1** (recorded in acetone-*d*₆) differed from those reported by Hu *et al.*⁴ for natural **1** (Fig. 3). Proton and carbon assignments



Scheme 4 An alternative route to **1** and structure assignment of **15**.

Position	1 natural		1 synthetic	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1		11.26 (s)		10.23 (s)
2	121.8		133.1	
3	110.8		102.2	
4	153.2		154.2	
4 α	116.0		118.0	
5	99.7	6.54 (d, 7.8)	99.5	6.52 (d, 7.6)
6	123.9	7.09 (dd, 8.2, 7.8)	122.7	7.01 (dd, 8.0, 7.6)
7	104.7	6.95 (d, 8.2)	104.7	6.94 (d, 8.4)
7 α	137.9		137.2	
α	14.9	4.10 (s)	13.9	4.05 (s)
β	119.5		119.2	
1'	87.5	4.33 (d, 9.1)	74.5	4.64 (d, 8.8)
2'	72.2	2.76 (m)	74.5	3.60-3.49 (m)
3'	77.8	3.13 (m)	78.4	3.60-3.49 (m)
4'	69.5	2.96 (m)	70.2	3.60-3.49 (m)
5'	81.0	3.13 (m)	80.7	3.60-3.49 (m)
6'	61.0	3.67 (m)	61.8	3.85 (d, 13.3)
		3.43 (m)		3.73 (dd, 12.0, 4.0)
OCH $_3$	69.6	3.88 (s)	54.7	3.91 (s)

Fig. 3 Comparison of NMR chemical shift data for natural (ref. 4) and synthetic **1** recorded in acetone- d_6 .

for synthetic **1** were obtained from COSY, HSQC, and HMBC experiments, which also provided support for the hydrogen-carbon and carbon-carbon connectivity of the molecule. To further confirm the structure of our synthetic material, compound **1** was converted to peracetate **15** (Ac_2O , Pyr, 95%) and COSY and NOESY experiments were performed. Analysis of the cross-peaks in the NOESY spectrum of **15** revealed a connectivity pattern consistent with the findings for compound **12**: a glucopyranosyl carbohydrate moiety is attached at the C.2 carbon atom of the indole ring.

Careful study of the spectroscopic data in Fig. 3 indicates that major differences in chemical shift between synthetic and natural

1 occur for protons on the carbohydrate substituent and for protons and carbon atoms near the site of attachment of the sugar moiety to the indole ring. The 9.1 Hz coupling constant^{16a} reported for H1' of the natural product suggests that the carbohydrate moiety is indeed a hexopyranose, and likely a diastereomer of synthetic **1** such as allose (the C-3 epimer) or galactose (the C-4 epimer).^{16b}

In summary, we have developed a concise route to indole-3-acetonitrile-4-methoxy-2-C- β -D-glucopyranoside, the proposed structure of a natural indole C-glycoside from *Isatis indigotica*. Comparison of spectroscopic data for synthetic and natural **1** indicate that the natural product likely contains a diastereomeric hexopyranose moiety. Preparation of the galactopyranose- and allopopyranose-containing indole C-glycosides is underway and comparisons of their spectroscopic data with that of the natural material will be reported in due course.

Acknowledgements

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Notes and references

- M. R. Hansen and L. H. Hurley, *Acc. Chem. Res.*, 1996, **29**, 249.
- U. Hackzell and G. D. Daves, *Prog. Med. Chem.*, 1985, **22**, 1.
- (a) M. J. Cairns and V. Murray, *Biochem. Mol. Biol. Int.*, 1998, **46**, 267; (b) L. C. Hardman and V. Murray, *Biochem. Mol. Biol. Int.*, 1997, **42**, 349; (c) V. Murray, A. G. Moore, C. Matias and G. Wickham, *Biochim. Biophys. Acta, Gene Struct. Expression*, 1995, **1261**, 195; (d) A. S. Prakash, A. G. Moore, V. Murray, C. Matias, W. D. McFadyen and G. Wickham, *Chem.-Biol. Interact.*, 1995, **95**, 17; (e) N. Kamei, T. Sekiguchi, M. Tsuboi, S. Ishii, E. Ohtsubo and Y. Maeda, *Nucleic. Acids Res.*, 1994, **31**, 87; (f) D. Sun, M. Hansen and L. Hurley, *J. Am. Chem. Soc.*, 1995, **117**, 2430.
- Y. Wu, Z.-X. Zhang, H. Hu, D. Li, G. Qiu, X. Hu and X. He, *Fitoterapia*, 2011, **82**, 288.
- C. S. Schindler, L. Bertschi and E. M. Carreira, *Angew. Chem., Int. Ed.*, 2010, **49**, 9229.
- (a) P. A. Gent and R. Gigg, *Carbohydr. Res.*, 1976, **49**, 325; (b) P. Rollin and P. Sinay, *J. Chem. Soc., Perkin Trans. 1*, 1977, 2513.
- A. J. Mancuso, S.-L. Huang and D. Swern, *J. Org. Chem.*, 1978, **43**, 2480.
- (a) T. Imamoto, *Pure Appl. Chem.*, 1990, **62**, 747; (b) J. Alzeer and A. Vasella, *Helv. Chim. Acta*, 1995, **78**, 177.
- T. Lowary, M. Meldal, A. Helmboldt, A. Vasella and K. Block, *J. Org. Chem.*, 1998, **63**, 9657.
- M. G. Banwell, M. T. Jones, D. T. Loong, D. W. Lupton, D. M. Pinkerton, J. K. Ray and A. C. Willis, *Tetrahedron*, 2010, **66**, 9252.
- K. Sonogashira, *J. Organomet. Chem.*, 2002, **653**, 46.
- A. Rodriguez, C. Koradin, W. Dohle and P. Knochel, *Angew. Chem., Int. Ed.*, 2000, **39**, 2488.
- J. Ezquerra, C. Pedregal, C. Lamas, J. Barluenga, M. Perez, M. A. Garcia-Martin and J. M. Gonzalez, *J. Org. Chem.*, 1996, **61**, 5804.
- V. Perez-Alvarez, M. S. Morales-Rios, E. Hong and P. Joseph-Nathan, *J. Pharm. Pharmacol.*, 1997, **49**, 246.
- W. M. Pearlman, *Tetrahedron Lett.*, 1967, 1663.
- (a) Hu *et al.* report two separate coupling constants for H1' in ref. 2: 9.1 Hz and 9.5 Hz (b) The >9 Hz coupling constant for H1' in the natural product is indicative of an equatorial hydroxyl group at C.2 of the carbohydrate; thus a mannopyranose unit is not a likely candidate for the carbohydrate substituent of the natural product.