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A convenient synthesis of (*Z*)-4-hydroxy-*N*-desmethyltamoxifen (endoxifen)

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ABSTRACT

A mixture of the (*Z*)- and (*E*)-isomers of 4-hydroxy-*N*-desmethyltamoxifen was conveniently prepared in four steps. These geometrical isomers were then neatly separated by semi-preparative Reverse Phase High Performance Liquid Chromatography (RP-HPLC) using specified conditions. Additionally, the isolated *E*-isomer could be equilibrated in aqueous strong acid in acetonitrile or trifluoroacetic acid/dichloromethane to give a clean 1:1 mixture of *Z*/*E* isomers that was re-subjected to HPLC separation. In this way, most of the undesired (*E*)-isomer could be readily converted to the desired (*Z*)-isomer providing quick access to over 200 mg quantities of pure endoxifen (*Z*-isomer), a potent antiestrogenic metabolite of tamoxifen traditionally used in breast cancer treatment.

1-[4-(2-Dimethylaminoethoxy)-phenyl]-1,2-diphenylbut-1(*Z*)-ene (tamoxifen (TAM)) is a non-steroidal antiestrogen drug widely used for breast cancer treatment.[1] The pharmacological profiles of tamoxifen indicate that it elicits its anti-cancer activity through its active metabolites 4-hydroxytamoxifen (4-OH-TAM) and its desmethyl analogue endoxifen that are generated by the action of hepatic CYP 2D6 and 3A4 isozymes on tamoxifen after hydroxylation followed by N-demethylation.[2,3] It is established that some patients do not derive therapeutic benefit from the administration of tamoxifen or even suffer relapses because of their inherent genotypic constraints.[3] Co-administration of certain drugs (e.g., paroxetine or fluoxetine) also have demonstrated interactive inhibitory effect on CYP 2D6 and other cytochrome P450 enzymes. This of lack of activity results from reduced availability of therapeutic levels of the active metabolite (*Z*)-desmethyl-4-hydroxytamoxifen (endoxifen).[4] Recently, Hawse and co-workers have shown that endoxifen is the actual anti-estrogenic drug that works by degrading the estrogen receptor and not by its inhibition.[3] Therefore, in order to facilitate human tissue studies as well to explore the possibility that it may be appropriate in special cases to consider a dose regimen that, instead of tamoxifen, includes the active metabolite endoxifen, relatively large quantities of endoxifen may be required. A search of the literature showed that while low level milligram quantities of the endoxifen have been purified by analytical HPLC,[5] synthesis and purification of relatively large quantities of endoxifen have not been reported. Herein, we report synthesis and purification protocols that have resulted in production of pure endoxifen in excess of 200 mg quantities for animal studies and tissue work in less than two weeks time. This work involved a short four-step synthesis of a mixture of endoxifen and the (*E*)-isomer and the use of semi-preparative reverse phase HPLC columns for their separation. Nevertheless, much larger quantities of endoxifen are expected to be conveniently generated by employing preparative columns.

Apart from a stereoselective synthesis of tamoxifen involving carbometalation of alkynylsilanes,[6] earlier reported syntheses of (*Z*)-4-hydroxytamoxifen, a precursor of endoxifen, were somewhat cumbersome and non-stereoselective.[7] A ground-breaking stereoselective synthesis of (*Z*)-4-hydroxytamoxifen by Gauthier and Labrie involved McMurry reaction as a key step.[8] Even though the synthesis of endoxifen was not reported, the authors managed to obtain a favorable 14:1 ratio of the (*E*)- and (*Z*)-isomers of 1-(4-hydroxyphenyl)-1-[4-(trimethylacetoxy)phenyl]-2-phenylbut-1-ene (**3b:4a**, Fig. 1) after reacting the monopivaloyl derivative of 4,4′-dihydroxybenzophenone (**1b**) with propiophenone in the McMurry reaction simply by manipulating proportions of $TiCl_4$ and Zn. The ratio 14:1 was enhanced to 100:1 by trituration of the crude with methanol. In our hands, the crude obtained after the McMurry reaction was directly chromatographed on silica gel to obtain desired (*E*)-stereoisomer in 88% yield. In this reaction, the phenolic and the ethyl components were shown to preferentially align *trans* to each other as precedented.[9] We carried out the reported four-step Gauthier–Labrie synthetic sequence through the intermediate **5** to (*Z*)-4-OH-TAM **3c**. Unfortunately, attempted demethylation of **5** or the (*Z*)-4-OH-TAM **3c** itself using drastic conditions (vinyl chloroformate in dioxane at 135–170 °C in sealed tube) as reported[5c] or ethyl chloroformate[5a,10a] in refluxing toluene produced a significantly stereoscrambled mixture of endoxifen and its (*E*)-isomer in low yield. In using some other milder carbamate-mediated *N*-demethylation conditions,[10b–d] it was noticed that lower yields and significant stereorandomization of the

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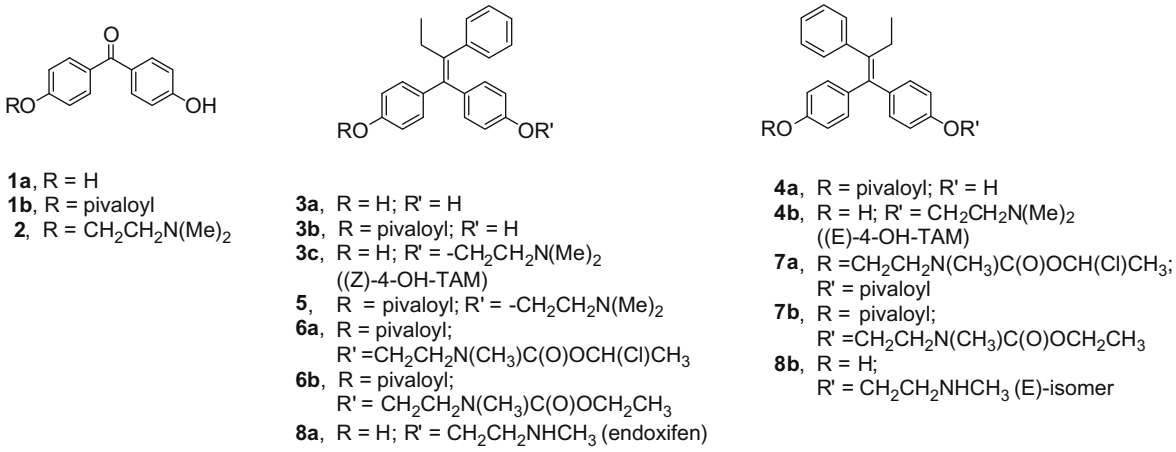
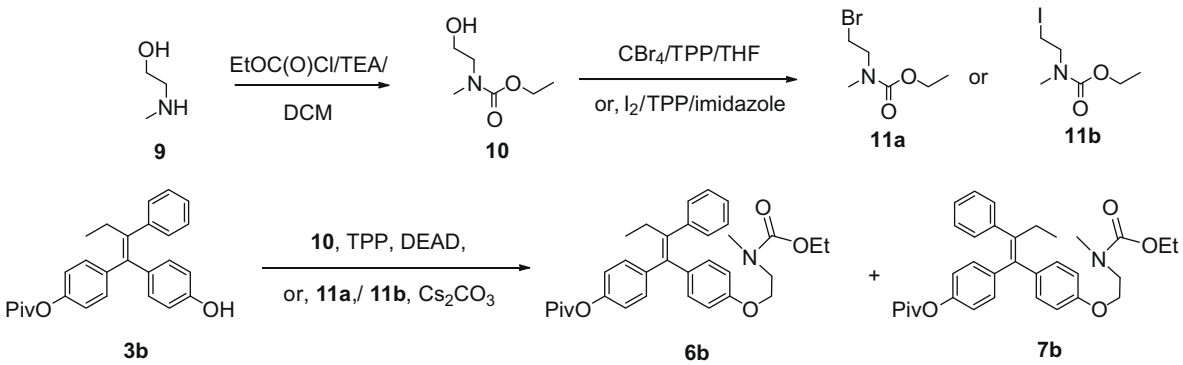


Figure 1. Endoxifen and reported intermediates for its synthesis.

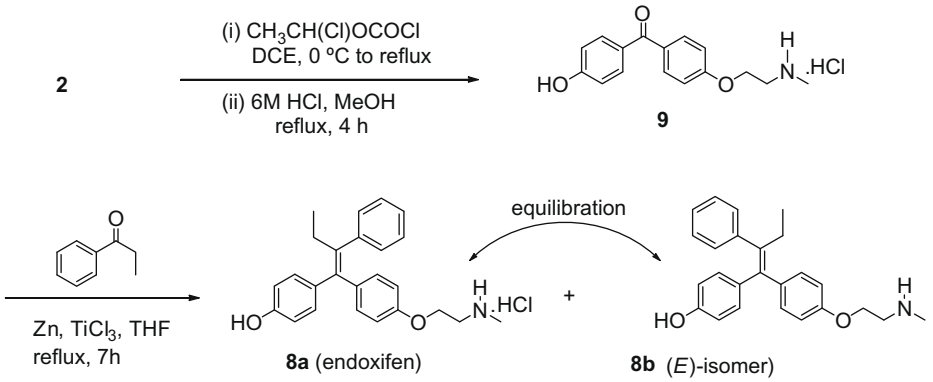
double bond could not be avoided. Of the various carbamate-mediated demethylation protocols, the best reaction conditions entailed heating a mixture of 2-chloroethyl chloroformate and (*Z*)-4-OH-TAM **3c** in dichloroethane which resulted in stereorandomization of the tetrasubstituted double bond to the extent of 20% furnishing a 4:1 mixture of the desired endoxifen carbamoyl precursor (**6a**) and its (*Z*)-isomer (**7a**) in 79% yield (Fig. 1). The mechanism of the isomerization during carbamate-mediated *N*-demethylation is currently speculative, albeit precedented.[5a] Since the components of this stereoisomeric mixture could not be separated, it was directly reacted with methyllithium at −78 °C in tetrahydrofuran which concomitantly cleaved carbamoyl and pivaloyl ester moieties to generate a 4:1 mixture of endoxifen (**8a**) and its (*E*)-isomer (**8b**). The overall six-step synthetic sequence furnished a mixture of endoxifen **8a** and its (*E*)-isomer **8b** in 26% combined yield.

With the hope of retaining stereochemical integrity during demethylation, we attempted to synthesize pure carbamoyl (*E*)-isomer of **6b** by reacting **3b** (Scheme 1) either with the ethyl (2-hydroxyethyl)(methyl)carbamate (**10**, prepared by reacting the alcohol with ethylchloroformate/triethylamine in DCM; cf. Supplementary data) under Mitsunobu conditions, or by S_N2 reaction between **3b** and 2-bromo or 2-iodo-derivative of ethyl (2-hydroxyethyl)(methyl)carbamate (prepared by reacting ethyl (2-hydroxyethyl)(methyl)carbamate **10** with CBr_4, triphenylphoshine/DCM to give **11a**, or with I_2/triphenylphoshine/imidazole/toluene to give **11b** under basic (Cs_2CO_3/DMF); cf. Supplementary data). While the Mitsunobu reaction completely retained stereochemistry of the substituted product **6b**, the basic conditions (Cs_2CO_3/DMF) employed during bromo- or iodo-displacement resulted in 30% stereoscrambling to give **7b**. This unwelcome reaction outcome suggested that both acidic (e.g., treatment with DCM/TFA mixture, vide infra) as well as the basic milieu compromised the stereochemical integrity of the electron-rich tetrasubstituted double bond. The labile nature of the stereochemistry under basic conditions was further evidenced when an attempt was made to achieve simultaneous removal of the pivaloyl and ethoxycarbonyl moieties from pure **6b** (derived from Mitsunobu reaction) with methyllithium under the usual mild (−78 °C) conditions. This reaction also produced ca. 30% undesired (*E*)-isomer **8b**. Similarly, when methyllithium was mixed with pure **8b** in THF at −78 °C, 30% of endoxifen **8a** was obtained. While the acid-catalyzed stereorandomization of the tetrasubstituted double bond is expected, the base-mediated partial isomerization during the ethoxycarbonyl removal may be explained by invoking the possibility of resonance in phenolate anion playing a part in generating a partial single bond character in the *para*-substituted sp^2-hybridized benzylic carbon of the phenol moiety.

Since it was important to rapidly produce over 200 mg of endoxifen for use in several ongoing anti-breast cancer investigations, the problem of separating the final 4:1 endoxifen:(*E*)-isomer mixture presented a challenge. Even though RP-HPLC separation of endoxifen from its (*E*)-isomer has been achieved in small quantities for in vitro studies,[5c–e] protocols for larger scale separation were not reported. Our attempts using the reported RP-HPLC conditions for larger scale separation using semi-preparative RP-HPLC columns and phosphate or triethylamine-containing buffers gave poor resolution of the endoxifen and its (*E*)-isomer. Additionally, silica gel chromatography using various eluents containing basic additives was unsuccessful. After considerable experimentation, success was finally realized when the RP-HPLC separation of the



Scheme 1.



Scheme 2. Modified synthesis of endoxifen.

stereoisomeric mixture was attempted with isocratic elution with a buffer containing 50% of 20 mM triethylammonium bicarbonate in acetonitrile at pH 8.8. This protocol separated the two peaks well apart even under significant column overloads (Vydac column, C-8, 2.2 × 25 cm, FR 8 mL/min: RT for endoxifen, 53 min; for (*E*)-isomer 81 min). The identities of the two geometrical isomers were confirmed by peak matching with reported NMR data[5a] as well as by its expected antiestrogenic activity.[3]

In spite of the fact that the six-step protocol for the synthesis of endoxifen can be achieved from the published synthetic procedures from its precursor 4-OH-TAM, the confounding problem of significant double bond isomerization during the demethylation still remained and necessitated HPLC purification. It was, therefore, deemed pragmatic to shorten the overall synthesis of endoxifen by doing away with protection/deprotection of the hydroxyl group of 4,4′-dihydroxybenzophenone (**1**) altogether. Our overall four-step strategy that continues to rely on the McMurry reaction[8] is given in Scheme 2. The *N*,*N*-dimethylethyl derivative 4,4′-dihydroxybenzophenone (**2**), made from 4,4′-dihydroxybenzophenone **1** in 46% yield was demethylated using 2-chloroethyl chloroformate-mediated demethylation methodology as described above in 83% overall yield. However, instead of decarbamoylation with methyllithium furnishing lower yield of the deprotected product, the intermediate 2-chloroethyl carbamate was decomposed with 6 M-HCl in refluxing methanol to give the secondary amine hydrochloride salt (**9**) in higher yield (83%, Scheme 2). The hydrochloride salt **9** was subjected to McMurry reaction with propiophenone furnishing a 90% chromatographed combined yield of a mixture of the endoxifen **8a** and the (*E*)-isomer **8b** in 1:3 ratio. Fortunately, this unfavorable ratio was readily and cleanly enhanced to 1:1 by heating the isomeric mixture in 6 N-HCl in aqueous acetonitrile for 6 h or by, more simply, stirring the mixture with 1:1 DCM/TFA for 1 h. The (*Z*)-and (*E*)-isomers were then separated using the RP-HPLC conditions as outlined above. Additionally, the undesired (*E*)-isomer (**8b**)-containing fractions obtained from the HPLC runs were combined and re-equilibrated cleanly to 1:1 mixture of endoxifen and **8b** either by heating with equal volume of 6 N-HCl at 60 °C for 4–6 h, or, by evaporating to dryness and stirring with 1:1 DCM/TFA at rt. In both cases, the equilibrated mixture was directly subjected to HPLC purification resulting in enhanced overall yield of the endoxifen. The remarkably large RT difference between the two isomers achieved under specified buffer conditions was critical to their successful larger scale separation because semipreparative RP-HPLC column could be safely overloaded. This protocol also enabled storage of large quantities of endoxifen as 1:1 *Z*/*E* mixture at −15 °C under dark for extended periods of time, pending fresh isolation of endoxifen as and when needed.

In sum, a short four-step methodology for rapid generation of endoxifen/(*E*)-isomer mixture, obtained in 34% overall yield, was combined with a highly efficient RP-HPLC protocol for separation of endoxifen from the (*E*)-isomer. This methodology was used to conveniently and rapidly prepare over 200 mg quantities of pure endoxifen as and when needed for animal and tissue studies.

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Supplementary data

Supplementary data (the spectroscopic and RP-HPLC chromatographic data as well as synthetic procedures for all the reported intermediates, and those of the final products) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.117.

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