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A convenient synthesis of 3-methyleneoxindoles: cytotoxic metabolites of indole-3-acetic acids

Sharon Rossiter*

Gray Cancer Institute, PO Box 100, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, UK

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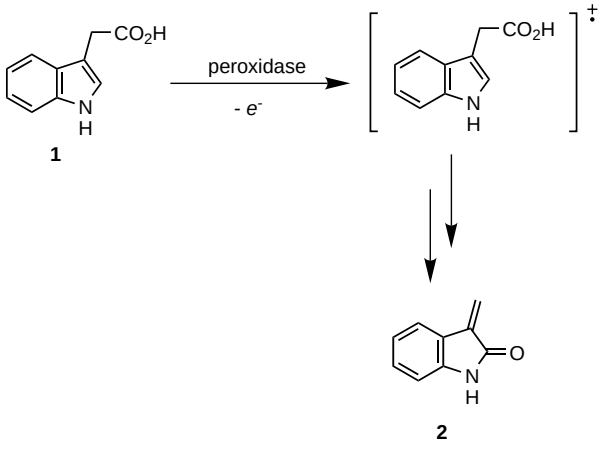
Abstract—3-Methyleneoxindole is a cytotoxic metabolite of indole-3-acetic acid with potential for use in cancer therapy. This species and ring-substituted analogues are conveniently synthesised from the corresponding isatins via a Peterson olefination.

1. Introduction

3-Methyleneoxindole **2** is a product of plant peroxidase oxidation of the naturally-occurring auxin indole-3-acetic acid, IAA **1** (Scheme 1).[1–3] This interesting species does not appear to have any auxin or anti-auxin properties,[4,5] but has been shown to be toxic to bacteria.[6] More recent studies have shown that oxidation of IAA with horseradish peroxidase generates a species believed to be **2** that exhibits toxicity to mammalian cells and causes damage to plasmid DNA;[7] therefore IAA, which is non-toxic, is potentially useful as a prodrug for enzyme targeted cancer therapy.[7,8] Our recent studies on the oxidation of substituted IAAs[8] have led to a number of questions about the mechanism of toxicity and the nature of the cytotoxic species in each case, and so we required pure samples of **2** and substituted analogues for further study.



Scheme 1. Horseradish peroxidase oxidation of IAA.

The previous literature synthesis of **2** involves the NBS bromination and oxidation of the parent IAA to give 3-bromooxindole-3-acetic acid, followed by aqueous or base-catalysed elimination to give **2**.[9] However, it is reported that **2** was only isolated at ca. 90% purity and is unstable in solutions above 10^{-4} M, hindering further purification.[9] In our hands this synthesis proved to be unreliable for the generation of high-quality samples of **2**.

Kornet attempted to make a number of related 3-methyleneoxindole precursors with a view to generating **2** by elimination under cellular conditions, but did not observe any cytotoxicity.[10] Other synthetic routes to this simple but interesting heterocycle do not appear to have been explored. Although other 3-alkylideneoxindoles have been synthesised by various methods, e.g. via a Wittig reaction of isatin[11] or condensation of an aldehyde with oxindole,[12] none of these routes appear to have been used for the synthesis of **2**, and in many cases the reaction conditions or the product purification methods would seem to rule out their suitability. Our preliminary investigation of the Wittig reaction between a methylenephosphorane and isatin under mild conditions was not successful.

Keywords: Peterson olefination; 3-methyleneoxindole; isatin.

* Present address: The Wolfson Institute for Biomedical Research, University College London, The Cruciform Building, Gower Street, London WC1E 6BT, UK. Tel.: +44 20 7679 6865; fax: +44 20 7679 6799; e-mail: s.rossiter@ucl.ac.uk

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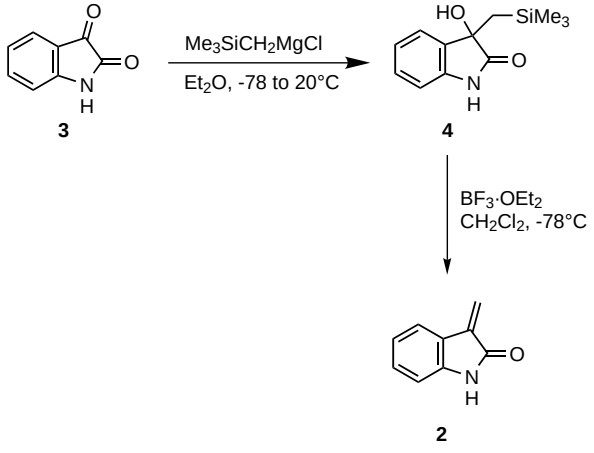
We report a simple synthesis that gives 3-methyleneoxindole in high purity and is easily adaptable for the synthesis of analogues corresponding to the oxidation products of substituted indole-3-acetic acids.

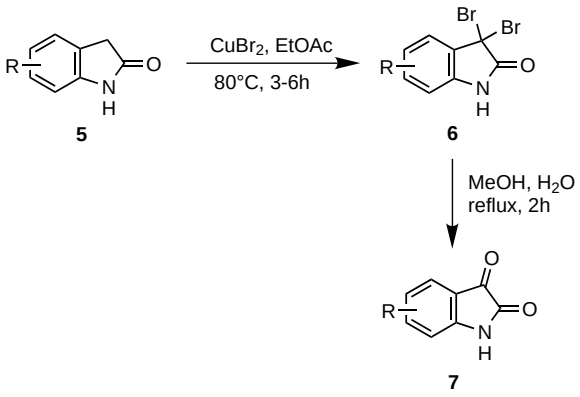
2. Results and discussion

The chosen two-step route utilises the versatile Peterson olefination of ketones via a β-silyl alcohol intermediate.[13] Reaction of isatin **3** with trimethylsilylmethylmagnesium chloride gave the β-silyl alcohol **4**. This is moderately stable (although aqueous workup should be avoided), and could be purified immediately by flash column chromatography. Pure 3-methyleneoxindole was then conveniently obtained by stirring **4** at −78 to 0°C with boron trifluoride (diethyl etherate complex) (Scheme 2). With careful workup, the product could be isolated in low to moderate yields at >99% purity (HPLC).

A number of analogues with electron-donating or electron-withdrawing groups were also synthesised (Table 1). Isatins that were not commercially available were synthesised from the corresponding oxindole **5** using an adaptation of Kraynack's method.[14] The 3,3-dibromooxindole **6** was obtained in almost quantitative yields by heating with copper(II) bromide,[15] which we found to be more convenient than the use of pyridinium tribromide, avoiding unwanted bromination on the benzene ring. The dibromooxindole was then heated in aqueous methanol to give the substituted isatin **7** (Scheme 3).



Scheme 2. Peterson olefination of isatin.



Scheme 3. Substituted isatins.

3. Methods

3.1. General procedure for the preparation of 3-hydroxy-3-trimethylsilylmethyloxindoles

Isatin (2–5 mmol) was suspended in dry diethyl ether (20 mL) and cooled to −78°C. Trimethylsilylmethylmagnesium chloride (2 equiv. of a 1 M solution in diethyl ether) was added, with stirring. The mixture was stirred at −78°C for 15 min, then allowed to warm to room temperature with stirring for a further 18 h. The reaction was quenched with methanol, and then the entire reaction mixture was concentrated in vacuo to give a red-brown solid. Purification by flash column chromatography (1:1 hexanes:ethyl acetate) gave 3-hydroxy-3-trimethylsilylmethyloxindole **4** as a white powder, yield 63%, mp 163–164°C; δH/ppm (60 MHz, DMSO-d_6) 7.49–7.14 (4H, m, ArH), 5.99 (1H, s, OH), 1.65 (2H, s, $SiCH_2$), 0.0 (9H, s, $SiMe_3$); m/z 235 (M^+), 220 (M^+−Me), 192; found: C, 61.29; H, 7.29; N, 5.95; $C_{12}H_{17}NO_2Si$ requires C, 61.24; H, 7.28; N, 5.95%.

3.2. General procedure for t he synthesis of 3-methyleneoxindoles

3-Hydroxy-3-trimethylsilylmethyloxindole (0.5–1 mmol) in dichloromethane (20 mL) was cooled to −78°C, and boron trifluoride diethyl etherate (5 equiv.) was added, with stirring. The mixture was stirred at −78°C for 2 h, and then at 0°C for a further 1 h. The mixture was poured into satd $NaHCO_3$, extracted with ether (2×100 mL), the organic layers washed with $NaHCO_3$ once more, dried over $MgSO_4$ and the solvent was evaporated to give pure 3-methyleneoxindole **2** (99–100% purity by HPLC) as a yellow powder, yield 38%, mp

Table 1. 3-Methyleneoxindoles prepared from isatins

Substituent	Yield, Grignard addition (%)	Yield, elimination step (%)	Mp (°C)
None	63	38	244–246 (dec.)
5-F	56	58	258–260 (dec.)
5-Cl	47	49	210–212 (dec.)
6-Cl	67	49	283–285 (dec.)
5-Me	55	36	244–250 (dec.)
4-Cl	57	41	264–268 (dec.)

244–246°C dec. (lit.[9] for a 90% pure sample 218°C dec.); δH/ppm (60 MHz, $CDCl_3$) 7.41–6.94 (4H, m, Ar-H), 6.39 (1H, s, methylene), 6.13 (1H, s, methylene); m/z 145 (100%, M^+). The UV spectrum was identical to that of 3-methyleneoxindole generated by the enzymatic oxidation of indole-3-acetic acid.

Our ability to synthesise a number of substituted 3-methyleneoxindoles at such high purity shows a significant advantage over the previous method.[9] We found that pure **2** could be stored for a few weeks and was sufficiently stable in aqueous solution at millimolar to micromolar concentrations to enable accurate concentration-dependent measurements of cytotoxicity and rates of reaction with cellular nucleophiles.[16]

4. Conclusions

We report a simple and flexible two-step synthesis of 3-methyleneoxindoles from readily available or easily synthesised isatins, furnishing these bioactive compounds at the high purity necessary for biological studies. The reactivity of these simple heterocycles could also be investigated further, possibly as part of a route to more complex indoles.

Acknowledgements

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