Total synthesis of indole-3-acetonitrile-4-methoxy-2-\(\beta\)-d-glucopyranoside. Proposal for structural revision of the natural product†

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Indole-3-acetonitrile-4-methoxy-2-\(\beta\)-d-glucopyranoside (1), a novel \(\beta\)-glycoside from Isatis indigotica with important cytotoxic activity, has been prepared in ten steps from ethynyl-\(\beta\)-C-glycoside 3 and 2-iodo-3-nitrophenyl acetate 6. Key steps in the synthesis include a Sonogashira coupling and a \(\text{CuI}\)-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. Numerous members of this family display potent antitumor, antiviral, and antibiotic activities, and there is ample experimental evidence that C-aryl glycosides bind duplex DNA.

Two novel alkaloids recently isolated from the roots of the plant Isatis indigotica possess an indole-\(\beta\)-glycoside core. Indole-3-acetonitrile-4-methoxy-2-\(\beta\)-d-glucopyranoside (1, Fig. 1) displays cytotoxic activity against human myeloid leukemia HL60 cells (IC\(_{50}\) = 1.3 mM) and human liver cancer HepG2 cells (IC\(_{50}\) = 2.1 mM). The structural isomer of 1, \(N\)-methoxy-indole-3-acetonitrile-2-\(\beta\)-d-glucopyranoside (2), shows cytotoxic activity against both HL60 cells (IC\(_{50}\) = 5.1 mM) and human myeloid leukemia Mata cells (IC\(_{50}\) = 12.1 mM). In view of its promising biological profile, and with the ultimate aim of exploring the DNA-binding properties of indole-\(\beta\)-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-\(\beta\)-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.

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

Allylation of dextrose under acidic conditions 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 hydrolysis furnished 2,3,4,6-tetra-O-benzyl glucose, which upon Swern oxidation provided lactone 5. Following literature precedent, reaction of 5 with lithium (trimethylsilyl)acetide in the presence of \(\text{CeCl}_3\) led to an intermediate lactol which was immediately reduced with \(\text{BF}_3\cdot\text{OEt}_2\) to provide the silyl-protected alkynyl glycoside. Subsequent treatment with aqueous \(\text{NaOH}\) gave rise to alkyne.

The synthesis of the aryl iodide coupling partner commenced from commercially available 2-amino-3-nitrophenol; diazotization in the presence of \(\text{NaI}\) furnished iodide 6a, which could be methylated (\(\text{K}_2\text{CO}_3\), \(\text{CH}_3\text{I}\), DMF) or acylated (\(\text{AcCl}\), \(\text{Et}_3\text{N}\), DCM, 0 °C) to give 6b or 6c, respectively.
Initial attempts at coupling methyl ether 6b with alkyne 3 under standard Sonogashira conditions 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). 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. 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. 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 catalyst 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, prepared in 67% yield from 9 (H2, Pd(OH)2; TBS-Cl, imid, DMF, 60 °C). Acetonitrile 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-d6) differed from those reported by Hu et al.4 for natural 1 (Fig. 3). Proton and carbon assignments...
experiments, which also provided support for the hydrogen moity of the sugar moiety to the indole ring. The 9.1 Hz coupling constant 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).

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.

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Notes and references

16 (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 C2 of the carbohydrate; thus a manno-pyranose unit is not a likely candidate for the carbohydrate substituent of the natural product.