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Synthesis and characterization of a metabolite of Tamoxifen

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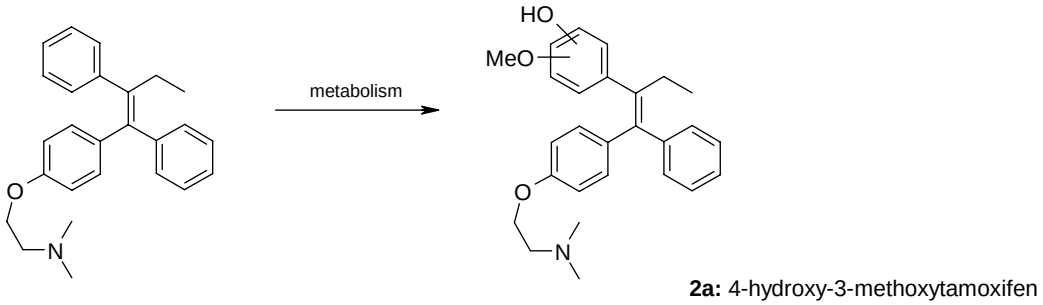
Introduction

The anti-estrogen Tamoxifen (**1**), generally used in the treatment of breast cancer, is listed as a doping substance on the WADA Prohibited List due to its selective estrogen receptor modulator capabilities. Treatment of the negative effects of anabolic androgenic steroid abuse is the rumoured reason for the ban of this substance.[1]

The structure of the main metabolite in urine has not been determined yet. GC-MS data suggested that hydroxymethoxytamoxifen is the most probable one. 4-Hydroxy-3-methoxytamoxifen (**2a**) has been successfully synthesized, but comparing this compound with a sample obtained from an excretion study, it could be shown that this compound was not the metabolite.[2] Therefore, it was suggested that 3-hydroxy-4-methoxytamoxifen (**2b**) should be synthesized in order to compare this compound with the sample obtained from an excretion study.



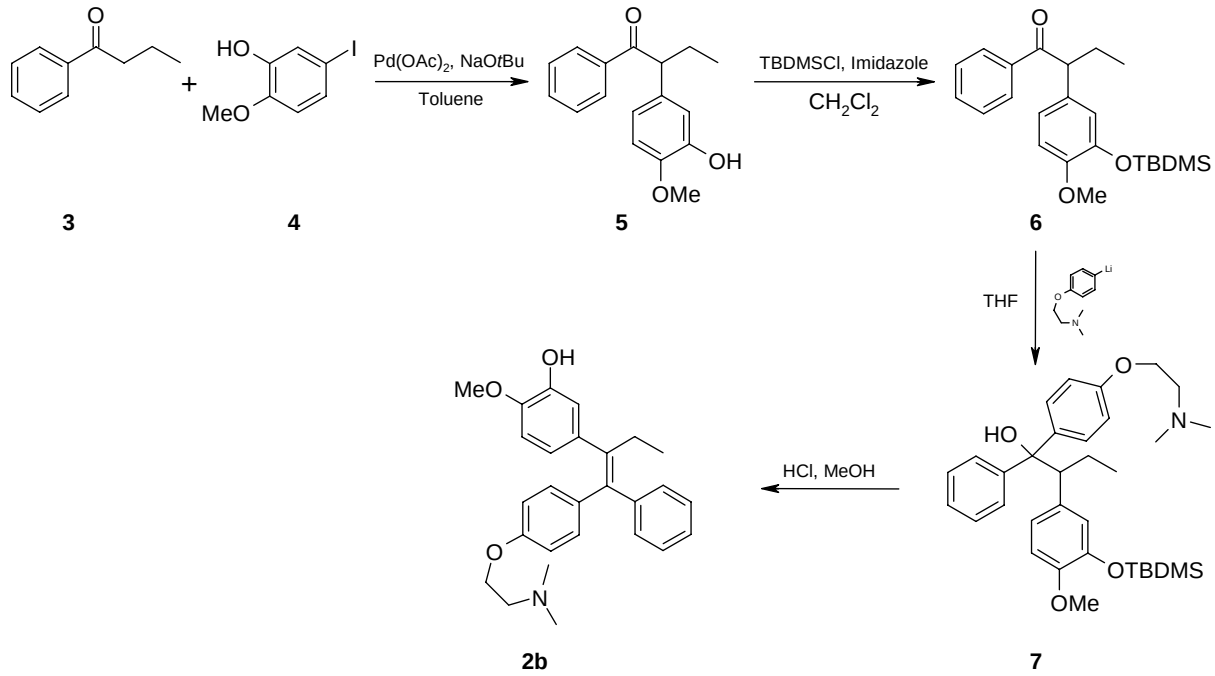
1 **2** **2a:** 4-hydroxy-3-methoxytamoxifen **2b:** 3-hydroxy-4-methoxytamoxifen

Synthesis of 3-hydroxy-4-methoxytamoxifen (2b)

Most Tamoxifen syntheses are based on the McMurry-coupling reaction of a benzophenon- and a propiophenon-derivative. These reactions usually result in good yields and are easy to be carried out.[3,4,5] In some cases it is even possible to carry out the synthesis in a stereospecific way and thus obtaining *E*- and *Z*-isomers selectively.[6] However, the three residues on the phenyl ring of the propiophenon-derivative were not stable enough for the

reaction conditions so that just decomposition of starting material could be observed in our case.

After experiments using Zn-organyls[3,7] or Wittig-reaction[7] and three-component-Suzuki-coupling,[8] the desired product could be obtained finally by the following "classical synthesis of Tamoxifen" pathway.[9,10] 5-Iodo-2-methoxyphenol (**4**) was reacted with *n*-butyrophenon (**3**) in a Pd-catalyzed reaction.[11] The following addition of a metal organyl was tested with several organyls, reaction conditions and additives – on compound **5** with the free hydroxy group as well as **6** with the silyl protected one. It turned out that the reaction could not be carried out successfully with Zn-organyls or Grignard-compounds, even by using HMPA, LiCl and $ZnCl_2$ as additives. By carrying out the reaction with lithium organyls, it could be shown that the reaction can be run successfully by using the protected educt **6** and LiCl as additive. After dehydration and deprotection of **7** catalyzed by HCl, the desired metabolite was obtained as an *E*/*Z* mixture, which could be separated by chromatography. The stereochemistry of the main product, which also was the desired one, could be determined by NOE NMR experiments.[6] Since an NOE between the ethyl group and the unsubstituted phenyl ring could be seen, the synthesized product was determined to have *Z*-configuration. Using this synthetic pathway, the desired metabolite could be obtained in an overall yield of 15.6 % starting from 5-iodo-2-methoxyphenol.

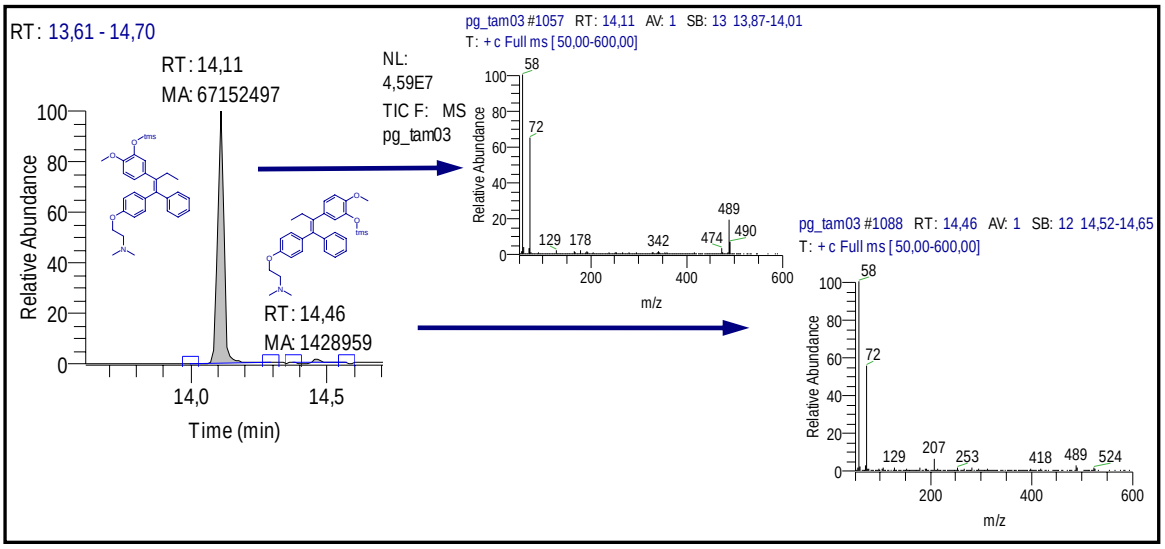


Analytical characterization of 3-hydroxy-4-methoxytamoxifen

GC-MS data of the mono-silyl derivative were recorded on a Thermo Quest Trace GC 2000 equipped with an AS 2000 autosampler and a Voyager mass detector. The conditions are summarized in the following table:

Flow parameters	Helium at 57 kPa
Injection parameters	3 µl at 10:1 split and 270°C
Temperature program	175°C, 3°C/min to 210°C; 25°C/min to 305°C for 2 min
Column	Restek RTX-1ms, 15m x 0.25 mm ID, 0.25 µm film
MS interface	280°C direct inlet
Ion source	EI+; 70 eV at 250°C
Quadrupol detection	Scan from 70 – 600; 2.5 scans/s, starting after 2 min

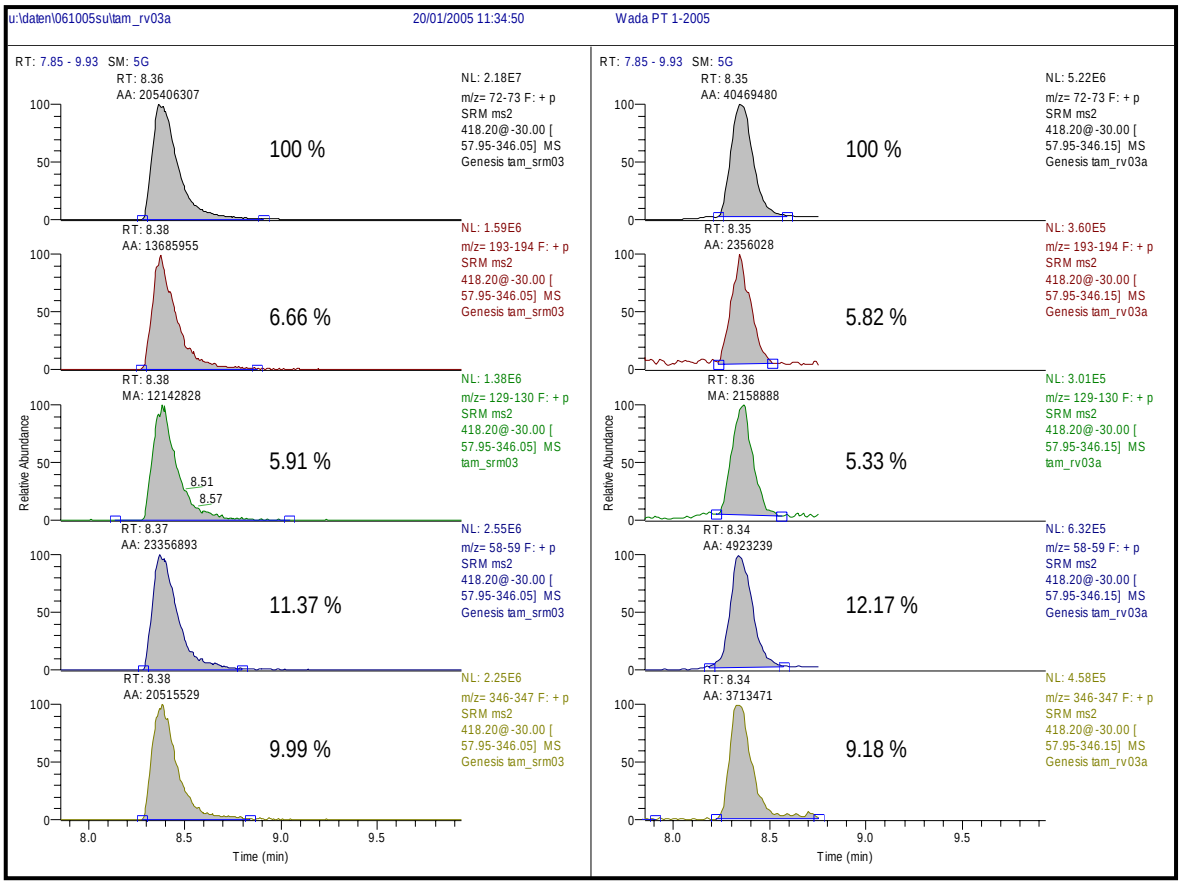
Two peaks were detected and could be characterized as *E* and *Z* – isoforms of 3-hydroxy-4-methoxytamoxifen. The ratio was detemined to be *Z* : *E* = 98 : 2.



Proof of authenticity by comparison with an excretion study

Mass transitions after LC-seperation of the main synthetic compound (*Z*-form) are compared with an excretion study with tamoxifen from the WADA 2005 PT-01 round. The criteria for identification according to the WADA technical document TD2003IDCR were applied.

Column (type and code):	Zorbax Eclipse XDB-C18 2.1x50mm 3.5µm
Precolumn (type and code):	Zorbax Eclipse XDB-C8 2.1x2.5mm 5 µm
Liquid phase	D: Water 0.2% Formic Acid, C: Methanol 0.1% Formic Acid
Internal Standard (ISTD):	Methyltestosterone
Instrument (type and code):	LC MS01 (Surveyor LC and Quantum Discovery MS)



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