Total Synthesis and Structural Verification of Isatindigotindoline C

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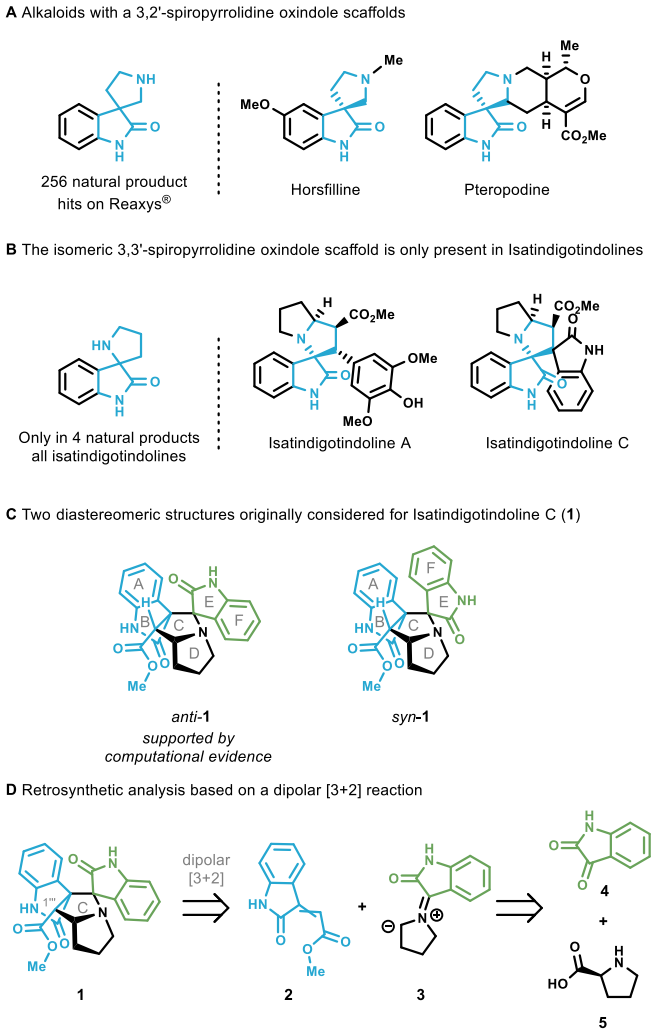
Abstract: Total synthesis of the polycyclic alkaloid isatindigotindoline C is achieved in two steps using an *exo*-selective [3+2]-dipolar cycloaddition. The synthesis verifies the originally computationally assigned relative stereochemistry.

Introduction

Isatindigotindolines are a structurally unique family of 3,3'-spiropyrrolidine oxindole alkaloids isolated by Huang and Song *et. al.* in 2018 from leaves of *Isatis indigotica* plant used in traditional and folk medicine in Asia.[1] The isatindigotindolines caught our attention due to our continued interest in the synthesis of bioactive nitrogenous compounds.[2] During the isolation studies, several isatindigotindolines were shown to inhibit β-amyloid aggregation.[1] Alongside with their demonstrated potential use in biology, the isatindigotindoline alkaloids *are the first examples of secondary metabolites* with a 3,3'-spiropyrrolidine oxindole core (Scheme B), this is a sharp contrast to the abundance of alkaloids with the isomeric 2,3'-spiropyrrolidine oxindole structure (Scheme 1A). In addition, the isatindigotindolines constitute a rare example of an entire alkaloid family occurring naturally as racemates.[3]

The structurally most complex member of this alkaloid family, (±)-isatindigotindoline C (**1**), has an *bis*-oxindole core. The relative stereochemistry of the *bis*-oxindole core of **1** proved non-trivial to assign during the original isolation and structure determination studies. Correlating experimental ^{13}C shifts with computed values gave very similar regressions for both *anti* (**1**) and *syn* (**1'**) bisoxindole diastereomers (R^2 = 0.9989 vs. R^2 = 0.9984 respectively). These NMR correlations, in combination with comparisons to

computed ECD curves allowed the relative configuration to be assigned *anti*.[1] To verify this computation-assisted stereochemistry assignment, and to gain synthetic access to the chemically and biologically intriguing family of isatindigotindolines, we embarked on the total synthesis of the proposed structure of **1**.



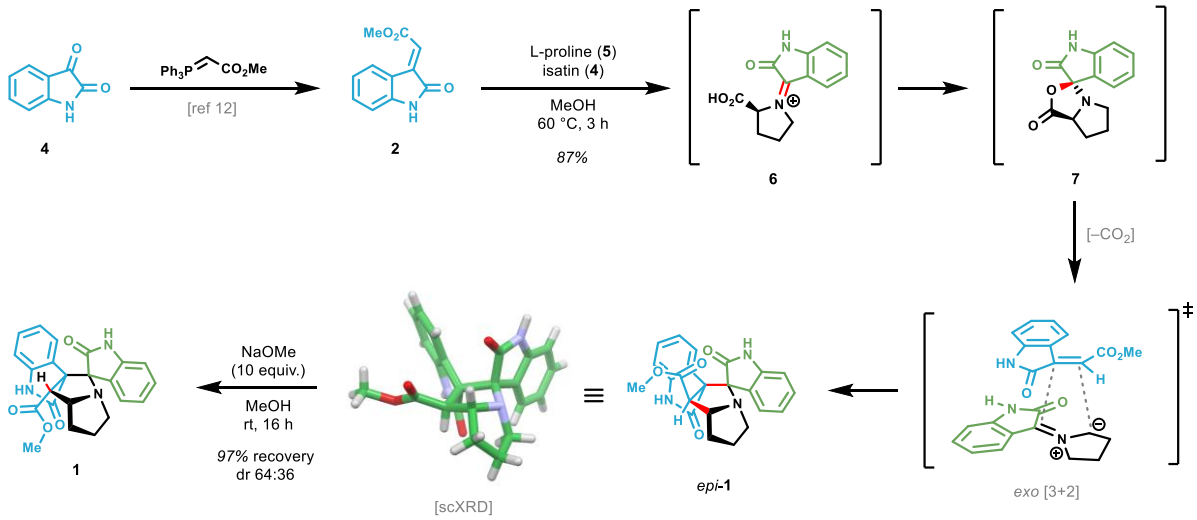
Scheme 1: A The 3,2'-spiropyrrolidine oxindole scaffold is a common motif in many alkaloids (256 hits on Reaxys database). **B** The isomeric 3,3'-spiropyrrolidine oxindole scaffold is present only in the 4 solitary isatindigotindoline alkaloids. **C** Two diastereomeric structures originally considered for isatindigotindoline C (*anti*-**1** and *syn*-**1**) differ in the relative configuration of the bisoxindole unit.[1] **D** Proposed retrosynthesis of the proposed structure of **1** based on a 1,3-dipolar cycloaddition.

Overall **1** displays a formidable synthetic challenge with four contiguous stereogenic centers, including a quaternary all-carbon stereogenic center, and six rings all compacted around a central pyrrolidine ring C (Scheme 1D). As a corollary, retrosynthetically disconnecting the principal ring C of **1** in a 1,3-dipolar cycloaddition fashion results in marked overall simplification in complexity (Scheme 1D). The resulting azomethine ylide **3** would be formed in a decarboxylative condensation between isatin (**4**) and proline (**5**).[4] This approach is in line with the hypothetical biosynthetic route and also agrees with the general tendency of racemic natural products to arise from the facile cyclizations of achiral precursors.[1,3] To clear the methyl ester α-stereogenic center of **1**, a (*Z*)-methyleneindoline ester (**2**) dipolarophile would be needed, however their preparation is tedious and requires harsh conditions.[5] Alternatively, we envisioned that an *exo*-1,3-dipolar cycloaddition between (*E*)-methyleneindoline ester **2**, easily prepared from isatin using Wittig olefination, and the azomethine ylide **3** would form *epi*-**1**, which could in turn be epimerized to natural Isatindigotindoline C (**1**) in base.[6]

Results and Discussion

With this plan at hand, we prepared the α,β-unsaturated methyleneindoline dipolarophile (*Z*)-**2** according to a known literature procedure.[7] Heating methyleneindoline **2** with proline (**5**) and isatin (**4**) (1:1:1 molar ratio) in MeOH readily precipitated the desired *exo* cycloaddition product *epi*-isatindigotindoline C (*epi*-**1**) as a white flocculant solid (1.7 g, 87% yield). The relative *anti* stereochemistry of the bisoxindole core, and the configuration of the methyl ester α-stereogenic center in *epi*-**1** were reliably established from single crystal x-ray data (Scheme 2).

Treating *epi*-**1** with K_2CO_3 in THF resulted only in partial epimerization (dr 96:4). Using a stronger base, NaOMe in MeOH, gave a synthetically viable dr of 64:36 (**1** to *epi*-**1**) with 97% mass recovery. Careful flash chromatography furnished an analytically pure sample of **1**. The ^{1}H and ^{13}C NMR data of synthetic Isatindigotindoline C (**1**) in were in full agreement with those reported for the natural product (see SI).[1]



Scheme 2: Total synthesis of *epi*-isatindigotindoline C (*epi*-**1**) and isatindigotindoline C (**1**).

Conclusion

In summary, we have achieved the first total synthesis of isatindigotindoline C (**1**) in two steps from the known ester **2**. Synthetic isatindigotindoline C (**1**) is identical to the natural product based on NMR data which, together with the single crystal X-ray structure of the precursor *epi*-**1**, corraborates the original computation-assisted structural assingment. The synthetic route also supports the postulated biosynthetic pathway, and suggests the key dipolar cycloaddition takes place in an *exo* fashion controlled by steric repulsion as opposed to secondary orbital interaction favored *endo* mode in the isoelectronic Diels–Alder reactions. Considering the ease at which the key dipolar cycloaddition proceeds, it is likely that additional secondary metabolites containing the 3,3‘-spiropyrrolidine oxindole scaffold remain to be discovered.

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